Déterminants de la structure des communautés fongiques dans les forêts de Corse : rôle des perturbations et de la composition forestière

Adrien Taudière

To cite this version:

Adrien Taudière. Déterminants de la structure des communautés fongiques dans les forêts de Corse : rôle des perturbations et de la composition forestière. Interactions entre organismes. École pratique des hautes études - EPHE PARIS, 2016. Français. NNT : 2016EPHE3066 . tel-02102366

HAL Id: tel-02102366
https://theses.hal.science/tel-02102366

Submitted on 17 Apr 2019

École Pratique des Hautes Études

École Pratique des Hautes Études
Mention « Systèmes intégrés, environnement et biodiversité »
École doctorale de l'École Pratique des Hautes Études
Centre d'Écologie fonctionnelle et Évolutive / Équipe interactions biotiques
Et
Université de Montpellier

Déterminants de la structure des communautés fongiques dans les forêts de Corse

Rôle des perturbations et de la composition forestière

Par Adrien TAUDIERE

Thèse de doctorat d'Écologie

Sous la codirection de :
M. Christopher CARCAILLET, Directeur d'Études EPHE
et de M. Franck RICHARD, Maitre de conférences à l'Université de Montpellier

Version pré-soutenance

Devant un jury composé de :

M. Christopher CARCAILLET, Directeur des Études EPHE, LEHNA (UMR 5023) - Lyon
M. Philippe CHOLER, Chargé de recherches CNRS, LEA (UMR 5553 CNRS) - Grenoble
M. Thomas CURT, Directeur de recherches IRSTEA, UR EMAX - Aix-en-Provence
M. Anders DAHLBERG, Professor at the Swedish University of Agricultural Sciences - Uppsala
M. Fréderic MEDAIL, Professeur à Aix-Marseille Université, IMBE (UMR 6372) - Aix-en-Provence
M. Franck RICHARD, Maitre de conférences a l'UM, CEFE (UMR 5175) - Montpellier
Mme Elisa THEBAULT, Chargée de recherches CNRS, IEES (UMR 7618) - Paris

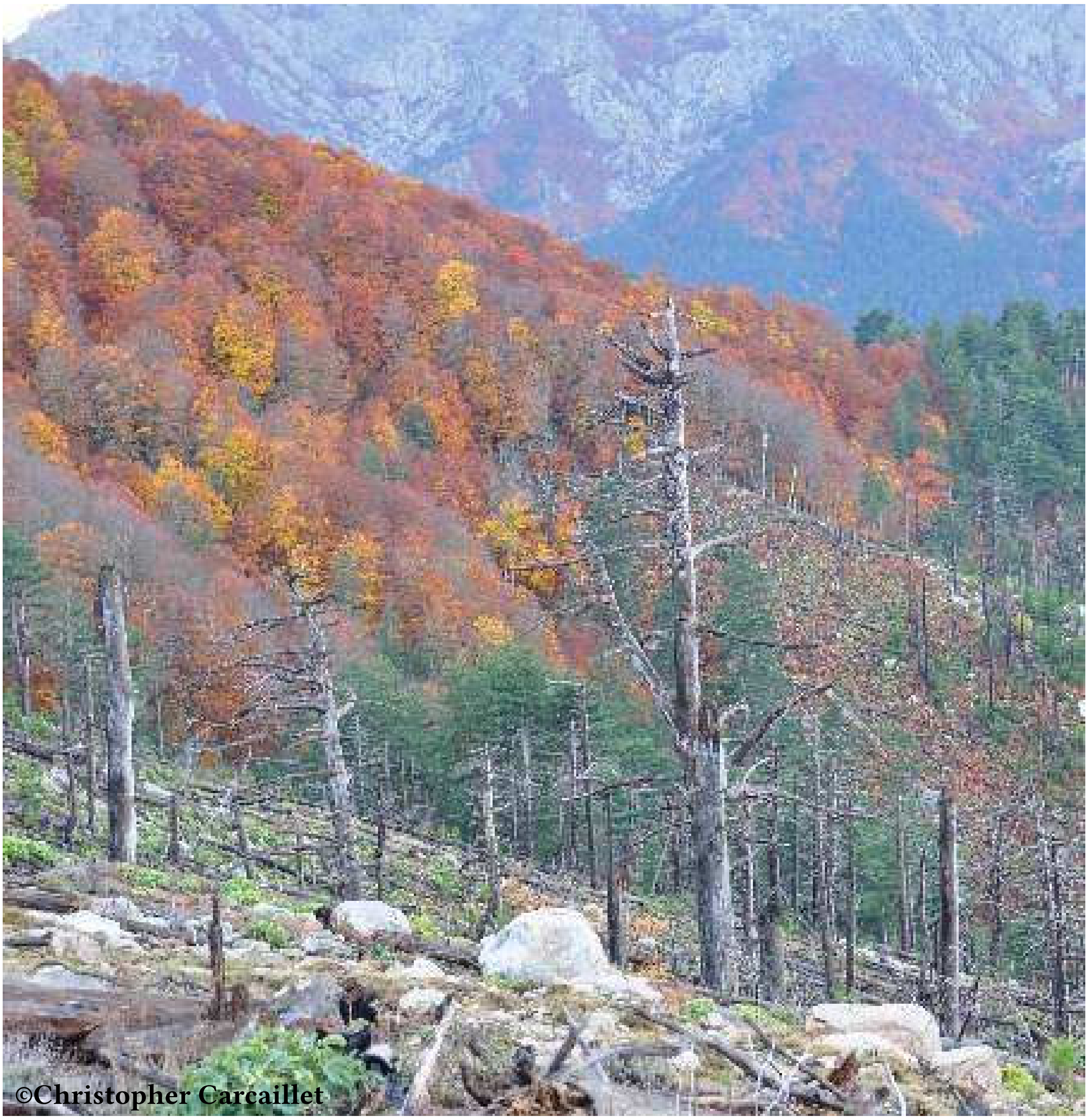
Table des Matières

Les termes suivis du symbole 📘 à la première occurrence (dans le corps de texte) sont présents dans le glossaire. Les photos sans copyrigth ni crédit photographique (par ex. Wikipedia) sont des photos de l’auteur sous licence 🅭 creative commons (CC BY-SA 4.0 FR).

Chapitre I

Introduction générale

> « *[...] les gens qui sont très préoccupés par le but de leur voyage s'amusent toujours plus que les autres aux milles détails de la route.* »
>
> Pierre Loty, *Pêcheur d'Islande*, 1886



Hêtraie-pinède surplombant la forêt de pins laricio de Rospa-sorba parcourue par le feu en 2000

Cette thèse s'intéresse à l'écologie des communautés de champignons à travers l'étude de plusieurs modes de vie fongiques. L'introduction commence par une description du groupe d'organismes que forment les **champignons** (section 1) et par une courte présentation de l'**écologie des communautés** (section 2). Nous présenterons ensuite les trois groupes écologiques étudiés que nous appellerons « guildes ». Les **champignons ectomycorhiziens** feront l'objet d'une première partie (section 3). Nous introduirons ensuite brièvement les caractéristiques des **champignons endophytiques** (section 4) puis des **champignons décomposeurs** également appelés saprotrophes (section 5). Nous évoquerons par la suite les outils utilisés dans cette thèse, et en particulier les **outils moléculaires** et d'**analyses de réseaux** dans la section 6. Enfin, nous exposerons dans la section 7 la problématique du **feu** en milieu méditerranéen et en particulier dans le cas des forêts de **pin laricio** (*Pinus nigra* [Arnold] subsp. *laricio* [Maire] var. *Corsicana* [Loudon] Hyl. [1913] [1]). Nous conclurons l'introduction par un exposé succinct de nos hypothèses de travail en mettant en lien les sujets des différentes sections qui précèdent (section 8).

1 Diversité des champignons

1.1 Qu'est ce qu'un champignon ?

Les Eumycètes (appelés communément « champignons vrais ») sont des **Eucaryotes** [2] et forment le groupe frère des Animaux dans le clade des Opisthokontes constitué d'organismes dont les cellules portent (ou dont les ancêtres portaient) des flagelles antérieurs (Lecointre et coll. 2006). Les Eumycètes forment un groupe monophylétique [3] d'organismes **hétérotrophes** [4] **absorbotrophes** [5] (Campbell et coll. 2012) très diversifié (Fig. I.1). La nutrition par absorption implique une sécrétion d'enzymes dans le milieu environnant (exodigestion) et un mode de nutrition **osmotrophe** [6].

Les champignons ont très peu de caractères morphologiques ou physiologiques partagés. La grande majorité des espèces d'Eumycètes disposent d'une paroi cellulaire à chitine ainsi que d'une voie métabolique de biosynthèse de la lysine complètement originale (Lecointre et coll. 2006). Pendant longtemps, les naturalistes ont délimité ce clade sur la base d'une absence de chlorophylle chez les organismes de ce clade – et donc sur leur hétérotrophie – couplée à leur absence de mobilité en

---

[1] Source : Tela Botanica

[2] Ensemble des êtres vivants caractérisés par des cellules à noyau contenant des organites. On appelle organites les structures du cytoplasme délimitées par une membrane et spécialisées – à l'instar des organes chez les êtres vivants – dans une ou des fonctions du métabolisme.

[3] Désigne un groupe phylogénétique qui comprend une espèce ancestrale et tous ses descendants (Lecointre et coll. 2006).

[4] Organismes qui se procurent le carbone en se nourrissant d'autres organismes, au contraire des organismes autotrophes qui se procurent le carbone via la photosynthèse (plantes et algues) ou plus rarement par la chimiosynthèse (bactéries et archées).

[5] Mode de nutrition par absorption des nutriments à travers la paroi extérieure de l'organisme.

[6] Les organismes osmotrophes se nourrissent à partir de substances dissoutes de leur environnement extérieur. On les oppose aux organismes phagotrophes, qui ingèrent la matière pour la digérer.

milieu terrestre. Il faut attendre Whittaker (1969) pour voir émerger le règne des Champignons, puis la phylogénie moléculaire pour démêler les relations évolutives de parenté au sein des champignons et délimiter le groupe des Eumycètes. Ainsi, certains organismes comme les Oomycètes ou encore les Myxomycètes (Fig. I.2a, g et m) ne font plus partie des Champignons au sens phylogénétique du terme (Eumycètes). En dehors de ces caractères communs, le règne des Eumycètes embrasse une immense diversité de formes et d'écologies.

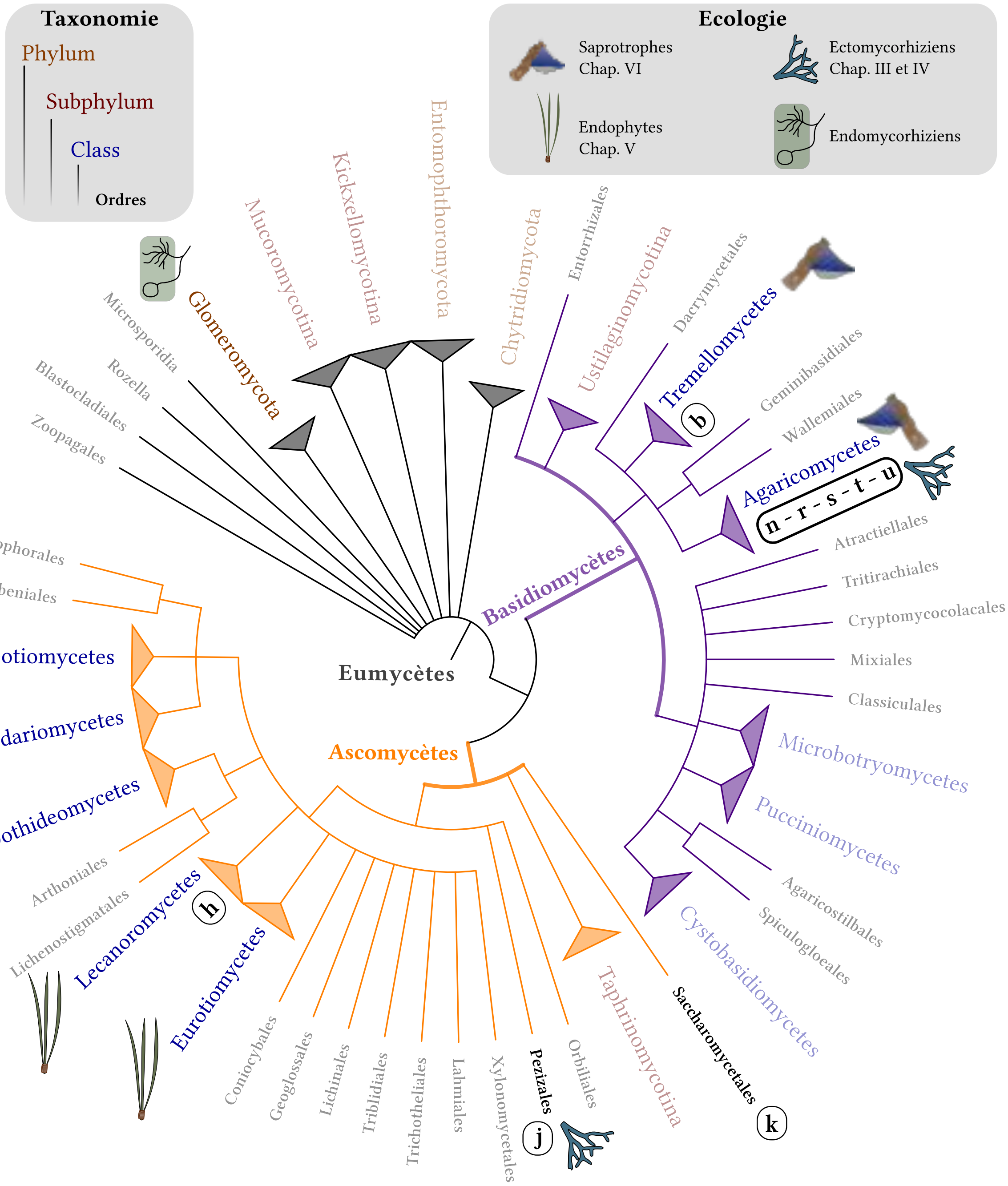


Figure I.1 – Classification phylogénétique des Eumycètes : Les symboles indiquent les principales guildes fongiques. Les lettres indiquent des photos de la figure I.2. Les données phylogénétiques proviennent de la classification de Hibbett et coll. (2007) mise à jour en octobre 2015 (v. 12). L'arbre a été dessiné grâce au logiciel iTOL (v.3, Letunic et Bork 2016).

1.2 Une diversité extraordinaire et mal connue

La diversité des Eumycètes s'exprime aussi bien au niveau taxinomique[7] et phénotypique qu'écologique. Ainsi, le groupe des Eumycètes contient :

- des levures unicellulaires (Fig. I.2k), mais aussi des armillaires dont le mycélium peut s'étendre sur plusieurs dizaines d'hectares (Smith et coll. 1992 ; Bendel et coll. 2006) ;
- des parasites de l'homme et des cultures, mais aussi des aliments très convoités et utilisés (par ex. les morilles Fig. I.2j ou encore la levure de bière Fig. I.2k), et des sources de médicaments (Kaul et coll. 2012) ;
- des champignons aquatiques (marins ou dulcicoles[8]) souvent mobiles (Richards et coll. 2012), mais aussi des champignons terrestres immobiles ;
- des organismes parasites, mais aussi symbiotiques ou encore décomposeurs (Fig. I.3 et I.13) ;
- des organismes dont la reproduction sexuée est inconnue, mais aussi des organismes qui investissent dans des stratégies sexuelles complexes. Dans tous les cas, ces structures de reproduction sont appelées spores. Les spores permettent la dispersion, la dormance et, dans le cas de la reproduction sexuée, les flux génétiques.

Une grande diversité spécifique

Le nombre d'espèces de champignon connues – très principalement des Eumycètes – était estimé à environ 100 000 en 2011 (Hawksworth 2012). La majeure partie des espèces d'Eumycètes appartiennent aux clades des **Ascomycètes** (c. 65 000 espèces) et des **Basidiomycètes** (c. 30 000 espèces). Les autres clades comptabilisent un peu plus de 2 000 espèces [Chytridiomycètes *sensu lato* : c. 950 ; Zygomycètes *sensu lato* : c. 1 050 ; Gloméromycètes : c. 160 ; chiffres issues de Kirk et coll. 2008 cités dans Blackwell 2011]. Les clades des Ascomycètes et Basidiomycètes sont caractérisés par des structures de reproduction particulières, respectivement des asques et des basides. La majorité des champignons connus du grand public sont des macromycètes[9] du clade des Basidiomycètes.

La richesse des champignons est évaluée conservativement à plus d'un million et demi d'espèces (Hawksworth 1991 ; Hawksworth 2001) mais atteint probablement plus du double (Blackwell 2011 ; Hawksworth 2012). Nous sommes donc capables de nommer moins de 7% de la diversité fongique. Et même pour les groupes taxinomiques les plus connus, les relations phylogénétiques et les délimitations d'espèces sont en constante évolution (par ex. les morilles, Loizides et coll. 2016).

---

[7] Nous avons choisis dans ce travail d'utiliser le terme « taxinomie » plutôt que « taxonomie » en suivant les conseils des linguistes (Tardieu 2011).

[8] Désigne les organismes qui vivent et se reproduisent en eau douce.

[9] Désigne les champignons dont les fructifications – ou sporophores – sont visibles à l'œil nu.

Une grande diversité phénotypique

Quelques Eumycètes – les levures – sont constitués de cellules uniques (Fig. I.2k). Les Eumycètes multicellulaires sont constitués de filaments de diamètre compris entre 1 et 100 μm : les **hyphes** (Fig. I.2v). Chez la majorité des Ascomycètes et des Basidiomycètes les hyphes ont un diamètre inférieur à 5 μm [10] (Howard et Gow 2013). Ces hyphes ont une croissance apicale et peuvent être cloisonnés ou non (hyphes coenocytiques). Les hyphes sont composés d'une paroi – solidifiée par la présence de chitine – qui entoure la membrane plasmique et les cytoplasmes cellulaires. Le réseau formé par les hyphes ramifiés est appelé mycélium (voir Fig. I.11 pour une illustration).

Les hyphes sont capables de se couper et de fusionner avec d'autres hyphes compatibles. Par conséquent, la notion d'individu chez les champignons est complexe (Johnson et coll. 2012). On distingue deux types d'individus : les individus génétiques, également appelés **génets** et les individus physiques, les **ramets**. Un génet – constitué d'hyphes issus d'une même spore ou de spores clonales – peut se découper en plusieurs ramets, et ces ramets peuvent fusionner à nouveau. Les génets peuvent mesurer plusieurs dizaines de mètres (Bonello et coll. 1998 ; Leake et coll. 2004). L'étude des ramets se situe à une petite échelle spatio-temporelle (échelle de la physiologie) et les outils de suivi sont en plein développement (par ex. Wallander et coll. 2013). D'un point de vue écologique, la notion de génet paraît par conséquent plus pertinente et opérationnelle (Douhan et coll. 2011).

Une grande diversité écologique

Derrière l'absorbo-hétérotrophie partagée des champignons se cache une grande diversité de modes de nutrition (Peay et coll. 2008). Certains champignons se nourrissent de matière organique vivante (parasites, mutualistes et même certains prédateurs) ou morte (décomposeurs) en provenance de plantes et d'animaux, y compris de champignons. Dans cette thèse nous nous limiterons à l'étude des champignons dont la nutrition est liée aux plantes (c.-à-d. en excluant les mutualistes, commensaux et pathogènes animaux ; voir Fig. I.4). La section suivante s'intéresse à la diversité des interactions plantes-champignons.

---

[10] La minceur de l'hyphe permet aux champignons d'explorer des volumes de substrat formidables au regard de leur biomasse. La présence de deux noyaux non fusionnés chez de nombreux Eumycètes dicaryotiques pourraient d'ailleurs trouver son origine dans la sélection des champignons dont les cellules sont particulièrement minces. En effet, la fusion des deux noyaux après plasmogamie pourrait entraîner un encombrement des hyphes minces qui ralentirait le transport intra-cellulaires des composés chimiques (Finn Kjellberg, com. pers.).

(I) Planche I : légende page 14

(II) Planche II : légende page suivante

Figure I.2 – Exemple de la diversité phénotypique des fructifications de champignons présents en Corse : Tous les champignons présentés sur ces planches sont présents en Corse (seule la photo **k** n'a pas été prise en Corse, mais la levure de bière y est sûrement présente… au moins dans la Pietra®). Ces photos représentent principalement les systèmes reproducteurs de macromycètes, c'est-à-dire de champignons dont les fructifications sont visibles à l'œil nu. Sont également représentés des organismes anciennement inclus dans les champignons (des Myxomycètes **a**, **g** et **m**), des formes végétatives (le lichen *Lobaria pulmonaria* **h** et la levure de bière *Saccharomyces cerevisiae* **k**) et une fructification invisible à l'œil nu de *Penicillium sp.* (**v**).

Détails planche I : a) Sporophore de *Diderma spumarioides* (Myxomycètes ; Amibozoaires) ; **b)** Sporophore de *Exidia glandulosa* (Basidiomycètes, famille des Auriculariaceae) ; **c)** Sporophore de *Diatrype stigma* (Ascomycètes, classe des Sordariomycètes), un champignon corticioïde (groupe paraphylétique caractérisé par des fructifications en forme de croûte) sur tronc de chêne vert (*Quercus ilex*) ; **d)** Sporophore d'une espèce inconnue (peut être le Myxomycète *Ceratiomyxa fruticulosa*) sur tronc de chêne vert ; **e)** Sporophore de *Panellus stipticus* (Basidiomycètes, famille des Mycenaceae) ; **f)** Sporophore de *Lentinellus castoreus* (Basidiomycètes, famille des Auriscalpiaceae) ; **g)** Sporophore de Myxomycètes appartenant sans doute au genre *Stemonitis* ; **h)** Sporophore de deux espèces de lichen. L'espèce la plus abondante est *Lobaria pulmonaria* (le nom latin du lichen correspond au nom du champignon) et la plus claire appartient au genre *Alectoria* ; **i)** Sporophore de *Sebacina spp* (Basidiomycètes, famille des Exidiaceae) ; **j)** Sporophore de morille (*Morchella sp* ; Ascomycètes, classe des Pézizomycètes) au pied d'arbousiers (*Arbutus unedo*) au printemps suivant un feu ; **k)** La levure de bière *Saccharomyces cerevisiae* vue au microscope à contraste interférentiel différentiel, *source* : W 🅭[nom d'utilisateur : Masur] ; **l)** Sporophore de *Mycena stipata* (Basidiomycètes, famille des Mycenaceae) sur un tronc de pin laricio (*Pinus nigra* subsp. *laricio*) en décomposition dans une zone brûlée intégralement 15 ans avant la photo.

Détails planche II : m) Plasmodes de *Physarum psittacinum* (Myxomycètes) ; **n)** Sporophore d'un polypore nid d'abeille (*Daedaleopsis nitida* ; Basidiomycètes, famille des Polyporaceae) sur tronc de chêne vert ; **o)** Sporophore de *Bjerkandera adusta* (Basidiomycètes, famille des Meruliaceae) ; **p)** Sporophore de champignon résupiné (Se dit d'un champignon dont la surface stérile est fixée à l'envers sur son substrat et la surface fertile, l'hyménium, est tournée vers l'extérieur ; source : Wiktionary), sans doute *Stereum hirsutum* (Basidiomycètes, famille des Stereaceae) ; **q)** Sporophore de *Xylobolus frustulatus* (Basidiomycètes, famille des Stereaceae) ; **r)** Sporophore de *Xylobolus subpileatus* (Basidiomycètes, famille des Stereaceae) sur chablis de chêne vert ; **s)** Sporophore de *Phanerochaete sp.* (Basidiomycètes, famille des Phanerochaetaceae) sur chablis de chêne vert ; **t)** Sporophore de *Phyllotopsis nidulans* (Basidiomycètes, famille des Tricholomataceae) ; **u)** Sporophore de *Fomitiporia robusta* (Basidiomycètes, famille des Hymenochaetaceae) ; **v)** Sporophore de *Penicillium crustaceum* (Ascomycètes, classe des Eurotiomycetes), *source* : modifié de W 🅭.

1.3 Les multiples relations plantes-champignons

Les écologues s'intéressent aux interactions qui concernent des organismes vivants, dont les interactions directes entre eux. Ces relations peuvent être classées en fonction de l'effet de l'interaction sur les partenaires (Cheng 1991 cité dans Thomas et coll. 2010). Ainsi, on distingue les interactions bénéfiques pour un seul organisme partenaire (parasitisme, prédation, commensalisme), pour les deux (mutualisme) ou pour aucun partenaire (compétition ou amensalisme). En plus de cet axe bénéfice/préjudice pour les partenaires, on distingue les interactions temporaires (par ex. la prédation) des interactions durables (par ex. le parasitisme). Enfin, les interactions écologiques diffèrent du point de vue de leur degré d'intimité[11] (Fontaine et coll. 2011).

---

[11] Degré de dépendance des partenaires à leur interaction. Par exemple, la pollinisation est moins intime que l'interaction mycorhizienne.

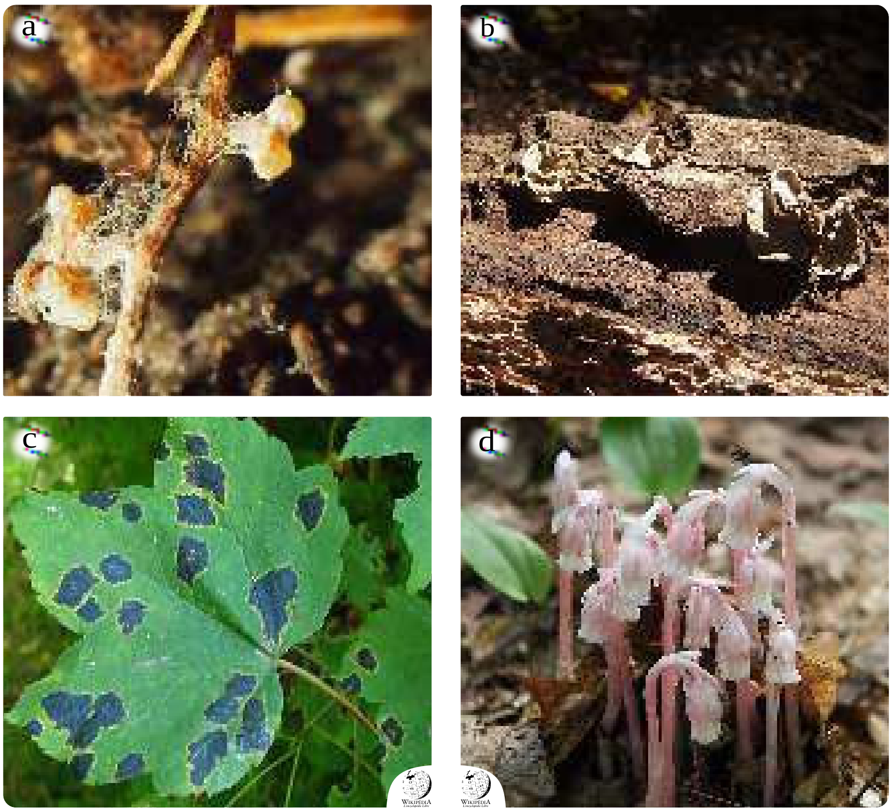


Figure I.3 – Quelques exemples d'interactions plantes-champignons : a) Une ectomycorhize de pin laricio, l'interface du mutualisme ECM ; **b)** Sporophore de *Xylobolus subpileatus* qui décompose un chablis de chêne vert (*Quercus ilex*) ; **c)** *Rhytisma acerinus* (appelé communément « tache goudronneuse ») parasite très courant des feuilles d'érable sycomore (*Acer pseudoplatanus*), *source* : W 🅭[nom d'utilisateur : Annabel] ; **d)** *Monotropa uniflora*, une plante myco-hétérotrophe obligatoire qui parasite des champignons de la famille des Russulaceae, *source* : W 🅭[nom d'utilisateur : Magellan nh].

Les relations plantes-champignons forment un continuum trophique (Hoeksema 2010) qui va du parasitisme fongique (symbiose asymétrique en faveur du champignon), à la mycohétérotrophie (symbiose asymétrique au profit de la plante) en passant par des interactions à bénéfices réciproques (Karst et coll. 2008 ; Fig. I.3 et I.4). Ce dernier cas concerne près de 90% des espèces de plantes terrestres à travers les **mycorhizes**, organes d'origine mixte résultant de la symbiose entre le mycélium d'un Eumycète[12] et les racines d'une plante (Fig. I.3a ; Selosse et coll. 2006). Les pathogènes fongiques des plantes affectent drastiquement la biodiversité mais aussi le fonctionnement des écosystèmes (Fisher et coll. 2012). Les champignons décomposeurs sont également des rouages particulièrement importants des processus écosystémiques (Kendrick 2001 ; voir section 5).

1.4 La conservation des champignons

On ne sait pas cultiver la majorité des espèces fongiques et leur structures sont souvent fugaces et invisibles à l'œil nu (Peay et coll. 2008). Cela pose des problèmes pour la conservation de la diversité fongique, que ce soit *ex situ* ou *in situ*. Même en mettant de côté les problèmes classiques rencontrés par ce type de conservation (par ex. Volis et Blecher 2010), la conservation *ex situ* des champignons

---

[12] Groupe monophylétique des champignons « vrais ».

est une solution partielle[13] qui concerne principalement les champignons dont la valeur économique est importante (Varese et coll. 2011).

Il existe deux grandes approches de conservation des champignons *in situ* : (i) les listes de protection et (ii) la protection des habitats. La première approche consiste à mettre en place de listes rouges d'espèces à partir de données de distribution et d'écologie des espèces (Dahlberg et coll. 2010 ; Dahlberg et Mueller 2011). La conservation basée sur la notion d'espèce pose cependant de nombreux problèmes en raison du nombre important d'espèces fongiques, de la difficulté à délimiter ces espèces et de la modestie des connaissances sur leur distribution et leur écologie[14]. En particulier, les critères des listes rouges de l'UICN (Union Internationale pour la Conservation de la Nature) incluent des critères populationnels (Dahlberg et Mueller 2011) très difficiles à appréhender chez les champignons (par ex. la taille des populations ou encore des extinctions locales). Cependant, des listes importantes d'espèces ont d'ores et déjà été construites dans les pays du nord de l'Europe (Dahlberg et coll. 2010 ; Dahlberg et Mueller 2011 ; Heilmann-Clausen et coll. 2015). De plus, les progrès constants des outils moléculaires modernes pourraient permettre de contourner certains de ces problèmes (par ex. Geml et coll. 2014). La seconde approche consiste à protéger les champignons en protégeant leurs habitats (Moore et coll. 2001), en particulier en adaptant la gestion humaine de ces habitats (Lonsdale et coll. 2008).

Au delà de la conservation des champignons pour eux-même, Heilmann-Clausen et coll. (2015) affirment que les champignons sont essentiels pour la science de la conservation car :

- Ils fournissent des habitats (Cockle et coll. 2012 ; Schigel 2012) et des interactions cruciales (Dighton 2003 ; Fernandez et Kennedy 2015a) pour d'autres organismes[15] ;
- Ils peuvent servir d'indicateur du fonctionnement et des trajectoires des écosystèmes (par ex. Lilleskov et coll. 2011) ;
- Ils peuvent caractériser des habitats à haute valeur de conservation (par ex. Norstedt et coll. 2001 ; Parmasto 2001) ;
- Ils forment des liens puissants entre les sociétés humaines et la nature grâce à leurs valeurs alimentaires, médicales et biotechnologiques ;
- Ils constituent un exemple de groupe très diverse dont la conservation pourra être la source de nouveaux outils et approches pour d'autres groupes taxinomiques (par ex. pour les bactéries).

* * *

---

[13] Voir Moore et coll. (2001) pour quelques discussions autour de la conservation *ex situ* des champignons.

[14] Lire Suz et coll. (2015) pour une proposition d'amélioration des données de distribution des champignons mycorhiziens.

[15] Par exemple, Cockle et coll. (2012) ont montré l'importance des champignons dans la dégradation du bois mort, permettant à de nombreux oiseaux de nicher dans des cavités du bois. Dans un autre registre, Fisher et coll. (2012) ont souligné les effets délétères des champignons sur d'autres organismes et sur la santé des écosystèmes.

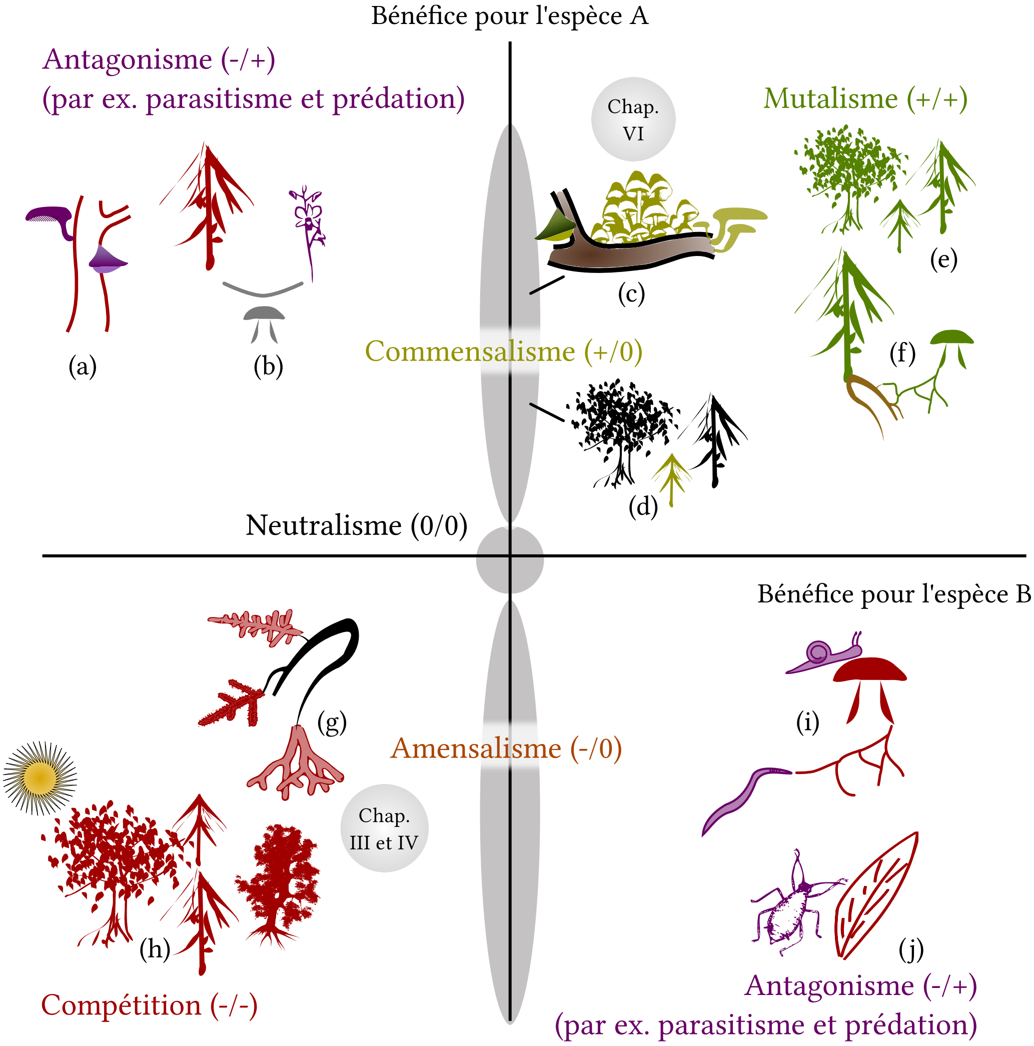


FIGURE I.4 – Typologie des interactions entre organismes vivants : Les interactions écologiques peuvent être classées en six grandes catégories du point de vue des bénéfices pour les partenaires (Selosse 2000 ; Morales-Castilla et coll. 2015).

L'antagonisme – interaction au bénéfice d'un partenaire et délétère pour l'autre (+/-) – inclut le parasitisme et la prédation. **a)** Le parasitisme des polypores sur les arbres (Norstedt et coll. 2001) ou encore **b)** la mycohétérotrophie (parasitisme entre plantes via des champignons mycorhiziens ; Roy et coll. 2008) sont des antagonismes impliquant les plantes et les champignons. D'autre cas d'antagonisme affectent séparément les champignons à travers la fongivorie du mycélium ou du sporophore (**i** ; Schigel 2012), et les plantes à travers l'herbivorie (**j** ; Coley et Barone 1996).

Le commensalisme – interaction au bénéfice d'un partenaire et sans effet pour l'autre (+/0) – est également une forme courante chez les plantes et les champignons. On peut citer **c)** la facilitation du recrutement des plantules par les plantes adultes qui maintiennent notamment une humidité bénéfique (effet nurse ; Gómez-Aparicio et coll. 2004) et **d)** la décomposition du bois mort par les champignons saprotrophes (Fukami et coll. 2010).

Le mutualisme – interaction à bénéfice réciproque (+/+) – est omniprésent dans les interactions entre plantes (**e** : facilitation ; Bruno et coll. 2003) mais également entre plantes et champignons (**f** : interaction mycorhiziennes ; Smith et Read 2008).

La compétition – interaction à préjudice réciproque (-/-) – est également très présente chez les champignons (**g** : compétition [par ex. compétition pour la ressource] entre champignons mycorhiziens ; Kennedy et coll. 2009) et chez les plantes (**i** : compétition pour la lumière ; Craine et Dybzinski 2013).

L'amensalisme – interaction délétère pour un partenaire et sans effet pour l'autre (-/0) – est plus rare. Enfin, **le neutralisme** désigne les interactions sans effet pour les deux partenaires (0/0). Notez que cette absence d'effet peut être le fait d'une compensation des avantages et des inconvénients que procure l'interaction aux partenaires (Selosse 2000).

RÉSUMÉ

(i) Le clade des **Eumycètes** (groupe monophylétique des champignons « vrais ») est constitué d'une grande diversité d'organismes **hétérotrophes absorbotrophes** caractérisés par une paroi cellulaire à **chitine** et une voie métabolique de biosynthèse de la lysine. Cette diversité est encore très mal connue et sa conservation en est à son balbutiement.

(ii) Les interactions plantes-champignons embrassent l'ensemble des interactions possibles quant aux bénéfices/coûts pour les partenaires. Ces interactions sont à la base de nombreux processus écosystémiques.

(iii) Cette thèse traite de trois cas d'interaction plantes-Eumycètes à travers les champignons **ectomycorhiziens** (mutualisme ; section 3), les champignons **endophytiques** (mutualisme, parasitisme, commensalisme ; section 4) et les champignons **décomposeurs** (principalement commensalisme, section 5).

SUMMARY

(i) The phylum of **Eumycetes** (monophyletic clade grouping the "true" fungi) consists of **heterotrophic** and **absorbotrophic** organisms able to synthesize lysine. Cell walls of the members of Eumycetes contain **chitine**. Eumycete diversity is tremendous but poorly known and it conservation is in its early days.

(ii) Plant-fungus interactions range from purely mutualistic relations to parasitic ones and all the intermediate types of interactions. These interactions are key for numerous ecosystemic processes.

(iii) This thesis deals with three cases of plant-Eumycete interactions : **ectomycorrhizal** fungi, (mutualism; section 3), **endophytes** (mutualism, parasitism and commensalism; section 4) and **saprobiotic** fungi (section 5).

2 L'écologie des communautés en bref

Préambule : Ma vision de l'écologie des communautés a fortement été stimulée par mes collaborations en écologie fonctionnelle sur le thème du rôle de la variation intra-spécifique des traits dans l'assemblage des communautés végétales (voir manuscrits K et L en annexe).

2.1 La communauté

Une communauté est définie comme l'ensemble des individus – appartenant à différentes espèces d'un même groupe [16] – qui coexistent dans un même lieu (Looijen et van Andel 1999 ; Vellend 2010). Il reste à définir ce qu'est un groupe dans cette définition. Parfois de façon implicite, la majorité des écologues réduisent une communauté à un ensemble d'individus qui interagissent potentiellement tous entre eux. Pour qu'il y ait interaction, il faut qu'il y ait soit une relation d'exploitation directe (*sensu lato* [17]), soit une (ou plusieurs) ressource(s) limitante(s) commune(s).

Certains chercheurs considèrent effectivement l'ensemble des individus en interaction comme formant une communauté (par ex. Bersier 2007, page 366). D'autres préfèrent simplifier le problème en se concentrant sur la compétition pour la ressource et en laissant de côté les relations d'exploitation à d'autres disciplines comme l'étude des réseaux trophiques. Dans cette thèse, nous avons choisi cette définition restreinte de la communauté pour étudier les communautés fongiques. Une définition plus large nous obligerait à décrire conjointement les communautés végétales et fongiques qui sont structurées à des échelles spatiales différentes.

Nous entendons donc un groupe au sens de guilde (Blondel 2003), c'est à dire un groupe d'organismes en compétition pour la ressource (*sensu lato*, c.-à-d. en incluant la ressource spatiale mais aussi les mutualistes et les prédateurs communs). Ce choix de cette définition restreinte est motivé (**i**) par l'histoire de l'étude de l'écologie des champignons qui s'est toujours basée sur la distinction de différentes guildes, (**ii**) par la faible quantité d'information sur les relations d'exploitations qui impliquent les champignons, et (**iii**) par la différence d'échelle spatio-temporelle entre les communautés des plantes et celles des champignons. Ce dernier point est fondamental. La taille minimum des arbres est beaucoup plus importante que celle des individus fongiques. Pour étudier les interactions directes (par ex. compétition pour la lumière) et indirectes (par ex. partage de parasite), il faut étudier des parcelles forestières alors que les interactions entre individus fongiques peuvent être très nombreuses à l'échelle de quelques centimètres.

La notion spatiale de *lieu* est donc à la fois primordiale et intentionnellement vague. L'idée est que le lieu doit être suffisamment petit pour que tous les individus puissent interagir, même

---

[16] Certaines définitions de la communauté ne font pas appel à cette notion de groupe. Mais la grande majorité des études en écologie des communautés s'intéressent à une sous partie seulement des individus présent sur un lieu. Certains délimitent ces groupes en s'intéressant à un clade monophylétique (par ex. les végétaux terrestres, les poissons osseux, les orchidées) ou à une forme écologique (par ex. les arbres, les microorganismes, les poissons).

[17] On entend ici l'exploitation directe au sens large, c'est à dire en incluant l'utilisation d'un autre organisme pour fournir aussi bien des ressources nutritives (interactions trophiques) qu'un habitat (par ex. nid d'oiseau dans un arbre) ou un service (par ex. pollinisation).

indirectement. La taille de cette entité spatiale – difficile à estimer – est rarement testée empiriquement avant d'analyser des communautés. Elle dépend de la biologie des organismes étudiés, et en particulier de la taille et de la mobilité de ces organismes (par ex. une parcelle forestière pour les arbres, le système racinaire d'un groupe d'arbres pour les champignons mycorhiziens, un tronc mort pour les champignons décomposeurs).

Nous retiendrons ici qu'une communauté est constituée d'un ensemble d'individus qui partagent au moins une ressource (*sensu lato*) limitante dans un espace donné (Laroche 2014).

2.2 La niche écologique

La niche est un concept qui décrit la façon dont une espèce tolère l'environnement (niche abiotique) et comment elle interagit avec les autres espèces présentes (niche biotique), en particulier celles de sa communauté. On peut distinguer deux types de niche[18] : la niche *grinnellienne* (Grinnell 1917) s'intéresse à la performance d'une espèce en fonction de la ressource tandis que la niche *eltonienne* (Elton 1927) s'intéresse à l'impact d'une espèce sur son environnement (Devictor et coll. 2010), en particulier *via* les relations trophiques (Soberón 2007). Hutchinson (1957) proposa une définition d'inspiration grinnellienne – intégrant également les idées d'Elton – qui est aujourd'hui la plus utilisée (Colwell et Rangel 2009). Selon Hutchinson, la niche d'une espèce[19] est un hypervolume à n dimension, où chaque dimension est une condition environnementale (abiotique ou biotique) qui limite la viabilité des individus de l'espèce.

Hutchinson (1957) apporta également une nuance importante en distinguant les niches fondamentales et réalisées (Colwell et Rangel 2009). La niche fondamentale est la région incluse dans l'espace de la niche où l'espèce peut exister indéfiniment, c'est un attribut intrinsèque de l'espèce. La niche réalisée est la région dans l'espace de la niche qui est réellement occupée par l'espèce en prenant en compte les interactions interspécifiques. C'est un attribut issu de l'interaction entre la niche fondamentale et l'environnement. Aujourd'hui, le concept de niche réalisée prend également en compte l'histoire des environnements propices (par ex. la dérive des continents) et les limites à la dispersion (Colwell et Rangel 2009).

2.3 Assemblage des communautés

Les mécanismes d'assemblage des communautés

Les mécanismes d'assemblage des communautés constituent l'ensemble des processus qui déterminent la coexistence des organismes et donc la structure des communautés observées. On nomme couramment « règles d'assemblage des communautés » l'ensemble des patrons[20] qui permettent d'inférer ces mécanismes d'assemblage (Weiher et Keddy 2001). On peut distinguer trois

---

[18] Voir Pocheville (2009) p.631-632 pour une critique de cette distinction

[19] Hutchinson est le premier à parler de la niche comme un attribut de l'espèce et non de l'environnement (Colwell et Rangel 2009).

[20] Weiher et Keddy (2001) parle plutôt de « contraintes » sur la formation et le maintien des communautés. Cependant, le terme de contrainte n'est pas équivalent à celui de mécanisme, et avec le temps, les règles d'assemblage des communautés désignent les descriptions de ces contraintes, c'est à dire les patrons.

échelles de processus d'assemblage : les processus biogéographiques, les processus externes à la communauté et les processus internes (Fig. I.5 ; Lortie et coll. 2004 ; Violle et coll. 2012). Une partie de la littérature récente cherche à dissocier les processus en utilisant les différentes échelles comme révélateur des différents processus [21] (Bernard-Verdier et coll. 2012 ; Chalmandrier et coll. 2013).

Cette distinction des processus selon l'échelle reflète les notions de niche α [22] et de niche β [23] proposées par Silvertown (2004). La notion de filtre ne doit pas masquer l'existence de retro-actions provenant de la composition des communautés réalisées (flèches oranges Fig. I.5). Ces rétro-actions – aussi bien biotiques qu'abiotiques – peuvent modifier le succès écologique de l'espèce responsable des modifications (construction de niche) ou d'autres espèces de la communauté (Erwin 2008).

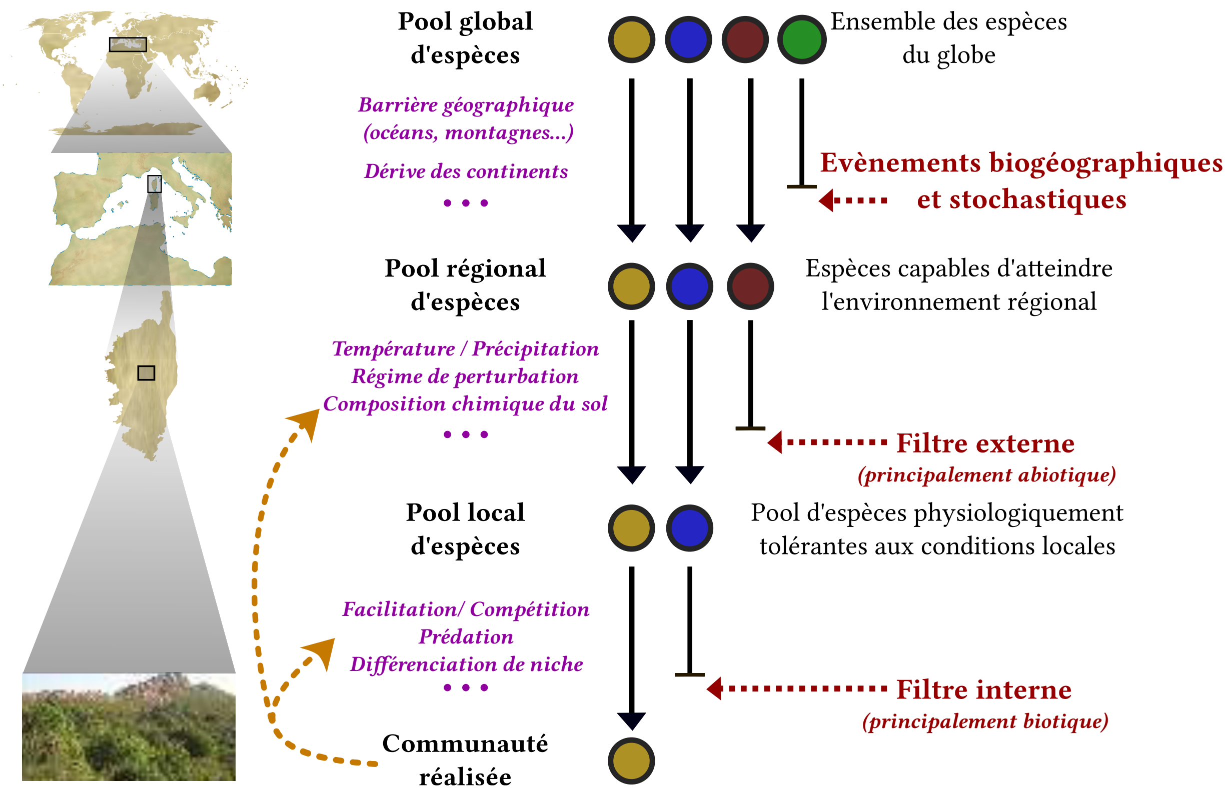


Figure I.5 – Mécanismes d'assemblage des communautés : L'écologie des communautés cherche à comprendre les mécanismes qui expliquent les communautés locales d'espèces (points colorés). Des exemples de processus d'assemblage sont indiqués en italique pour les trois échelles de processus (on parle aussi de filtres) indiqués en rouge. Les flèches oranges indiquent des rétro-actions par modification de l'environnement, aussi bien biotique qu'abiotique, par les organismes de la communauté réalisée. Ce schéma peut également être compris au niveau des individus plutôt que des espèces (voir Violle et coll. 2012 pour une revue de la question).

---

[21] Ainsi, les écologues parlent classiquement de filtre environnemental (sous entendu *abiotique*) pour les processus externes à la communauté, et d'interactions biotiques pour les processus internes. Voir Violle et coll. (2012) et Taudiere et Violle (2016) pour une discussion autour de cette classification.

[22] La niche α est la région de la niche de Hutchinson qui comprend les dimensions de la niche qui varient à une échelle spatiale plus petite que celle de la communauté.

[23] La niche β est la région de la niche de Hutchinson qui comprend les dimensions de la niche qui varient à une échelle spatiale plus grande que celle de la communauté.

Les quatre classes de processus d'assemblage des communautés (Vellend 2010)

De façon transversale à la notion de filtre externe et interne (voir Violle et coll. 2012 pour une explication et le manuscrit K en annexe pour une application), Vellend (2010) propose quatre classes de processus pour l'écologie des communautés : la **sélection**, la **dérive**, la **spéciation** et la **dispersion** (Fig. I.6).

La notion de **sélection** inclut tous les processus qui font appel à des différences déterministes de valeurs sélectives entre espèces[24]. Autrement dit, la notion de sélection regroupe tous les processus d'assemblage des communautés qui sont la conséquence déterministe des interactions locales entre des espèces fonctionnellement distinctes et leurs environnements. Ainsi, la sélection inclut à la fois les filtres d'habitat et les interactions (en particulier par compétition, facilitation et prédation).

La **dérive** comprend tous les processus stochastiques qui agissent sur l'abondance des espèces (par ex. démographie ; Hubbell 2001). La formation de nouvelles espèces à partir d'un ancêtre commun – la **spéciation** – modifie le pool régional d'espèces et induit donc des modifications des compositions spécifiques des communautés. Enfin, l'importance de la **dispersion** entre communautés est depuis longtemps reconnue, et le cadre conceptuel des métacommunautés (Holyoak et coll. 2005) a facilité depuis plus de 10 ans l'incorporation de la dispersion en écologie des communautés.

Le rôle des perturbations dans l'assemblage des communautés

Les perturbations modifient à la fois les processus de sélection (par ex. via une mortalité différentielle des espèces), de spéciation (par ex. en favorisant la spéciation allopatrique par fragmentation spatiale), de dispersion (par ex. par fragmentation spatiale entraînant la diminution des capacités de dispersion), et de dérive (par ex. par diminution du nombre d'individus de la communauté).

Les perturbations jouent un rôle prépondérant dans de nombreux écosystèmes (voir l'exemple du feu détaillé dans la section 7 de l'introduction). Les discussions autour du rôle des perturbations ont joué un rôle important dans l'histoire de l'écologie. Ainsi, la controverse autour des notions de succession végétale et de climax[25] qui opposa les idées de Clements (1916) et Gleason (1926) fournit un bon exemple. Clements (1916) voit la communauté comme un « super-organisme » dont les espèces sont les organes, la succession son ontogénie, et le *climax* son stade à l'équilibre quand la succession succède. La notion de climax implique par essence les perturbations comme des forces négatives responsables d'une régression de l'écosystème. Gleason (1926) oppose à la vision des communautés « super-organismes » de Clements (1916) la notion d'association végétale centrée sur l'individu. Avec ce concept, Gleason (1926) propose l'idée que les associations de plantes sont plus probablement dues à la coïncidence qu'à des phases de développement prédéfinies, ce qui réduit l'aspect négatif

---

[24] Si Vellend (2010) propose un cadre théorique au niveau spécifique pour simplifier son propos, il admet que ces quatre classes de processus s'appliquent également à différents niveaux d'organisation (par ex. les populations, les individus). D'ailleurs, le cadre conceptuel qu'il propose est lui même fortement inspiré de celui de la génétique des populations.

[25] Définition du CNRTL : « Terme d'une progression, point culminant ; en particulier, en biogéographie, état naturel (d'ailleurs plus ou moins théorique) vers lequel tendent sol, végétation et faune en un point donné ».

des perturbations en écologie. En raison de la notion de climax, les perturbations, et en particulier le feu, ont longtemps eu une image péjorative en écologie (Krebs et coll. 2010).

* * *

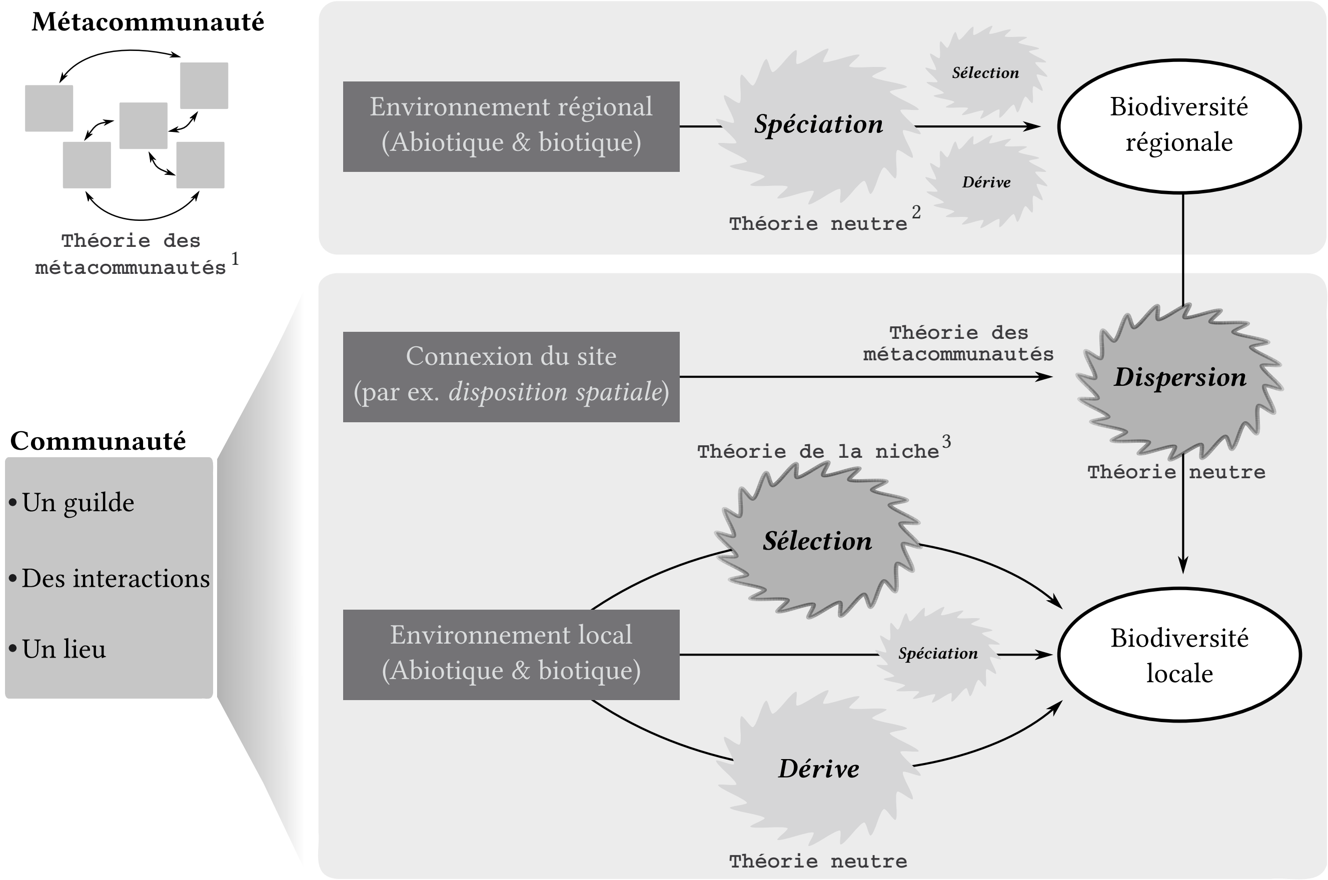


Figure I.6 – Classification des processus d’assemblage des communautés selon Vellend (2010) : Illustration des processus (formes dentées) d’assemblage des communautés proposés par Vellend (2010) et de leurs interactions avec les conditions du milieu (rectangles gris) et la diversité biologique à l’échelle des métacommunautés et des communautés (formes arrondies). La taille des formes dentées correspond à l’importance que revêtent classiquement les processus à différentes échelles. Ce travail s’intéresse principalement au processus de dispersion et de sélection à l’échelle des communautés (formes dentées plus sombres). Trois théories non exclusives en écologie (théorie des métacommunautés, théorie de la niche et théorie neutre) sont positionnées en fonction des processus sur lesquelles elles insistent. Voir tableau 2 de Vellend (2010) pour la classification d’autres théories. Même si les processus évolutifs ne sont pas explicitement représentés sur le schéma, ils sont à la fois moteurs (par ex. de la spéciation) et résultats (par ex. via la sélection et la dérive) des processus d’assemblage des communautés. Figure inspirée de la figure 4 de Vellend (2010) et de la figure 2 de Laroche (2014).

1 : Holyoak et coll. (2005) ; **2** : Hubbell (2001) ; **3** : Hutchinson (1957)

Résumé

(i) L'écologie des communautés s'intéresse aux **mécanismes** responsables des **compositions et structures** des communautés d'organismes vivants.

(ii) Une **communauté** est constituée d'un ensemble d'individus qui partagent au moins une **ressource limitante** (*sensu lato*) dans un **espace** donné.

(iii) On peut classer les processus d'assemblage en fonction de plusieurs paramètres : selon le caractère **abiotique** ou **biotique** de l'agent responsable du processus, selon l'échelle spatiale à laquelle se déroule le processus – **filtre biogéographique**, **filtre externe** et **filtre interne** (sous entendu « à la communauté ») – , selon la position du processus sur un gradient de **stochasticité** vers le **déterminisme** (par ex. les processus auxquels fait appel la théorie neutre *vs* ceux impliqués dans la théorie de la niche).

(iv) Vellend (2010) propose quatre grandes classes de processus d'assemblage : la **sélection** (dont le filtre d'habitat, l'action des perturbations et les interactions biotiques), la **dérive**, la **spéciation** et la **dispersion**. Dans ce travail nous ciblerons particulièrement les processus de sélection et dans une moindre mesure de dispersion (voir tableau I.5).

Summary

(i) The aim of community ecology is to understand **mechanisms** that drive the **composition and structure** of communities of biological organisms.

(ii) A **community** is the set of individuals sharing at least one **limiting resources** (*sensu lato*) in a given **place**.

(iii) Community assembly processes can be classified considering to various parameters. One can classify processes according to their **biotic** or **abiotic** nature. Others prefer to emphasize the gradient from **determinism** (*e.g.*, niche processes) to pure **stochastic** processes (e.g. neutral theory). Finally, processes can be classified according to their spatial scale of influence: **biogeographic filter**, **external filter** (implied to the community) and **internal filter** (implied to the community).

(iv) Vellend (2010) suggests four classes of community assembly processes: **selection** (including habitat filtering and biotic interactions), **drift**, **speciation** and **dispersion**. In this work, we will focus on selection processes and, in a lesser extent on dispersal processes (*cf.* table I.5).

3 Le mutualisme ectomycorhizien (ECM)

3.1 Les interactions mycorhiziennes : omniprésentes et polymorphes

Définition historique

La découverte de l'interaction mycorhizienne est récente à l'échelle de la biologie (Fig. I.7). Une des raisons principales semble être l'invention puis la démocratisation des microscopes indispensables à la compréhension de cette symbiose. Theodor Hartig est le premier à observer ces structures aux alentours de 1840 (Garbaye et Fortin 2013). Les frères Tulasne postulent un an plus tard la nature fongique de l'interaction. La nature ubiquiste[26], mutualiste, et les variations morphologiques des interactions mycorhiziennes sont découvertes à la fin du XIXe siècle sous l'impulsion d'Albert Bernhard Frank. Frank (1887) (cité dans Koide et coll. 2004) instaure la distinction, encore utilisée aujourd'hui, entre les ecto- et les endo-mycorhizes (Fig. I.10). Au début du XXe, Rayner (1926-1927) confirme la nature bénéfique de l'interaction pour l'hôte dans une suite d'articles (commençant par Rayner 1926 et finissant par Rayner 1927).

Il faut attendre le développement de la biologie moléculaire (Gardes et Bruns 1993 ; Horton et Bruns 2001) pour commencer à étudier l'écologie de l'interaction. Les fructifications constituaient jusqu'alors la seule partie connue du cycle de vie des champignons mycorhiziens. En complément de la biologie moléculaire, le déploiement de nouvelles techniques expérimentales, notamment celles utilisant les isotopes (Simard et coll. 1997), s'est avéré crucial dans notre compréhension de la symbiose mycorhizienne.

Une autre raison peut être invoquée pour expliquer le décalage entre l'importance des mycorhizes dans le monde vivant et l'intérêt récent qu'elles suscitent. La nature ubiquiste de cette interaction rend difficile à concevoir les effets de l'absence de l'interaction. Autrement dit, vu que la très grande majorité des plantes forment des mycorhizes partout et tout le temps, il est difficile de savoir s'il s'agit d'une interaction mutualiste, parasitaire, commensaliste ou amensaliste (Fig. I.4). Et donc, il est difficile d'étudier l'importance de l'interaction sans certains outils aujourd'hui accessibles. De plus, cette interaction obligatoire rend complexe la culture des champignons impliqués, ce qui rend l'identification des partenaires fongiques difficile sans outils moléculaires (Koide et coll. 2004).

La connaissance d'un groupe d'organismes aussi large que les champignons dépend beaucoup des angles de vue permis par les outils d'identification disponibles à un instant *t*. Tout comme la découverte et l'amélioration du microscope ont permis une meilleure compréhension de la symbiose ectomycorhizienne, l'amélioration des techniques d'identification, qu'elles soient morphologiques ou moléculaires (Fig. I.9), a entraîné et entraîne toujours des changements de vision du modèle d'étude. Pendant longtemps, les champignons ectomycorhiziens ont été classés grâce à leur parties reproductives souvent aériennes : le sporophore[27]. Cette méthode d'identification est encore utilisée

---

[26] « Qui peut vivre partout, qui s'adapte facilement aux milieux les plus divers » (CNRTL).

[27] Le terme sporophore est synonyme de carpophore ou de fructification. Il s'agit de l'organe en charge de la reproduction chez les champignons (littéralement « qui porte les spores »). L'ensemble des sporophores d'un individu constitue sa fructification. En anglais la traduction consacrée est le terme « *sporocarp* ».

en raison de sa facilité et de son faible coût technique. En revanche, l'identification des fructifications requiert un savoir naturaliste qui peine à se transmettre aux nouvelles générations de chercheurs.

Theodor Hartig publie les premières observations de mycorhizes grâce à un microscope

Les frères Tulasne (Charles sur la photo) identifient le caractère fongique de la symbiose mais postulent une interaction parasitique.

Franz Kamienski étudie les mycorhizes de Monotropa hypopitys.

Albert Bernhard Frank introduit le terme "Mykorrhiza". En plus d'émettre clairement l'hypothèse du statut de symbiose de l'interaction, il va démontrer l'ampleur de ce mutualisme dans les systèmes racinaires de nombreuses plantes.

ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts

Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes

David S. Hibbett, Luz-Beatriz Gilbert & Michael J. Donoghue

Net transfer of carbon between ectomycorrhizal tree species in the field

Suzanne W. Simard, David A. Perry, Melanie D. Jones, David D. Myrold, Daniel M. Durall & Randy Molina

The molecular revolution in ectomycorrhizal ecology: peeking into the black-box

MYCORRHIZAL SYMBIOSIS

Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning

Jonathan Leake, David Johnson, Damian Donnelly, Gemma Muckle, Lynne Boddy, and David Read

Rooting theories of plant community ecology in microbial interactions

LETTERS

The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis

Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi

Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide

FIGURE I.7 – Chronologie simplifiée de l'étude des mycorhizes : Le choix des publications est évidement subjectif. Les publications récentes citées ici traitent principalement des ectomycorhizes, mais des publications analogues concernant les autres types de mycorhizes existent.

De plus, cette vision aérienne de l'association mycorhizienne s'est révélée très éloignée de ce qui se passe sous-terre, du point de vue de la diversité et de l'abondance relative des espèces (Gardes et Bruns 1996 ; Dahlberg et coll. 1997). Il s'est donc avéré nécessaire d'utiliser également des méthodes d'identification à partir des apex, c'est à dire de la mycorhize au sens strict (Fig. I.8).

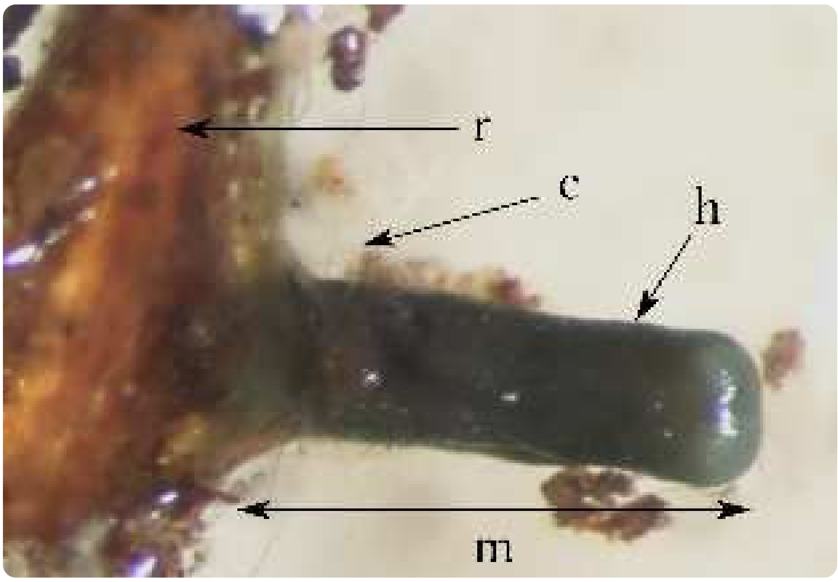


Figure I.8 – Ectomycorhize (m) sur racine de pin laricio (r) : Le mycélium du partenaire fongique (**c**) donne sa couleur bleue à la mycorhize (**m**) qui mesure environ 15 mm. Le mycélium entoure la racine en formant le réseau de Hartig (**h**). La séquence d'ADN fongique issue de ce champignon est identique à 100% avec une séquence attribuée à *Wilcoxina rehmii* dans GenBank.

Le morphotypage[28] et les méthodes type RFLP (*Restriction Fragment Length Polymorphism*, polymorphisme des longueurs de fragments de restriction) ont permis de grandes avancées dans la décennie 1995–2005 sous l'impulsion respective de Agerer (1987) et Gardes et coll. (1991) avant d'être abandonnés au fur et à mesure (Fig. I.9). En revanche, la richesse de description des morphotypes (par ex. initiative deemy) est aujourd'hui un atout majeur pour l'écologie fonctionnelle des champignons ectomycorhiziens (Moeller et coll. 2013).

Les méthodes T-RFLP (Dickie et coll. 2002) et la combinaison du morphotypage avec le séquençage de type Sanger (Gardes et Bruns 1996) ont pris le relais dans les années 2000 pour quelques années seulement (Fig. I.9). Le morphotypage est une méthode complexe à apprendre, longue à appliquer, et qui ne permet pas de discerner de nombreuses espèces cryptiques (par ex. Rubini et coll. 2011). Le séquençage direct (type Sanger) continue à être beaucoup utilisé. La tendance générale est une augmentation très importante du nombre de publications utilisant des nouvelles techniques de séquençage (acronyme anglais : NGS pour *next-generation sequencing*, également appelées HTS pour *High Throughput Sequencing*). Ces techniques de séquençage permettent une profondeur de description bien plus importante que les techniques plus anciennes. Le nombre de séquences obtenues par échantillon va de plusieurs milliers (par ex. pyrosequençage 454), à plusieurs centaines de milliers (par ex. plateforme illumina Mi-Seq). Pour plus de détails sur ces méthodes, se référer à la section 6 de l'introduction.

Le passage d'une vision aérienne (identification des sporophores) à souterraine (identification des mycorhizes) et la prépondérance des techniques moléculaires sur les techniques morpho-anatomiques jouent un rôle important dans la vision actuelle de l'interaction ectomycorhizienne. La

---

[28] Méthode de classification des champignons ECM via les caractéristiques morpho-anatomiques de la mycorhize (voir Fig I.11 pour une illustration).

propension actuelle des chercheurs à privilégier largement les techniques moléculaires s'oppose à la nécessité de connaître la physiologie et l'écologie des organismes que l'on étudie, notamment par la mesure de traits phénotypiques. La complémentarité des approches moléculaires et phénotypiques est maintenant bien reconnue mais les applications sont encore assez rares.

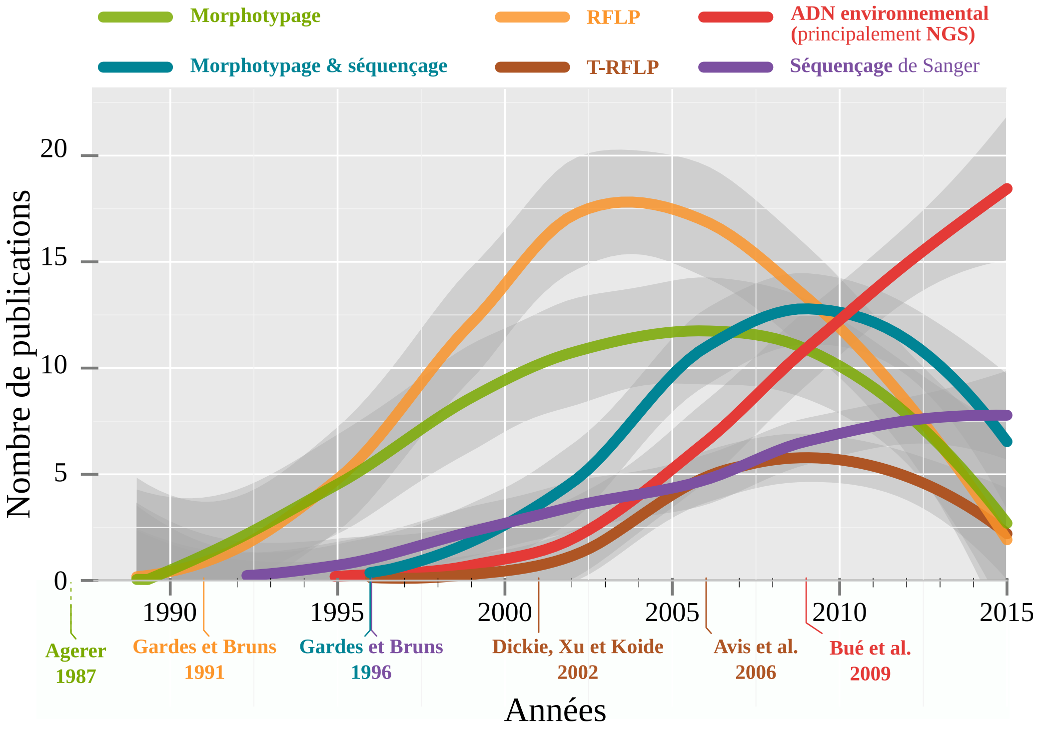


Figure I.9 – Tendances des méthodes d'études des champignons ectomycorhiziens : Les courbes ont été calculées par la fonction geom_smooth du package ggplot2 avec l'argument method="loess". Les données proviennent de recherche sur Web of Science™ (20 janvier 2015) en recherchant dans le sujet (« topic ») pour toutes les années jusqu'à 2015 : **Morphotypage** : « morphotyp* AND ectomycorrhiz* NOT sequenc* NOT molecular » ; **Séquençage** : « "sanger sequencing" AND ectomycorrhiz* OR direct sequencing AND ectomycorrhiz* » ; **Morphotypage & séquençage** : « morphotyp* AND ectomycorrhiz* AND sequenc* OR morphotyp* AND ectomycorrhiz* AND molecular » ; **RFLP** : « RFLP AND ectomycorrhiz* » ; **T-RFLP** : « T-RFLP AND ectomycorrhiz* OR TRFLP AND ectomycorrhiz* » ; **NGS** : « "environmental sequencing" AND ectomycorrhiz* OR NGS AND ectomycorrhiz* ». Notez que le nombre de publications de l'année 2015 est sûrement sous-estimé en raison des délais de Web of Science™. Cependant, les ratios des différentes méthodes d'identification restent valables.

Définition anatomique et physiologique

La mycorhize est un organe mixte résultant de l'interconnexion des tissus végétaux et fongiques (Fig. I.8 et I.10). Cet organe, qui donne son nom à la symbiose mycorhizienne, est un lieu d'échange entre les partenaires. Il permet le transfert de carbone, de vitamines et d'hormones de la plante vers le champignon. En « contrepartie », le partenaire fongique procure de l'eau, des minéraux et des hormones. Cette symbiose est également impliquée dans l'adaptation des plantes à leur milieu biotique et abiotique. Les champignons mycorhiziens fournissent une protection racinaire accrue, en particulier contre les pathogènes fongiques (Maherali et Klironomos 2007) et contre la dessiccation

(Smith et Read 2008). Le partenaire fongique peut également protéger la racine contre les composés polluants comme l'aluminium (Egli et Brunner 2002).

Il existe plusieurs types d'interactions mycorhiziennes caractérisées par des morphologies, des écologies et des histoires évolutives différentes (Fig. I.10). Les types mycorhiziens sont traditionnellement divisés en deux groupes : les interactions mycorhiziennes où les tissus fongiques s'infiltrent à l'intérieur des cellules de l'hôte (les endomycorhizes) et celles où la surface d'échange est extra-cellulaire (les ectomycorhizes ; ECM). En réalité, dans les endomycorhizes les hyphes fongiques ne pénètrent pas directement la cellule végétale mais provoquent des invaginations de la paroi des cellules de l'hôte, ce qui induit cet aspect d'interpénétration des partenaires (Smith et Read 2008).

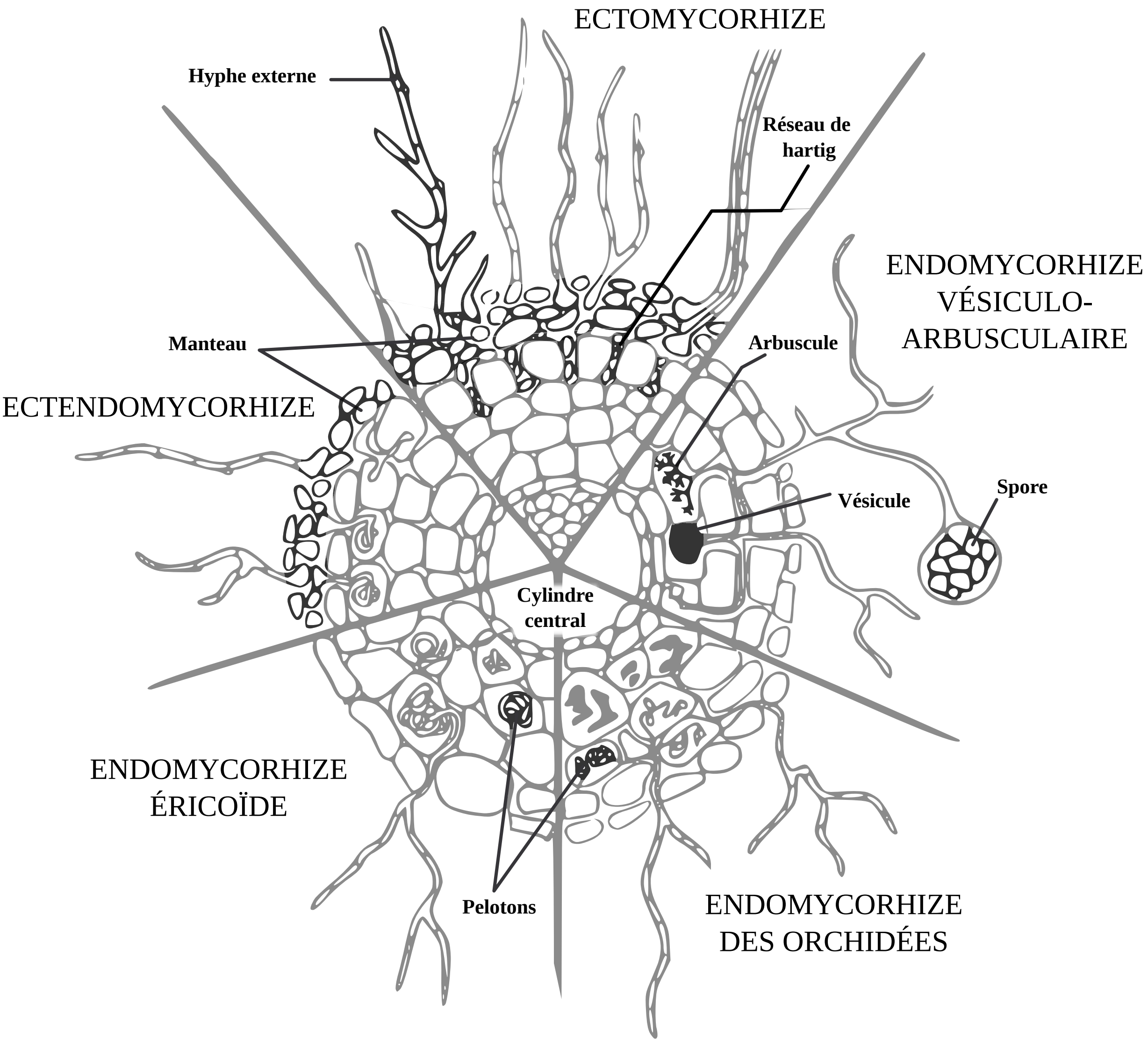


FIGURE I.10 – Schéma des différents types morphologiques de mycorhizes : Modifié de Selosse et Le Tacon (1998). Les cellules rectangulaires sont des cellules végétales, les autres sont des cellules fongiques. Les zones noires indiquent les structures fongiques importantes et légendées.

Parmi les **endomycorhizes**, la forme la plus abondante est la forme vésiculo-arbusculaire qui concerne approximativement 85 % des familles de plantes et des espèces d'Angiospermes (Wang et Qiu 2006). Les **endomycorhizes vésiculo-arbusculaires** impliquent donc une très grande diversité de plantes (environ 200 000 espèces, van der Heijden et coll. 2015), en revanche les partenaires fongiques sont peu nombreux (entre 300 et 1 600 espèces, van der Heijden et coll. 2015) et appartiennent tous à la division des Gloméromycètes. La symbiose entraîne la formation d'arbuscules qui constituent une grande surface d'échange, et de vésicules qui ont sans doute un rôle de stockage (Fig. I.10 ; Smith et Read 2008). Les **endomycorhizes des orchidées** impliquent des plantes de la famille des Orchidacées et des champignons appartenant au phylum des Basidiomycètes (Smith et Read 2008). Les **endomycorhizes éricoïdes** concernent les plantes de la famille des Éricacées (sous famille des Éricoïdées) et des champignons Ascomycètes ou Basidiomycètes (Smith et Read 2008).

Les **ectomycorhizes** (ECM) impliquent des espèces de plusieurs familles de plantes. En milieu boréal et tempéré, les hôtes appartiennent principalement à la famille des Pinacées, Fagacées, Bétulacées, Myrticacées, Salicacées, Rosacées et Cistacées. En milieu tropical, certaines familles de plantes sont également ectomycorhiziennes comme les Diptérocarpacées, les Euphorbiacées, les Fabacées ou encore les Polygonacées (Wang et Qiu 2006 ; Smith et Read 2008). Les hyphes des champignons ECM (Basidiomycètes, Ascomycètes et quelques Zygomycètes) forment un manteau autour des cellules racinaires. Les hyphes pénètrent entre les cellules corticales en formant un réseau appelé réseau de Hartig (Smith et Read 2008 ; Fig. I.8). Le cylindre central de la plante, délimité par l'endoderme, n'est jamais colonisé (Selosse 2000 ; Fig. I.10). À l'extérieur de l'ectomycorhize, les hyphes fongiques explorent le sol environnant et peuvent ainsi pourvoir la plante en eau et en minéraux (Fig. I.11).

Enfin, les **ectendomycorhizes** forment des réseaux de Hartig mais présentent également des structures intra-cellulaires (Smith et Read 2008). Les ectendomycorhizes sont mal connues et bien moins répandues que les ectomycorhizes et que les endomycorhizes vésiculo-arbusculaires.

Définition écologique et évolutive

L'interaction mycorhizienne est durable[29], intime[30] et mutualiste[31]. Cette association peut être vue comme une extension de la niche des plantes impliquées, c'est-à-dire comme une part du phénotype étendu des plantes (au sens de Dawkins 1989). Symétriquement, les niches écologiques des champignons mycorhiziens sont façonnées par leurs hôtes potentielles.

Les premiers champignons mycorhiziens, de type endomycorhiziens vésiculo-arbusculaires ont probablement joué un rôle prépondérant dans l'adaptation de premiers végétaux au milieu terrestre (Field et coll. 2015). Ainsi, des traces d'arbuscules et de vésicules ont été retrouvées sur des fossiles d'*Aglaophyton* (Remy et coll. 1994 ; Taylor et coll. 2005). La forte capacité des champignons d'acquisition et de transport de l'eau a sans doute été indispensable aux plantes dans ce nouveau milieu où l'hygrométrie était le facteur limitant principal.

Au delà de l'écologie des partenaires, les symbioses mycorhiziennes participent à de nombreux processus écosystémiques (Courty et coll. 2010 ; Averill et coll. 2014 ; van der Heijden et coll. 2015).

---

[29] C'est d'ailleurs le sens anglophone du mot symbiose.

[30] Les deux partenaires sont morphologiquement étroitement liés via la pénétration des tissus du champignon dans les tissus de la plante.

[31] L'interaction est à bénéfice réciproque, voir figure I.4.

Ainsi, le mycélium des mycorhizes est impliqué dans la décomposition de la matière organique (Langley et coll. 2006 ; Talbot et coll. 2008), la qualité de la litière (Langley et Hungate 2003), l'agrégation des composées pédologiques (Rillig et Mummey 2006), le cycle du carbone (Cairney 2012), de l'azote (Wu 2011), du phosphore (Cairney 2011) et de l'eau (Allen 2007), ou encore dans l'activité bactérienne de la rhizosphère[32] (Langley et Hungate 2003).

Le rôle des symbioses mycorhiziennes a également été mis en évidence dans les processus d'invasion biologique (Pringle et coll. 2009 ; Nuñez et coll. 2013), de facilitation des plantules (van der Heijden et Horton 2009) et plus généralement dans l'assemblage des communautés végétales (Bever et coll. 2010 ; Hoeksema et coll. 2010 ; Klironomos et coll. 2011 ; van der Heijden et coll. 2015). Les mycorhizes sont parfois les vecteurs des transferts de carbone entre plantes, notamment via les CMN (« Common Mycorrhizal Networks », Simard et coll. 1997, voir encadré 4). L'implication des mycorhizes dans les activités humaines comme l'agriculture et la foresterie sont des directions prometteuses pour la recherche appliquée (Leake et coll. 2004 ; Smith et Read 2008).

3.2 La symbiose ECM en forêt méditerranéenne

Caractéristiques de l'interaction ECM

L'interaction ECM implique des plantes ligneuses (autour de 6 000, van der Heijden et coll. 2015), principalement tempérées, et des macromycètes[33] dont l'appareil végétatif ne pénètre pas les parois cellulaires (estimées à plus de 20 000 espèces, van der Heijden et coll. 2015). Les champignons ectomycorhiziens sont constitués de trois parties (Fig. I.11) :

- Les **mycorhizes** au sens strict, également appelées **apex**, sont caractérisées morphologiquement par la présence de deux structures (Fig. I.10 et I.11) : un **manteau** mycélien qui engaine la partie racinaire de la mycorhize, et, en dessous du manteau, un **réseau de Hartig** constitué de mycélium qui pénètre entre les cellules racinaires en formant un réseau visible en coupe.
- Une structure végétative leur permet d'explorer le sol environnant à la recherche d'eau et de nutriments : **le mycélium extraracinaire**. Certaines espèces forment des structures composées de mycélium agglutiné : les **rhizomorphes**. Ces rhizomorphes supportent des transferts de molécules (C, N, P, hormones, etc.) à longue distance autorisant ainsi une exploration efficace des zones éloignées de la mycorhize (Boddy 1993 ; Agerer 2001). Certaines espèces forment des structures de résistance, souvent sous la forme de **sclérotes** (Smith et coll. 2015).
- Des **structures reproductives** sexuées ou asexuées. Les **sporophores** produisent les **spores** sexuées (méiospores) et, beaucoup plus rarement chez les champignons ECM (Hutchison 1989), des **conidies** produisent des spores asexuées (mitospores ; par ex. chez les pézizes, Healy et coll. 2013).

---

[32] Désigne la région du sol directement sous influence des racines.

[33] Désigne un champignon dont le sporophore est visible à l'œil nu. Ils sont appelés communément champignons supérieurs. C'est un groupe polyphylétique qui comprend principalement deux grands groupes d'Eumycètes : les Basidiomycètes et les Ascomycètes.

Encadré 1 : Donneurs, esclaves, tricheurs ou assureurs ? Une interaction complexe et protéiforme.

Globalement, les champignons mycorhiziens sont bénéfiques à l'hôte et ont besoin de cet hôte pour vivre (Smith et Read 2008). L'interaction semble donc à première vue un échange équilibré entre **donneurs**. Pourtant, certains champignons ce sont spécialisés sur une seule ou quelques espèces de plantes alors que les plantes les plus spécialistes ont des dizaines d'espèces de partenaires fongiques potentiels (par ex. les aulnes ; Rochet et coll. 2011). L'obligation pour les champignons de s'associer à une plante peut donc s'apparenter à une prison dorée pour les champignons spécialistes. Cette « prise d'otage évolutive » (Thomas et coll. 2010, p. 561) renvoie plutôt l'image d'un champignon **esclave** de l'interaction dont la survie dépend de celle de l'espèce hôte.

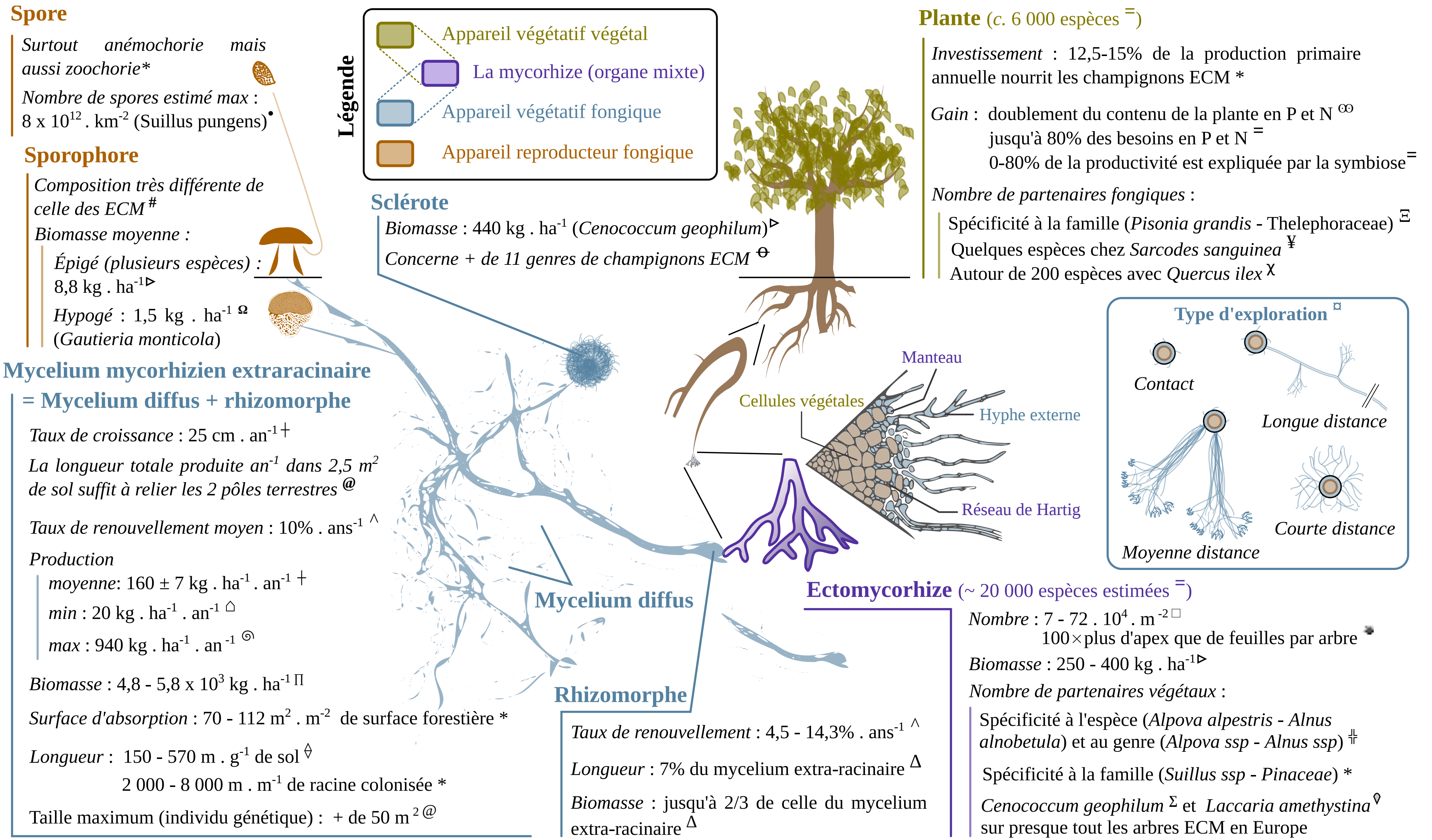
Face à ce constat et compte tenu de l'échelle de l'évolution biologique, il semble logique de voir apparaître de nombreuses formes de tricherie dans l'interaction. En effet, l'individu **tricheur**, c'est à dire celui qui prend les bénéfices de l'interaction sans donner de contrepartie, devrait voir sa valeur sélective augmenter. La tricherie devrait se propager dans la population et éroder les bénéfices du mutualisme (Jones et coll. 2015). Se pose donc la question suivante : comment se maintient ce mutualisme malgré les avantages sélectifs clairs apportés par le fait de tricher ?

Cette question est complexe puisque la mesure expérimentale de la tricherie dépend du contexte et que les communautés de champignons mycorhiziens sont diversifiées (Jones et coll. 2015). De plus, les bénéfices de l'interaction ECM sont de plusieurs natures, ce qui complique encore la mesure de ce rapport bénéfices/coûts pour les partenaires. Les mécanismes stabilisant les mutualismes mycorhiziens les plus invoqués sont le phénomène de récompense réciproque (carbone contre phosphate, Kiers et coll. 2011 ; carbone contre azote, Fellbaum et coll. 2014) et la punition des mauvais partenaires (Steidinger et Bever 2014). Cette question est un front de recherche concernant l'association mycorhizienne (van der Heijden et coll. 2015) mais aussi le mutualisme en général (Jones et coll. 2015).

Image issue du manuscrit page 107

Les champignons mycorhiziens se positionnent sur un gradient mutualisme/parasitisme (Johnson et coll. 1997). Même si le bénéfice réciproque est la règle globale, dans certains cas (environnement biotique et abiotique, stade de vie des partenaires, saison...) la relation peut se transformer temporairement en parasitisme. C'est l'augmentation de la valeur sélective sur la durée entière de l'interaction qui permet de définir le mutualisme ou le parasitisme. Les partenaires ont alors un rôle d'**assureur** : même si durant un moment un des partenaires est floué, il récupérera sans doute ce déficit à un autre moment de la vie de l'interaction. On peut alors imaginer qu'au cours de l'interaction, la symbiose mycorhizienne peut occuper l'ensemble du schéma de la figure I.4 selon l'environnement dans lequel l'interaction a lieu.

Les travaux récents militent pour un cadre plus complet de l'étude de la symbiose mycorhizienne qui prenne en compte toutes ces facettes de l'interaction ECM (voir Bender et coll. 2014, manuscrit A).



Spore

Surtout anémochorie mais aussi zoochorie[*]

Nombre de spores estimé max : 8 x 10^{12} . km^{-2} (Suillus pungens)[•]

Sporophore

Composition très différente de celle des ECM[#]

Biomasse moyenne :

- *Épigé (plusieurs espèces) :* 8,8 kg . ha^{-1}[▷]
- *Hypogé* : 1,5 kg . ha^{-1}[Ω] (*Gautieria monticola*)

Légende

- 🟩 Appareil végétatif végétal
- 🟪 La mycorhize (organe mixte)
- 🟦 Appareil végétatif fongique
- 🟧 Appareil reproducteur fongique

Sclérote

Biomasse : 440 kg . ha^{-1} (*Cenococcum geophilum*)[▷]

Concerne + de 11 genres de champignons ECM[Θ]

Plante (*c.* 6 000 espèces[=])

Investissement : 12,5-15% de la production primaire annuelle nourrit les champignons ECM [*]

Gain : doublement du contenu de la plante en P et N [ꝏ]
jusqu'à 80% des besoins en P et N [=]
0-80% de la productivité est expliquée par la symbiose[=]

Nombre de partenaires fongiques :

- Spécificité à la famille (*Pisonia grandis* - Thelephoraceae) [Ξ]
- Quelques espèces chez *Sarcodes sanguinea* [¥]
- Autour de 200 espèces avec *Quercus ilex* [χ]

Type d'exploration [¤]

Mycelium mycorhizien extraracinaire
= Mycelium diffus + rhizomorphe

Taux de croissance : 25 cm . an^{-1} [+]

La longueur totale produite an^{-1} dans 2,5 m^2 de sol suffit à relier les 2 pôles terrestres [@]

Taux de renouvellement moyen : 10% . ans^{-1} [^]

Production

- *moyenne*: 160 ± 7 kg . ha^{-1} . an^{-1} [+]
- *min* : 20 kg . ha^{-1} . an^{-1} [⌂]
- *max* : 940 kg . ha^{-1} . an^{-1} [◎]

Biomasse : 4,8 - 5,8 x 10^3 kg . ha^{-1} [Π]

Surface d'absorption : 70 - 112 m^2 . m^{-2} de surface forestière [*]

Longueur : 150 - 570 m . g^{-1} de sol [◊]
2 000 - 8 000 m . m^{-1} de racine colonisée [*]

Taille maximum (individu génétique) : + de 50 m^2 [@]

Rhizomorphe

Taux de renouvellement : 4,5 - 14,3% . ans^{-1} [^]

Longueur : 7% du mycelium extra-racinaire [Δ]

Biomasse : jusqu'à 2/3 de celle du mycelium extra-racinaire [Δ]

Ectomycorhize (~ 20 000 espèces estimées [=])

Nombre : 7 - 72 . 10^4 . m^{-2} [□]
100×plus d'apex que de feuilles par arbre [✸]

Biomasse : 250 - 400 kg . ha^{-1}[▷]

Nombre de partenaires végétaux :

- Spécificité à l'espèce (*Alpova alpestris - Alnus alnobetula*) et au genre (*Alpova ssp - Alnus ssp*) [╫]
- Spécificité à la famille (*Suillus ssp - Pinaceae*) [*]
- *Cenococcum geophilum* [Σ] et *Laccaria amethystina* [⚲] sur presque tout les arbres ECM en Europe

[Ω] Hunt et Trappe, 1987
[#] Gardes et Bruns, 1993
[+] Dahlberg et Stenlid, 1994
[Δ] Rousseau et al., 1994
[▷] Dahlberg et al., 1997
[¥] Kretzer et al., 2000
[¤] Agerer, 2001
[◊] Bidartondo et al., 2001
[Σ] Horton et Bruns, 2001
[□] Taylor, 2002
[ꝏ] Read et Perez-Moreno, 2003
[@] Leake et al., 2004
[Π] Wallander et al., 2004
[⌂] Nilsson et al., 2007
[◎] Parrent et Vilgolys, 2007
[Ξ] Chambers et al., 2005
[⚲] Roy et al., 2008
[*] Smith et Read, 2008
[╫] Moreau et al., 2011
[•] Peay et al., 2012
[^] Ekblad et al., 2013
[χ] Taudiere et al., 2015
[Θ] Smith et al., 2015
[=] Van der Heijden et al., 2015
[✸] Franck Richard (com. pers.)

FIGURE I.11 – L'interaction ectomycorhizienne en quelques chiffres : Ces valeurs illustratives peuvent varier drastiquement selon les espèces en interaction et l'environnement de l'interaction. Se référer au corps de texte et aux publications d'origine pour plus de détails.

Spécificité et préférences de l'interaction ECM

Chez les champignons ectomycorhiziens, il existe un gradient de spécificité, depuis l'association exclusive jusqu'au généralisme en passant par des degrés d'association à tous les niveaux taxinomiques. *Cenococcum geophilum* et *Laccaria amethystina* ont été retrouvés sur la plupart des arbres ectomycorhiziens en Europe (Horton et Bruns 2001 ; Roy et coll. 2008). Au contraire *Alpova alpestris* n'est connu que sur *Alnus alnobetula* (Moreau et coll. 2011) et les espèces du genre *Suillus* s'associent exclusivement à des plantes de la famille des Pinaceae (Wu 2000). Les préférences d'association sont souvent liées à la phylogénie des partenaires. D'ailleurs les livres de mycologie indiquent très souvent lorsque les espèces de champignon poussent plutôt dans les forêts de résineux (Gymnospermes) ou de feuillus (Angiospermes). Un exemple d'adaptation à un nouvel hôte a été très bien documenté en Amérique du Nord (Pringle et coll. 2009 ; Wolfe et coll. 2010 ; Wolfe et Pringle 2012). *Amanita phalloide* a été introduit au États Unis au XX^{ème} siècle sans doute à partir de plants venus d'Europe. Cette espèce de champignon ECM interagit très principalement avec des chênes dans sa zone géographique d'origine. Sur la côte Est des États-Unis, l'amanite phalloïde s'associe avec des pins mais présente une distribution très limitée alors que sur la côte Ouest, cette espèce s'est adaptée à un hôte endémique : le chêne de Californie (*Quercus agrifolia*).

Au delà de la spécificité à l'échelle de l'espèce, le degré de préférence de l'interaction pour les paires d'espèces partenaires est mal connu. Pour une même espèce, deux espèces partenaires peuvent varier fortement en matière de nombre d'interactions, de degré de dépendance et de degré de mutualisme. La quantification de ces préférences d'association permettrait d'aller plus loin dans notre compréhension de la symbiose que l'étude des spécificités à l'échelle des espèces. Mais ces quantifications sont très difficiles à mesurer dans les systèmes complexes formés par les arbres et leurs symbiontes fongiques racinaires.

Histoire évolutive des plantes et des champignons ECM

Le plus ancien fossile d'ectomycorhize date du milieu de l'Éocène (c. 45 millions d'années, LePage et coll. 1997) mais les reconstructions moléculaires et les études biogéographiques proposent des dates plus anciennes (autour de 135 millions d'années, Smith et Read 2008). Le caractère ectomycorhizien a évolué plusieurs fois chez les champignons à partir de précurseurs décomposeurs (Bruns et coll. 1998 ; Hibbett et coll. 2000 ; James et coll. 2006). De nombreux champignons ECM ont des capacités de décomposition héritées de leurs ancêtres saprotrophes (Shah et coll. 2015).

Même si ces capacités enzymatiques sont moindres que celles d'espèces à mode de vie uniquement décomposeur (Kohler et coll. 2015), les champignons ECM pourraient donc obtenir une partie de leur carbone et de leurs nutriments grâce à une décomposition active (Koide et coll. 2008). Certains groupes ectomycorhiziens ont conservé des capacités similaires à celles des champignons responsables des pourritures blanches (par ex. les espèces du genre *Paxillus*) ou brunes (par ex. les espèces du genre *Cortinarius*). Cependant, Lindahl et Tunlid (2015) indiquent que même si les champignons ECM sont capables d'obtenir de l'azote et du phosphore grâce à la décomposition, ils n'obtiennent en revanche que très peu de carbone via la décomposition.

Encadré 2 : De la nécessité d'être ectomycorhizé : histoires d'un amour indispensable.

Un phénomène permet de se rendre compte de l'importance de la mycorhization pour les plantes : l'introduction d'espèces. Lors de la plantation d'essences d'arbres exotiques, de très nombreux essais au cours du XX^e^siècle ont mis en exergue le poids de l'interaction ECM dans la réussite des plantations (Briscoe 1959 ; Mikola 1970). L'importance de l'inoculation des plantules chez les plantes ECM est renforcée par la forte spécificité dans l'interaction ECM [a] (Reinhart et Callaway 2006). Ces patrons ont surtout été observés dans les plantations de conifères, qui ont un cortège ECM particulier. Cette difficulté d'établissement en plantation est également observée dans des conditions naturelles : les pins ont des difficultés pour coloniser de nouvelles régions (Thiet et Boerner 2007).

Plus récemment, Nuñez et coll. (2009) ont montré pour trois espèces de Gymnospermes invasives (*Pseudotsuga menziesii*, *Pinus ponderosa* et *P. contorta*) que les plantules s'établissaient mieux et grandissaient plus vite aux abords des plantations. De plus, la croissance des individus loin des plantations est améliorée lorsque l'on plante ces individus avec du sol des plantations – et donc, avec les champignons associés. Les espèces de champignons très présentes dans la plantation mais absentes des plantules éloignées de la plantation (*Wilcoxina mikolae* (voir photo I.8) et *Rhizopogon sp.*) sont connues pour faire peu de dissémination à longue distance. *Wilcoxina mikolae* se reproduit surtout végétativement ou par chlamydospore [b] tandis que les espèces du genre *Rhizopogon* ont des fructifications souterraines qui nécessitent une dissémination endozoochore [c]. Au contraire, *Suillus luteus*, connu pour ses fructifications aériennes massives, est retrouvé sur les pins loin des plantations. La limitation de la dispersion des champignons ECM semble donc contenir l'invasion des pins dans ces forêts dominées par *Nothofagus dombeyi* (Nothofagaceae ; principalement ectomycorhiziens) et *Austrocedrus chilensis* (Cupressaceae ; endomycorhiziens).


©J. H. Parkins - Nature

Pour autant, l'image ci-dessus (publiée par Whitfield 2007) est trompeuse. Le résultat de l'interaction d'un point de vue de la valeur sélective n'est pas toujours favorable aux partenaires (voir encadré 1 et manuscrit A). De plus, l'association ECM peut être utilisée par les plantes pour s'exclure. Ainsi, l'alliaire officinale (*Alliaria petiolata*) est une plante invasive qui inhibe le développement des symbiotes racinaires des plantes natives (Hale et coll. 2016), notamment des champignons ECM (Wolfe et coll. 2008).

---

[a] Par exemple par rapport à l'interaction endomycorhizienne.
[b] Spore résistante grâce à sa large paroi. Les chlamydospores sont quasiment toujours asexuées.
[c] Mode de dispersion impliquant le passage dans le tube digestif d'un animal.

Certains auteurs proposent l'existence de cas de retour à la vie libre à partir d'un mode de vie mycorhizien (Hibbett et coll. 2000), mais cet avis est contesté (Bruns et Shefferson 2004). Quel que soit le résultat du débat, le nombre de passages de l'état décomposeur à un mode de vie ectomycorhizien est beaucoup plus important que la transition inverse (Bruns et coll. 1998 ; Hibbett et coll. 2000 ; James et coll. 2006). Cela conduit à penser que la perte de certaines fonctions liées au mode de vie libre lors de la transition vers la symbiose est difficilement réversible. Cette irréversibilité du mode de vie induit de fortes contraintes évolutives chez les taxons ectomycorhiziens qui pourraient jouer un rôle important dans la stabilité de l'interaction ectomycorhizienne (Wolfe et coll. 2012).

Encadré 3 : Mesurer des traits fonctionnels fongiques pour dépasser la « malédiction du binôme latin » (Raffaelli 2007)

L'objectif de l'écologie fonctionnelle (McGill et coll. 2006 ; Violle et coll. 2007) est de dépasser cette vision uniquement taxinomique en recherchant des traits [a] chez les partenaires qui permettent de prédire leurs réseaux d'interactions (Koide et coll. 2014). Par exemple, on peut s'attendre à ce que les champignons associés à des arbres à système racinaire pivotant soient différents de ceux associés à des arbres à système racinaire fasciculé. De même, la stratégie écologique de la plante (voir manuscrit page 112) et son habitat (voir manuscrit page 127) sont susceptibles de contraindre les communautés fongiques associées. On peut également utiliser les distances phylogénétiques des partenaires comme des indicateurs, des *proxies*, de leur dissimilarité de traits (Mouquet et coll. 2012). On fait alors l'hypothèse que l'évolution de ces traits subit une inertie phylogénétique. Les mycologues ont très tôt utilisé ce concept en différenciant les champignons qui poussent dans les forêts de résineux (Gymnospermes) et ceux qui poussent dans les forêts de feuillus (Angiospermes).

---

[a] Un trait est défini ici comme tout caractère mesurable, qu'il soit morphologique, chimique ou encore comportemental.

Diversité des partenaires ECM en Méditerranée

Le climat méditerranéen, marqué par des étés chauds et secs et par des hivers doux, caractérise le biome méditerranéen. Le pourtour de la Méditerranée concentre la majeure partie de ce biome qui abrite de nombreuses espèces végétales dont un grand nombre sont endémiques (Médail et Quézel 1999), formant un point chaud de biodiversité (Myers et coll. 2000). Les essences d'arbres ECM – qui constituent la majorité des forêts méditerranéennes – n'échappent pas à la règle. Les forêts méditerranéennes abritent une grande diversité fongique (Richard et coll. 2005 ; Shahin et coll. 2013 ; Buscardo et coll. 2015) à la fois aérienne (détermination ou séquençage des sporophores) et souterraine (morphotypage/séquençage des mycorhizes ou séquençage à haut débit du sol).

Rôle de l'interaction ECM dans la régénération et la survie des arbres en milieu méditerranéen : notions de réseau commun mycélien et de réseau mycorhizien écologique

L'interaction ECM facilite l'établissement des plantules (Richard et coll. 2009 ; Teste et coll. 2009) et la coexistence des espèces (Selosse et coll. 2006 ; McGuire 2007) dans les communautés végétales. La symbiose ECM contribue aux dynamiques de successions végétales primaires (Nara 2006) et secondaires (van der Heijden et Horton 2009 ; Bingham et Simard 2012). Un effet de facilitation par les ectomycorhizes a été démontré dans le cas de successions secondaires en Corse (*Arbutus unedo* facilitant l'établissement de *Quercus ilex*, Richard et coll. 2009), et en Californie (établissement de *Pseudotsuga menziesii* [Pinaceae] facilité par la présence d'*Arctostaphylos* [Ericaceae], Horton et coll. (1999)).

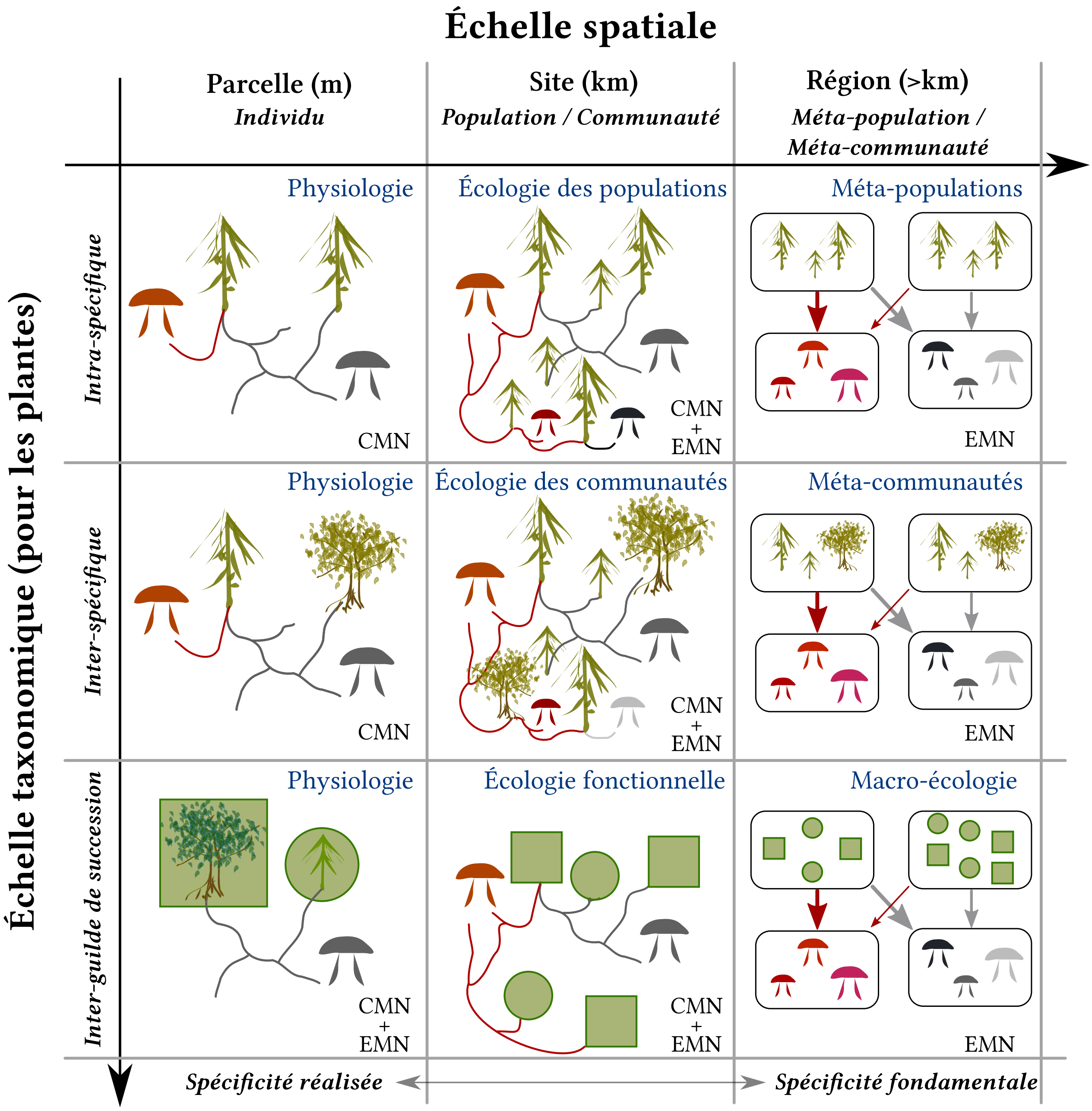


Figure I.12 – Échelle spatiale et taxinomique des réseaux plantes-champignons ECM : L'échelle spatiale des interactions ECM est indiquée en abscisse. L'axe des ordonnées représente l'échelle taxinomique des plantes divisée en trois parties : (i) les interactions entre individus végétaux de la même espèce, (ii) les interactions entre individus appartenant à des espèces différentes, et (iii) les interactions entre groupes fonctionnels avec le cas des guildes de succession. Les couleurs symbolisent des espèces différentes. Les cercles et les carrés représentent respectivement des espèces de début et de fin de succession. Le texte en bleu indique la discipline qui correspond le plus à l'échelle indiquée. **CMN** : Réseau mycélien commun (*Common Mycorrhizal Network*) ; **EMN** : Réseau mycorhizien écologique (*Ecological Mycorrhizal Network*).

Encadré 4 : Réseau mycorhizien commun : définition physiologique (CMN) contre définition écologique (EMN)

Les travaux de Suzanne Simard et son équipe (Simard et coll. 1997 ; Simard et Durall 2004 ; Simard et coll. 2012), ont mis en évidence des transferts de carbone entre plantes et ont démontré que ces transferts sont absents lorsque l'on perturbe le mycélium mycorhizien extra-racinaire qui relie les plantes (**CMN** pour « Common mycorrhizal Network » ; Fig. I.12). À plusieurs reprises dans l'évolution est apparue la capacité de certaines plantes à obtenir une partie (myxotrophie) voire tout (mycohétérotrophie) leur carbone d'autres plantes (Selosse et Roy 2009). Cette capacité à utiliser les champignons mycorhiziens pour parasiter d'autres plantes renforce l'idée que les transferts de carbone entre plantes sont essentiels dans l'écologie des plantes.


Régénération de plantules de pin laricio sous des adultes. Photo C. Carcaillet

Au delà des transferts de carbone, les plantes tirent parti des champignons mycorhiziens pour faciliter les échanges d'hormones, de minéraux, d'enzymes, ou encore d'informations (Simard et coll. 2012). Ainsi, Babikova et coll. (2013) ont démontré que des plantes attaquées par des pucerons émettaient un signal relayé par le réseau mycélien commun. Cette communication souterraine permet à la plante réceptrice de produire des composés de défense avant même d'avoir été attaquée. Néanmoins, la significativité écologique de ces transferts fait encore débat (Robinson et Fitter 1999 ; van der Heijden et coll. 2015) en particulier à cause des autres chemins potentiels de transferts entre plantes qu'il est difficile de contrôler en milieu naturel.

Les champignons ECM ont un rôle prépondérant dans l'établissement des plantules chez de nombreux arbres. Chez les plantes autotrophes, le transfert de carbone a surtout été démontré à partir d'arbres adultes en direction des plantules. La quantité de carbone transférée est d'autant plus forte que les conditions abiotiques sont stressantes (Simard et coll. 2012). Les CMNs pourraient être un des mécanismes de facilitation des plantules dans les milieux stressants comme le milieu méditerranéen. Mais il existe d'autres hypothèses non exclusives.

L'interaction rapide de la plantule avec des partenaires fongiques en bonne santé (**EMN** pour *Ecological Mycorrhizal Network* ; Taudiere et coll. 2015 ; Fig. I.12) est une hypothèse plus parcimonieuse puisqu'elle ne postule pas la continuité physique du mycélium. C'est d'ailleurs dans ce sens que Molina et Trappe (1982) ont utilisé en premier le terme de « *common mycelial networks* » en faisant l'hypothèse que *Arbutus menziesii* et *Arctostaphylos uva-ursi* favorisent l'établissement des plantules de pin qui suivent dans la succession car ces plantules peuvent « exploiter les réseaux mycorhiziens supportés par les deux plantes arbutoïdes [a] ». Les effets des champignons ECM sur la structure du sol et des communautés d'organismes édaphiques pourraient également favoriser l'établissement des plantules.

Dès lors que l'on s'intéresse à l'écologie des partenaires, les effets physiologiques précis qui sous-tendent les effets de facilitation via les champignons ECM ne nous intéressent pas. Dans cette thèse nous nous intéressons au résultat de l'ensemble des effets physiologiques dirigés par la présence de champignons ECM partagés entre plantes. Pour résumer, la symbiose ECM joue un rôle dans l'assemblage des communautés végétales (Bever et coll. 2010 ; van der Heijden et coll. 2015 ; plus de détails page 36). La recherche des processus physiologiques à l'œuvre [b] n'est pas du domaine de ce travail.

---

[a] Phrase originale : "[...] these plants [*Arbutus menziesii* and *Arctostaphylos uva-ursi*] maintain EM fungal diversity in forest ecosystems following disturbance and benefit seedling establishment of later-seral Pinaceae because seedlings can exploit the mycorrhizal networks supported by these arbutoid plants."(cité dans Molina et Horton 2015).

[b] Par exemple les transferts de carbone de nutriments d'informations via le mycélium, la présence d'inoculum en bonne santé lors de l'établissement ou encore le partitionnement des ressources via des partenaires différents.

Comme détaillé dans l'encadré 4, les plantes interagissent via les champignons ECM soit (i) directement par lien physique entre individus qui permet le transfert de matière entre plantes (CMN pour *Common Mycorrhizal Network*, Simard et coll. 1997), soit (ii) indirectement lorsque ces plantes sont capables de former des ECM avec des partenaires fongiques identiques (EMN pour *Ecological mycorrhizal network* ; Taudiere et coll. 2015). Trois processus pourraient donc favoriser la régénération des plantules grâce à la végétation déjà présente :

- La présence d'inoculum compatible pour les plantules. Certains champignons ECM présentent des limites à la dispersion (Nuñez et coll. 2009 ; Peay et coll. 2012). Ainsi, leur présence sur un site grâce à la végétation déjà présente pourrait favoriser la rapidité de l'interaction ECM et donc l'établissement des plantules (notion de réseau mycélien écologique : EMN, voir encadré 4).
- Parmi ces champignons compatibles, certains ramets (individus physiques) sont déjà bien développés grâce à l'investissement des plantes ECM déjà présentes. La plantule a donc rapidement accès à des réseaux mycéliens conséquents et aux avantages qu'ils procurent (notion de réseau mycélien écologique : EMN, voir encadré 4).
- Enfin, parmi ces ramets compatibles et déjà développés, certains peuvent relier différents arbres physiquement via leur réseau mycélien et ainsi entraîner la mise en place d'un réseau mycélien commun (CMN ; voir encadré 4 et Fig. I.12 ; Simard et coll. 1997 ; van der Heijden et Horton 2009).

Les réseaux mycorhiziens écologiques (EMN) décrivent à la fois la potentialité de la mise en place d'un flux de carbone entre plantes, mais aussi la présence de cette source d'inoculum pouvant favoriser d'autres plantes ECM. Un réseau mycorhizien écologique est défini comme un réseau d'interactions mutualistes reliant un ensemble de plantes et de champignons, quel que soit le niveau biologique étudié (par ex., individus, populations, espèces), capables d'établir des connexions mycorhiziennes dans au moins un contexte écologique (Fig. I.12 ; Taudiere et coll. 2015).

3.3 Les communautés de champignons ECM sont structurées par les hôtes, mais pas seulement

Tout n'est pas partout[34] …

L'importance de la dispersion dans l'assemblage des communautés de champignons ECM a été soulignée relativement récemment (Peay et coll. 2010). Peay et coll. (2007) ont montré une corrélation positive entre la diversité des champignons ECM et la taille des patchs d'arbres ainsi qu'avec la distance des hôtes à la forêt. En appliquant les préceptes de la théorie de la biogéographie insulaire de MacArthur et Wilson (1967), ils attribuent ce patron à un effet important de la dispersion sur les communautés fongiques ECM. Peay et Bruns (2014) ont mesuré la dispersion des spores de Basidiomycètes en utilisant des pièges à spores. Ils ont confirmé l'influence de la dispersion sur les communautés de spores ECM mais également sur la colonisation des plantules. Une autre

---

[34] En référence à la célèbre phrase de Baas Becking (1934). Plus d'information dans la section sur la dispersion des champignons endophytiques page 43.

caractéristique de la dispersion des champignons ECM est la forte variance de capacité de dispersion des espèces (Peay et coll. 2012). Tous les travaux cités dans le paragraphe précédent ont pour cible des champignons anémochores[35]. Or certains champignons ECM sont ecto-zoochores (par ex. *Tomentella sublilacina* ; Lilleskov et Bruns 2005) ou endo-zoochores (par ex. via des mammifères [Claridge 1992 ; Frank et coll. 2009 ; Nuñez et coll. 2013] et des arthropodes [Lilleskov et Bruns 2005]). C'est particulièrement le cas des champignons hypogés[36], comme la truffe, qui fructifient sous-terre.

… et l'environnement sélectionne

En plus de la structuration des communautés fongiques ECM par leurs hôtes (discuté précédemment), plusieurs autres facteurs jouent un rôle clé dans l'assemblage des communautés de champignons ECM. Les facteurs abiotiques tels que la température, l'humidité ou encore la composition chimique du sol peuvent affecter différentiellement les espèces (ou les individus) fongiques en fonction de leur degré de résistance à la sécheresse, ou de leurs capacités enzymatiques (Smith et Read 2008 ; voir Courty et coll. 2016, annexe page 368).

Les espèces fongiques ECM sont réparties préférentiellement dans les profils pédologiques (Landeweert et coll. 2003 ; Shahin et coll. 2013 ; Taylor et coll. 2014). En plus de leur répartition différentielle, les espèces fongiques ECM à mycélium hydrophobique acquièrent leur azote plus profondément que les espèces à mycélium hydrophylique (Hobbie et coll. 2014). Pickles et coll. (2015) ont montré que le filtre d'habitat (différents sols) rentre en interaction avec le filtre d'hôte. Dans la même idée, Moeller et coll. (2013) ont montré qu'en présence d'un sol pauvre en nutriment, les pins (*Pinus muricata*) deviennent pygmées et abritent des communautés ECM taxinomiquement et fonctionnellement très différentes. Peu d'études ont réussi à séparer de manière satisfaisante l'effet direct de l'environnement sur les communautés fongiques ECM des effets indirects via l'hôte. Récemment, Courty et coll. (2016) ont mis en évidence un filtre environnemental lié à l'adéquation entre la composition chimique du sol et les capacités enzymatiques des espèces.

* * *

---

[35] Du Grec ancien *anemos* (« vent ») et *khôrein* (« se mouvoir »). Qualifie un mode de dispersion des propagules (ici les spores) par le vent.

[36] Du Grec ancien *hypo* (« sous ») et *gaia* (« la terre »).

Résumé

(i) Les associations mycorhiziennes – découvertes au cours de la deuxième moitié du XIX^{e} siècle – impliquent les racines de la grande majorité des plantes terrestres et diverses espèces de champignons. Les partenaires entretiennent une interaction durable, intime et, le plus souvent, mutualiste.

(ii) Une des formes d'interaction mycorhizienne, l'ectomycorhize (ECM), est caractéristique des plantes ligneuses, en particulier en milieu tempéré et boréal. L'interaction ECM est impliquée dans la physiologie et la dynamique des populations et des communautés des partenaires. De plus, la symbiose ECM est prépondérante dans le fonctionnement de nombreux processus écosystémiques.

(iii) Les communautés de champignons ECM sont façonnées par les plantes hôtes en raison du degré de spécificité très variable des plantes et des champignons associés. D'autres facteurs responsables des structures des communautés de champignons ECM incluent les perturbations (par ex. le feu, les chablis), la composition du sol et les processus de dispersion.

Summary

(i) Mycorrhizal interactions were discovered during the second half of the XIX^{e} century. They involved roots of most terrestrial plants and various fungal species. Mycorrhizal partners form durable, intimate and mutualistic interactions.

(ii) Ectomycorrhizal (ECM) interactions is a mycorrhizal interactions type that engaged fungi with woody plants, in particular in Temperate and Boreal ecosystems. ECM interactions are implicated into physiology, population and community dynamics of partners. Moreover, ECM symbiosis is crucial in the functioning of numerous ecosystemic processes.

(iii) ECM fungal communities are shaped by their host due to the highly variability in the level of specificity of the symbiosis. Others factors drive community structures of ECM fungi including disturbance (*e.g.*, fire), soil chemical composition, as well as dispersal processes.

4 Les champignons endophytiques foliaires

4.1 Vivre dans la plante : l'endophytisme est un mode de vie partagé par une grande diversité de micro-organismes

On retrouve des micro-organismes partout sur et dans les plantes, des feuilles (phyllosphère) aux racines (rhizosphère) en passant par les tiges, les fleurs, l'écorce et les fruits. Les principaux organes examinés par les chercheurs sont les feuilles et les racines (voir tableau I.1). À l'instar du phénotype (Clemente et coll. 2012) et du comportement humain (Dinan et coll. 2015) qui sont influencés par son microbiote intestinal, les plantes sont des co-constructions en partenariat avec les micro-organismes (Partida-Martinez et Heil 2011 ; Lebeis 2015 ; Vandenkoornhuyse et coll. 2015). Pour comprendre le phénotype étendu (*sensu* Dawkins 1989) des plantes – et donc leur écologie et leur évolution – on se doit de comprendre l'écologie et l'évolution de ces endophytes.

Sous l'angle taxinomique, les endophytes sont très diverses. Les Archées et surtout les Bactéries sont présents dans les tissus végétaux. En particulier, les Proteobactéries, Actinobactéries, et Firmicutes sont pléthoriques dans les plantes (Hardoim et coll. 2015). Chez les Eucaroytes, le groupe majoritaire est celui des Eumycètes (champignons « vrais »), en particulier chez les Dothideomycètes (Ascomycètes), Sordariomycètes (Ascomycètes), Agaricomycètes (Basidiomycètes) et chez les Gloméromycètes (Hardoim et coll. 2015). De nombreuses revues de la littérature portant sur les différents groupes taxinomiques existent (tableau I.1). Ici nous nous intéressons tout particulièrement aux champignons endophytiques foliaires (CEF) et à leur écologie.

4.2 L'écologie des communautés fongiques endophytiques foliaires

La structure des communautés de champignons endophytiques foliaires [37] (CEF) est déterminée par de nombreux facteurs abiotiques (par ex. la température) et biotiques [38] (par ex. la teneur en carbone des feuilles). Dans le cadre théorique de Vellend (2010), les règles d'assemblage étudiées ici appartiennent aux groupes des mécanismes de sélection (par ex. la taxinomie de l'hôte) et de dispersion (par ex. la distance spatiale).

Les processus de dispersion chez les micro-organismes et champignons endophytes foliaires

En ce qui concerne les processus de dispersion, un paradigme a longtemps été de mise concernant la biogéographie des micro-organismes (Fenchel et Finlay 2004), dont celle des CEF (champignons endophytiques foliaires). Ce paradigme se résume par la célèbre phrase : « *Everything is everywhere,* but, *the environment selects* » [39] (Baas Becking 1934, cité dans de Wit et Bouvier 2006).

---

[37] On parle ici des feuilles au sens large, c.-à-d. y compris les aiguilles des conifères.

[38] Lire Hardoim et coll. (2015) pour une discussion des déterminants des communautés endophytiques bactériennes.

[39] La version originale en allemand (« *alles is overal :* maar *het milieu selecteert* ») utilise bien l'italique pour mettre en valeur le mot marr (« *but* » ; de Wit et Bouvier 2006)

Quelques articles ont corroboré cette hypothèse, par exemple chez les Ascomycètes du sol (Green et coll. 2004) et chez les champignons associés aux racines (Queloz et coll. 2011). Pourtant, de nombreux exemples récents contredisent le cosmopolitisme des micro-organismes (voir Martiny et coll. (2006) pour une revue de la littérature).

Une structuration spatiale est connue chez les champignons endomycorhiziens (Wolfe et coll. 2007 ; Mummey et Rillig 2008 ; Dumbrell et coll. 2010a), ectomycorhiziens (Lilleskov et coll. 2004 ; Peay et coll. 2007, 2012) et endophytiques foliaires des herbacées (Higgins et coll. 2014). Les CEF des arbres ne dérogent pas à la règle (tableau I.2). Ainsi, les communautés de CEF sont structurées à l'échelle des branches (Cordier et coll. 2012), des arbres (Cordier et coll. 2012), des régions (Coince et coll. 2014) et des continents (U'Ren et coll. 2012). La latitude est un facteur important structurant les communautés de CEF (Arnold et Lutzoni 2007 ; Bálint et coll. 2015 ; Millberg et coll. 2015), mais les mécanismes potentiels derrière ce patron latitudinal sont nombreux. Finalement, Oono et coll. (2014) ont mis en évidence des structures populationnelles chez certaines souches de *Lophodermium australe* aux États-Unis, une espèce de CEF courante dans les aiguilles de pin en bonne santé.

Cependant une structuration dans l'espace ne s'oppose pas obligatoirement à la phrase de Baas Becking (1934). En effet, une disposition spatiale particulière peut être causée par des processus de dispersion ou par une variation de l'environnement spatialement distribuée. Dans le cas où les processus de dispersion entraînent des patrons spatiaux, la phrase de Baas Becking (1934) ne fonctionne pas puisque *tout n'est pas partout*. En revanche, dans la deuxième partie de la phrase

Tableau I.1 – Tableau des revues de la littérature traitant des endophytes : La colonne *focus* indique l'angle particulier sous lequel la revue traite les endophytes.

Habitat	**taxinomie**	**Références**	***Focus***
Endosphère racinaire, phyllosphère et rhizosphère	Bactéries (Champignons)	Bulgarelli et coll. 2013	Structure et fonctionnement
Phyllosphère	Bactéries	Vorholt 2012	Structure et fonctionnement
Endosphère foliaire, phyllosphère	Bactéries	Griffin et Carson 2015	Forêt tropicale et agro-écosystème
Endosphère foliaire et racinaire	Bactéries, Champignons	Hardoim et coll. 2015	Structure et fonctionnement
Endosphère foliaire et racinaire	Champignons	Rodriguez et coll. 2009	Délimitation de groupes fonctionnels
Endosphère foliaire	Champignons	Arnold et Lutzoni 2007	taxinomie et écologie
Endosphère foliaire et racinaire	Champignons	Porras-Alfaro et Bayman 2011	Écologie et application
Endosphère foliaire et raméale	Champignons	Sieber 2007	Gradient mutualisme-parasitisme
Phyllosphère	Bactéries (Champignons)	Rastogi et coll. 2013	Structure et fonctionnement
Phyllosphère	Bactéries	Farré-Armengol et coll. 2016	Interaction avec les composés organiques volatiles

– *mais l'environnement sélectionne* – Baas Becking (1934) souligne l'importance de l'environnement sur le développement local des micro-organismes. Donc, si les variables environnementales (par ex. les variables climatiques ou la présence de prédateurs/parasites) sont spatialement structurées (par ex. corrélées à la latitude), un patron spatial peut émerger sans contredire l'idée de Baas Becking (1934).

Les processus de sélection des champignons endophytiques foliaires : filtre environnemental et influence de l'hôte

Les processus de sélection (*sensu* Vellend 2010) des communautés de champignons endophytiques foliaires (CEF) sont nombreux I.2. Les études récentes ont mis en avant le rôle des filtres environnementaux (pluviométrie, température moyenne, feu ; Zimmerman et Vitousek 2012 ; Coince et coll. 2014 ; Huang et coll. 2016). Dans certains cas, les conditions abiotiques responsables de ce processus de sélection des espèces dans la communauté locale sont induites par la plante. Ainsi, Coince et coll. (2014) ont mis en évidence des différences de communautés d'endophytes entre des feuilles dont le PH était différent. Yang et coll. (2016b) ont quant à eux montré un effet de la teneur en carbone foliaire sur les communautés d'endophytes.

Les CEF présentent des préférences d'association envers les plantes au niveau spécifique (par ex. Kembel et coll. 2014) et populationnel (Bálint et coll. 2015). De plus, ces organismes sont sensibles à l'âge des individus (Oono et coll. 2015) et des feuilles (Millberg et coll. 2015). La structuration des communautés en fonction de l'âge peut être expliquée par deux processus : (i) les feuilles d'âges différents ont des compositions chimiques différentes, et (ii) une feuille plus âgée est un écosystème dans lequel de nombreuses espèces sont arrivées et les espèces pionnières ont pu être remplacées par des espèces compétitrices[40].

Enfin, Millberg et coll. (2015) ont constaté que les feuilles étaient associées à des communautés différentes selon leur état de santé. Dans ce dernier cas, il est difficile de connaître le sens de causalité. Soit les feuilles en mauvaise santé sélectionnent effectivement des espèces fongiques endophytiques différentes, soit certaines espèces ou assemblages d'espèces sont responsables de la santé des feuilles.

Plusieurs processus de sélection, connus chez les plantes mais également chez les champignons, n'ont pas encore été testés. En particulier, la limitation de la similarité est encore très peu étudiée chez ces organismes hyper-diverses dont l'écologie reste mal connue (mais voir Courty et coll. 2016 : manuscrit N en annexe). À notre connaissance, aucune étude ne s'est intéressée aux effets de la dérive et de la spéciation sur les communautés de CEF (voir discussion).

* * *

---

[40] On fait alors appel au concept de succession et de groupe fonctionnel de Grime (1977).

Tableau I.2 – Revue des déterminants des structures de communautés de champignons endophytiques foliaires : Ce tableau récapitule les résultats de la littérature concernant les déterminants des structures des communautés de champignons endophytiques foliaires, parfois conjointement avec les champignons de la phyllosphère (en l'absence de stérilisation de la surface de la feuille avant séquençage).

	Références	**Méthodes**	**Séq.**	**Hôte(s)**	**OTUs**	**Taxons dominants**	**Lieu**	**Éch.**	**Séq./éch.**	**Déterminant de la diversité α et β**
Sans stérilisation de la surface des feuilles	Abdelfattah et coll. 2015	454	19 719	*Olea Europae*	124 (Blast)	Dothideomycetes *Aureobasidium* *Devriesia* *Pseudocercospora*	Calabre, Italie	72	274	Différents organes de plantes α(+) β(++)
	Coince et coll. 2014	454	149 946	*Fagus sylvatica*	1 457 (Uclust)	Cryptococcus Taphrina	France	33	4 544	Région α(=) β(++) PH α(=) β(+) Température moyenne α(=) β(++)
	Cordier et coll. 2012	454 + CE-SSCP	96 130	*Fagus sylvatica*	1 604 (Uclust)	*Lalaria inositophila* *Taphrinales Tremellales*	Rhônes-Alpes, France	27	3 560	Échelle spatiale (de l'arbre à la feuille) β(++)
	Jumpponen et Jones 2009	454	18 020	*Quercus macrocarpa*	360 (95%, CAP3)	*Microsphaeropsis sp* *Alternaria sp*	Kansas, États-Unis	54	334	Parcelles urbaines vs rurales α(++) β(++)
	Millberg et coll. 2015	454	75 855	Isolat	598 (98 % SCATA)	Artoniomycetes Dothideomycetes Leothiomycetes	Suède	69	1 099	Latitude α(=) β(+) Parcelles de plantation vs naturelles α(+) β(++) Âge des aiguilles α(=) β(+) Santé des aiguilles α(++) β(++)
Avec stérilisation de la surface des feuilles	Arfi et coll. 2012	454	209 544	*Avicennia marina* *Rhizophora stylosa*	2 877 (98% CD-HIT-EST)	Dothideomycetes Lecanoromycetes Sordariomycetes	Nouvelle Calédonie	12	17 462	Taxonomie de l'hôte α(+) β(++) Immergé vs émergé β(++)
	Bálint et coll. 2015	Illumina	1 759 757	*Populus balsamifera*	2 022 (Uclust, min nb seq= 10)	Dothideomycetes Mycosphaerella	Canada & Alaska	150	11 732	Réchauffement α(+) β(++) Latitude α(++) β(+) Génétique de l'hôte α(+) β(+)
	Bálint et coll. 2013	454	126 402	*Populus balsamifera*	179 Amplicon Noise, avec singletons)	Pleosporales Leptosphaerulina	Canada & Alaska	25	5 056	Génétique de l'hôte β(++)
	Botella et Diez 2011	Isolat	229	*Pinus halepensis*	38	*Cyclaneusma minor* *Lophodermium pinastri* *Fusarium proliferatum* *Alternaria sp*	Espagne	Isolat	Isolat	Aucun
	Bullington et Larkin 2015	Illumina + 454	2 540 729	*Pinus monticola*	1 358 (Uclust)	Dothideomycetes Davidiella	Idaho, États-Unis	57	44 574	Aucun
	Gazis et Chaverri 2015	Isolat	2 346	*Hevea*	710 (99 %)	*Trichoderma*	Basin Amazonien	Isolat	Isolat	Parcelles de plantation vs naturelles α(+) β(++)
	Huang et coll. 2016	Isolat & Sanger	1 184	*Juniperus deppeana;* *Quercus spp.*	95	Sordariomycetes Leothiomycetes Dothideomycetes	Sud-Ouest des États-Unis	Isolat	Isolat	Feu α(++) β(++) Taxonomie de l'hôte β(++)

Suite du tableau

Avec stérilisation de la surface des feuilles	Kembel et Mueller 2014	Illumina	4 131 371	57 espèces	11 848 (Uclust)	Sordariomycetes Dothideomycetes Eurotiomycetes	Île de Barro Colorado, Panama	100	41 314	Taxonomie de l'hôte β(++) Traits fonctionnels des feuilles β(+) Proximité phylogénétique des hôtes β(=)
	Kemler et coll. 2013	Ion Torrent	168 775	*Eucalyptus grandis*	2 415 (Uclust)	Mycosphaerellaceae Botryosphaeriaceae	KwaZulu-Natal, Afrique du Sud	147	1 148	Aucun
	Oono et coll. 2015	Isolat & Sanger	491	*Pinus taeda*	118 (99 %)	Dothideomycetes Leothiomycetes	North Carolina, USA			Adultes vs plantules α(++) β(++)
	Siddique et Unterseher 2016	Illumina	820 441	*Fagus sylvatica*	414 (Uclust, min nb seq= 5)	Helotiales Capnodiales Pleosporales	Bavière, Allemagne	30	27 348	Altitude α(++) β(++) Feuilles sénescentes vs normales α(=) β(+)
	Unterseher et coll. 2013	Isolat & Sanger	390	*Vasconcellea microcarpa; Tillandsia spp.* et *Hevea brasiliensis*	155	Hypocreales Xylariales Pleosporales	Pérou	Isolat	Isolat	Altitude α(=) β(=) Taxonomie de l'hôte α(+) β(++)
	U'ren et coll. 2014	454	33 310	*Pinus leiophylla*	19 (95 % Mothur)	Dothideomycetes Sordariomycetes	Arizona, États-Unis	108	308	Aucun
	Vincent et coll. 2016	Isolat & Sanger	2 079	11 espèces d'arbres	94 (95 % Mothur)	NA	Nouvelle Guinée	Isolat	Isolat	Taxonomie de l'hôte β(+) Échelle spatiale (locale et régionale) β(=)
	Yang et coll. 2016	454	1 079 386	*Betula hermanii*	1 265 (Usearch)	Dothideomycetes	Province de Jilin, Chine	54	19 989	Altitude α(++) β(++) Teneur en carbone des feuilles α(++) β(++)
	Zhang et Tao 2015	454	76 691	4 espèces	250 (Uclust)	Helotiales Pleosporales Capnodiales	Archipel de Svalbard, Arctique	12	6 391	Taxonomie de l'hôte α(=) β(++) Écosystème Arctique vs (sub)tropical β(++)
	Zimmerman et Vitousek 2012	454	665 155	*Metrosideros polymorpha*	2 552 (95% Esprit)	Dothideomycetes	Mauna Loa Volcano, Hawai'i	130	5 117	Pluviométrie α(=) β(++) Altitude α(=) β(++)
	Taudiere et coll. *in prep*	Illumina	~8 200 000	*Pinus nigra* subsp. *laricio*	662 (Uparse) - 4 150 (Uclust)	Dothideomycetes *Cyclaneusma minor*	Corse, France	81	101 235	Sites α(+) β(++) Âge des aiguilles α(=) β(+) Hauteur dans l'arbre α(=) β(=)

Résumé

(i) Les endophytes sont des organismes qui habitent – au moins pour une partie de leur cycle de vie – à l'**intérieur des tissus végétaux** sans créer de maladie apparente.

(ii) Une multitude de micro-organismes habitent **l'intérieur des feuilles** d'arbres, principalement des **champignons** dont on connaît encore très mal l'écologie.

(iii) On sait que les communautés de champignons endophytiques foliaires sont structurées par l'**environnement local** – en particulier via la plante **hôte** – et par leur capacité de **dispersion**. En revanche la quantification nécessaire à la comparaison des différentes règles d'assemblage des communautés manque encore.

Summary

(i) Endophytic organisms live, at least during a part of their life cycle, **inside plant tissues** without creating apparent disease.

(ii) A multitude of micro-organisms lives **inside leaves** of tree, mainly **fungi** whose ecology is poorly known.

(iii) Both **environmental filter** – including **host** mediated filter – and **dispersion** processes structure fungal endophytic foliar communities. But we are still ignorant about the relative magnitude of the different community assembly rules governing those communities.

5 Les champignons décomposeurs

5.1 Le mode de vie décomposeur

Les Eumycètes ont des capacités de décomposition très importantes. Les champignons sont les seuls organismes capables de dégrader certaines molécules complexes (Romaní et coll. 2006). En particulier, dans de nombreux écosystèmes, seuls quelques Basidiomycètes sont capables de décomposer la lignine (Hammel 1997 ; Jeffery et coll. 2013) bien que quelques taxons bactériens disposent de capacités lininolytiques (Bugg et coll. 2011). Par ailleurs, les champignons sont bien meilleurs que les bactéries pour la décomposition en milieu acide. De plus, à l'inverse des Bactéries et des Archées, les champignons ont des structures macroscopiques (dont les rhizomorphes, Boddy 1993 ; Fig. I.15a) qui jouent un rôle important dans la redistribution de l'eau et des nutriments dans le sol à l'échelle de plusieurs mètres.

On peut distinguer les champignons décomposeurs en trois grandes guildes en fonction de leur habitat et de leur source de carbone (tableau I.2). Les champignons **saprohumicoles** sont présents dans la litière et sont impliqués dans les premiers stades de décomposition de la matière organique. Les champignons **lignicoles** (littéralement « qui habitent dans le bois ») et **saproxyliques** sont capables de décomposer le bois y compris pour certains la lignine (champignons lininolytiques). Enfin, de nombreux champignons décomposeurs habitent – au moins pour une partie du cycle de vie – dans le sol (litière non incluse). Dans ce travail, nous nous intéressons à l'écologie des **champignons lininolytiques** (manuscrit I) et des champignons **décomposeurs édaphiques** (projet de manuscrit J).

5.2 La niche d'habitat et la niche trophique chez les champignons

Les champignons se nourrissent de nombreuses manières (voir introduction). Leur source de nourriture forme une partie de leur niche. Mais la niche trophique est insuffisante pour comprendre l'écologie des champignons. Par exemple, les endophytes foliaires et les champignons ectomycorhiziens, bien que se nourrissant du carbone des végétaux en symbiose, ont indéniablement des niches écologiques différentes. Même si des membres des deux guildes peuvent interagir via leur hôte, il est difficile de concevoir de nombreuses interactions directes entre eux.

Un deuxième sous ensemble de la niche est la niche d'habitat. En fonction de son lieu de vie, l'individu fongique va éprouver un environnement biotique et abiotique différent. La niche d'habitat et la niche trophique ne sont pas indépendantes (Fig. I.14) : difficile pour un champignon mycorhizien de vivre dans les feuilles des arbres durant une longue partie de son cycle de vie. La niche d'habitat recouvre un grand nombre de dimensions de la niche y compris les axes de niche liés à l'hôte, aux perturbations et aux variabilités spatio-temporelles des conditions abiotiques (tableau I.3). Ces différences de niches trophiques et d'habitat sont impliquées dans les règles d'assemblage des communautés. C'est sur la base du mode de nutrition et de l'habitat que sont définis les guildes fongiques qui déterminent les différents chapitres de cette thèse.

Tableau I.2 – Définition des termes associés aux champignons décomposeurs : Les relations entre les différents termes sont illustrées dans la figure I.14.

Termes	Définitions
Décomposition	Transformation biologique de la matière organique morte à la fois par désagrégation en molécules plus petites et par modification de la composition chimique (Lindahl et Tunlid 2015).
Lignicole	Adjectif qualifiant les organismes qui habitent dans le bois (Gobat et coll. 2010) sans obligatoirement s'en nourrir. Par exemple, on retrouve fréquemment les sporophores de certaines espèces de Cortinaires dans des troncs d'arbres en décomposition malgré leur statut de champignon ECM. La ressource en eau – souvent limitante dans la phase de fructification des champignons – dans le bois mort pourrait expliquer ce phénomène. Une étude de Ottosson et coll. (2015) a démontré la présence de nombreux groupes écologiques dans les grumes en décomposition.
Litière	Ensemble des matières organiques à différents stades de décomposition. Dans un sens plus restreint, on utilise ce terme pour désigner les débris végétaux tombés sur le sol qui forment l'horizon OL (Gobat et coll. 2010).
Pourriture	Ensemble des symptômes visibles associés au développement d'un ou plusieurs champignons saproxyliques (Gobat et coll. 2010).
Pourriture blanche (fibreuse)	Faciès de décomposition (Fig. I.13) caractérisé par la dégradation de la lignine et de la cellulose (Lindahl et Tunlid 2015). Les organismes capables de cette double dégradation sont **lininolytiques** et appartiennent principalement au clade fongique des Basidiomycètes (Jeffery et coll. 2013).
Pourriture brune (cubique)	Faciès de décomposition (Fig. I.13) caractérisé par la dégradation de la cellulose et de l'hémicellulose uniquement (Lindahl et Tunlid 2015) ou presque (Bugg et coll. 2011). Les organismes capables de dégrader uniquement la cellulose sont appelés cellulolitiques.
Pourriture molle	Faciès de décomposition (Fig. I.13) caractérisé par la dégradation de la cellulose uniquement. Les champignons responsables de pourriture molle attaquent souvent des bois plus humides et avec moins de lignine que les champignons provoquant des pourritures blanches et brunes (Goodell et coll. 2008).
Saprophytes	Du Grec ancien *sapros* (« putride ») et *phyton* (« plante »). Organismes hétérotrophes dont la majorité du carbone provient de la décomposition de matière organique morte végétale.
Saprotrophes	Du Grec ancien *sapros* (« putride ») et *trophicos* (« nourrissant »). Organismes hétérotrophes dont la majorité du carbone provient de la décomposition de matière organique morte (Lindahl et Tunlid 2015). On utilise souvent ce terme pour les organismes absorbotrophes (on parle sinon d'organismes détrivores ou saprophages).
Saproxyliques	Du Grec ancien *sapros* (« putride ») et *xylon* (« bois »). Organismes hétérotrophes dont la majorité du carbone provient de la décomposition de matière organique morte issue du bois.

5.3 Écologie des communautés de champignons décomposeurs

Le cas des champignons saprotrophes présents dans le sol

La composition de la végétation détermine la diversité et la composition des champignons décomposeurs édaphiques par plusieurs chemins. Tout d'abord, il existe des préférences voire des spécialisations des champignons décomposeurs en fonction des espèces (Zhou et Hide 2001 ; Ushio et coll. 2008 ; par ex. aiguille de conifère, feuille d'arbre caducifolié), des génotypes (Schweitzer et coll. 2008), des organes (par ex. feuille, branchage) et de la composition chimique du sol (Allison et coll. 2009 ; Ayres et coll. 2009). La végétation peut également affecter indirectement la communauté fongique de décomposeurs édaphiques, par exemple par une modification de la communauté mycorhizienne qui interagit fortement avec les décomposeurs (Averill et coll. 2014 ; Fernandez et Kennedy 2015a).

Figure I.13 – Faciès de décomposition d'origine fongique : a, b) Faciès de décomposition de type pourriture blanche (ou molle) causée par la dégradation de la lignine et de la cellulose. **c)** Souche de pin laricio comportant un gradient de faciès de décomposition du bas (pourriture blanche, flèche blanche) vers le haut (pourriture cubique, flèche orange) en relation avec un gradient hydrique. **d** et **e)** Faciès de décomposition typique de la pourriture brune (ou cubique) causée par la dégradation de la cellulose et de l'hémicellulose sans décomposition de la lignine. *Source* d : W 🅭[nom d'utilisateur : Beentree]. **f)** La décomposition du bois est un travail d'équipe. Si les micro-organismes, dont les champignons, sont indispensables, les animaux tels que les oiseaux (par ex. les pics) et les insectes (par ex. les termites et les Coléoptères saproxyliques) participent activement à ce processus. **g** et **h)** Faciès de décomposition en *nid d'abeille* de *Xylobolus subpileatus.*

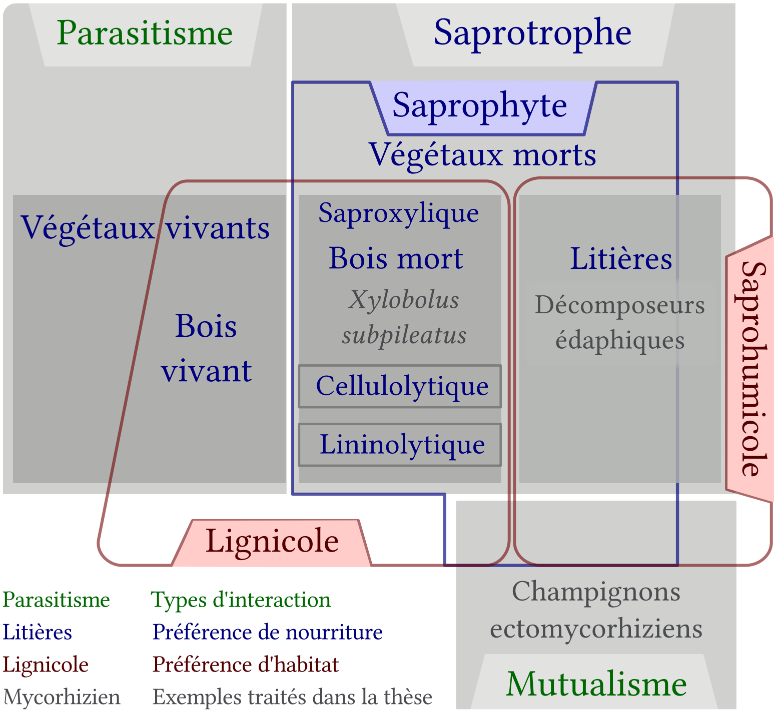


FIGURE I.14 – Illustration des relations entre niche d'habitat et niche trophique à travers la terminologie associée : Les types d'interactions sont indiqués en vert, les préférences trophiques en bleu et les préférences d'habitat en rouge. Les guildes étudiées dans cette thèse sont indiquées en gris excepté celle des endophytes foliaires.

Les perturbations structurent également les communautés de champignons décomposeurs édaphiques. Le feu est une des perturbations naturelles majeures sur terre (Moritz et coll. 2014, section 7 de l'introduction). Les incendies réduisent fortement la biomasse fongique (Dooley et Treseder 2012 ; Holden et Treseder 2013) et modifient la composition des champignons du sol, que ce soit dans le cas de feux prescrits (Oliver et coll. 2015) ou de feux naturels (Holden et coll. 2016). Les procédés par lesquels le feu influence ces communautés incluent la mort des micro-organismes ou d'autres espèces en interactions [41], mais aussi les modifications à plus ou moins long terme des caractéristiques physico-chimiques du sol (Holden et coll. 2013). D'autres perturbations altèrent les communautés fongiques en particulier les activités humaines. Il en est du management forestier à court terme (Paillet et coll. 2010) comme de l'usage des sols à long terme (Dupouey et coll. 2002). Nous verrons également dans le manuscrit I que les chablis sont aussi des déterminants de la structure des communautés de plusieurs guildes de champignons dont les champignons saprotrophes et saproxyliques.

Taylor et coll. (2014) ont identifié des champignons du sol qui sont des indicateurs d'habitat (sec *vs* humide ; acide *vs* non acide ; organique *vs* minéral). Ils trouvent de nombreux indicateurs opposés au sein d'un même genre ou d'une même guilde. Ils en déduisent un partitionnement de la niche plus fort avec déplacement de caractères [42] après spéciation.

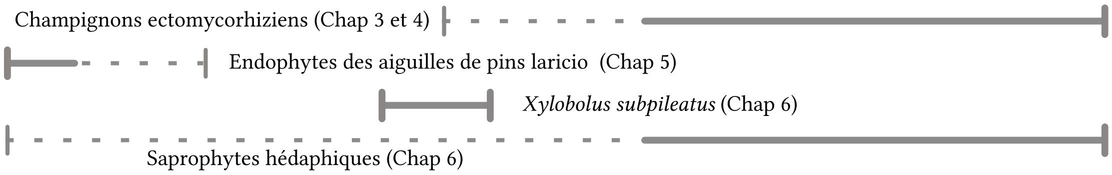
---

[41] Par exemple, des modifications de la composition des plantes et des prédateurs peuvent transformer les communautés fongiques du sol (Neary et coll. 1999 ; Mataix-Solera et coll. 2009 ; Dooley et Treseder 2012).

[42] Le déplacement de caractère est une accentuation évolutive des différences de traits entre espèces sympatriques, causé par la compétition pour la ressource (Silvertown 2004). On parle aussi de limitation de la similarité quand on veut souligner l'aspect observationnel (patron) plutôt que mécaniste (processus).

Tableau I.3 – Niche d'habitat et influence relative hypothétique des règles d'assemblage des communautés : Les colonnes correspondent aux préférences d'habitats et les lignes à quatre déterminants potentiels des communautés fongiques. L'importance relative est indiquée par le nombre d'étoiles. Les positions des quatre guildes traitées dans ce travail sont indiquées en dessous du tableau.

	Plantes						Sol		
	Feuilles (phyllosphère)		Bois		Racines (rhizosphère)				
	Endophytes foliaires	Surface foliaire	Bois vivants	Bois morts	Endophytes racinaires	Surface racinaire	Litière	Horizon organique	Horizon minéral
Diversité des conditions abiotiques	**	**	**	*	**	***	***	***	***
Variabilité temporelle abiotique	*	***	*	**	**	*	**	**	*
Influence de l'hôte (taxinomie, physiologie, ...)	***	*	***	**	*	***	**	*	*
Influence du feu	**	***	***	***	**	*	***	**	*



Le cas des champignons saproxyliques

On connaît l'écologie des champignons décomposeurs du bois principalement sous l'angle des fructifications. Les communautés de champignons saproxyliques des forêts tempérés dépendent de nombreux paramètres. La taille du matériel à décomposer (par ex. branche ou tronc), son âge (temps depuis la mort et depuis le contact avec le sol) et l'exposition au soleil structurent les communautés de champignons saproxyliques. Certaines espèces de champignons préfèrent les trouées de grandes tailles sans végétation au-dessus (Heilmann-Clausen 2005). Le rôle de l'histoire, c'est-à-dire de l'ordre d'arrivée des espèces dans la communauté, est également important chez les champignons saproxyliques (Fukami et coll. 2010).

Durant le processus de décomposition, les propriétés physico-chimiques du bois changent graduellement. Les champignons sont en partie responsables de l'augmentation de la teneur en eau et en lignine et donc de la diminution de la biomasse et de la densité du bois pendant le processus de décomposition (Rajala et coll. 2012). Les espèces fongiques se succèdent sur le bois (Rayner et Boddy 1988). Au début on trouve surtout des Ascomycètes responsables de pourriture molle (def. dans tableau I.2) qui sont remplacés par des champignons – surtout des Basidiomycètes – capables de dégradation de type pourriture blanche et brune (def. dans tableau I.2). La succession est donc fortement dirigée par le basculement entre des espèces capables de dégrader uniquement la cellulose vers de espèces capables de dégrader des substrats plus riches en polyphénols, dont fait partie la lignine (Daniel et Nilsson 1998). Pendant cette altération du bois, la richesse spécifique des champignons présents dans le bois tend à augmenter (Kubartová et coll. 2012). Par conséquent, les interactions interspécifiques augmentent en complexité avec l'âge du substrat. Comme signalé dans cette section, la niche trophique n'est pas suffisante pour étudier l'écologie des communautés des champignons saproxyliques. En effet, on trouve du mycélium, et parfois des fructifications, de champignons ECM dans le bois (Ottosson et coll. 2015) et leur abondance relative augmente avec la décomposition (Rajala et coll. 2012).

* * *

Figure I.15 – Illustration des organes visibles chez les macromycètes saproxyliques : (a) Rhizomorphes (voir glossaire) d'armillaires (*Armillaria mellea*) sur tronc de hêtre. (**b-d**) Fructification du polypore *Phylloporia pini* sur pin maritime. (**c**) Fructification de *Lentaria subcaulescens* avec faciès de décomposition particulier en lambeaux perpendiculaires. (**e**) Fructification de *Stereum insignitum* sur tronc de hêtre au sol. (**f**) Fructification de *Xylobolus subpileatus* sur chêne vert. La surface du champignon est en partie recouverte par un myxomycète (plasmode jaune appartenant sans doute à l'espèce *Badhamia utricularis*) en pleine fructification.

RÉSUMÉ

(i) Les champignons décomposeurs jouent un rôle central dans le fonctionnement des écosystèmes, notamment de part la capacité de certains Basidiomycètes à dégrader la lignine.

(ii) La composition des communautés de champignons décomposeurs du sol (saprotrophes édaphiques) est déterminée par la taxinomie de la végétation, par la composition chimique du sol, mais aussi par les perturbations comme le feu. Il semble également que la compétition entre espèces structure ces communautés.

(iii) Les communautés de champignons décomposeurs du bois (saproxyliques) sont structurées par l'ordre d'arrivée des espèces et suivent une succession au cours du processus de décomposition. De plus, le type et l'origine du substrat jouent un rôle primordial dans la composition de ces communautés.

SUMMARY

(i) Saprotrophic fungi are central in ecosystem functioning, especially due to the capacity of some Basidiomycetes to degrade lignin.

(ii) Community composition of soil saprotrophic fungi is driven by above-ground vegetation taxonomy, soil chemical composition, competition, and disturbances such as fire.

(iii) Communities of wood saproxylic fungi are structured by order of arrival on the wood. Moreover, those communities follow a succession along the decaying process. Lastly, the nature and origin of the substrate undoubtedly shape those communities.

6 L’écologie fongique à l’heure de la biologie moléculaire et de la science des réseaux

6.1 La biologie moléculaire chez les champignons

La majorité des champignons sont difficiles à identifier en raison de leur taille microscopique – au moins durant une grande partie de leur cycle de vie – et de leurs milieux de vie compliqués à explorer (par ex. le sol, l’eau ou l’intérieur des tissus d’autres organismes). Même pour les macromycètes (voir note 33), deux siècles de mycologie n’ont pas suffi à explorer leur diversité sur la base de critères morphologiques uniquement. La brièveté de certains épisodes de fructification, les fortes ressemblances morpho-anatomiques et la difficulté pour cultiver la majorité des macromycètes entraînent une dépendance très forte des mycologues envers les outils moléculaires (voir le cas pour les champignons ectomycorhiziens, Fig. I.9 page 28). En particulier, l’identification à différentes échelles taxinomiques dépend aujourd’hui fortement du séquençage des organismes fongiques. On utilise pour cela des marqueurs moléculaires (encadré 5) que l’on compare à des bases de données de séquences assignées taxinomiquement.

Le marqueur actuellement le plus utilisé chez les champignons provient de la région de l’ADN ribosomique ITS1-5.8S-ITS2, ou ITS (voir encadré 5 et Fig. I.16 ; Gardes et Bruns 1993 ; Ryberg et coll. 2009 ; Abarenkov et coll. 2010). Pour cibler la zone du génome à séquencer, on utilise des amorces, courtes séquences d’ADN flanquant la région d’intérêt. Historiquement, les premières amorces permettant le séquençage de l’ITS sont les amorces ITS1 et ITS5 (White et coll. 1990). Ces amorces sont la plupart du temps spécifiques à un groupe taxinomique. Ainsi, l’amorce ITS1-F (Gardes et Bruns 1993) est spécifique des champignons dans leur ensemble, tandis que les amorces ITS4-B (Gardes et Bruns 1993) et LB-W (Tedersoo et coll. 2008) ont été créées pour discriminer les champignons ECM appartenant aux Basidiomycètes (Lindahl et coll. 2013).

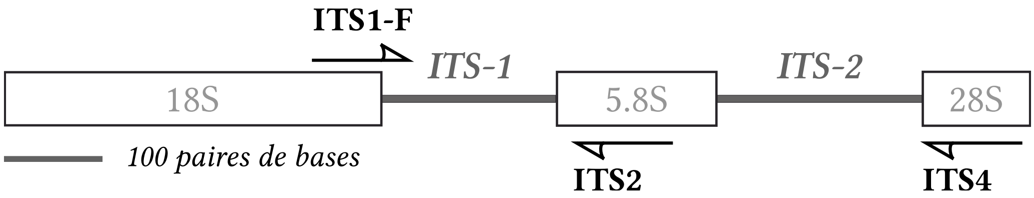


Figure I.16 – Schéma de la position de l’ITS dans l’ADN ribosomique : Les noms en noir correspondent aux amorces utilisées dans ce travail. La longueur des séquences est variable. À titre d’illustration, nous avons utilisé les valeurs de la base de données Unite (Kõljalg et coll. 2013).

6.2 Les techniques de séquençage à haut débit (NGS)

Les techniques de séquençage à haut débit (NGS [43]) sont aujourd’hui très utilisées en mycologie et en particulier en écologie des communautés fongiques (Peay et coll. 2008 ; Bálint et coll. 2016).

---

[43] NGS pour *Next-Generation Sequencing* également appelées HTS pour *High Throughput Sequencing* ; voir Fig. I.17 pour une comparaison de la dynamique temporelle d’utilisation des deux abréviations. Nous avons choisi le terme de

Encadré 5 : L'ITS (*Internal transcribed spacer*), le code-barre génétique des champignons

Un marqueur est un code-barre, une courte région du génome que l'on séquence grâce à des amorces spécifiques. Pour classifier – phylogénétiquement ou taxinomiquement – à moindre coût, il est nécessaire d'identifier des régions à séquencer chez un grand nombre d'organismes. Chez les animaux, la région identifiée est celle de la sous-unité 1 de la cytochrome oxidase (*CO1*, ADN mitochondrial ; par ex. Hebert et coll. 2003), tandis que chez les plantes Hollingsworth et coll. (2009) conseille l'utilisation couplée de deux régions (*rbcL* + *matK*).

Chez les champignons le marqueur principal est l'ITS [a]. Ce choix est le fruit de travaux de comparaison des marqueurs (par ex. Schoch et coll. 2012). Un bon marqueur doit être (i) suffisamment variant à l'échelle des champignons dans son ensemble pour différencier les espèces, (ii) suffisamment conservé pour pouvoir comparer des champignons très éloignés phylogénétiquement, et (iii) amplifiable facilement pour l'ensemble des champignons [b]. Pour maximiser les deux premières conditions, il faut que l'écart entre les distances génétiques intra- et inter-spécifiques (le *barcode gap* en anglais ; par ex. Puillandre et coll. 2012) soit élevé. Schoch et coll. (2012) ont démontré que l'ITS est une région qui respecte assez bien ces trois conditions. Aujourd'hui, la suprématie de l'ITS est confortée par la qualité et la quantité des séquences ITS présentes dans les bases de données (en particulier dans la base de données UNITE ; Abarenkov et coll. 2010).

Chez les Eucaroytes, l'ITS se trouve entre la petite sous-unité ribosomique (SSU 16S/18S) et la grande sous-unité (LSU 23S/25S/28S). L'ITS est découpé en deux parties (Fig. I.16) par le gène 5.8S : l'ITS-1 et l'ITS-2 qui sont de longueur très variables. Cette région est transcrite mais laissée de côté lors de l'assemblage des ribosomes. L'ITS, en tant qu'ADN ribosomique, est présent en grand nombre de copies dans les cellules (Selosse et coll. 2016). Sa présence dans une région fortement conservée (l'ADN ribosomique) permet à l'ITS d'être informatif à relativement large échelle taxinomique. Mais, comme l'ITS ne code pas pour de l'ARN fonctionnel, il présente suffisamment de variations pour permettre la différenciation des espèces dans de nombreux cas (Lindahl et coll. 2013).

---

[a] À quelques exceptions près. Par exemple, les champignons impliqués dans les endomycorhizes, les Gloméromycètes, sont souvent étudiés à l'aide de la séquence qui code pour la petite sous-unité ribosomique (SSU ; Öpik et coll. 2010 ; Hart et coll. 2015).

[b] Cette condition n'est pas vérifiée pour certains groupes et certains marqueurs (voir site internet de UNITE).

Ces techniques ont trois avantages sur les techniques classiques de séquençage de type Sanger (Shokralla et coll. 2012 ; Lindahl et coll. 2013) :

- Le nombre de séquences par échantillon en sortie du séquenceur est beaucoup plus important ;
- Le coût par séquence est très inférieur ;
- Le séquençage de type Sanger ne permet que le séquençage des individus un par un alors que les *NGS* permettent de séquencer la communauté d'un échantillon environnemental. On parle de métagénomique pour désigner la discipline qui étudie ces échantillons d'ADN environnemental.

Le grand nombre de séquences obtenu par les *NGS* entraîne un traitement bioinformatique important pour sélectionner les séquences de qualité et les regrouper en unités qui ont un sens biologique (voir section Biologie moléculaire et bioinformatique). Les méthodes de regroupement les plus utilisées font appel à la notion d'OTU (de l'anglais « Operational taxonomic Unit). Ce concept repose sur la notion de distance génétique [44] comme marqueur de différence écologique/phénotypique.

NGS pour deux raisons dans cette thèse. Tout d'abord, l'utilisation de ce terme augmente plus vite que l'utilisation de *HTS* et le terme *NGS* est plus souvent utilisé en génomique environnementale.

[44] Le plus souvent la distance seuil est fixée à 3% pour l'ITS (Nilsson et coll. 2008).

Cette notion n'est que partiellement reliée à la notion d'espèce, d'où la notion d'unité taxinomique « opérationnelle ». La notion d'espèce – un segment d'une lignée évolutive – est aujourd'hui assez consensuelle (De Queiroz 2007), y compris chez les champignons (Giraud et coll. 2008). En revanche les critères pratiques de délimitation sont beaucoup plus variables selon les chercheurs et les disciplines (De Queiroz 2007 ; Giraud et coll. 2008). Le lien entre les OTUs issues de séquençages à haut débit et les espèces taxinomiques présentes dans les bases de données peut se faire en comparant les séquences. De nombreux problèmes peuvent survenir si l'on confond la notion d'OTU avec celle d'espèce[45], mais le concept d'OTU a indéniablement permis des avancées cruciales dans nos connaissances de l'écologie des micro-organismes.

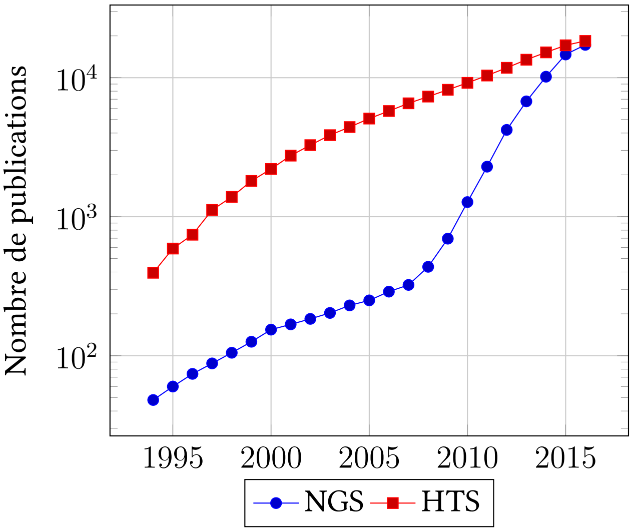


Figure I.17 – Tendance temporelle de l'utilisation des termes *Next-generation sequencing* (NGS ; en bleu) et *High-throughput sequencing* (HTS ; en rouge) : Résultat de la recherche sur Web Of Science™ dans le *topic* de « "Next-generation sequencing" OR NGS » et « "High-throughput sequencing" OR HTS » le 13 Août 2016.

6.3 De nombreux réseaux écologiques d'interactions impliquent des champignons

Qu'est qu'un réseau d'interactions ?

Un réseau d'interactions est un objet constitué d'au moins deux classes d'éléments : des **nœuds** et des **liens** représentant une interaction entre les deux nœuds qu'ils relient. Ces éléments peuvent être caractérisés par de multiples attributs comme par exemple le sens et le force de l'interaction qu'un lien représente, ou encore le poids d'un nœud dans le réseau. Les réseaux d'interactions sont des objets d'étude importants dans de nombreuses disciplines, en physique statistique, informatique, sociologie, épidémiologie et biologie (dont l'écologie). Les méthodes et les caractéristiques des réseaux étudiés varient en fonction des disciplines, mais de nombreux transferts fructueux ont émaillé la récente émergence de la science des réseaux. De nombreux ouvrages sont disponibles sur la science

---

[45] Ainsi, les variations intra-clonales (Lindner et Banik 2011) et intra-spécifiques (Nilsson et coll. 2008) sont très importantes, ce qui entraîne une inadéquation entre le concept d'OTUs et celui d'espèce.

des réseaux (par ex. Boccaletti et coll. 2006 ; Newman 2010 ; Barabási 2016) et son implication en écologie (Bascompte et Jordano 2007 ; Vazquez et coll. 2009 ; Sueur 2015).

Barabási (2012) résume le développement de la science des réseaux ainsi : « Reductionism, as a paradigm, is expired, and complexity, as a field, is tired. Data-based mathematical models of complex systems are offering a fresh perspective, rapidly developing into a new discipline: network science. » que l'on peut traduire ainsi : « Le réductionnisme, en tant que paradigme, expire, et la complexité, en tant que champs disciplinaire, est fatigué. Les modèles mathématiques de systèmes complexes basés sur les données offrent une perspective rafraîchissante, qui se développe rapidement en une nouvelle discipline : la science des réseaux. »

Utilisation des réseaux en écologie

La science des réseaux a d'abord été utilisée en écologie pour comprendre la structure des réseaux trophiques (Paine 1980 ; Pimm et coll. 1991 ; Dunne et coll. 2002) et s'est ensuite étendue à d'autres types de réseaux écologiques d'interactions en particulier les réseaux mutualistes[46] (Bascompte et coll. 2003).

En s'appuyant sur les concepts et les outils de la science des réseaux, les écologues ont utilisé divers indices et mesures pour comparer les réseaux entre eux. L'objectif de ces comparaisons est autant de trouver des variations biologiquement intéressantes que de chercher des invariants (par ex. Jordano et coll. 2003). Les indices utilisés sont alors des indices globaux, c-à-d. calculés à l'échelle du réseau. Parmi ces indices, on trouve la **connectance**[47], l'**imbrication** (*nestedness* en anglais ; Bascompte et coll. 2003) et la **modularité** (Olesen et coll. 2007). L'imbrication d'un réseau mesure à quel point le réseau est structuré sous forme de poupées russes, c-à-d. à quel point les partenaires des espèces spécialistes sont également des partenaires des espèces généralistes. La modularité mesure la structuration du réseau en sous-ensemble de nœuds qui interagissent plus entre eux qu'avec des nœuds d'autres groupes que l'on appelle modules.

D'autres indices sont utilisés en écologie, comme la distribution des différents motifs[48] (Milo 2002), le diamètre du réseau[49] ou encore la distribution des degrés[50]. Rien que dans le cas particulier des réseaux bipartis[51], Dormann et coll. (2009) ont étudiés plus de 20 indices globaux et les relations que ces indices entretiennent.

---

[46] Un réseau mutualiste est un réseau d'interactions où un lien représente une association mutualiste (c-à-d. à bénéfices réciproques) entre les espèces/individus qu'il relie.

[47] Proportion de liens possibles qui sont effectivement présents dans le réseau. Correspond dans la majorité des réseaux au nombre de liens divisé par le carré du nombre de noeuds.

[48] Un motif est un groupe de nœuds dont les liens forment une structure particulière (par exemple trois nœuds reliés par un triangle de lien est un motif).

[49] Le diamètre d'un réseau correspond à la distance minimale entre toutes les paires de nœuds du réseau.

[50] Le degré d'un nœud est le nombre de lien qu'il établit avec d'autres nœuds.

[51] Un réseau biparti est constitué de deux classes de partenaires qui n'interagissent qu'avec des partenaires de l'autre classe. C'est par exemple le cas des réseaux d'interactions plantes-pollinisateurs ou acteurs-films. C'est un cas particulier de réseau multiparti que l'on oppose aux réseaux unipartis.

Tableau I.4 – Publication sur les réseaux écologiques mycorhiziens : Les publications qui traitent des réseaux mycéliens (CMN ; voir encadré 4 et Fig. I.12) ne sont pas incluses dans ce tableau. *Pl.* : nombre de taxons de plantes ; *Ch.* : nombre de taxons fongiques ; ∅ Valeur non spécifiée dans la publication ; *Liens* : nombre de liens ; *Séq.* : nombre de séquences pour les séquençages à haut débit (NGS) ; *PDI* : indice de spécialisation (*Paired Difference Index* ; Poisot et coll. 2012) ; la colonne *Réseau(x)* donne des indications sur les réseaux étudiés et les méthodes d'échantillonnage de la diversité fongique ; enfin, la colonne *Mesures* indique les différentes facettes auxquelles la publication s'est intéressée. Les abréviations *AM* ; *ECM* et *OM* correspondent respectivement aux interactions endomycorhiziennes vésiculo-arbusculaires, ectomycorhiziennes et endomycorhiziennes des orchidées. Les valeurs accompagnées d'une astérisque (*) sont des valeurs moyennes sur les réseaux étudiés. Les deux astérisques (**) indiquent que la valeur reportée est calculée pour un regroupement des OTUs à 3%.

Publications	**Type**	**Pl.**	**Ch.**	**Liens**	**Séq.**	**Réseau(x)**	**Mesures**
Jacquemyn et coll. 2010	OM	5	12	26	-	Qualitatif (Sanger sur les racines)	Imbrication
Jacquemyn et coll. 2011	OM	16	23	69	-	Réseau global (11 régions échantillonnées en Europe ; Sanger sur les racines)	Imbrication, Phylogénie des plantes
Martos et coll. 2012	OM	73	95	210	-	Qualitatif (Sanger sur les racines)	Imbrication, Modularité, Écologie et phylogénie des plantes
Jacquemyn et coll. 2015	OM	20	96	178	137 183	Quantitatif (454 sur les racines)	Imbrication, Modularité, Rôles des espèces
Chagnon et coll. 2012	AM	10	47	209	138 919	Qualitatif (454 sur les racines)	Imbrication, Modularité
Montesinos-Navarro et coll. 2012	AM	35	61**	194	-	Qualitatif (Sanger sur les racines)	Imbrication, Modularité, Connectance, Abondance des plantes
Chagnon et coll. 2015	AM	8	25	89	∅	Qualitatif (454 sur les racines)	Modularité, Traits et phylogénie des plantes
Encinas-Viso et coll. 2016	AM	18	15	-	-	Réseau spatial de co-occurence (Identification morphologique des spores)	Imbrication, Modularité, Connectance
Fodor 2013	ECM	7	87	246	-	Qualitatif (fructifications aériennes)	Imbrication, Modularité, C-score, Asymétrie, Connectance, Rôles des espèces
Bahram et coll. 2014	ECM	4.8*	89.9*	-	-	10 réseaux locaux	Imbrication, Modularité
Taudiere et coll. 2015	ECM	16	234	993	-	Régional et qualitatif (mélange de données naturalistes et moléculaires)	Modularité, Écologie des plantes
Roy-Bolduc et coll. 2016	ECM	4	200	687	34 192	Quantitatif (454 sur sol et racines)	Imbrication, Modularité, PDI

Dans ce travail, nous ne nous intéressons pas à la comparaison entre réseaux, nous utilisons donc des indices locaux, c'est-à-dire les indices calculés au niveau d'un lien ou d'un nœud. Le nombre de partenaires dans le réseau – le **degré** – est un indice souvent utilisé. Certains indices locaux ne nécessitent pas de faire appel à la théorie des graphes et aux méthodes associées. Ainsi, le degré est un simple comptage du nombre de partenaires qui ne capture pas d'« effet réseau ». L'utilisation de la terminologie des réseaux pour calculer des indices locaux aussi simples participe du « machisme statistique[52] » à l'œuvre en Écologie aujourd'hui.

Dans le cadre de ce travail, nous avons également utilisé des indices de niveaux intermédiaires, c'est-à-dire des indices calculés sur les nœuds du réseau mais qui prennent en compte explicitement la structure du réseau. Par exemple, nous avons calculé deux indices locaux en lien avec la modularité (caractéristique global) du réseau. Ces indices décrivent l'importance des nœuds dans la structure du réseau (Guimerà et coll. 2004 ; Guimerà et Amaral 2005). Le premier indice est le **coefficient de participation** 📘 qui mesure l'équitabilité de la distribution des liens du nœud considéré vers les différents modules. En d'autres termes, plus un nœud est lié équitablement aux différents modules, plus son coefficient de participation est important. Le deuxième indice est le **degré relatif intra-module** 📘 qui mesure l'importance du nœud dans son module.

Utilisation de la notion de réseau pour mieux comprendre les interactions mycorhiziennes

Nous avons déjà vu dans la section 3 de l'introduction l'importance de la notion de réseau en écologie des champignons (en particulier dans l'encadré 4 et sur la figure I.12). À travers la comparaison entre réseaux communs mycéliens (CMN) et réseaux écologiques mycorhiziens (EMN ; encadré 4) nous avons déjà illustré deux utilisations diamétralement opposées de la notion de réseaux (réseau concret d'interactions physiquement réalisées [CMN] *vs* représentation abstraite d'interactions écologiques potentielles [EMN]).

Dans le cas des CMN, il s'agit d'un réseau concret formé par le mycélium fongique qui permet de relier physiquement au moins deux individus végétaux. Ainsi, les études sur les CMN s'intéressent principalement au partage de ramets[53] entre les plantes. L'intégrité physique du ramet étant compliquée à vérifier, les études sur les CMN utilisent soit les génets[54] comme indicateur de ramets (par ex. Beiler et coll. 2015), soit des marqueurs isotopiques (par ex. Simard et coll. 1997). Ces deux méthodes produisent peu d'interactions et de partenaires en sortie. La théorie des graphes[55] – développée pour des réseaux complexes de tailles conséquentes – ne semble pas d'un grand secours dans le cadre de l'étude des CMN.

---

[52] B. Bolker propose la notion de « machisme statistique » sur le blog dynamic ecology pour dénoncer la tendance en Écologie à utiliser des méthodes statistiques (trop) complexes alors que des tests simples suffiraient. Voir la discussion sur le sujet page 275.

[53] Individu fongique physiquement indivisé ; voir glossaire et ce paragraphe page 11.

[54] Individu fongique génétique ; voir glossaire et ce paragraphe page 11.

[55] Théorie mathématique et informatique dont l'objet principal est le graphe, également appelé réseau. La théorie des graphes est fortement intégrée dans la science des réseaux.

En revanche, les EMN sont des représentations abstraites des interactions potentielles entre plantes et champignons ECM. Les EMN nous renseignent sur les potentialités de formation de CMN (par ex. Ebenye et coll. [*in press*] en annexe), mais pas seulement (voir encadré 4). Le nombre d'études qui travaille sur la topologie ou la position des partenaires dans les réseaux écologiques d'interactions mycorhiziennes est assez faible. Le tableau I.4 liste la grande majorité des publications qui analysent des réseaux écologiques d'interactions ECM (principalement interspécifiques) en utilisant certains concepts de la science des réseaux. Trois types d'interactions mycorhiziennes sont équitablement étudiés : les mycorhizes des orchidées (OM), les endomycorhizes vésiculo-arbusculaires (AM) et les ectomycorhizes (ECM). Notez que dans plusieurs cas, le réseau est un réseau de coexistence [56] et que les auteurs parient – sans toujours le dire clairement – qu'il s'agit d'un bon *proxy* d'un réseau d'interactions (Caruso et coll. 2012).

Le faible nombre de publications et de jeux de données, associé à la disparité de la taille des réseaux (nombre d'interactions possibles [57]), empêchent une comparaison des valeurs de modularité et d'imbrication des différentes publications. De plus, on peux s'interroger sur la validité de tels indices développés pour des graphes de grandes tailles au regard de la (très) faible taille de la majorité des réseaux étudiés. Le calcul de l'imbrication et de sa significativité sur des réseaux constitués de quatre plantes (par ex. dans Bahram et coll. 2014 et Roy-Bolduc et coll. 2016) ne me semble ni correct statistiquement ni intéressant biologiquement. Dans un article récent, Chagnon (2016) met en garde contre les conclusions rapides et fallacieuses établies à partir de l'analyse des réseaux d'interactions mycorhiziennes. En particulier, il rappelle que les réseaux écologiques mycorhiziens sont des représentations graphiques d'observations, et qu'il reste une étape cruciale avant la compréhension des processus. Pour cet auteur, le passage des patrons au processus d'assemblage des communautés et de fonctionnement des écosystèmes reste compliqué dans l'état de nos connaissances et est trop souvent contourné.

* * *

---

[56] On définit une interaction entre c et p lorsque l'on trouve de l'ADN du champignon c dans le sol près de la racine (par ex. Toju et coll. 2015) ou dans la racine de la plante p. Caruso et coll. (2012) soulignent que la présence de mycélium de a proche des racines de b n'est pas toujours synonyme d'interaction mycorhizienne entre a et b.

[57] Un réseau est d'autant plus grand que la matrice d'interactions est grande. Dans le cas d'un réseau uniparti, la taille correspond au nombre de nœuds au carré. Dans le cas des réseaux bipartis, la matrice d'interactions peux avoir des nombres différents de lignes et de collonnes, la taille correspond alors au nombre de partenaires d'une classe multiplié par le nombre de partenaires de la deuxième classe.

Résumé

(i) L'étude de la diversité fongique bénéficie depuis 25 ans de la biologie moléculaire. Notre connaissance de la diversité fongique a été facilitée dernièrement grâce à l'instauration de l'ITS (*Internal Transcribed Spacer*) comme marqueur universel fongique couplée à la mise en place de bases de données et à la mise au point de méthodes de séquençage à haut débit (NGS).

(ii) L'écologie des champignons fait appel à la notion de réseau à différentes échelles (par ex. réseau morphologique du mycélium, réseau écologique d'interactions interspécifiques). Des outils de la théorie des graphes (par ex. la modularité et l'imbrication) ont été récemment utilisés pour mieux comprendre la structure des réseaux d'interactions mycorhiziennes.

Summary

(i) Study of fungal diversity benefits from molecular biology since 25 years. And this gain has been accelerated recently thanks to the institution of ITS (*Internal Transcribed Spacer*) as universal fungal barcode, the establishment of data base and the tuning of next-generation sequencing (NGS).

(ii) Fungal ecologists use the concept of network at different scales (*e.g.*, morphological network of mycelium, ecological network of interspecific interactions). Graph theory brings some tools (*e.g.*, modularity and nestedness) to fungal ecologists interesting in the structure of ecological mycorrhizal networks.

7 Les feux en milieu méditerranéen et le cas du pin laricio en Corse

7.1 Le feu : un processus prépondérant dans la structure des communautés végétales…

Au niveau planétaire, les feux de végétation sont une composante majeure de la biosphère (Bond et Keeley 2005). En effet, les feux déterminent une grande part de l'organisation des communautés végétales (Bond et Keeley 2005) ou animales (Neary et coll. 1999), de l'histoire évolutive du vivant (Pausas et Keeley 2009 ; Pausas 2015) et de nombreux processus associés (par ex. le cycle de l'azote, Wan et coll. 2001). Le passage d'un feu a des effets directs sur le sol : érosion par ruissellement, lessivage, et migration des cations (Certini 2005). En modifiant les paramètres environnementaux, le feu induit des réponses variables selon les espèces y compris à travers la modification des interactions intra- et interspécifiques.

Les plantes ont des stratégies diversifiées pour recoloniser le milieu après feu (Pausas et coll. 2016). Ainsi, on distingue les espèces capables de rejeter (« *post-fire resprouters* ») et celles dont les graines résistent au feu et germent en profusion après un passage de feu qui va lever leur dormance (« *post-fire seeders* »). Les espèces qui n'ont pas survécu au feu localement (y compris sous forme de graines) mais régénèrent grâce à des graines qui viennent de zones voisines non brûlées sont appelées « *postfire colonizers* ».

Au regard de ces stratégies face au feu dans le monde végétal, on peut se demander (i) si les stratégies écologiques des plantes façonnent les communautés de champignons associées (manuscrit B), et (ii) si les champignons arborent des stratégies écologiques face au feu (manuscrit F et projets de manuscrit G et J).

7.2 … et des communautés de micro-organismes du sol

La température considérée comme seuil pour les racines des plantes – et donc la diversité microbienne qui en dépend – est évaluée à 48°C (Neary et coll. 1999). Pour les champignons en générale cette valeur est plus élevée (60°C dans un soil sec ; 70°C dans un soil humide ; 94°C dans le cas des mycorhizes vésiculo-arbusculaires ; Neary et coll. 1999). Pour indication, les feux de forêts induisent des températures au niveau de la surface du sol souvent comprise entre 200°C et 300°C mais ces températures décroissent rapidement avec la profondeur. Ainsi, une température de 400°C en surface est d'environ 50°C à 5 cm de profondeur (Neary et coll. 1999).

C'est souvent d'autres facteurs suivant le feu qui affectent le plus les communautés de micro-organismes édaphiques. Par exemple, la modification chimique du sol, sa mise à nu pouvant induire une sécheresse et le lessivage du sol, et le changement de composition aérienne entraînent des modifications de la composition des organismes du sol (Neary et coll. 1999 ; Certini 2005).

7.3 Le feu en région méditerranéenne

En région méditerranéenne, les régimes de feux sont peu connus et l'incidence sur la biodiversité des termes temporels de ces régimes (fréquence, intervalle depuis le dernier feu, etc.) ne fait l'objet que de rares travaux (Scarascia-Mugnozza et coll. 2000 ; Capitanio et Carcaillet 2008) alors même que les risques d'érosion de cette diversité sont exacerbés par les changements climatiques qui influencent les régimes de feu (Pausas et Fernández-Muñoz 2012).

Moritz et coll. (2014) partagent le bassin méditerranéen en deux régions eu égard à la dynamique du régime de feu et de la végétation. Le nord de la région méditerranéenne est ainsi caractérisé par la déprise agricole et le dépeuplement des zones rurales. Ajouté aux programmes de reboisement dans cette région, le couvert forestier y croît (Moritz et coll. 2014). De plus, les politiques relatives au feu privilégient la suppression directe des feux[58] (North et coll. 2015), entraînant l'augmentation de combustibles et, par voie de conséquence, des « méga-feux » (San-Miguel-Ayanz et coll. 2013). Au contraire, le sud et l'est de la région se distinguent par une diminution du couvert végétal attribuable à une surexploitation des terres. Le risque qui pèse sur ces écosystèmes est bien plus la désertification que le feu (Moritz et coll. 2014).

Les premiers travaux sur les régimes récents de feux montrent que ces derniers sont fréquents en forêt de pins noirs tant en Corse (Leys et coll. 2013), qu'en Espagne (Fulé et coll. 2008) ou en Grèce (Christopoulou et coll. 2013).

7.4 La place du pin laricio en Corse

Une essence montagnarde à forts enjeux de conservation

Le pin laricio (*Pinus nigra* Arnold subsp. *laricio* (Poiret) Maire var. *Corsicana* (Loudon) Hyl.) est une essence de montagne (Fig. I.18) caractérisée par un tronc gris clair et veiné de profondes fissures noires (Fig. I.19b). Les aiguilles vertes sombres sont géminées et mesurent entre 12 et 15 cm (Rameau et coll. 2008). Les pommes de pins (Fig. I.19c) sont relativement petites (4-8 cm) et subsessiles (Tela Botanica). Le pin laricio était déjà présent il y a plus de 15 000 ans en Corse autour du lac de Créno (Leys et coll. 2014).

Il peut vivre plusieurs centaines d'années (Rameau et coll. 2008) et atteindre des circonférences importantes (Fig. I.19a et I.19b). Le pin laricio domine les paysages à l'étage montagnard et dans une moindre mesure à l'étage supraméditerranéen (900 m – 1 800 m ; Rameau et coll. 2008 ; Fig. I.19e et Fig. I.18). Il y constitue des peuplements purs, et des peuplements mélangés, en particulier avec le pin maritime (*Pinus pinaster*) et le hêtre (*Fagus sylvatica*). Le pin laricio est une sous espèce endémique des forêts montagnardes de Corse. Les peuplements de cette essence emblématique sont considérés comme prioritaires au sens de la Directive Habitats (CEE 92/43, 1992, code Natura 2000 : 9530). La

---

[58] La suppression directe décrit l'action d'éteindre les feux le plus rapidement possible. On oppose souvent cette méthode au brûlage dirigé et à l'éclaircissage. Ces deux dernières techniques ont pour objectif de limiter le combustible et ainsi d'éviter les feux de couronne de forte intensité, très compliqués à circonscrire. Lire North et coll. (2015) pour un appel à l'augmentation de la prévention des feux en lieu et place de la lutte soudaine et subie.

Encadré 6 : Le feu et son régime

Un feu, ou un ensemble de feux, peut être caractérisé par une collection de paramètres qui constitue son régime. Le mot régime, d'abord utilisé par les scientifiques français, a été adopté dans la littérature internationale anglophone concomitamment à un changement de paradigmes au début des années 1960 (Krebs et coll. 2010). Le contexte social, culturel et scientifique favorisait dans le monde occidental une vision plus complexe des systèmes naturels. Le feu passe du statut péjoratif de « dictateur [a] » des écosystèmes à celui de perturbation majeure nécessaire au fonctionnement de certains écosystèmes (Krebs et coll. 2010).

Le régime de feu *stricto sensu* est l'ensemble des paramètres qui décrivent où, quand et de quel type sont le(s) feu(x) (Krebs et coll. 2010). Certains incluent également dans le régime (*sensu lato*) les conditions de feux (par ex. la quantité et l'inflammabilité du combustible ou encore les conditions météorologiques) et les effets immédiats (par ex. la sévérité 📘 pour les organismes ou pour les sociétés humaines). Voici une liste non exhaustive de paramètres couramment mesurés pour caractériser les feux.

- Les paramètres spatiaux :
  - La **taille** du feu (surface brûlée) ;
  - Le type de feu : **feu de surface** 📘 [b] ou **feu de couronne** 📘 [b]. Les feux de surface brûlent la litière et les plantes de sous bois (Fig. II.2c-d), infligeant parfois des dégâts importants aux troncs des arbres. Au contraire, les feux de couronne vont également brûler les branches et le feuillage des arbres. Le plus souvent, les feux qui entraînent un replacement complet de la végétation sont des feux de couronne (Fig. II.2e-f).
- Les paramètres temporels :
  - La **vitesse de propagation** du feu ;
  - La **durée** du feu ;
  - Le **temps écoulé depuis le dernier feu** [b] ;
  - La **fréquence de feu**, et sa **prédictibilité** (variance de la fréquence).
- Les paramètres d'effet :
  - L'**intensité** 📘 du feu est souvent mesurée par la température au sol. Un feu intense dégage plus d'énergie qu'un feu peu intense.
  - La **sévérité** [b] du feu mesure l'importance de son effet sur l'écosystème (souvent regardé par le prisme des plantes). La sévérité dépend de la vitesse de propagation, de l'intensité du feu, des paramètres climatiques locaux et des écosystèmes/espèces impactés. Les feux de surface sont moins intenses et moins sévères que des feux de couronne.

---

[a] Le terme régime de feu était alors souvent associé à un registre de vocabulaire autour de la soumission et de l'échappement (Krebs et coll. 2010).

[b] Ces paramètres de régime sont considérés dans ce travail.

forte pluviométrie de son biotope, en interaction avec la géologie de la Corse, rend les sols acides et riches en matière organique.

Les peuplements de pin laricio supportent une diversité originale. Ainsi, le seul oiseau endémique de France métropolitaine, la citelle corse (*Sitta whiteheadi*) habite les troncs morts de ce pin (Thibault et coll. 2016). Un autre exemple provient de la grande diversité de polypores qui se nourrissent de cette essence, dont de nombreux sont vulnérables (Norstedt et coll. 2001).

Une ressource, des services et un emblème

En plus de sa dimension écologique, le pin laricio a une position centrale dans la nature corse tant d'un point de vue économique que culturel.

Le pin laricio fournit des **services d'approvisionnement**. Cette essence est un bois d'œuvre important (25 100 ha de production ; Plan Pluriannuel Régional de Développement Forestier de Corse). C'est même l'essence principale dans les forêts relevant du régime forestier (Tome 1 de la « Contribution à la conduite des peuplements de pin laricio et habitats associés »). Le pin laricio est la troisième essence de reboisement utilisée en France continentale grâce à sa rusticité, sa plasticité et sa productivité. Aujourd'hui, il est principalement utilisé pour la production de structure, parquet, bardage, chevrons et coffrage. Le pin laricio fournit également des huiles essentielles commercialisées.

Les arbres de Corse ont longtemps permis aux habitants de se chauffer et de construire des bâtiments, mais il faut attendre que la Corse soit rattachée à la France en 1768 pour voir apparaître une exploitation organisée au niveau de l'île, en particulier pour le pin laricio. En 1772, le premier acte forestier de l'île est édicté. « L'ordonnance du Roy sur la matière des bois et forêts pour l'isle de Corse » compte remédier au « mauvais état de ces bois » dû aux « abroutissements, incendies et guerres ».

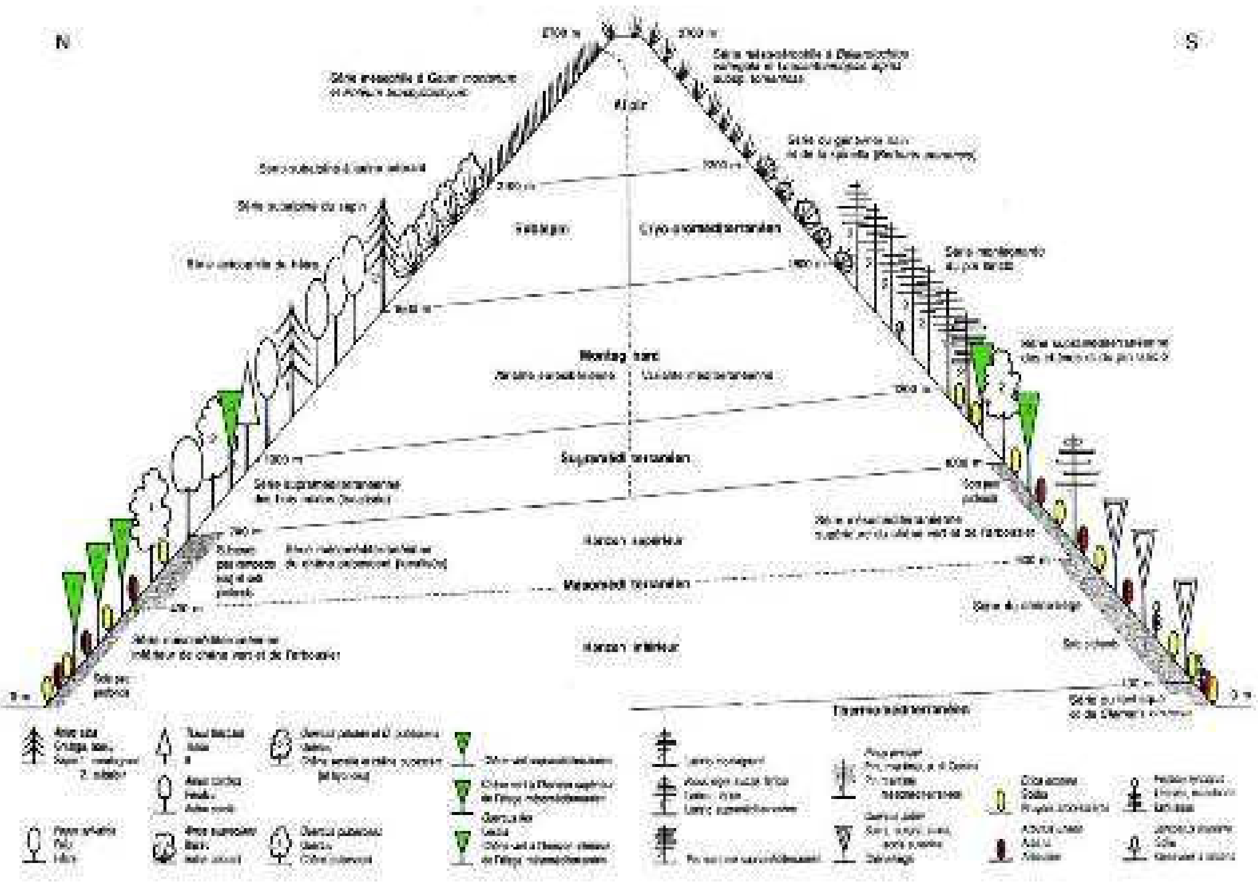


Figure I.18 – **Étages de végétation en Corse et répartition des principales espèces végétales structurant les écosystèmes (à partir de Gamisans 1999).**

En 1842, le pin laricio est l'arbre d'intérêt productif le plus abondant avec un nombre d'individus de diamètre supérieur à 35cm estimé à 1 398 000 (Bourcet, 1996, *Aperçu sur deux siècles d'histoire forestière en Corse* Revue Forestière Française. 48(6) :563-580 cité dans Collectif 2006a). Bourcet (1996) estime que ce nombre a augmenté de 37% entre 1842 et 1982. L'exploitation forestière à grande échelle ne prendra vraiment de l'importance qu'au XIX^e^ siècle avec la construction de grandes routes forestières et les accords Blondel (1852), du nom du commissaire qui a clarifié le foncier forestier endistribuant les forêts publiques entre l'État et les communes.

Durant l'ancien régime le pin laricio servait à la construction de bateaux (par ex. des mâts, des barques de pêche), de meubles et de bâtiments (poutres, canaux d'irrigation, toitures en bardeau). La résine du pin servait d'emplâtre pour calmer les douleurs, soigner les coupures de couteau et ôter les épines de la peau. Enfin, des morceaux de tronc chargés en résine (bois gras) servaient à allumer les feux. En plus d'une valeur économique directe, les forêts de pins laricio procurent de nombreux **services de régulations environnementales**. Ces forêts protègent les zones montagnardes de l'érosion et des avalanches tout en régulant le cycle de l'eau.

Les services **écosystémiques culturels** incluent les valeurs esthétiques, éducatives, touristiques, religieuses ou encore philosophiques portées par la nature (Daniel et coll. 2012). Les forêts de pins laricio sont porteuses de ces valeurs et produisent ainsi des services écosystémiques culturels. Ainsi, le pin laricio est souvent mis en avant par les guides touristiques en particulier à l'égard des randonneurs. Pour comprendre la valeur culturelle du pin laricio, il suffit de regarder les photos de forêt corse dans google™ image (recherche de « forêt corse ») : sur les 50 premières photos où l'on aperçoit des arbres, 35 photos (70%) contiennent un ou plusieurs pin(s) laricio. On retrouve d'ailleurs le pin laricio sous la plume de Maupassant (1882) :

> « *Le chemin montait doucement au milieu de la forêt d'Aïtône. Les sapins*[59] *démesurés élargissaient sur nos têtes une voûte gémissante, poussaient une sorte de plainte continue et triste, tandis qu'à droite comme à gauche leurs troncs minces et droits faisaient une sorte d'armée de tuyaux d'orgue d'où semblait sortir cette musique monotone du vent dans les cimes.* »

Guy de Maupassant, *Un bandit corse*

Texte publié dans *Gil Blas* le 25 mai 1882 sous la signature de Maufrigneuse.

---

[59] On nommait couramment le pin laricio « sapin » à cette époque.

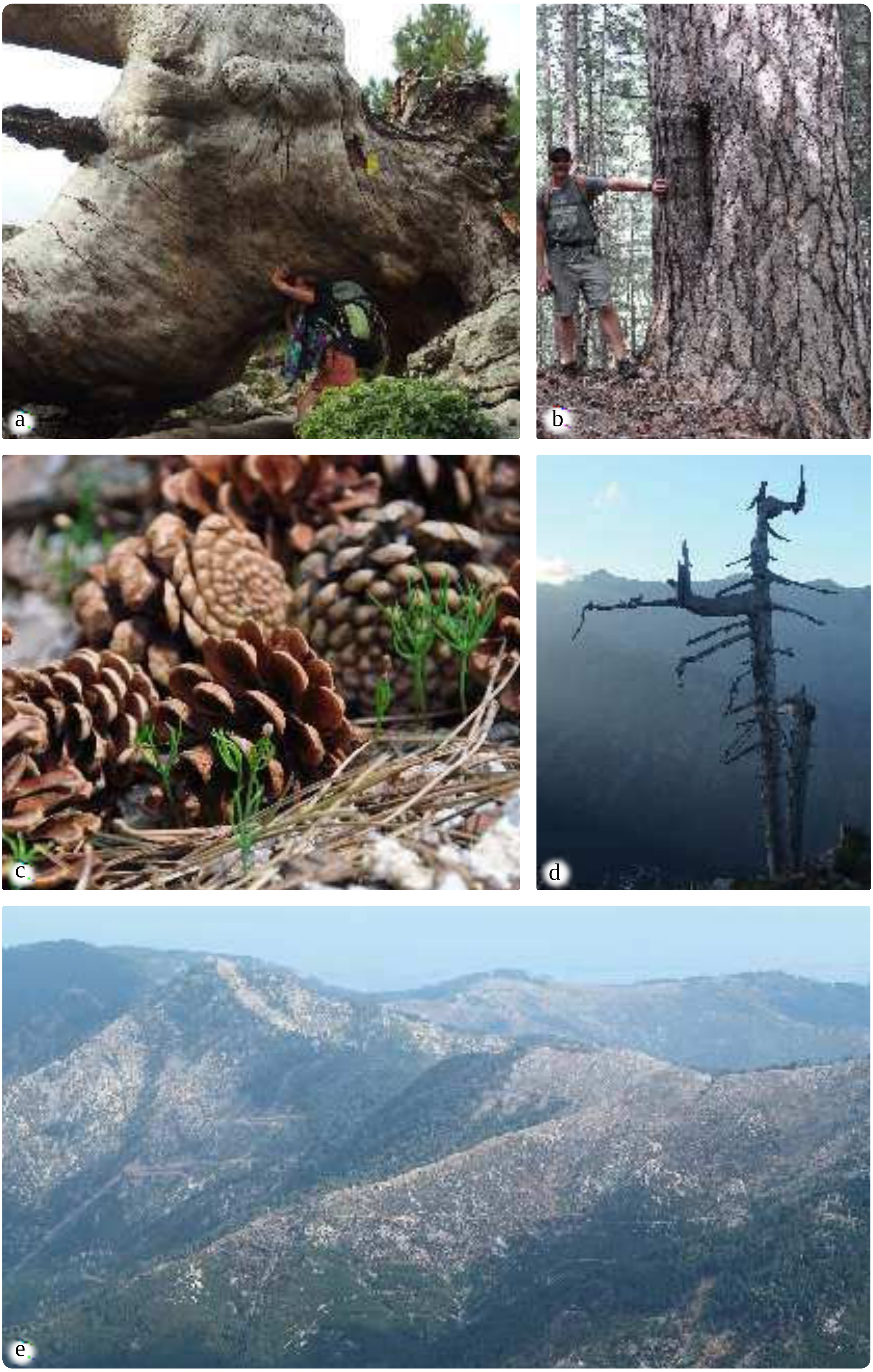


Figure I.19 – Photos de pins laricio : (a) Individu mort dans la vallée du tavignano. Merci à E. Le Fur pour l'échelle. **(b)** Individu vivant avec trace de cicatrice dans la vallée du Verghello. Merci à P. Fulé pour l'échelle. **(c)** Régénération de plantules au milieu des cônes de pin. **(d)** Vestige d'un individu victime du passage de feu de Rospa sorba en 2000. Photo prise en 2016. **(e)** Vue (en 2016) du col de Rospa sorba victime d'un feu en 2000.

7.5 Le feu, menace principale sur le pin laricio ?

Le nombre et la surface des feux ont augmenté en méditerranée au cours des dernières années (Pausas et coll. 2008). Les raisons sont multiples et incluent la lutte contre le feu depuis des décennies et les changements d'usage des terres (Moritz et coll. 2014). Il est dès lors indispensable de connaître la résilience des écosystèmes face au feu. Ainsi, les maquis, les garrigues et les forêts de chênes présentent une forte résilience après feu que ce soit par recrutement des graines ou par régénération (Pausas et coll. 2008). En revanche, les forêts de pins noirs sont sensibles au feu et peu résilientes (Pausas et coll. 2008).

Les pins noirs – dont le pin laricio – ne produisent pas de cônes sérotineux[60] et ont des capacités de dispersion limitées (Ordóñez et coll. 2006). Cela explique en partie leur faible capacité de régénération après feu (Pausas et coll. 2008). En revanche, les pins noirs ont une écorce épaisse et les arbres matures n'ont pas de branches basses. Les pins noirs résistent donc régulièrement aux feux de faibles intensités et peuvent présenter des cicatrices de feu (Fulé et coll. 2008 ; Leys et coll. 2014 ; Fig. I.19b et Fig. II.4c). Les feux capables de détruire des peuplements de pin laricio sont par conséquent des feux de couronne souvent intenses. Pour autant, grâce à une étude des macrofossiles d'arbres dans un lac corse, Leys et coll. (2014) ont montré que le pin laricio survit depuis plus de 15 000 ans près du lac, et ce malgré des fréquences de feu localement plus importantes qu'aujourd'hui.

Durant l'été 2000, 11 000 hectares ont été parcourus par le feu en Corse. Parmi les zones parcoures, de nombreuses populations de pin laricio ont brûlé aussi bien au sein de peuplements éparses que de forêts pures ou en mélange (Soulères 2000 ; Fig. I.19e). Au total, près de la moitié des surfaces de pin laricio ont été parcourues par le feu cette année là (11 000 ha[61] sur 22 000 ha[62]). Au delà de la mortalité directe ou indirecte des hôtes, le feu provoque des modifications de l'environnement des arbres (par ex. modification de la composition et de la communauté microbienne du sol) et de leur physiologie (par ex. modification des flavonoïdes produits ; Lavoir et coll. 2013).

Même si l'essence n'est pas menacée en elle-même par le feu (Leys et coll. 2014), les incendies comme ceux de 2000 provoquent des réactions sociales fortes. Les agents ONF, en première ligne face au feu, sont particulièrement sensibles à la protection de la forêt face aux incendies. Ainsi, le technicien forestier Beretti, chef du secteur de la commune de Ghisoni regrette les atermoiement des pompiers durant le feu de 1980 en illustre l'attachement des forestiers à leur forêt :

> *« Le feu "musardant", en l'absence de vent, dans la partie détruite par l'incendie de 1970, ne présentait aucun danger malgré les épais tourbillons de fumée émanant de la combustion d'étocs résineux dans le vide incendié, le Chef de la subdivision PAOLACCI après avoir fait une reconnaissance du front, insiste à nouveau auprès de M. GIORGI pour l'inviter à "occuper" ces hommes sur ce front. Le Chef du centre refuse d'intervenir arguant le "trop grand danger" pour les sauveteurs, mais exécute les ordres donnés dans le même sens par le commandant*

---

[60] La sérotinie est une adaptation des végétaux face au feu. Les graines des plantes sérotineuses sont conservées dans les cônes (par ex. les cônes des pins) jusqu'à ce qu'un feu fasse fondre la résine des cônes en les libérant (Pausas 2015).

[61] Soulères 2000

[62] Collectif 2006b

VERDEVOYE venant sur les lieux. Cette heure de perdue a permis au feu d'endommager un petit bouquet d'arbres qui auraient pu être facilement épargnés. »

C. Beretti, Chef de secteur de la forêt de Ghisoni
Rapport d'incendie de l'ONF du 17 septembre 1980, p. 2 - 3

« *Que d'efforts gaspillés sur des fronts pelés [...] alors que les fronts menaçant les peuplements forestiers sont souvent abandonnés laissant la forêt sans défense. Et qui connaît mieux la forêt que le forestier qui la sillonne chaque jour, qui l'aménage, qui la cultive et qui l'aime ? Ce forestier qui est considéré comme un intrus lorsqu'il vient défendre "sa" forêt qui brûle ! !* » *Ibid.* p. 4

* * *

Résumé

(i) On peut caractériser un feu ou un ensemble de feux par son régime. Parmi les nombreux paramètres qui constituent un régime de feu, nous nous intéressons dans cette thèse à la sévérité et au temps depuis le dernier feu.

(ii) Le pin laricio est une espèce endémique de Corse vivant entre 900 et 1 800 m d'altitude. Cette essence concentre d'importants intérêts écologiques, économiques et sociaux.

(iii) Historiquement, le feu n'est pas le talon d'Achille du pin laricio, mais l'année 2000 a vu le feu parcourir la moitié des peuplements de pin laricio. Le pin laricio présente une certaine résistance aux feux de faibles intensités mais une faible résilience.

Summary

(i) We can characterize a fire or an collection of fire by its regime. Among the numerous parameters which constitute fire regime, this thesis is interested in severity and time since fire.

(ii) Corsican pine is an endemic species in Corsica (France) growing between 900 to 1 800 m of altitude. This species concentrates significant ecological, economical and sociological interests.

(iii) Historically, fire is not the Achilles' heel of the Corsican pine. Nevertheless, fire runs through half of Corsican pine forests in 2000. This pine species is fire-resistant for low intensity fire but display weak resilience.

8 Résumé des hypothèses

8.1 Les communautés de champignons ECM dépendent fortement de la composition de la végétation régionale et locale

- **Les espèces de plantes ECM abritent des communautés fongiques différentes à l'échelle locale** : projet de manuscrit D 🗎
  - Les préférences d'associations sont suffisamment fortes pour que différents hôtes – toutes choses égales par ailleurs, en particulier l'environnement local – abritent des communautés fongiques différentes.
- **Les individus d'espèces de plantes ECM différentes qui coexistent partagent plus de partenaires fongiques localement que des individus de ces deux espèces qui ne coexistent pas** : projet de manuscrit D 🗎
  - Les individus qui coexistent rencontrent des conditions environnementales proches. On peut donc s'attendre à un filtre environnemental commun de leurs communautés de champignons ECM.
  - Indépendamment de l'environnement abiotique, les individus qui coexistent « piochent » dans la même communauté fongique potentielle (mycélium, sclérote ou spore présent localement). Une structure spatiale des espèces de champignons indépendantes de l'environnement (c.-à-d. médiée par la dispersion des champignons) pourrait entraîner une ressemblance des communautés d'individus qui coexistent.
  - Au contraire, la coexistence pourrait être facilitée par le relâchement de la compétition via la distribution des partenaires ECM entre les individus d'espèces différentes. Les individus qui coexistent seraient alors capable de se « partager » les partenaires localement en deux cortèges relativement bien distincts.
- **Les espèces de plantes ECM qui coexistent beaucoup partagent plus de partenaires fongiques que les plantes qui coexistent peu** : manuscrit C 🗎
  - Les espèces de plantes qui coexistent beaucoup – et donc leur partenaires fongiques – rencontrent des conditions environnementales proches. On peut donc s'attendre à un filtre environnemental commun et donc des communautés proches chez ces plantes.
  - Étant donné la facilitation entre plantes via les ECM, on peut postuler qu'une pression de sélection sur les plantes engendre une tendance au partage local de leur cortèges.
  - Au contraire, des processus de déplacement de caractères pourraient entraîner la tendance inverse avec les plantes qui coexisteraient plus facilement si elles se partagaient les ressources à travers des partenaires fongiques différents.
- **Le réseau d'interactions ECM à l'échelle de la Corse est structuré par les hôtes et leur relations phylogénétiques** : manuscrits B 🗎, C 🗎

- Les préférences d'associations sont suffisamment fortes pour que différents hôtes abritent des communautés fongiques différentes à l'échelle de la région.

➲ **Les espèces spécialistes s'associent préférentiellement à des partenaires généralistes** : manuscrit B 🗎

- Au vue de la nécessité de trouver des partenaires à la fois pour les plantes et les champignons ECM, on s'attend à ce que les espèces spécialistes interagissent avec des partenaires largement distribués. On s'attend également à ce que les espèces largement distribuées soient généralistes pour trouver des partenaires facilement sur un grand territoire.
- Les espèces de plantes pionnières pourraient avoir intérêt à interagir avec des partenaires spécialistes pour s'établir rapidement dans une communauté. Les espèces de plantes pionnières vivant peu de temps, on peut s'attendre à ce que leur cortège fongique soit relativement pauvre.

8.2 Le feu modifie les structures des communautés fongiques

➲ **Les feux – en particulier les feux de couronne – favorisent la diversité fongique à l'échelle du paysage en modifiant les structures des communautés fongiques pour toutes les guildes par plusieurs processus** : manuscrit F 🗎 et projets de manuscrit G 🗎 et J 🗎

- Effet direct de la température sur le mycélium (effet attendu plus fort sur l'horizon A que B) ;
- Effet indirect par changement de la composition physico-chimique du sol (effet attendu plus fort sur l'horizon A que B) ;
- Effet indirect à travers l'effet sur l'hôte (mort, stress, diminution de la compétition avec d'autres plantes, ...).
- Au contraire, les feux réduisent la diversité fongique du paysage par la disparition d'une partie de la communauté sans remplacement par de nouvelles espèces. La composition des communautés après feu serait alors emboîtée dans celle des communautés présentent longtemps après un feu.

8.3 Les règles d'assemblage des communautés de champignons dépendent de leurs préférences d'habitats et de ressources

➲ **Les communautés fongiques de différentes guildes ont des caractéristiques écologiques dissemblables, ce qui entraîne des règles d'assemblage des communautés relatives aux guildes** : voir l'ensemble des manuscrits et la discussion générale.

- On peux séparer la communauté des champignons saprotrophes et celle des champignons ECM en fonction des ressources limitantes partagées (par ex. la cellulose et la lignine dans un cas, le carbone de l'hôte et les nutriments pour l'autre). Par ailleurs, les feux de surface impactent la litière et la matière organique mais peu les arbres. En revanche, les feux de couronne détruisent complètement la végétation en produisant en partie de la matière organique à décomposer.

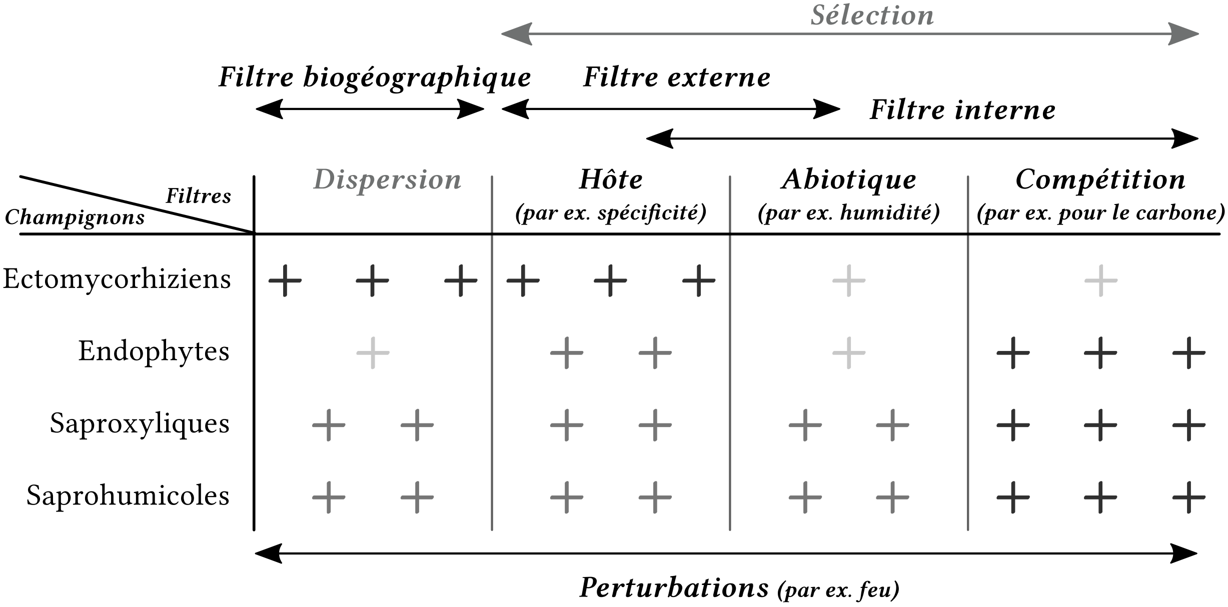
Par conséquence, on peut s'attendre à ce qu'un feu de surface affecte plus les champignons saprotrophiques qu'ECM alors qu'un feu de couronne marque plus les communautés de champignons ECM que saprotrophiques.

- Les champignons ectomycorhiziens (chapitres III et IV) sont toujours des organismes multicellulaires, pour beaucoup de plus grande taille et avec des cycles de vie plus long que les champignons endophytiques foliaires (CEF ; manuscrit H 🗎). On peux s'attendre à des facilités de dispersion moins fortes chez les champignons ECM que chez les CEF et donc une importance de la dispersion plus forte dans l'assemblage de ces communautés (tableau I.5).
- Le dialogue complexe de l'interaction entre la plante et les champignons mycorhiziens (Martin et coll. 2001 ; Martin et coll. 2008) milite pour une spécificité d'hôte plus forte que chez les endophytes, et donc un filtre externe (voir Fig. I.5), induit par l'hôte, plus important pour la symbiose ECM (tableau I.5).

➲ **Hypothèse alternative** : Les champignons en général suivent des règles d'assemblage similaires quelles que soient leur préférences d'habitats et de ressources. Dans ce cas, il est probable que les ressemblances évoquées dans l'introduction suffisent à rendre les différences entre guildes de champignons inconsistantes en ce qui concerne les règles d'assemblage des communautés.

Tableau I.5 – Importance hypothétique des règles d'assemblage en fonction des guildes de champignons : Les classifications des processus d'assemblage sont issues des figures I.5 (doubles flèches noires) et I.6 (en gris).

Voir aussi tableau I.3.



Filtres *Champignons*	*Dispersion*	*Hôte (par ex. spécificité)*	*Abiotique (par ex. humidité)*	*Compétition (par ex. pour le carbone)*
Ectomycorhiziens	+ + +	+ + +	+	+
Endophytes	+	+ +	+	+ + +
Saproxyliques	+ +	+ +	+ +	+ + +
Saprohumicoles	+ +	+ +	+ +	+ + +

* * *

Avancement des travaux - *Works progress*

Une série de séquençage est encore en cours sur la plateforme Illumina en Allemagne. Cette série comporte tous les échantillons d'apex mycorhiziens et quelques échantillons de sol concernant la question du feu. Les résultats des manuscrits D et G sont donc préliminaires [63] et incomplets [64].

We are waiting for the second run of Illumina sequencing containing all samples of mycorrhizal root tips and several soil samples of Corsican pine. Drafts D *et* G *are therefore preliminary and incomplete.*

- Publications en auteur principal (Premier ou dernier auteur) - *publications in first/last author*
  - Manuscrit A : "Mycorrhizas: Dynamic and complex networks of power and influence"
  - Manuscrit B : "Beyond ectomycorrhizal bipartite networks: Projected networks demonstrate contrasted patterns between early- and late-successional plants in Corsica"
  - Manuscrit K : "cati: An R package using functional traits to detect and quantify multi-level community assembly processes"
- Manuscrits à soumettre sous peu (dans l'ordre de soumission prévu) - *Manuscripts to submit shortly (in the order of planned submission)* :
  - Manuscrit F : "Review on fire effects on ectomycorrhizal symbiosis, an unachieved work for a scalding topic"
  - Manuscrit I : "*Xylobolus subpileatus*, a hyper-specialized basidiomycete functionally linked to old canopy gaps"
  - Manuscrit E : "Using ectomycorrhizal bipartite networks for the conservation of fungal diversity: an indices-based approach"
  - Manuscrit C : "Why two plant species share as many ectomycorrhizal species at regional scale? A history of phylogeny, coexistence but not climate."
  - Manuscrit H : "Finding fungi in a needle stack: contrasted α- and β-diversity of endophytic Ascomycetes in Mountainous pine forests"
- Manuscrit en cours d'écriture, analyses terminées - *Manuscript in writing, data analysis done* :
  - "Soil saprobic fungal community assembly: effect of fire and forest species composition" (Projet de manuscrit J) (draft in progress in appendix)
- Manuscrits en attente de données complémentaires - *Waiting for additional data* :
  - "Effect of fire on ECM fungal communities in soil and roots of *Pinus nigra* subsp. *laricio* (Corsica, France)" (Projet de manuscrit G)
  - "Effect of host taxonomy and coexistence on ectomycorrhizal fungal communities" (Projet de manuscrit D)
- Publications en collaboration - *Publications in collaboration*
  - Manuscrit L : "A global meta-analysis of the relative extent of intraspecific trait variation in plant communities"
  - Manuscrit M : "Whose truffle is this? Distribution patterns of ECM fungal diversity in *Tuber melanosporum* brûlés developed in multi-host Mediterranean plant communities"
  - Manuscrit N : "Into the functional ecology of ectomycorrhizal communities: environmental filtering of enzymatic activities"
  - Manuscrit O : "Ectomycorrhizal fungi are shared between seedlings and adults in a monodominant *Gilbertiodendron dewevrei* rainforest in Cameroon"

---

[63] Il manque quelques échantillons de sol, en particulier pour la question du temps de résilience des communautés fongiques face au feu

[64] Il manque les échantillons des apex

Chapitre II

Échantillonnages et analyses

> « […] je me figurais volontiers que la source devait être dans ce nom inconnu et si doux de « Champi » qui mettait sur l'enfant, qui le portait sans que je susse pourquoi, sa couleur vive, empourprée et charmante. »
>
> Marcel Proust, *Du côté de chez Swann*, 1913

Ectomycorhizes sur racines de pin laricio

Préambule : En ce qui concerne l'échantillonnage des endophytes du pin laricio et des champignons décomposeurs du genre *Xylobolus*, se référer aux manuscrits pages 194 et 225. Merci à nos amis insulaires de Corse (CBNC) et d'Outre Manche (British Mycological Society) pour leur aide décisive sur le terrain.

1 Échantillonnages de la diversité fongique en relation avec le feu et la composition des forêts

1.1 Choix des sites en Corse

Campagne de terrain préliminaire

Nous avons effectué une première campagne de terrain afin d'identifier les sites potentiels et d'évaluer les difficultés d'échantillonnage inhérentes aux prélèvements de terrain dans des systèmes aussi complexes que les écosystèmes forestiers. Pour cette mission en Corse d'une semaine (10 - 17/06/2015), nous étions cinq : mes deux directeurs de thèse et moi-même avons eu la chance d'être accompagnés de deux chercheurs spécialistes du feu : Thomas Curt (Directeur de recherche, IRSTEA) et Peter Fulé (Professeur, Northern Arizona University). Peter Fulé est un spécialiste de la reconstruction des histoires de feu dans les forêts de *Pinus nigra* (e.g Fulé et coll. 2008 ; Christopoulou et coll. 2013) et sa connaissance des traces de feu a été cruciale dans la détermination des sites (Fig. II.1) et dans notre compréhension de l'impact des feux sur les pins noirs.

Choix des sites et des parcelles

Pour chaque variable (intensité et date du feu, profondeur et composition de la végétation dominante), nous avons effectué cinq carottes dans trois parcelles différentes et dans plusieurs massifs forestiers (les « sites »). L'ensemble du plan d'échantillonnage est résumé dans la figure II.9. Les sites, et à l'intérieur des sites les parcelles, ont été choisis en homogénéisant au maximum les variables environnementales telles que l'altitude[1], la végétation de sous bois[2], l'absence de coupes récentes à proximité ou encore l'âge des arbres[3].

Choix des forêts ayant subi des feux de sévérité différente

Les sites sélectionnés pour étudier l'effet du feu sur les ectomycorhizes ont été choisis grâce à la campagne de terrain préliminaire et à des discussions avec les membres de la DFCI (Défense de la forêt contre les incendies) d'Ajaccio : Fabrice Torre, le commandant Jérôme Mozziconacci et

---

[1] En moyenne les sites sont situés à 1 089m ± 158 m, avec un maximum de 1 529 m dans une Hêtraie-pinède et un minimum de 661 m correspondant à des parcelles de pin maritime pure. Pour les parcelles de pin laricio seul, l'altitude moyenne est de 1 087m ± 181m (min : 715 m, max : 1 450 m).

[2] En évitant particulièrement les autres espèces de plantes ectomycorhiziennes.

[3] En dehors des zones détruites par les feux, les prélèvements étaient toujours effectués auprès d'arbres matures et éloignés de plantules ou d'arbres de diamètre (à hauteur de poitrine) inférieur à 10 cm.

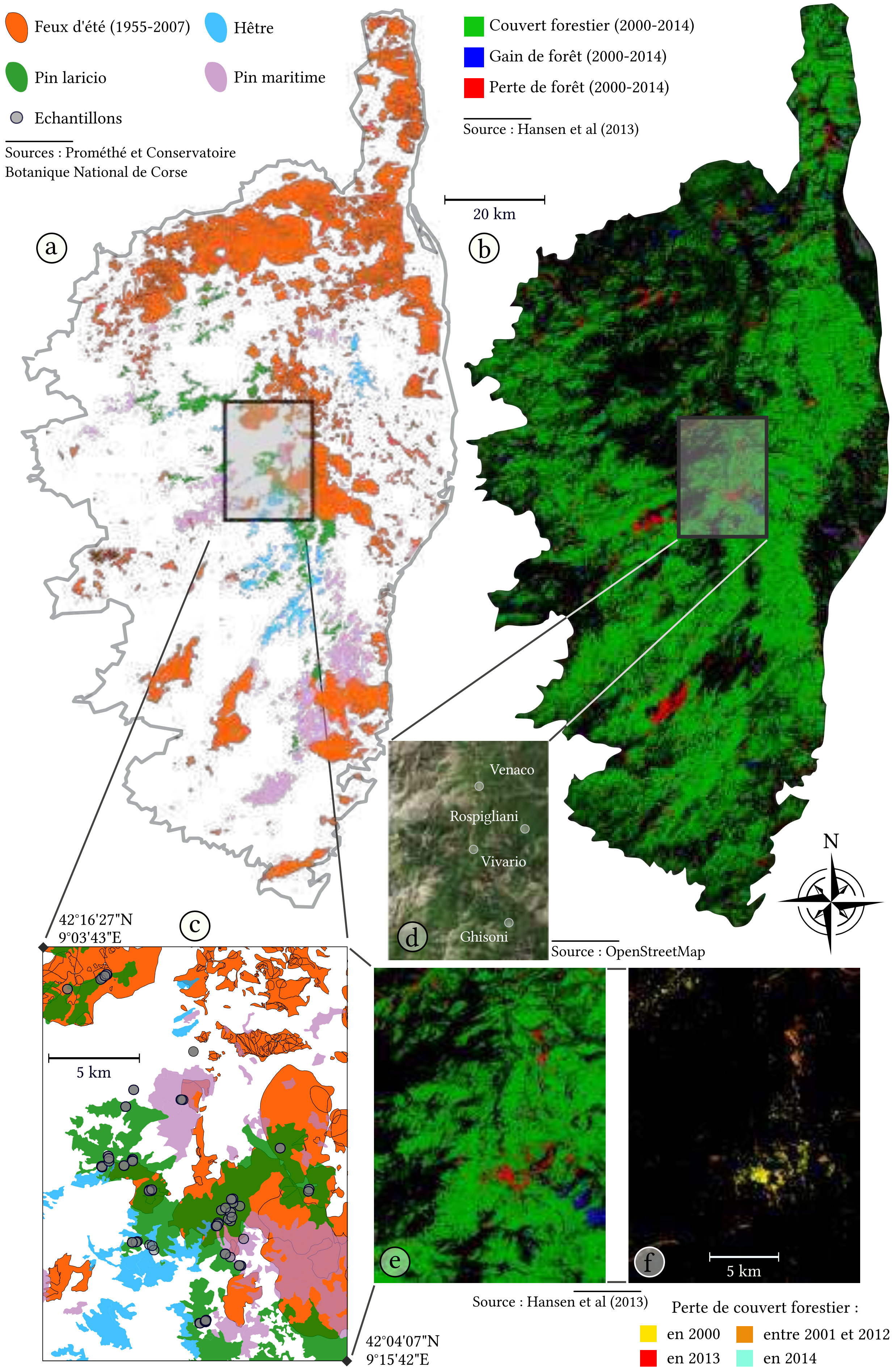
Phillippe Caramelle et les agents forestiers de l'ONF Pierre-Jean Pace, Germain et Antoine Paolacci, Alain Chavenon et Stéphane Muracciole .

Trois catégories d'intensité de feu ont été établis. Les parcelles témoins (Fig. II.2a) n'ont aucune trace visible de feu et aucun feu n'est répertorié dans les bases de données des forestiers. Les parcelles parcourues par le feu (Fig. II.2b) sont constituées de pins ayant survécu au passage du feu et présentant des traces de feu (voir Fig. II.4c). Enfin, les parcelles détruites par le feu (Fig. II.2c) sont constituées de plantes qui ont recolonisées après feu, avec parfois des traces de feu encore visibles, notamment sur les troncs au sol. Les zones parcourues ou détruites se situent dans des zones indiquées comme telles dans les bases de données (voir Fig. II.4b).

Choix des forêts monospécifiques et des forêts mixtes

Les sites sélectionnés pour étudier l'effet de la coexistence des arbres sur les communautés de champignons ECM remplissent trois conditions. (i) Ce sont des massifs forestiers incluant les trois types de forêts monospécifiques (pin laricio, hêtre et pin maritime ; figure II.3a-e) et les deux forêts en mélange (hêtre-pin laricio et pin maritime-pin laricio ; Fig. II.3d et f). (ii) Les parcelles ne devaient contenir en espèces de plantes ectomycorhiziennes que les essences d'intérêts. Par exemple, nous avons évité des sites et des parcelles contenant des chênes verts (*Quercus ilex*) en sous bois. (iii) Les forêts échantillonnées devaient être constituées de nombreux arbres âgés (dbh > 30 cm environ) pour éviter un effet confondant de l'âge des plantes sur les communautés de champignons ECM.

Au sein des parcelles de forêt mixte, les échantillons de sol et de racine ont été prélevés à proximité d'adulte des deux essences. De plus, les carottes de sol dans lesquelles seule une essence était présente sous forme de racine étaient laissées de côté et remplacées par une nouvelle carotte.



Sources : Prométhé et Conservatoire Botanique National de Corse

Source : Hansen et al (2013)

Source : OpenStreetMap

Source : Hansen et al (2013)

FIGURE II.1 – Contexte biogéographique des points d'échantillonnages : légende page suivante.

Contexte biogéographique des points d'échantillonnage (légende de la figure page précédente) : **a)** Carte de la distribution des feux (orange), du pin laricio (*Pinus nigra* subsp *laricio*, vert), du hêtre (*Fagus sylvatica*, bleu) et du pin maritime (*Pinus pinaster*, violet) en Corse. La distribution des feux est issue de la base de données Prométhée pour les années 1955-2007 (extraction du Conservatoire National Botanique de Corse). Les distributions des essences forestières sont issues du Conservatoire National Botanique de Corse à partir de données IGN (Institut Géographique National). **b)** Distribution du couvert forestier (en vert) et de son évolution en Corse entre 2000 et 2014. L'augmentation du couvert forestier (en bleu) est moins importante que la perte de forêt (en rouge) qui est principalement causée par les feux. Les données proviennent de l'étude *High-Resolution Global Maps of 21st-Century Forest Cover Change* qui utilise les images Landsat pour établir la distribution et l'évolution du couvert forestier (Hansen et coll. 2013) . **c)** Agrandissement de la zone d'étude et position des points d'échantillonnage (points gris). Légende identique à celle du panel a. **d)** Carte de situation de la zone d'étude (source : Open Street Map). **e)** Agrandissement de la zone d'étude montrant la distribution et les changements de couvert forestier. Légende identique à celle du panel b. **f)** Distributions temporelles de perte de couvert forestier dans la zone d'étude. Les pertes importantes de couvert forestier de l'année 2000 (en jaune) correspond aux nombreux feux de forêts ayant eu lieu en 2000 en Corse. Source identique aux panels b et e.

Figure II.2 – Modalités d'intensité de feu de forêt : Les forêts témoins (**a** et **b**) ne présentent pas de trace de feu. Au contraire les forêts parcoures (**c** et **d**) présentent de nombreuses traces de feu mais de nombreux arbres ont survécus. Enfin, dans les forêts détruites par le feu (**e** et **f**) la végétation présente est issue d'une colonisation post-feu et des traces du feu sont souvent encore visibles. La photo **c** présente des traces du feu de Tatonne en 2008, les photos **d**, **e** et **f** montrent des traces des feux de 2000 dans le massif de Ghisoni. Crédit photographique photo (**e**) : Christopher Carcaillet.

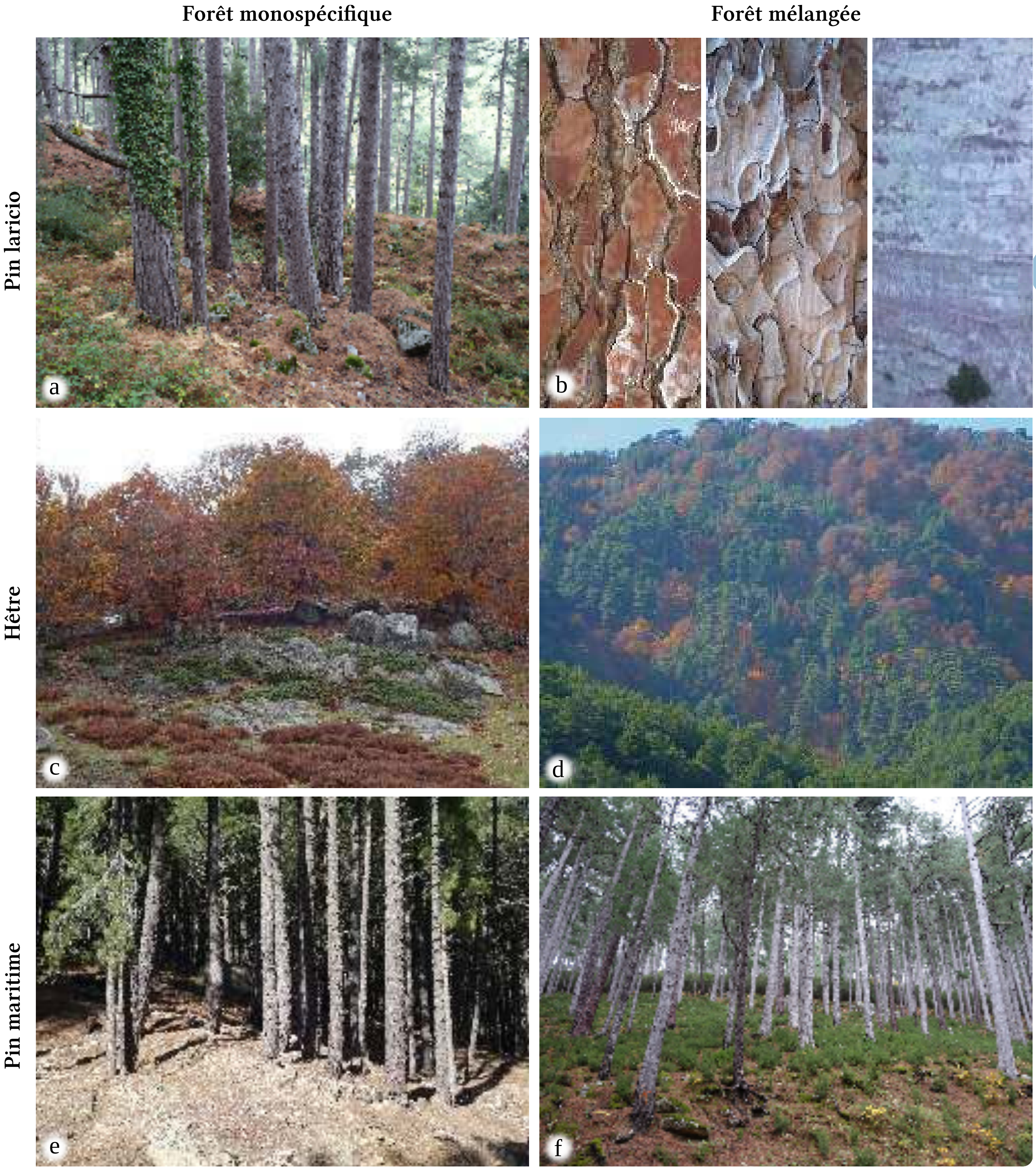


Figure II.3 – Forêts échantillonnées monospécifiques ou en mélange : Forêts monospécifiques de pin laricio (**a**), hêtre (**c**) et pin maritime (**e**) dans le massif de Ghisoni. Les trois essences ont des écorces caractéristiques (**b** ; de gauche à droite : pin maritime, pin laricio et hêtre). Le massif de Ghisoni abrite également des forêts mixtes hêtre-pin laricio (**d**) et des mélanges pin maritime-pin laricio (**f**).

1.2 Datation des feux

Il existe de nombreuses méthodes de datation des feux qui dépendent des données disponibles, des objectifs de recherche, et de l'étendue des dates considérées. Dans le cadre de cette thèse, les feux les plus anciens (1981) sont relativement récents. Nous avons utilisé trois méthodes de datation :

- Des bases de données (ONF et SDIS) complétées par des entretiens avec les pompiers et les gestionnaires forestiers (Fig. II.4a) ;
- Des cartes des feux dressées par les gestionnaires forestiers (Fig. II.4b) ;
- Des cicatrices de feux présentes à la base des troncs (Fig. II.4c).

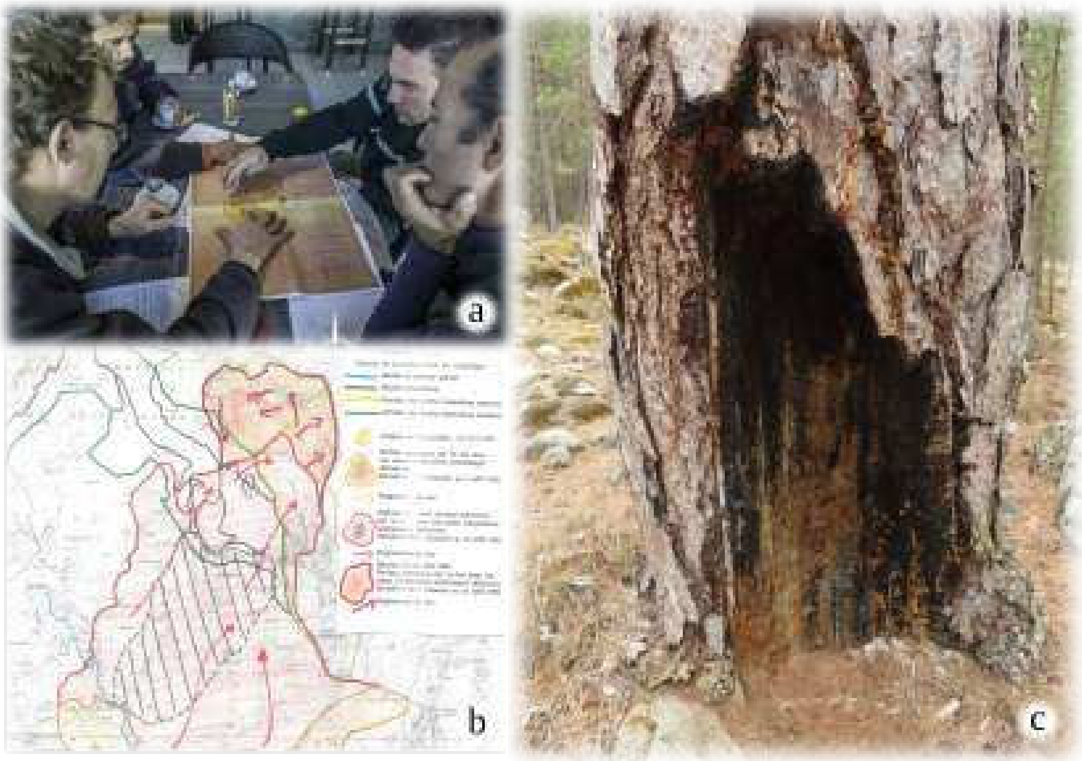


Figure II.4 – Méthodes de datation des feux : a) Discussion avec deux agents de l'ONF (Office national des forêts) à propos d'une carte représentant des polygones de feu sur le territoire de Ghisoni. **b)** Un exemple de carte de feu utilisée pour connaître la date du dernier feu sur les zones échantillonnées. Les différentes couleurs indiquent notamment les aires parcourues et les aires détruites par le feu. **c)** Cicatrice sur tronc de pin laricio due au passage du feu. On peut parfois observer plusieurs cicatrices emboîtées qui témoignent de plusieurs passages de feux. La hauteur de la cicatrice donne également une idée de la sévérité du feu. Le passage d'un feu sur un site s'établit à l'aide de plusieurs arbres portant des cicatrices concordantes.

Un des enjeux majeurs dans les études synchroniques est de connaître la date du dernier événement d'intérêt, dans notre cas la date du dernier feu. Nous avons confronté les trois méthodes citées plus haut dès que possible afin de n'omettre aucun feu. Seule la datation par cicatrice (Fig. II.4c) n'a pas été systématique. Au cours d'un séjour de terrain avec Peter Fulé, spécialiste de la reconstruction des histoires de feux chez les pins noirs, nous avions établi que de telles analyses nécessiteraient le temps d'une thèse complète. Aussi, les datations par cicatrice n'ont servi qu'a corroborer les dates préalablement établies via les documents d'archives, les bases de données et les entretiens.

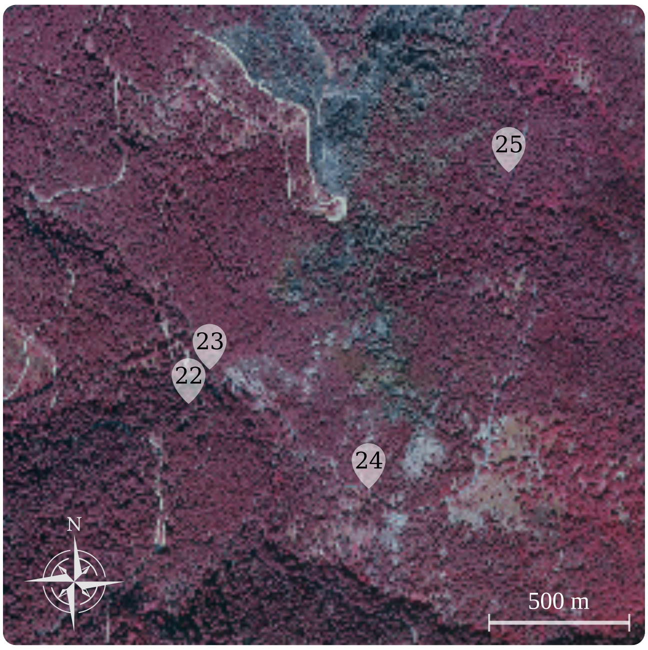


Figure II.5 – Identification par satellite des arbres touchés par le feu : Le site se trouve au dessus du village de Vivario. Les numéros correspondent à des numéros d'échantillons. Les arbres dont la capacité chlorophyllienne a été supprimée par le feu sont de couleur rouge. Les zones vertes sont constituées d'arbres ayant peu subit les effets du feu. On remarque bien l'effet des routes qui contraignent la propagation du feu et permettent aux pompiers d'atteindre facilement un front de combat.

1.3 Prélèvements de sol et d'apex ectomycorhiziens

Prélèvement de sol

Pour chaque modalité de variable (feu de surface, forêt pure de hêtre,…), trois parcelles par massif forestier ont été échantillonnées (Fig. II.9 pages 92 à 95). Dans chaque parcelle, nous avons effectué cinq prélèvements de sol aléatoirement répartis mais distants d'au moins 15 m[4]. La parcelle correspond à une zone homogène en cœur de peuplement pour éviter les effets de lisière. De plus, les communautés de champignons – en particulier les communautés de champignons ECM – sont connues pour changer fortement en fonction de l'âge des hôtes. Aussi, les parcelles étaient situées dans des peuplements âgés et nous avons effectué les prélèvements toujours à proximité d'adultes d'âge bien avancé.

Les communautés de champignons de la litière sont différentes de celles du sol, en particulier chez les champignons ECM (Shahin et coll. 2013 ; Clemmensen et coll. 2015) dont de nombreux taxons sont absents de la litière. Par conséquent, nous avons, préalablement à chaque prélèvement de sol, retiré la litière pour ne prendre que du sol. Dans le cas des forêts de pins laricio, nous avons divisé les carottes en deux horizons : l'horizon A (holo-organique) et l'horizon B (organo-minéral). Ce prélèvement en deux profondeurs contrastées permet de tester les effets du feu sur deux zones du sol qui subissent différemment les effets directs du feu.

Prélèvement des apex

Les apex (Fig. II.7) ont été piqués, c'est-à-dire échantillonnés à la pince à dissection sous une loupe binoculaire. Les racines étaient préalablement rincées puis mises dans l'eau sous la loupe binoculaire pour ôter la terre et les particules qui entourent les racines. Pour les gros amas de terre,

---

[4] Cette valeur est basée sur les travaux de Lilleskov et coll. (2004) qui ont montré que les champignons ECM pouvaient présenter des auto-corrélations spatiales. Ils ont proposé la valeur de 15 mètres comme la valeur sous laquelle 95% des espèces ne présentent plus d'auto-corrélation spatiale.

nous avons utilisé le jet d'eau de la pipette pour ne pas endommager les mycorhizes. Pour chaque échantillon, 200 apex ont été prélevés sans morphotypage. Autrement dit, les 200 premiers apex vus étaient échantillonnés même s'il s'agissait d'apex qui se ressemblaient fortement. Les prélèvements ont été effectués par Franck Richard et Adrien Taudière entre le 9 novembre 2014 et le 9 décembre 2014. Nous avons régulièrement comparé nos résultats pour éviter au maximum les biais d'observateur. Le biais observateur sera également pris en compte dans les analyses statistiques des séquences issues des apex.

Dans le cas de l'étude des forêts mixtes, les racines des différentes espèces étaient séparées selon leur morphologie. Pour chaque échantillon, nous avons piqué les mycorhizes sur environ 30 cm linéaire de racine. De plus, nous avons échantillonné au minimum trois fragments racinaires. Le nombre d'apex étaient alors compris entre 75 et 200. Les analyses des apex sont dans l'attente des données Illumina (voir section avancement des travaux).

Analyses chimiques des sols

En raison de contraintes budgétaires seuls 32 échantillons de sol ont été caractérisés chimiquement. 15 g de sol ont été tamisés à 2 mm puis broyés avec un broyeur à billes (particules de 250 µm). Ce travail et l'analyse des données ont été réalisés par Ananda Christophe dans le cadre de son stage de Master 1 (Annexe p. 385). Les concentrations en phosphore, carbone organique et azote total ont été mesurées par le laboratoire d'analyses des sols de l'INRA à Arras.

1.4 Résumé du plan d'échantillonnage

Pour résumer, nous avons établi le protocole d'échantillonnage de sol et d'apex ECM en Corse pour répondre à deux grands objectifs : (i) Caractériser le cortège de champignons ECM du pin laricio, et étudier en particulier l'effet du feu sur ce cortège et (ii) comprendre l'effet de la coexistence des plantes sur leur cortège de champignons ECM.

Les quatre planches de la figure II.9 résument le plan d'échantillonnage global. Tout d'abord, de nombreuses forêts monospécifiques de pin laricio ont été échantillonnées dans des zones ne présentant aucune trace de feu, ni sur le terrain, ni dans les archives. Les échantillons de ces forêts servent de témoins pour les deux grandes questions transversales (effet du feu et effet de la coexistence sur le cortège ECM ; Planche IV de la figure II.9). Pour tester la pertinence de notre plan d'échantillonnage à posteriori, nous avons sur-échantillonné un des sites avec 15 échantillons au lieu de 3 pour chacune des deux profondeurs (voir cartouche en bas de la planche II de la figure II.9). La variation inter-carotte (intra-parcelle) est présente mais plus faible que la variation inter-parcelle (Fig. II.8).

En ce qui concerne l'échantillonnage du cortège du pin laricio en relation avec le feu, nous avons échantillonné le sol et les apex à deux profondeurs (couche A et B) dans des parcelles qui diffèrent par la sévérité et/ou l'âge du feu qu'elles ont subi. Enfin, nous avons échantillonné le sol et les racines de forêt monospécifiques et mixtes en séparant les racines entremêlés des espèces en coexistence. Au total, 175 échantillons de sol et 163 échantillons d'apex (c. 25 000 apex) ont été échantillonnés dans cette thèse et envoyé à séquencer.

Figure II.6 – Illustration de l'échantillonnage du sol : Pour chaque échantillon de sol extrait à la truelle et au tournevis (**a**) nous avons prélevé du sol et des racines afin d'obtenir les mycorhizes. Pour la question du feu (**b**) nous avons retiré la litière (**li**) et séparé les échantillons en deux suivant l'horizon de sol (horizon organique noté **A** et horizon minéral **B**). Dans les forêts mixtes (**c**), nous avons séparé les racines des deux essences d'intérêt (flèches bleues : pin laricio et flèche rouge : pin maritine). Dans certains cas, les mycorhizes étaient déjà visibles sur les racines (flèches oranges ; photos **d**-**f**). Le sol et les racines étaient stockés dans des sachets de congélation (**g**) avant d'être stockés au congélateur.

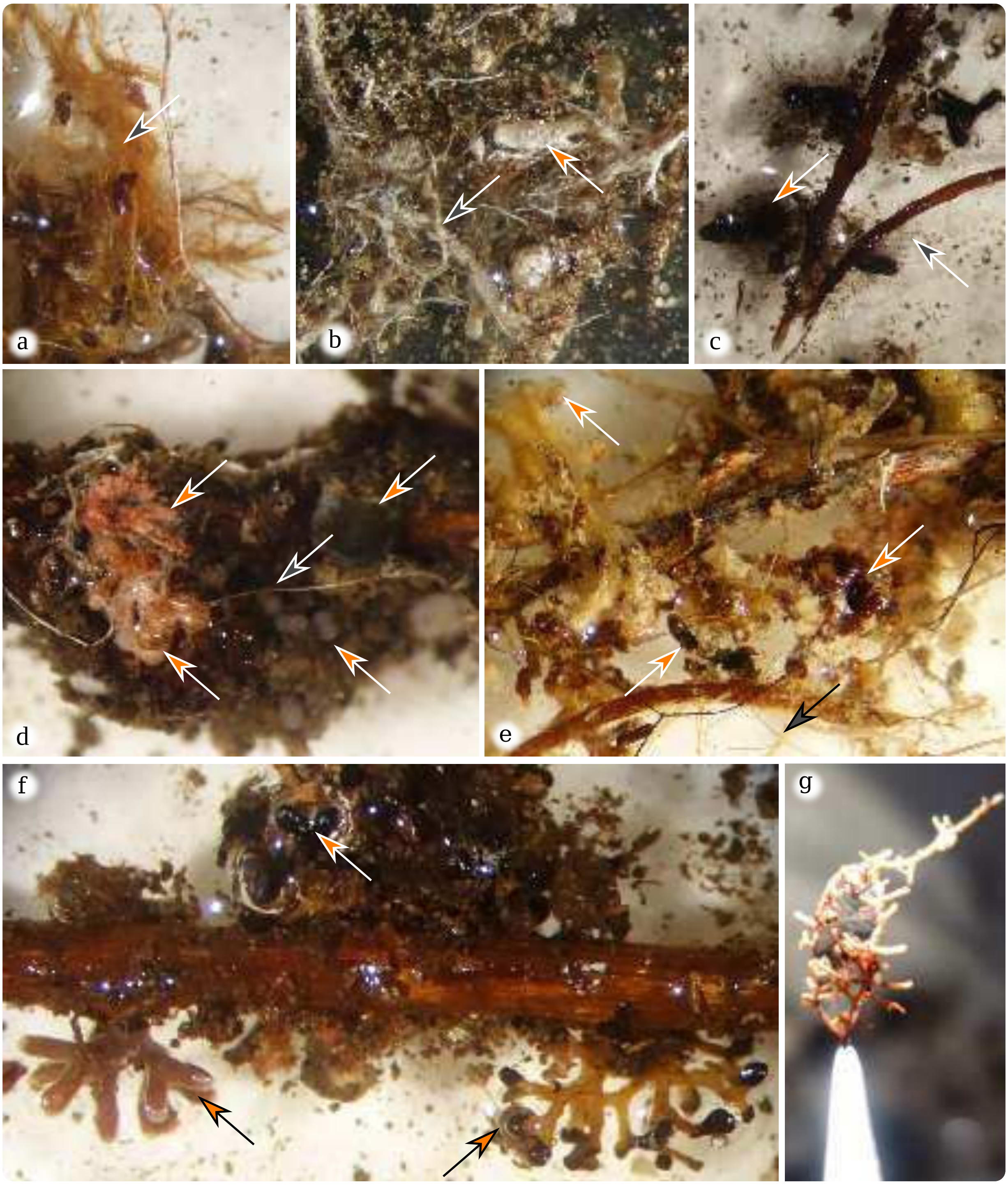


Figure II.7 – Illustration du prélèvement (piquage) des mycorhizes : Les photos (a-f ; prises à la loupe binoculaire en grossissement 5×) illustrent la diversité des mycorhizes (flèches oranges) échantillonnées. On peut observer des filaments mycéliens qui se rejoignent en panache (flèches grises) en particulier dans le cas des mycorhizes de cortinaires (**a**), d'amanites (**b**) et de *Cenococcum geophilum* (**c**). Une partie des nombreux filaments fongiques présents sur la photo **e** appartient sans doute à des champignons décomposeurs au vu du faciès de décomposition relativement avancé de la racine. La photo **f** illustre la grande diversité de formes qui se côtoient sur quelques centimètres de racines. Enfin, un amas de mycorhizes au bout d'une pince à dissection est illustré sur la photo **g**.

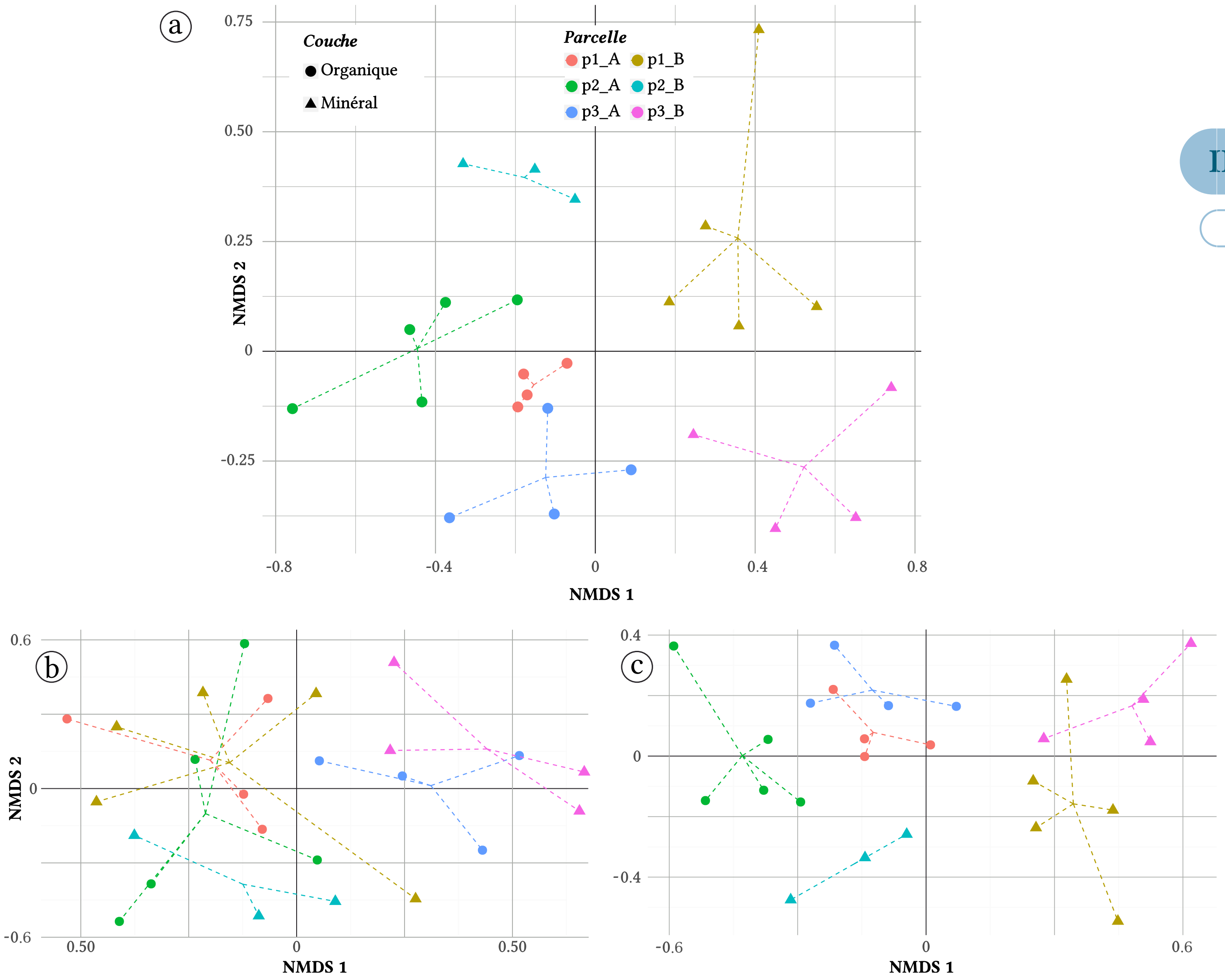
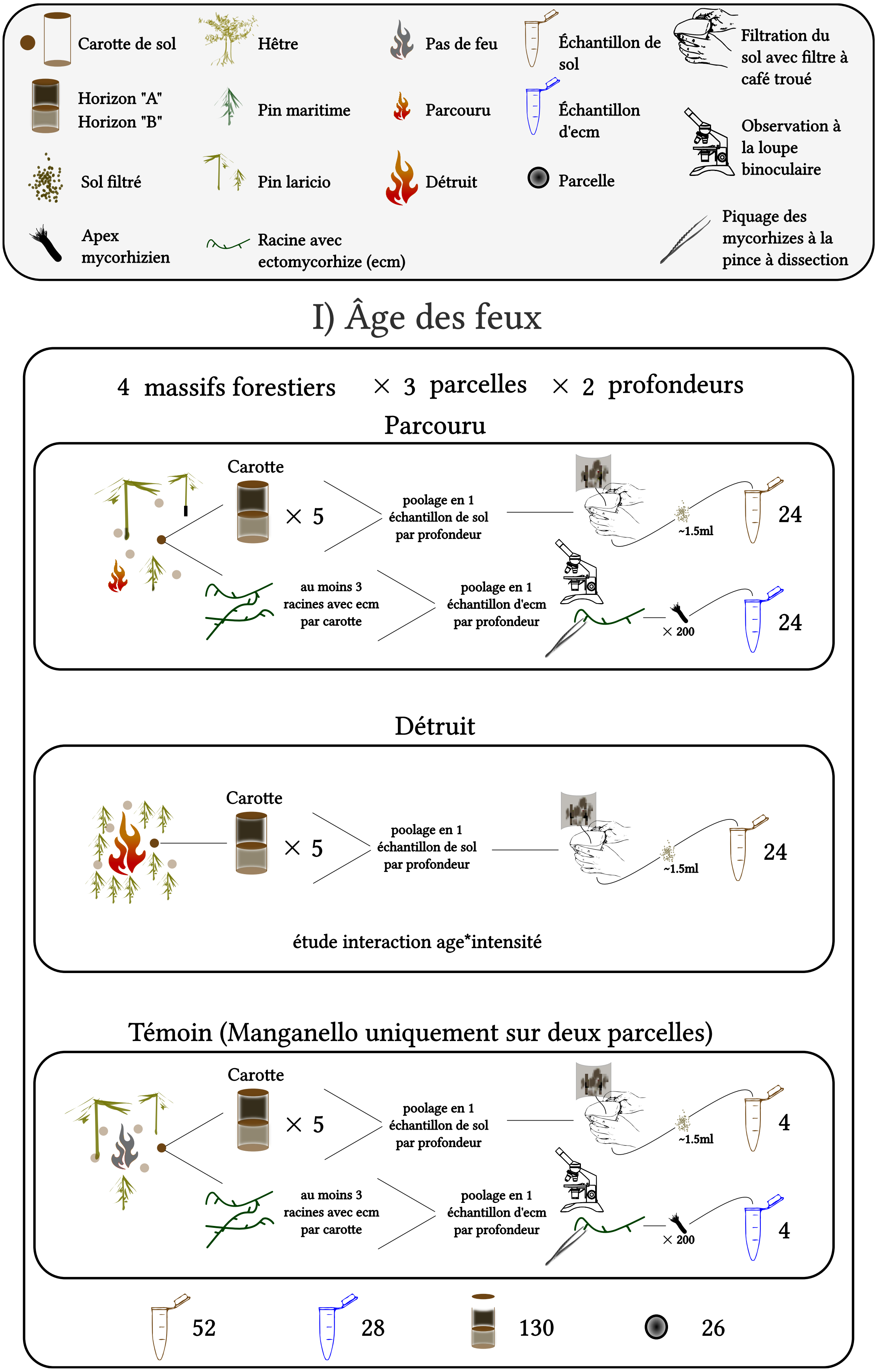
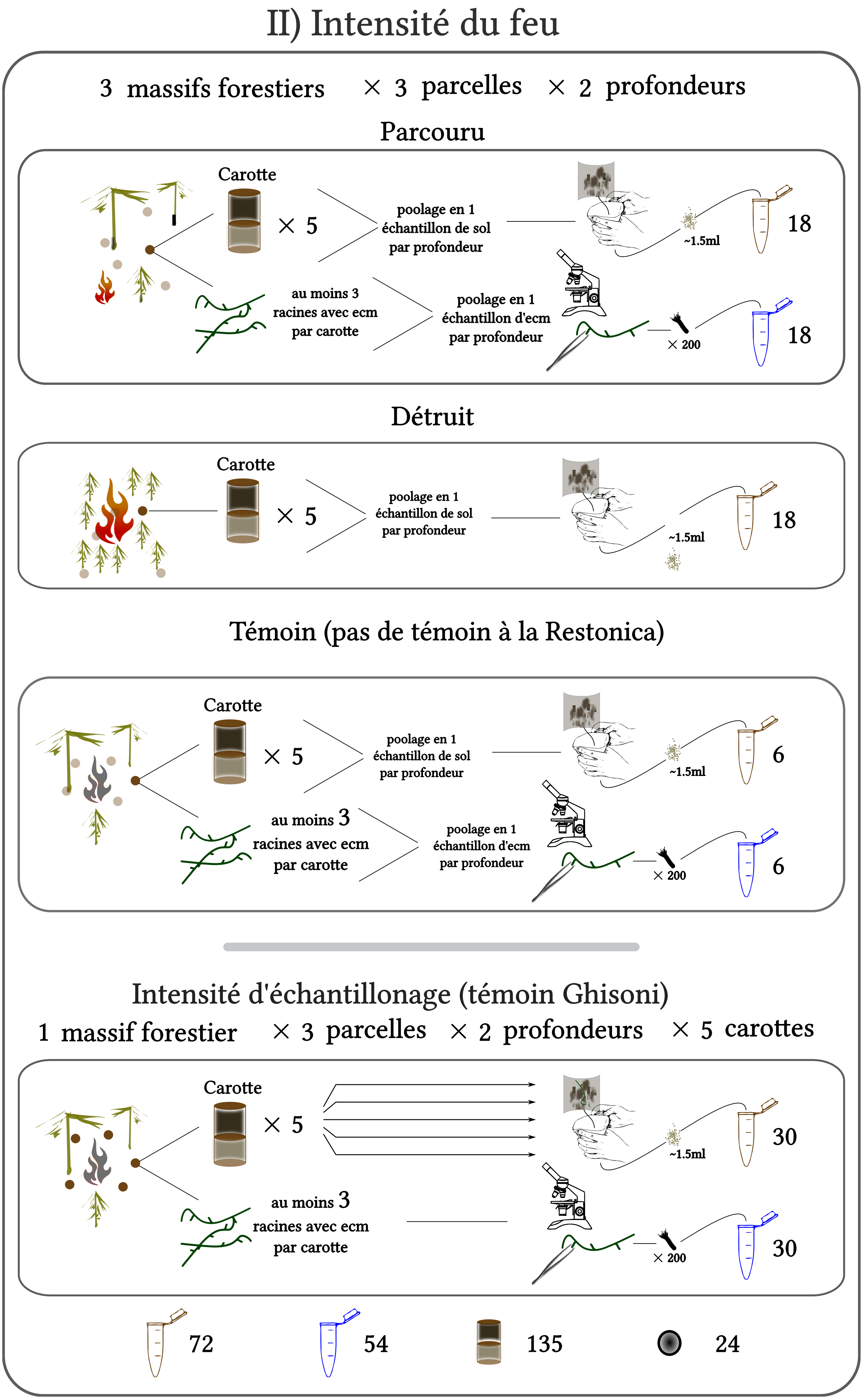


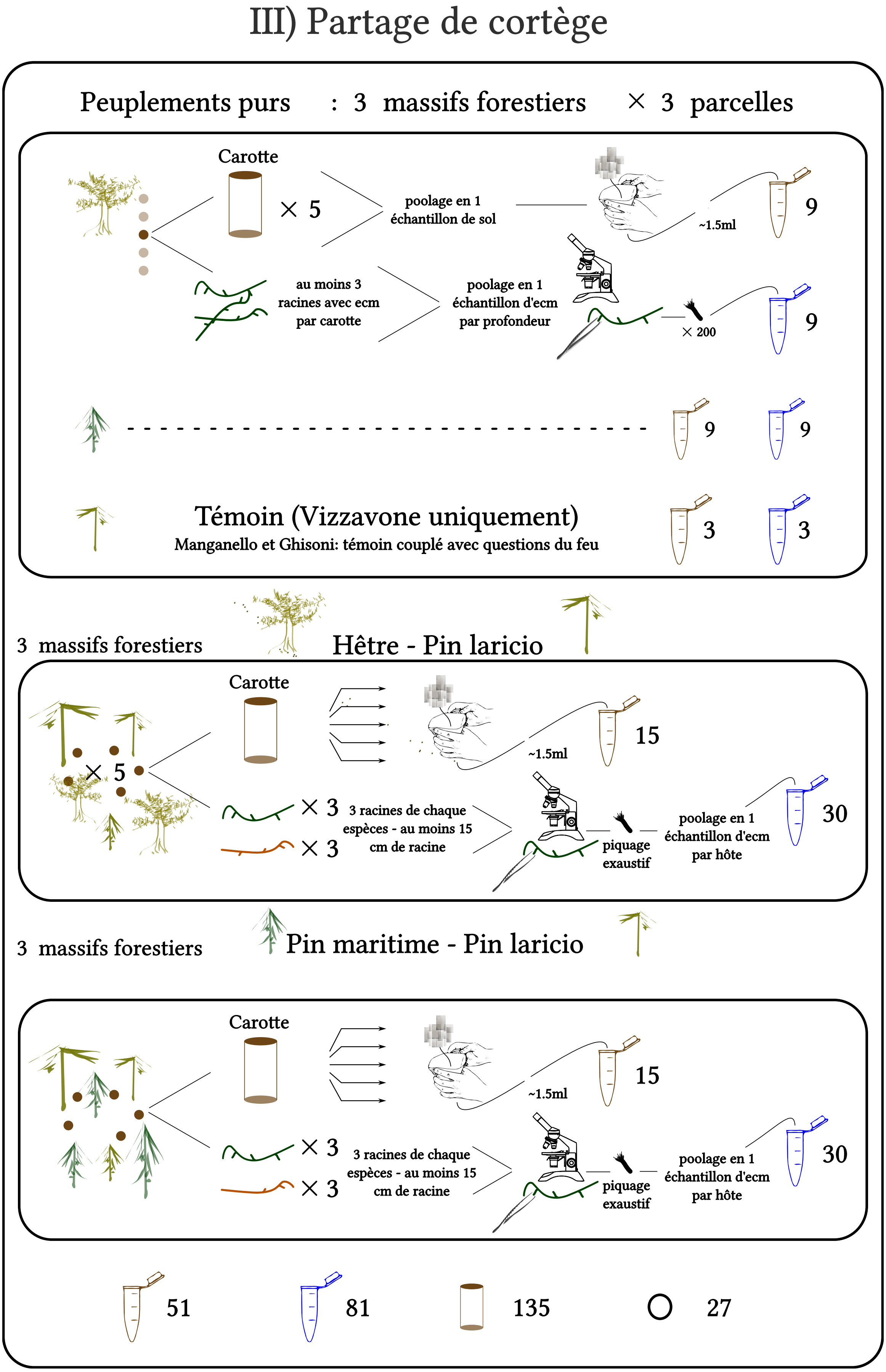
Figure II.8 – Variation intra-parcelle (inter-carottes) de la composition des communautés de champignons : Analyse de l'ensemble des champignons (**a**), des champignons ECM uniquement (**b**) et des champignons saprotrophes uniquement (**c**). Résultat de l'analyse multivariée des communautés des couches organiques (cercles) et minérales (triangle) des carottes des trois parcelles témoins (différentes couleurs) de pin laricio situées dans le massif de Ghisoni. L'analyse utilisée est un positionnement multidimensionnel non métrique (*Non-metric MultiDimenstional Scaling* [NMDS]) sur une distance de Bray-Curtis.



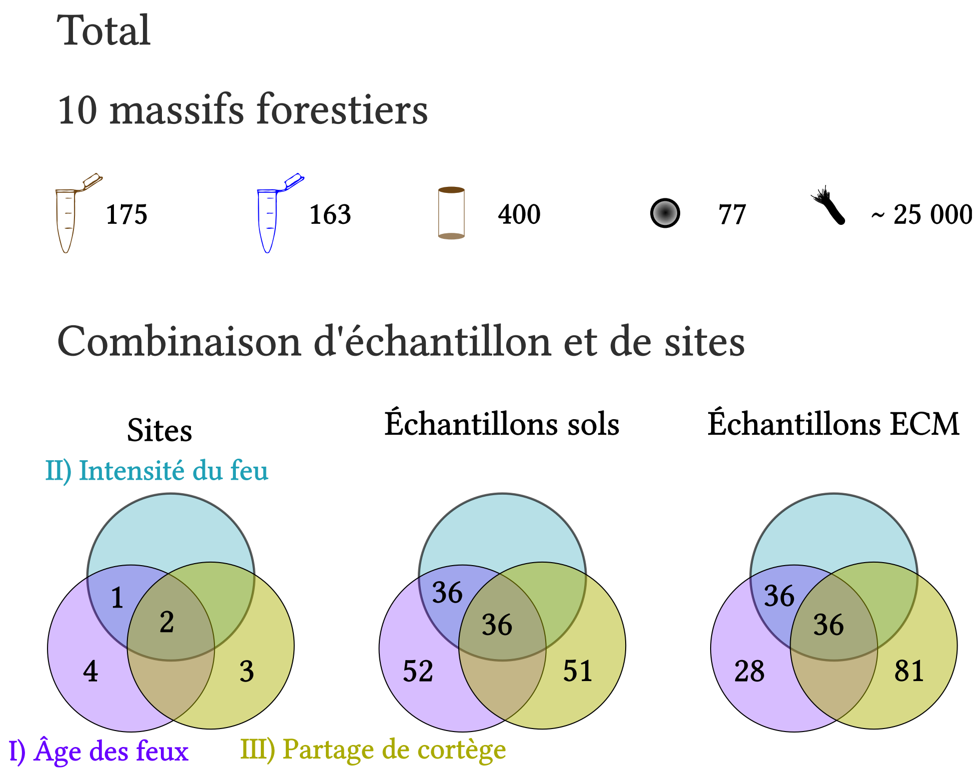
(I) Planche I : légende page 95



(II) Planche II : légende page 95



(III) Planche III : légende page 95



(IV) Planche IV : légende ci-dessous

Figure II.9 – Plan d'échantillonnage de terrain pour chacune des trois questions : effet de l'âge des feux (planche **I**), de l'intensité du feu (planche **II**) et de la taxinomie des arbres dominants (planche **III**) sur la composition fongique du sol et sur la composition des apex ECM. La planche **IV** décrit le nombre total de sites, parcelles, échantillons et mycorhizes échantillonnés. La légende des symboles est indiquée dans la zone grisée en haut de la planche **I**.

* * *

2 Biologie moléculaire et bioinformatique

2.1 Biologie moléculaire : stockage et extraction

Les sols filtrés et les tubes d'apex triés ont été conservés au réfrigérateur puis congelés dès le retour au laboratoire (entre 2 et 13 jours après le prélèvement). Les échantillons dont l'ADN devait être extrait (sol et apex) ont suivi ensuite le même traitement avec le kit FastDNA© SPIN Kit for Soil. La procédure est résumée dans la figure II.10. Brièvement, les échantillons (sol ou apex) étaient broyés, puis nettoyés des protéines grâce à des tampons et des centrifugations. La matrice de fixation permet ensuite de récupérer l'extrait d'ADN. Les concentrations en ADN des échantillons ont ensuite été mesurées sur un spectrophotomètre Nanodrop 8000 du labex CEMEB. Les résultats du spectrophotomètre indiquant la présence de contaminant protéique, nous avons lavé les échantillons à l'éthanol en suivant le protocole conseillé par l'entreprise MPBIO et disponible en ligne. Les concentrations mesurées par le spectrophotomètre après le protocole de précipitation à l'éthanol étaient beaucoup plus satisfaisantes (tableau II.1).

Les extractions ont été menées par groupe de douze échantillons afin de laisser un espace libre dans la centrifugeuse entre chaque échantillon pendant les centrifugations. Pour chacune des séries de douze, onze échantillons étaient extraits et un témoin négatif d'extraction était préparé. Les concentrations d'ADN dans les témoins négatifs étaient très inférieures à celles des autres extraits (tableau II.1). Après extraction et précipitation à l'éthanol, les échantillons ont été envoyés à l'équipe de Sebastian Leidel[5] pour séquençage sur plateforme Illumina *Miseq*.

Tableau II.1 – Concentrations d'ADN (en ng/ul) des échantillons et des témoins négatifs d'extraction.

	N	Minimum	1ier quartile	Médiane	Moyenne	3ème quartile	Maximum
Extrait de sol	193	0,993	14,070	20,650	32,62	31,38	320,50
Témoins d'extraction	25	-0,176	0,701	1,046	1,197	1,539	3,194

2.2 Bioinformatique

La procédure de bioinformatique présentée dans cette section reçoit en entrée les séquences brutes issues du séquençage *Miseq*. L'objectif est de rendre intelligible ces millions de séquences via leur regroupement en complexe de séquences proches. On cherchera ensuite dans des bases de données des informations concernant ces unités taxinomiques opérationnelles (OTUs pour *Operational Taxonomic Unit* ; concept abordé dans la section 6 de l'introduction). Bien entendu, il est nécessaire de trier les séquences chimériques et les séquences qui ne correspondent pas à de séquences du marqueur choisi (ici l'ITS, voir encadré 5).

---

[5] Max Planck Research Group for RNA Biology, Max Planck Institute for Molecular Biomedicine, Von-Esmarch-Strasse 54, 48149 Muenster, Germany

J'ai réalisé deux *pipelines*[6] bioinformatiques pour répondre aux spécificités des deux jeux de données issus de séquençage Illumina : l'analyse du sol et des apex des forêts d'altitudes corses (Fig. II.11) d'une part et l'analyse des endophytes foliaires du pin laricio d'autre part (Fig. II.12 ; manuscritH). La plus grande différence se situe dans les amorces utilisées (Fig. I.16) et donc la longueur des séquences. Pour les endophytes, seule la zone de l'ITS-2 a été séquencée soit des séquences d'environ 200 paires de bases. Cela implique que les séquences *forward* et *reverse* sont entièrement joignables. Au contraire, les séquences du sol et des apex incluent à la fois l'ITS-1 et l'ITS-2 ce qui a nécessité quelques adaptations. Je détaillerai ici la procédure pour les échantillons de sol et d'apex en utilisant les pieds de pages pour présenter les particularités du jeu de données sur les endophytes. Tout ce travail méthodologique trouve place dans les annexes des articles.

Nettoyage et formatage des données NGS

Après un filtre des séquences de mauvaises qualités (avec le logiciel Sickle ; étape 1, Fig. II.11), nous avons fusionné[7] les deux jeux de données issues des lectures *forward* et *reverse*. Puis, nous avons orienté les séquences dans le sens 5'-3' et séparé des séquences en deux jeux de données, que nous avons par la suite étudiés en parallèle (ITS-1 et ITS-2 ; étape 3 et 4, Fig. II.11).

Assignation des séquences aux échantillons et sélection des séquences ITS non chimériques

Les séquences sont ensuite assignées à leur échantillon grâce à des tags (séquences courtes rajoutées avant séquençage) propres à chaque échantillon (étape 5 et 6, Fig. II.11). Pour limiter les mauvaises assignations, nous avons ensuite sélectionné uniquement les séquences pour lesquelles les tags des deux extrémités correspondaient exactement à la séquence référençant un même échantillon.

Après suppression des amorces et des tags les séquences sont dérépliquées c'est-à-dire que les séquences exactement identiques sont fusionnées (étape 8, Fig. II.11). Le décompte du nombre initial de séquences est conservé dans le nom des séquences dérépliquées. Par la suite, les séquences chimériques et contenant des artefacts d'amorces sont retirées (étape 9, Fig. II.11). Le logiciel ITSx (Bengtsson-Palme et coll. 2013) permet ensuite d'éliminer les séquences qui ne sont pas des ITS (étape 10, Fig. II.11).

Dans ITSx, l'utilisateur peut choisir à quelle base de données on souhaite comparer nos séquences (par ex. champignons, plants, haptophytes). Les résultats du logiciel ITSx étaient étranges lorsque nous comparions les séquences à l'ensemble des bases de données. En effet, la sortie du logiciel indiquait de nombreuses séquences végétales, qui pour la plupart sont indéniablement d'origines

---

[6] En langage informatique, un *pipeline* est une chaîne de processus, la sortie d'un processus alimentant directement l'entrée du suivant.

[7] Vu que la séquence amplifiée – l'amplicon – a une longueur d'environ 200-250 bases, la fusion complète par paire des séquences *reverse* et *forward* est possible pour le jeu de données endophytes (étape 1, Fig. II.12). Cela permet d'avoir un contrôle qualité supplémentaire pour chaque paire de bases. Le filtre de qualité a lieu juste après (étape 2, Fig. II.12).

fongiques (Blast à 100% sur le site NCBI). Nous avons pris le parti de sélectionner uniquement la base de donnée fongique pour la suite des analyses [8].

Regroupement des séquences en OTUs

L'étape de regroupement des séquences en OTUs est une étape cruciale dans le *pipeline* bioinformatique (étape 11, Fig. II.11). Les différents algorithmes de regroupement se singularisent par leur puissance, leur sensibilité mais aussi par leur type d'approche. Les méthodes traditionnelles cherchent à faire des groupes de séquences plus proches entre elles qu'une valeur seuil (souvent 97%, plus rarement 99% d'identité). C'est le cas des logiciels Mothur (Schloss et coll. 2009), Uparse (Edgar 2013) et Uclust (Edgar 2010). Ce dernier est le logiciel de regroupement par défaut de la plate-forme d'analyse de données de séquençage Qiime. Parmi ces méthodes de regroupement par seuil, on distingue celles qui utilisent des bases de données externes pour agréger les OTUs (par ex. fonction `pick_closed_reference_otus.py` dans Qiime) de celles qui n'utilisent que les données séquencées (on parle de regroupement *de novo*). Bien entendu ces deux types de regroupement peuvent coexister au sein d'une même méthode (par ex. fonction `pick_open_reference_otus.py` dans Qiime).

Le choix d'un seuil d'identité que l'on sait erroné pour de nombreux groupes taxinomiques, ajouté à la ressource importante requise par les algorithmes à seuil, a entraîné l'apparition de méthodes de regroupement alternatives comme Swarm (Mahé et coll. 2014, 2015) et DADA 2 (Callahan et coll. 2016). Swarm repose sur l'analyse de réseau entre séquences. Mahé et coll. (2014) proposent de regrouper dans un même groupe toutes les séquences qui appartiennent à un réseau dont les liens sont formés entre séquences identiques ou avec une différence de une paire de bases (voir illustration à l'étape 9a de la figure II.12). DADA 2 propose quant à lui de ne jamais regrouper les séquences des différents échantillons et de fusionner les étapes de filtre de qualité et de regroupement des séquences en OTUs. Nous appelons abondance moléculaire le nombre de séquences par OTUs.

Nous avons comparé les résultats écologiques pour plusieurs méthodes de regroupement en OTUs dans les annexes des manuscrits qui utilisent la notion d'OTU. Dans la grande majorité des cas, les conclusions taxinomiques et écologiques (par ex. effet des facteurs d'intérêt sur la diversité, α et β) restent très proches. Nous avons également comparé les résultats issus de l'ITS-2 à ceux de l'ITS-1.

Utilisation de bases de données pour informer la taxinomie et l'écologie des OTUs

Une fois les OTUs formées, la séquence de référence de chaque OTUs [9] permet d'assigner des informations taxinomiques et écologiques (étape 12, Fig. II.11). Nous avons utilisé la base de donnée Unite (Kõljalg et coll. 2013) associée à plusieurs méthodes mises à disposition par le logiciel Qiime (Caporaso et coll. 2010 ; RDP classifier, uclust, mothur et blast). Les différentes méthodes donnant des résultats très proches, nous avons opté pour RPD classifier (Wang et coll. 2007). Le logiciel

---

[8] Pour l'analyse des endophytes, nous avons fait l'analyse avec et sans ces séquences « végétales » sans que cela ne change les résultats (matériels supplémentaires du manuscrit H).

[9] Selon les algorithmes utilisés, la séquence référence est soit une séquence consensus, soit – le plus souvent – la séquence qui est représentée par le plus grand nombre de copies à l'intérieur de l'OTU.

FUNGuild (Nguyen et coll. 2015b) et sa base de données permettent à partir de cette taxinomie (principalement via les noms de genre) de renseigner des informations écologiques, en particulier la guilde (ectomycorhizien, décomposeur, mycoparasite, …) à laquelle appartiennent les OTUs. Enfin, la création de la liste d'OTUs (matrice d'abondance échantillons × OTUs) fait appel au logiciel Usearch (étape 13, Fig. II.11).

* * *

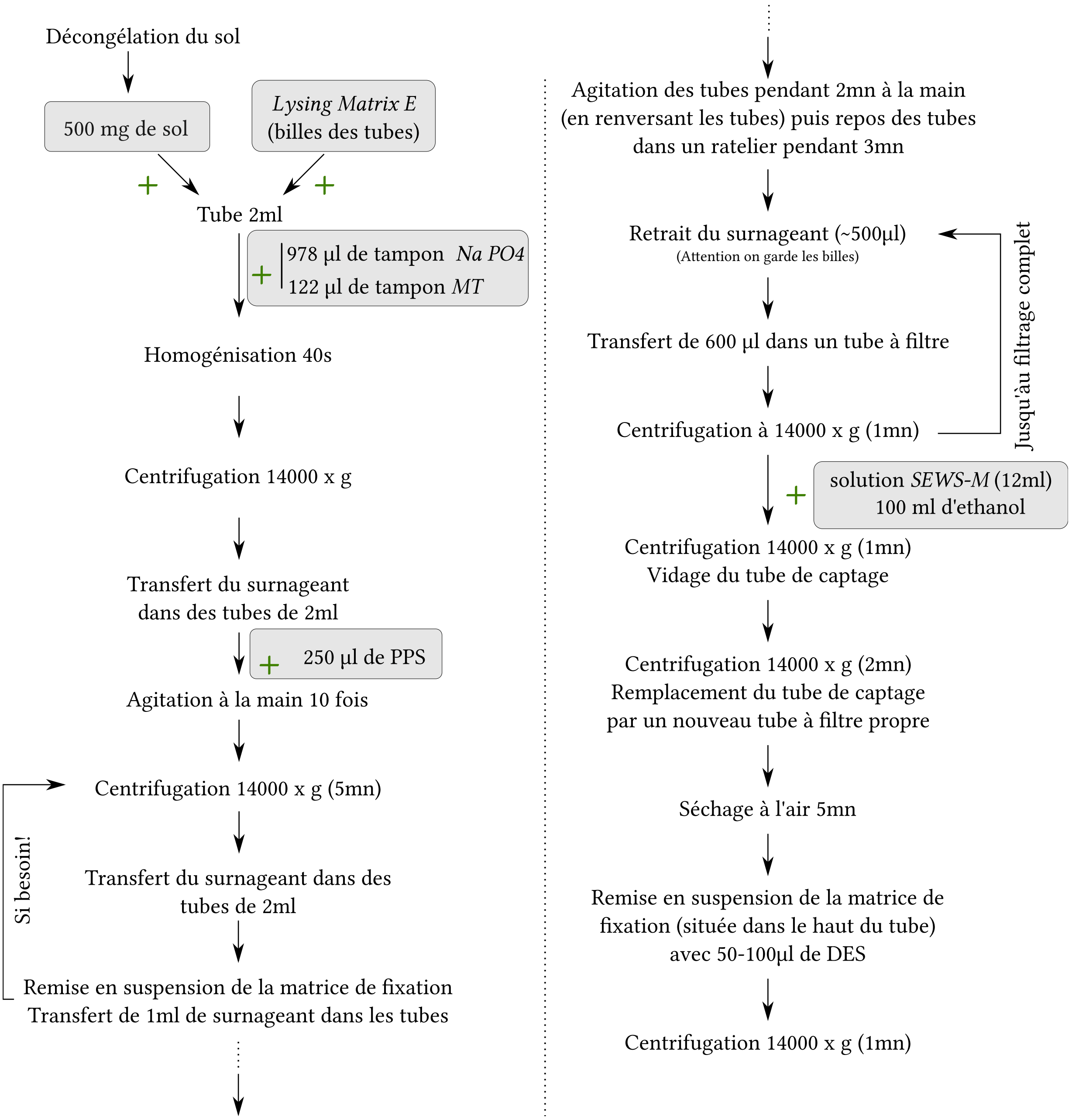
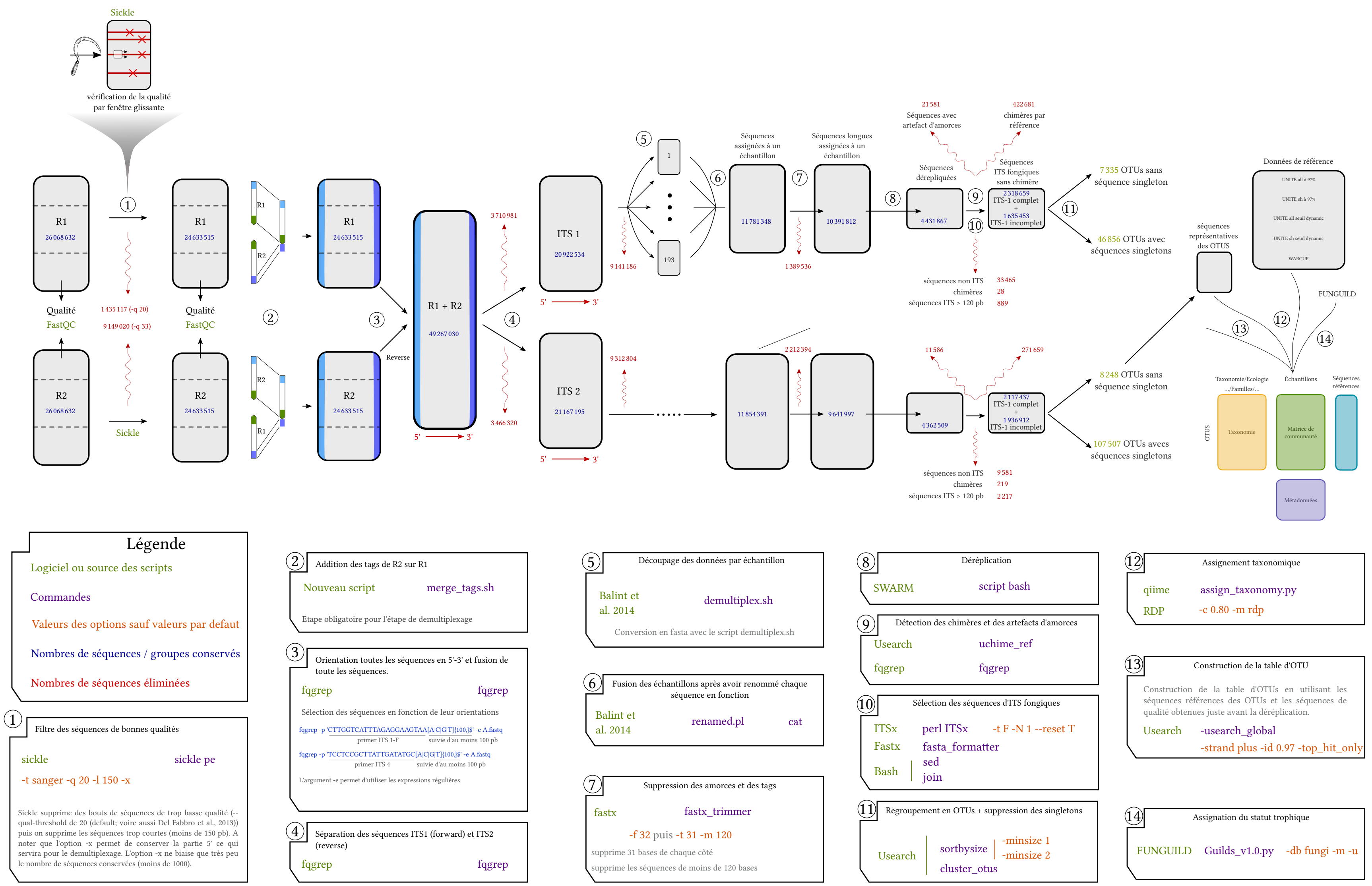


FIGURE II.10 – Méthodologie d'extraction d'ADN : Se reporter au manuel du kit FASTDNA© SPIN KIT FOR SOIL pour plus de détails.



Légende

Logiciel ou source des scripts

Commandes

Valeurs des options sauf valeurs par defaut

Nombres de séquences / groupes conservés

Nombres de séquences éliminées

① Filtre des séquences de bonnes qualités

sickle — sickle pe

-t sanger -q 20 -l 150 -x

Sickle supprime des bouts de séquences de trop basse qualité (--qual-threshold de 20 (default; voire aussi Del Fabbro et al., 2013)) puis on supprime les séquences trop courtes (moins de 150 pb). A noter que l'option -x permet de conserver la partie 5' ce qui servira pour le demultiplexage. L'option -x ne biaise que très peu le nombre de séquences conservées (moins de 1000).

② Addition des tags de R2 sur R1

Nouveau script — merge_tags.sh

Etape obligatoire pour l'étape de demultiplexage

③ Orientation toutes les séquences en 5'-3' et fusion de toute les séquences.

fqgrep — fqgrep

Sélection des séquences en fonction de leur orientations

```
fqgrep -p 'CTTGGTCATTTAGAGGAAGTAA[A|C|G|T]{100,}$' -e A.fastq
           primer ITS 1-F           suivie d'au moins 100 pb
fqgrep -p 'TCCTCCGCTTATTGATATGC[A|C|G|T]{100,}$' -e A.fastq
           primer ITS 4          suivie d'au moins 100 pb
```

L'argument -e permet d'utiliser les expressions régulières

④ Séparation des séquences ITS1 (forward) et ITS2 (reverse)

fqgrep — fqgrep

⑤ Découpage des données par échantillon

Balint et al. 2014 — demultiplex.sh

Conversion en fasta avec le script demultiplex.sh

⑥ Fusion des échantillons après avoir renommé chaque séquence en fonction

Balint et al. 2014 — renamed.pl — cat

⑦ Suppression des amorces et des tags

fastx — fastx_trimmer

-f 32 puis -t 31 -m 120

supprime 31 bases de chaque côté

supprime les séquences de moins de 120 bases

⑧ Déréplication

SWARM — script bash

⑨ Détection des chimères et des artefacts d'amorces

Usearch — uchime_ref

fqgrep — fqgrep

⑩ Sélection des séquences d'ITS fongiques

ITSx — perl ITSx — -t F -N 1 --reset T

Fastx — fasta_formatter

Bash — sed
join

⑪ Regroupement en OTUs + suppression des singletons

Usearch — sortbysize — -minsize 1
-minsize 2
cluster_otus

⑫ Assignement taxonomique

qiime — assign_taxonomy.py

RDP — -c 0.80 -m rdp

⑬ Construction de la table d'OTU

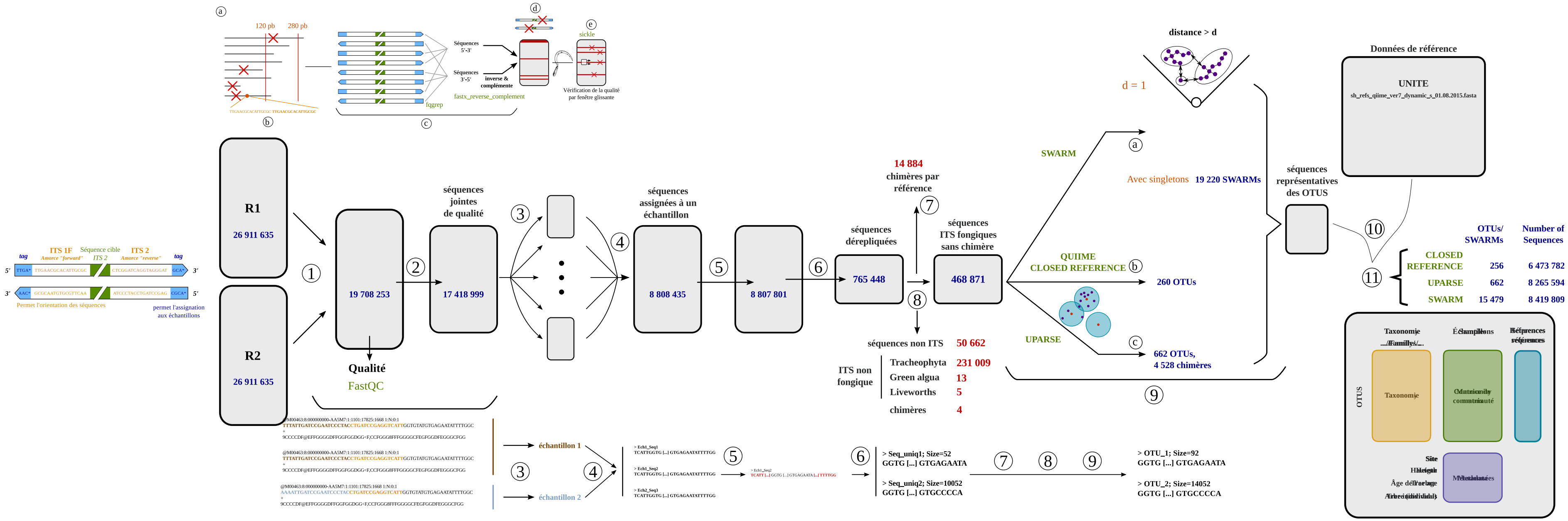
Construction de la table d'OTUs en utilisant les séquences références des OTUs et les séquences de qualité obtenues juste avant la déréplication.

Usearch — -usearch_global
-strand plus -id 0.97 -top_hit_only

⑭ Assignation du statut trophique

FUNGUILD — Guilds_v1.0.py — -db fungi -m -u

FIGURE II.11 – Procédure bioinformatique concernant les séquences d'extraits de sol



Légende

Logiciel ou source du script

Commande

Valeurs des options quand différentes des valeurs par défaut.

Nombre de séquences restantes

① **Fusion des séquences forward et reverse**

qiime join_paired_ends.py **19 708 253**

② **Filtre de qualité et orientation**

ⓐ **Suppression des séquences trop courtes (180 pb) et raccourcissement des séquences trop longues (280pb)**

fastx fastx_trimmer
-f 1 -l 281 **19 708 253**
-t 1 -m 180 **19 527 566**

ⓑ **Suppression des artefacts d'amorces**

Suppression des séquences avec amorces multiples

Balint et al. 2014 remove_multiprimer.py **19 519 016**

Suppression des séquences contenant une amorce fw et une rev

Linux grep **19 519 007**

ⓒ **Réorientation en 5'-3' de toutes les séquences**

fqgrep fqgrep **17 643 226**

fastx fastx_reverse_complement

ⓓ **Suppression des chimères "doubles sens"**

fqgrep fqgrep **17 627 890**

ⓔ **Suppression des séquences ce mauvaises qualitées**

sickle sickle
-q 33 **17 418 999**

③ **Découpage des données par échantillons (81 échantillon)**

Balint et al. 2014 demultiplex.sh

Tag forward **13 206 612**

Tag forward + reverse **8 808 435**

Conversion en fasta avec le script demultiplex.sh

④ **Fusion des échantillons après avoir renommer chaque séquence en fonction**

Balint et al. 2014 renamed.pl **8 808 435**

⑤ **Suppression des amorces et des tags**

fastx fastx_trimmer
-f 32 **8 808 435**
-t 31 -m 120 **8 807 801**

Suppression de 31 bases de chaque côté et des séquences de moins de 120 bases

⑥ **Déréplication des séquences**

SWARM script bash **765 448**

⑦ **Détection des chimères par référence**

Usearch uchime_ref **750 564**

⑧ **Sélection des séquences d'ITS fongiques**

ITSx perl ITSx **468 871**

⑨ **Regroupement en OTUs**

ⓐ **Regroupement en favorisant les distances entre OTUs**

SWARM ./swarm
-f -t 4 -d 1 **19 220**

ⓑ **Regroupement en groupe à 97% via Qiime**

qiime pick_closed_otus **260**
-s 0.97 -m uclust

ⓒ **Regroupement en groupe à 97% via Uparse**

Usearch sortbysize
-minsize 1

Usearch cluster_otus **662**
--maxaccepts 0
--maxrejects 0

⑩ **Assignement taxonomique**

ⓐ qiime assign_taxonomy.py
RDP -c 0.80 -m rdp

ⓑ qiime assign_taxonomy.py
uclust -similarity 0.90 -m uclust

ⓒ qiime assign_taxonomy.py
mothur -c 0.80 -m mothur

ⓓ qiime assign_taxonomy.py
blast -e 0.001 -m blast

⑪ **Formatage des résultats pour phyloseq**

Création de la table d'OTUs à partir des séquences de références des OTUs et des séquences propres obtenue juste avant la déréplication

Usearch -usearch_global
-strand plus -id 0.97 -top_hit_only

Conversion en format biom

Usearch uc2otutab.py
biom convert

Figure II.12 – **Procédure bioinformatique concernant les séquences d'extraits d'aiguilles de pin**

Résumé

(i) L'ADN des échantillons de sol et d'apex a été extrait puis envoyé pour séquençage Illumina des régions ITS-1 et ITS-2.

(ii) Les séquences obtenues ont été filtrées puis les séquences d'ITS propres ont été regroupées en groupes de séquences : les OTUs (*Operational Taxonomic Unit*). Ce travail de bioinformatique a été effectué en utilisant plusieurs *pipelines*.

(iii) À l'aide de bases de données, les OTUs ont été enrichies d'informations taxinomiques (UNITE) et écologiques (FUNGuild).

Summary

(i) DNA of soil and root tips samples were extracted and sent for Illumina sequencing of ITS1 and ITS2.

(ii) Sequences were quality filtered and clustered into operational taxonomic units (OTUs) using several pipelines.

(iii) We add taxonomic and ecological information to OTUs using UNITE and FUNGuild databases.

Chapitre III

Les réseaux d'interactions ectomycorhiziens en Corse

> « *Mycorrhizas, not roots, are the chief organs of nutrient uptake by land plants [...]* »
>
> Sally E. Smith & David Read, *Mycorrhizal Symbiosis*, 2008

Kapitu kapitu par Ngipi Ward © *Musée du quai Branly*

Ce tableau aborigène représente des points d'eau dans le désert qui sont reliés par les traces des animaux.

1 Synopsis

Ce chapitre comporte cinq manuscrits qui traitent des réseaux d'interactions ectomycorhiziennes à différentes échelles.

Le manuscrit A s'intéresse au caractère mutualiste de l'interaction mycorhizienne à travers un rapport de congrès organisé par le *New Phytologist*. Ce rapport de congrès souligne la forte variabilité spatio-temporelle et contextuellede l'interaction ECM et de son influence sur la valeur sélective des deux partenaires. Cette publication n'apporte pas de nouveaux résultats sur le sujet mais se fait l'écho des nombreux travaux sur le sujet présentés lors du symposium. Une fois accepté que les associations mycorhiziennes sont mutualistes à l'échelle des espèces mais pas toujours à l'échelle des individus « ici et maintenant », on peut étudier les liens entre individus d'espèces différentes.

La question des réseaux entre individus végétaux via les champignons trouve son fondement dans la littérature récente qui a mis en exergue le rôle des réseaux mycéliens communs (CMN ; encadré 4) dans le partage de carbone (Simard et coll. 2012) et dans l'échange de signaux (Babikova et coll. 2013) entre plantes, y compris entre individus d'espèces différentes. De plus, plusieurs publications postulent que l'interaction ECM est cruciale dans les processus de successions primaires et secondaires (Nara 2006 ; Richard et coll. 2009). Par ailleurs, les espèces de champignons ECM diffèrent en termes de spectres d'hôtes compatibles (Molina et Horton 2015) et de traits fonctionnels (par ex. les capacités enzymatiques [Courty et coll. 2016] et le type d'exploration du sol [Agerer 2001]). Ces différences entraînent des différences de phénotype étendus des plantes via leur champignons mycorhiziens. Par conséquent, la symbiose ECM pourrait être un facteur important de la coexistence des plantes en médiant des mécanismes de facilitation et de compétition directement [1] et indirectement [2].

Il est donc fondamental de comprendre quels sont les mécanismes à l'origine du partage de cortège ECM chez les plantes. Nous avons formulé et testé plusieurs hypothèses dans les manuscrits présentés B, C et D (incomplet). Les cortèges de champignons ECM varient entre les espèces. Mais, le niveau de partage de cortège fongique entre espèces de plantes varie également en fonction des distances phylogénétiques (degré de parenté, manuscrits B et C), des stratégies écologiques (espèces pionnières *vs* non pionnières ; manuscrit B) et des niveaux de coexistence (manuscrit C).

Le manuscrit D a pour objectif d'explorer le partage de cortège ECM entre plantes, non pas entre espèces à l'échelle régionale, mais entre individus d'espèces différentes dont les racines sont entremélées. Les résultats de ce manuscrit sont encore incomplets (voir section Avancement des travaux - *Works progress*). Pour finir, dans le manuscrit E nous developpons une approche et des outils à portée opérationnelle, dérivés de l'analyse des les réseaux ECM décrits en Corse, pour appréhender conjointement les champignons ECM, leurs hôtes et les processus écologiques qui s'appuient – au moins partiellement – sur la symbiose ECM (par ex. les successions végétales).

---

[1] Par exemple, facilitation grâce à des transferts de carbones et compétition pour les partenaires les plus performants.

[2] Par exemple, facilitation des plantules par les plantes présentes qui supportent un réseau mycélien déjà bien développé (voir encadré 4), relâchement de la compétition par partage des ressources via des partenaires fongiques qui diffèrent en termes de stratégie d'acquisition des minéraux.

2 Manuscrit A : "Mycorrhizas: Dynamic and complex networks of power and influence"

IN A NUTSHELL

Rationale: This paper is the meeting report of the 33rd New Phytologist Symposium entitled "*Networks of Power and Influence: ecology and evolution of symbioses between plants and mycorrhizal fungi*". This meeting report underlines the dynamic nature of mycorrhizal interactions and the inherent complexity of this symbiosis that arise from recent research presented during the symposium.

Type of interaction: Mycorrhizal symbiosis, mostly mutualistic but outcome for both partners seems more complex than previously thought.

Ecological filters of fungal communities: Dialogue between fungal species and plant host

Main conclusions: Mycorrhizal symbiosis are complex and dynamic.

New Phytologist

Forum

Meetings

Mycorrhizas: dynamic and complex networks of power and influence

33rd New Phytologist Symposium – Networks of Power and Influence: ecology and evolution of symbioses between plants and mycorrhizal fungi, Zürich, Switzerland, May 2014

Plants and mycorrhizal fungi form complex symbiotic relationships belowground that can result in beneficial or disadvantageous outcomes for one or both of the symbiotic partners. It is one of the most ancient and widespread symbioses on earth (Smith & Read, 2008). Since the research field on mycorrhizas developed in the middle of the last century, the scientific community has generated an enormous amount of knowledge on the ecology, evolution and striking importance of mycorrhizas for ecosystems worldwide. However, many key questions still remain unresolved, that new tools in genomics, isotopic analyses, and statistical approaches are only now making tractable.

The 33rd New Phytologist Symposium on 'Networks of Power and Influence: ecology and evolution of symbioses between plants and mycorrhizal fungi', a title inspired by a paper of Leake *et al.* (2004), was held in Zürich, Switzerland. The meeting provided one of the rare opportunities where the global community of mycorrhizal researchers could get together to present the latest advances in the field and discuss striking questions like: How did the mycorrhizal symbiosis evolve? Which conditions determine whether the symbiosis turns out to be mutualistic or parasitic? What is the mycorrhizal contribution to the sustainability of ecosystems and can mycorrhizal fungi be used for sustainable plant production? How can we increase the awareness of the mycorrhizal importance for ecosystem functioning worldwide?

> *'. . . To fully understand the nature of the symbiosis, it is essential to consider all of the temporal, spatial, and environmental conditions symbiotic partners may experience . . .'*

There was huge interest in the meeting with over 100 people on the waiting list, extensive and high level discussions, and high quality presentations including > 100 posters. Here we highlight four of the most exciting topics that were discussed during the meeting.

Providers, cheaters or insurers? – mycorrhizal phenotypes

While the mycorrhizal symbiosis is usually considered a mutualistic relationship, many examples exist where plants are apparently not benefitting from the mycorrhizal association. The same may hold true for the fungal partners, although this is far less well studied, in particular due to the obligate nature of the association for the majority of mycorrhizal fungi. This variation in responses of the symbiotic partners was described by the term 'mutualism–parasitism continuum' (Johnson *et al.*, 1997). During the meeting, several examples were shown of how the mycorrhizal symbiosis, not only directly but also indirectly, affects plant performance by modifying the wider biotic and abiotic environment. Mycorrhizal effects on herbivore–plant interactions (Babikova *et al.*, 2013), soil structure (Rillig & Mummey, 2006), other plant symbionts (Larimer *et al.*, 2014), or multiple ecosystem functions (Wagg *et al.*, 2014) can indirectly affect plant performance and provide benefits for the plants that are not easy to identify. Also plant fitness, that is, the effects of the symbiosis on the three components of individual plant performance (growth, reproduction and survival) is rarely addressed.

Beyond the multi-dimensionality of potential benefits, the spatio-temporal variation along the continuum is also important. Symbioses can be beneficial 'here and now' but can reduce the symbionts' fitness in another environmental context (e.g. stress conditions) or ontogenic stage (e.g. immature seedlings vs reproductive individuals). For example, John Klironomos (University of British Columbia, Canada) presented data which suggested that mycorrhizal fungi may provide benefits to the plants under extreme environmental conditions, while under optimal conditions the symbiosis may represent a net cost for the plant. Therefore, the mycorrhizal symbiosis could be regarded as an insurance policy for the plant.

It becomes increasingly clear that the mycorrhizal symbiosis is much more complex than a simple nutrient-for-carbon trading business. The use of the term parasitism might give an unbalanced view of the far-reaching complexity of plant–fungal relationships. It was therefore proposed by Sally Smith (University of Adelaide, Australia) to move away from the mutualism–parasitism continuum, and instead speak of a 'responsiveness continuum'. To fully understand the nature of the symbiosis, it is essential to consider all of the temporal, spatial, and environmental conditions symbiotic partners may experience during one, or even more lifespans.

Facing complexity with curiosity: importing concepts and methods from other disciplines

Another key aspect arising from the Symposium was the complexity of mycorrhizal associations from a physiological, ecological, or evolutionary point of view. To face this complexity, researchers are importing concepts and methods from other areas of research. Toby Kiers (Vrije Universiteit Amsterdam, the Netherlands) presented how economic market theory can be adapted to explain the evolution and stability of cooperation in the mycorrhizal system. The paradox of cooperation between both partners of the mycorrhizal symbiosis can be partially explained by the ability of the host to dedicate more carbon to their best partners (Kiers *et al.*, 2011) and of the symbiont to differentially allocate nutrients to individual plant hosts (depending on the amount of carbon they provide) in a common mycorrhizal network (Fellbaum *et al.*, 2014). This ability to impact the fitness of the partner, with regard to the benefits it provides in terms of nutrient exchange, seems to play a key role in the stabilization of mycorrhizal interactions. Another example on how the symbionts can control the fitness of their partners was presented by Francis Martin (INRA Nancy, France) showing how fungal effector proteins can control plant immunity (Plett *et al.*, 2014). These results stimulated the discussion whether the logo of the Symposium, showing fungi and plants shaking hands, should rather be modified to both wearing boxing gloves or even carrying knives (Fig. 1).

Mathematics and computer science can also provide a source of new ideas, as argued by Alicia Montesinos-Navarro (Universidad Nacional Autonóma de México, Mexico). She introduced ecological network theory to reveal the underlying processes of how multi-specific interactions between arbuscular mycorrhizal fungi and plants influence plant community structure. This network shows a high modularity, which is new evidence of selectivity in mycorrhizal interactions at the community level. Network theory was also mentioned by Ian Dickie (Lincoln University, New Zealand) and colleagues as a relevant tool to understand the driving forces behind mycorrhizal community structure through ecosystem development. Using a 120 000 yr chronosequence of temperate rainforest ecosystem development dominated by arbuscular mycorrhizal plants, they found no change in network nestedness along the succession. Interestingly, the network position (degree of centrality) of partners partially predicted new links during the ecosystem development.

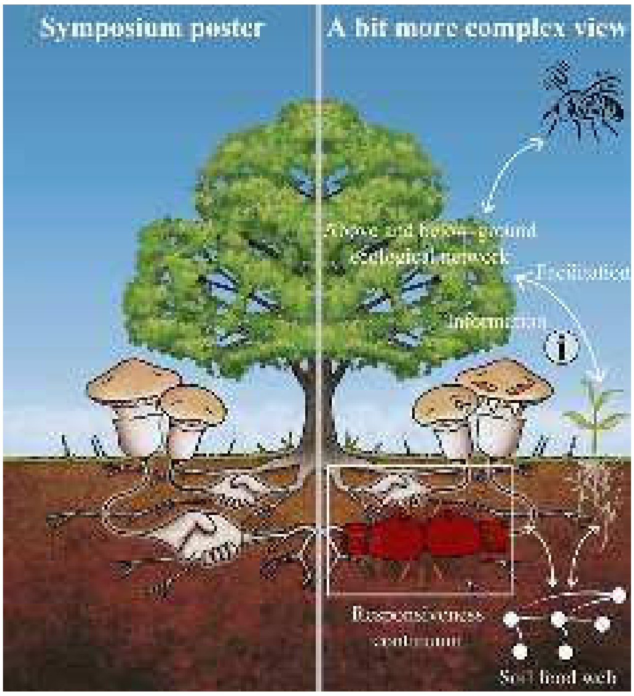


Fig. 1 The original conference poster (left) and a slight modification paying respect to the complexity of mycorrhizal associations and their environment (right).

Finally, there is a general call for the use of functional traits in mycorrhizal research, as the current categorical frameworks (e.g. saprotroph, pathogen, endophyte), are unsatisfactory to infer ecological strategies and to categorize fungi because several categories may apply to the same organism. Indeed, functional ecology allows for going beyond a taxonomic view of biodiversity and was proven to be very informative at the community and ecosystem scale. The call for using functional traits is not new (see van der Heijden & Scheublin, 2007; Parrent *et al.*, 2010), but recent technological advances (e.g. enzymatic activity profiling, isotopic tracing, etc.) may pave the way for incorporating functional ecology in modern mycorrhizal science, as argued by Carlos Aguilar-Trigueros (Freie Universität Berlin, Germany).

Mycorrhizal genomics, evolution and food security

The repeated emergence of mycoheterotrophy in plant evolution potentially challenges the sustainability of mycorrhizal networks, as discussed by Marc-André Selosse (Muséum national d'Histoire naturelle, Paris, France). By taking advantage of these networks, these plants without any photosynthetic capability are able to receive carbon from neighboring plants and survive in a low-light environment. Examples of fully mycoheterotrophic plants from Ericaceae and Orchidaceae were shown together with examples of plants in intermediate evolutionary steps with a mixotrophic metabolism, but interestingly the dependency of mixotrophic species on photosynthesis for seed production may limit their evolution to full mycoheterotrophy (Gonneau *et al.*, 2014). The ability of orchids to acquire carbon from mycorrhizal fungi is indeed a key process in their metabolism and recent transcriptomic and proteomic data are finally shedding some light on the molecular mechanisms underlying orchid mycorrhizal symbiosis (Perotto *et al.*, 2014; Valadares *et al.*, 2014).

The results emerging from the '-omic' sciences were the basis for discussion in the last session of the meeting. Francis Martin and David Hibbett (Clark University, Worcester, MA, USA) showed the progress of the Mycorrhizal Genomics Initiative, which has already publicly released the draft genome for 31 mycorrhizal fungi. Comparative analyses of ectomycorrhizal genomes suggested parallels between the evolution of ectomycorrhizas and 'brown rot' wood decay. However, ectomycorrhizal species are polyphyletic in origin, and while converging on a symbiotic lifestyle, each ectomycorrhizal clade has retained a unique array of decay-related enzymes, suggesting that they possess diverse decomposing capabilities. Also noteworthy was the genome analysis of ectomycorrhizal root tips (Plett & Martin, 2011). This analysis indicates that convergent ectomycorrhizal evolution is probably related to the deployment of a 'symbiosis genetic toolkit', which includes the loss of plant cell wall degradation enzymes and the gain of mycorrhiza-induced small secreted effector-like proteins such as MiSSP7 that control plant development and immunity (Plett *et al.*, 2014).

Ian Sanders (University of Lausanne, Switzerland), promoted exciting discussions by coupling evolutionary genomics of mycorrhizal fungi with field experiments. During his talk, he demonstrated how genetic variation in *Rhizophagus irregularis* (also addressed on the poster by Tania Wyss (University of Lausanne, Switzerland) who was awarded the Symposium poster prize) can be used to increase yields of cassava in real-world agricultural settings, demonstrating the importance of mycorrhizal fungi for food security in developing countries.

Respect mycorrhizas! Raising the awareness in other disciplines

By providing a platform for researchers working on soil biodiversity and plant–soil interactions in general, the Symposium paid respect to the fact that mycorrhizas should not be considered away from their wider biotic environment. Wim van der Putten (Netherlands Institute of Ecology, Wageningen, the Netherlands) presented the concept of 'plant–soil feedback' as a useful approach to explain the structure of ecosystems, and addressed the importance of disentangling the underlying network interactions among soil biota to enable the track down of causal effects. Bernhard Schmid (University of Zürich, Switzerland) presented new insights into how plant biodiversity effects can be influenced by soil biota, suggesting that the co-evolution of plant–soil communities is an intrinsic feature of ecosystems.

However, it also turned out that there is a discrepancy between the mycorrhizal research community and other disciplines in the recognition of the mycorrhizal symbiosis. While mycorrhizal researchers widely accept the fundamental role played by mycorrhizal fungi in terrestrial ecosystems, these organisms still attract little attention in current soil food-web theory (see e.g. Neutel *et al.*, 2007). It was discussed that this might be attributed to the fact that many of the data on mycorrhizal contributions to ecosystem performance have been generated from model systems under glasshouse conditions, often using sterilized soils and artificial plant–soil–fungus combinations. It was suggested by Wim van der Putten that we should plan experiments based on 'what you find in nature'. By conducting glasshouse experiments using combinations that can be found in real situations, ecological realism could be enhanced, and the undoubted important role mycorrhizal fungi play worldwide would receive further recognition.

Conclusions

The emergence of new approaches and technologies applied to the mycorrhizal symbiosis is constantly increasing our knowledge about plant–fungal interactions. However, as we learn more, new and amazing questions are raised. Overall, it is necessary to strengthen the dialogue among disciplines in order to capture the complexity of mycorrhizal interactions. Meetings like the 33rd New Phytologist Symposium are crucial to achieve this objective.

Acknowledgements

The authors would like to thank Michael Panagopulos, Marcel van der Heijden, Marc-André Selosse, Francis Martin and Ian Sanders for the organization of the meeting and Ian Dickie, Francis Martin and Marc-André Selosse for helpful comments on the manuscript. S. Franz Bender was funded by the Swiss National Science Foundation (project nos. 143097 and 125428); Rafael Valadares was funded by the New Phytologist Trust and the Brazilian 'National Counsel of Technological and Scientific Development' (CNPq); Adrien Taudiere was funded by the New Phytologist Trust and by the 'Conservatoire botanique national de Corse'.

S. Franz Bender[1]*, Rafael Borges da Silva Valadares[2] and Adrien Taudiere[3]

[1]Plant–Soil-Interactions, Institute for Sustainability Sciences ISS, Agroscope, Zürich, Switzerland;
[2]Cell and Molecular Biology Laboratory, Center for Nuclear Energy in Agriculture CENA, University of São Paulo USP, Piracicaba, SP, Brazil;
[3]Ecole Pratique des Hautes Etudes, Centre d'Ecologie Fonctionnelle et Evolutive, Montpellier, France
(*Author for correspondence: tel +41 58 468 7288; email franz.bender@agroscope.admin.ch)

References

Babikova Z, Gilbert L, Bruce TJA, Birkett M, Caulfield JC, Woodcock C, Pickett JA, Johnson D. 2013. Underground signals carried through common mycelial networks warn neighbouring plants of aphid attack. *Ecology Letters* **16**: 835–843.

Fellbaum CR, Mensah JA, Cloos AJ, Strahan GE, Pfeffer PE, Kiers ET, Bücking H. 2014. Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. *New Phytologist* **203**: 646–656.

Gonneau C, Jersáková J, Tredern E, Till-Bottraud I, Saarinen K, Sauve M, Roy M, Hájek T, Selosse MA. 2014. Photosynthesis in perennial mixotrophic *Epipactis* spp.(Orchidaceae) contributes more to shoot and fruit biomass than to hypogeous survival. *Journal of Ecology.* doi: 10.1111/1365-2745.12274.

van der Heijden MGA, Scheublin TR. 2007. Functional traits in mycorrhizal ecology: their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning. *New Phytologist* **174**: 244–250.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist* **135**: 575–586.

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* **333**: 880–882.

Larimer AL, Clay K, Bever JD. 2014. Synergism and context dependency of interactions between arbuscular mycorrhizal fungi and rhizobia with a prairie legume. *Ecology* **95**: 1045–1054.

Leake JR, Johnson D, Donnelly DP, Muckle GE, Boddy L, Read DJ. 2004. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. *Canadian Journal of Botany* **82**: 1016–1045.

Neutel A-M, Heesterbeek JAP, van de Koppel J, Hoenderboom G, Vos A, Berendse F, Kaldeway C, de Ruiter PC. 2007. Reconciling complexity with stability in naturally assembling food webs. *Nature* **449**: 599–602.

Parrent JL, Peay K, Arnold AE, Comas LH, Avis P, Tuininga A. 2010. Moving from pattern to process in fungal symbioses: linking functional traits, community ecology and phylogenetics. *New Phytologist* **185**: 882–886.

Perotto S, Rodda M, Benetti A, Sillo F, Ercole E, Rodda M, Girlanda M, Murat C, Balestrini R. 2014. Gene expression in mycorrhizal orchid protocorms suggests a friendly plant–fungus relationship. *Planta* **239**: 1337–1349.

Plett JM, Daguerre Y, Wittulsky S, Vayssieres A, Deveau A, Melton SJ, Kohler A, Morrell-Falvey JL, Brun A, Veneault-Fourrey C *et al.* 2014. Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. *Proceedings of the National Academy of Sciences, USA* **111**: 8299–8304.

Plett JM, Martin F. 2011. Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes. *Trends in Genetics* **27**: 14–22.

Rillig MC, Mummey DL. 2006. Mycorrhizas and soil structure. *New Phytologist* **171**: 41–53.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis.* Boston, MA, USA: Academic Press.

Valadares R, Perotto S, Santos E, Lambais M. 2014. Proteome changes in *Oncidium sphacelatum* (Orchidaceae) at different trophic stages of symbiotic germination. *Mycorrhiza* **24**: 349–360.

Wagg C, Bender SF, Widmer F, van der Heijden MGA. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences, USA* **111**: 5266–5270.

Key words: ecology, evolution, mycorrhiza, soil food-web, symbiosis.

3 Manuscrit B : "Beyond ectomycorrhizal bipartite networks: Projected networks demonstrate contrasted patterns between early- and late-successional plants in Corsica"

In a Nutshell

Rationale: There is a growing interest of plant ecologists in both mycorrhizal symbiosis and network analysis. Ectomycorrhizal (ECM) interaction is crucial in most forests of the nemoroboreal zone where it supports highly diverse plant-plant interactions. Here we applied ECM network derived features to analyze a high-resolution plant-fungi ECM matrix of association assembled at the Corsica Island scale.

The first peculiarity of this publication is the nature of the dataset. We built the ECM plant-fungi interactions matrix at the entire scale of Corsica by assembling molecular and field data including all validated occurrences published in the "gray literature". Mixing data of different nature was necessary to inform an network at the regional scale. The second innovation of this research is the use of the projected networks to provide a systemic view of indirect plant-plant interactions across landscapes and ecological successions. Instead of measuring global metrics such as nestedness and modularity, we focus on the position of plants in the network related to their number of partners and their ecology.

Type of interaction: ECM symbiosis at regional scale, mostly mutualistic

Ecological filters of fungal communities: Plant functional group (early- *vs* late stage); Host taxonomy and phylogeny

Main conclusions: There is no trade-off between the specialization of ECM plants and the specialization of their fungal partners. Moreover, the plant-plant projected network is highly saturated. Finally, there is a significantly lower-than-expected sharing of partners between early- and late-successional plant species, with fewer fungal partners for early-successiona ltrees and shrubs as compared to late-succession ones, and similar average specialization of symbionts interacting with early- and late-successional plant species.

frontiers
in Plant Science

ORIGINAL RESEARCH
published: 20 October 2015
doi: 10.3389/fpls.2015.00881

Beyond ectomycorrhizal bipartite networks: projected networks demonstrate contrasted patterns between early- and late-successional plants in Corsica

Adrien Taudiere[1]*, François Munoz[2,3], Annick Lesne[4,5], Anne-Christine Monnet[1], Jean-Michel Bellanger[1], Marc-André Selosse[6], Pierre-Arthur Moreau[7] and Franck Richard[1]

[1] UMR 5175, CEFE – CNRS – Université de Montpellier – Université Paul Valéry Montpellier – EPHE – INSERM, Montpellier, France, [2] UM2, UMR AMAP, Montpellier, France, [3] French Institute of Pondicherry, Pondicherry, India, [4] CNRS, LPTMC UMR 7600, Université Pierre et Marie Curie-Paris 6, Sorbonne Universités, Paris, France, [5] CNRS, IGMM UMR 5535, Université de Montpellier, Montpellier, France, [6] CNRS, Muséum National d'Histoire Naturelle, UMR 7205, Origine, Structure et Evolution de la Biodiversité, Paris, France, [7] Département de Botanique, Faculté des Sciences Pharmaceutiques et Biologiques, Université Lille, Lille, France

OPEN ACCESS

Edited by:
Sergio Rossi,
Université du Québec à Chicoutimi, Canada

Reviewed by:
Vicky Martine Temperton,
Plant Science (Institute of Bio- and Geosciences IBG-2) – Forschungszentrum Jülich, Germany
Raffaella Balestrini,
Consiglio Nazionale delle Ricerche, Italy

***Correspondence:**
Adrien Taudiere
adrien.taudiere@cefe.cnrs.fr

Specialty section:
This article was submitted to Functional Plant Ecology, a section of the journal Frontiers in Plant Science

Received: 26 May 2015
Accepted: 05 October 2015
Published: 20 October 2015

Citation:
Taudiere A, Munoz F, Lesne A, Monnet A-C, Bellanger J-M, Selosse M-A, Moreau P-A and Richard F (2015) Beyond ectomycorrhizal bipartite networks: projected networks demonstrate contrasted patterns between early- and late-successional plants in Corsica. Front. Plant Sci. 6:881. doi: 10.3389/fpls.2015.00881

The ectomycorrhizal (ECM) symbiosis connects mutualistic plants and fungal species into bipartite networks. While links between one focal ECM plant and its fungal symbionts have been widely documented, systemic views of ECM networks are lacking, in particular, concerning the ability of fungal species to mediate indirect ecological interactions between ECM plant species (projected-ECM networks). We assembled a large dataset of plant–fungi associations at the species level and at the scale of Corsica using molecular data and unambiguously host-assigned records to: (i) examine the correlation between the number of fungal symbionts of a plant species and the average specialization of these fungal species, (ii) explore the structure of the plant–plant projected network and (iii) compare plant association patterns in regard to their position along the ecological succession. Our analysis reveals no trade-off between specialization of plants and specialization of their partners and a saturation of the plant projected network. Moreover, there is a significantly lower-than-expected sharing of partners between early- and late-successional plant species, with fewer fungal partners for early-successional ones and similar average specialization of symbionts of early- and late-successional plants. Our work paves the way for ecological readings of Mediterranean landscapes that include the astonishing diversity of below-ground interactions.

Keywords: bipartite networks, projected networks, host-specificity, ecological strategies, Mediterranean forests, ectomycorrhiza, ecological mycorrhizal network

INTRODUCTION

Evaluating the extent and functions of the ecological links that soil biota create among terrestrial plants is a fascinating challenge in ecology. In temperate and boreal forests, ectomycorrhizal (ECM) symbiosis ecologically binds together 3% of terrestrial plant species and more than 6000 filamentous fungal species. Below-ground mycelia directly connect short roots to soil resources, and provide pathways for reciprocal nutrient fluxes and water exchanges among associated individuals (Smith and Read, 2008). ECM symbiosis contributes to indirect interactions among trees through shared fungus partners (Bingham and Simard, 2012), and these interactions facilitate seedling establishment (Richard et al., 2009) and species coexistence (Selosse et al., 2006) in plant communities. ECM symbiosis thereby contributes to plant community dynamics during both primary (Nara, 2006) and secondary ecological succession (Simard et al., 1997; Richard et al., 2009; van der Heijden and Horton, 2009; Bingham and Simard, 2012).

One of the most striking and consistently observed properties of ECM symbioses is the variation over three orders of magnitude of the number of ECM fungal species associating with a plant species, ranging from a few fungal species associating with *Sarcodes sanguinea* and *Neottia nidus-avis* (Kretzer et al., 2000; Selosse et al., 2002) to over 1800 for *Pseudotsuga menziesii* (Molina et al., 1992).

Ectomycorrhizal fungal species also display large variation in the number of associated plant species, e.g., *Cenococcum geophilum* and *Laccaria amethystina* have been found on most European ECM trees (Horton and Bruns, 2001; Roy et al., 2008) whereas *Alpova alpestris* only associates with one tree species in the genus *Alnus* (Moreau et al., 2011). Phylogenetically constrained interactions have been shown in some specialized lineages, e.g., *Suillus* sp. associate only with Pinaceae, *Leccinum* with Betulaceae/Salicaceae, and *Alnicola* with *Alnus* (Wu, 2000; den Bakker et al., 2004; Rochet et al., 2011). Hereafter, specialism and generalism are used *sensu* Öpik and Moora (2012), i.e., referring to the ability of organisms to associate with large (interaction generalists; see Glossary in **Box 1**) or small (interaction specialists) numbers of partners.

Demonstrations of the ecological and evolutionary advantages of specialism vs. generalism in ECM symbiosis are lacking, for several reasons: most ECM fungal lineages are refractive to *in vitro* cultivation, fungal species may appear less host-specific than they really are due to cryptic diversity, and determinants of fungal host-specialization are still controversial (Hawksworth, 2001; Bruns et al., 2002). It is commonly accepted that generalism may enable ECM plants to extend the habitats in which they can establish as seedlings (Bruns et al., 2002; Botnen et al., 2014). Thus, generalist plants may gain access to a greater reservoir of compatible ECM inoculum and may therefore establish more easily than specialist plants. The ability of ECM plants to colonize new areas more rapidly may result from plant ability either to associate with numerous distantly related partners (direct plant generalism), or to associate with a set of fungi that do so (indirect plant generalism through fungal generalism). During the first steps of ecological succession, generalism may primarily drive vegetation dynamics. Early-successional plants first establish in newly available habitats through fungi-mediated facilitation processes (e.g., Nara, 2006), while late-successional tree species colonize (e.g., Selosse et al., 2006; Richard et al., 2009) and outcompete (Bruns et al., 2002) in pioneer vegetation through facultative epiparasitism. At the end of the succession, ECM communities are hyper-diverse assemblages classically associated with long-lived late-successional tree species in old stands (Visser, 1995; Dahlberg et al., 1997; Smith et al., 2002; Richard et al., 2005; Walker et al., 2005). It has been hypothesized that this pattern of fungal diversity enrichment with forest aging may be driven by a late accumulation of host-specific fungi (Last et al., 1983; Smith et al., 2002; Twieg et al., 2007).

Two questions arise. First, is there a trade-off between the number of fungal partners and their specialization at the plant species level? Second, do early- and late-successional plant species differ in their association patterns, with late-successional species accumulating more specialized fungal species than early-successional plants? We here explore these questions by analyzing the correlation between the number of symbionts of a plant species and the average specialization of these symbionts, in regard to the successional status of the plant species in Corsica.

The analysis of bipartite interaction networks has provided important insights into ecological (Bascompte and Jordano, 2007; Chagnon et al., 2014) and evolutionary questions (Ives and Godfray, 2006; Krasnov et al., 2012). Analyses have demonstrated that the topology of the network depends on whether the interactions are mutualistic or antagonistic (Thébault and Fontaine, 2010). To date, most research on bipartite ecological networks has focused on plant-pollinator or plant–herbivore interactions (Thébault and Fontaine, 2010). The structure of local plant–fungi bipartite networks has been investigated at the species level in the context of endomycorrhizal (Chagnon et al., 2012; Montesinos-Navarro et al., 2012; Öpik and Moora, 2012) and orchid mycorrhizal associations (Martos et al., 2012). In the context of the ECM symbiosis, ever-increasing information is available on below-ground associations to inform the links between ECM fungal and plant species. However, studies examining these interactions in a network perspective are lacking (but see Bahram et al., 2014). Moreover, most studies have considered the ECM community as a static biotic component of the plant's ecological niche.

We here argue that investigating the structure of ECM interspecific networks is a path to explore the ecological role of ECM symbiosis at a systemic level. We have analyzed an ECM ecological bipartite network (see Glossary) constructed from a unique qualitative dataset that exhaustively assembled field and molecular records on ECM plant–fungi associations in the island of Corsica, France. ECM links between plant and fungal species are considered at the scale of the whole island. More specifically, our aim was to understand the potential of ECM symbiosis for creating fungi-mediated ecological interactions (e.g., facilitation or competition) between plant species, and reciprocally between ECM fungal species by means of shared host plants. These indirect interactions among species of the same kind (plants vs. fungi) mediated by species of the other kind constitute *projected networks* (see Glossary; Latapy et al., 2008; Nacher and Akutsu, 2011).

Our study investigated three main questions. First, whether there is a trade-off between the number and the specialization of fungal species associated with a plant species; second, whether plant species associated with fewer fungal species in the bipartite network are linked to fewer plant species in the projected

BOX 1 | Glossary.

Bipartite network: a network linking two distinct sets of nodes (with no links among nodes of the same set). **Figures 1** and **2A** illustrate the ectomycorrhizal (ECM) ecological network linking plant species with fungal species. Bipartite networks are also termed 2-mode networks or bimodal networks.

Common mycorrhizal network (CMN): below-ground network where fungal mycelia physically connect roots of different plant individuals.

Degree (*k*): in network terminology the degree k_n of a node n is the number of links it has established with other nodes. Here, the degree of a species (either a fungal or a plant species) corresponds to the number of its symbiotic partners. It measures its interaction specialization.

Ecological mycorrhizal network: mutualistic interaction network linking together plant and fungal entities (e.g., individuals, populations, species) able to establish a mycorrhizal connection in at least one ecological context and during one ontological stage. The ecological ECM network studied here at the species level (**Figure 1**) is qualitative (binary links) and only informs on the potentiality of two species to interact.

Interaction specialization: tendency to interact with few or lot of partners. A species that interacts with many species (high degree k) is termed an **interaction generalist** and a species that interacts with few species (low degree k) is termed an **interaction specialist**. In the case of the ECM ecological network, fungal interaction specialization is often called host-specificity of the fungal species in mycological literature.

Modular network: a modular network is made of subsets of nodes highly connected between them and poorly connected to others. These subsets are called modules or clusters. Modularity in an ecological network may reflect ecological (e.g., spatial or successional position for the plant species) and evolutionary (e.g., coevolution) processes.

Partner specialization (*c*): for a focal node n, we defined c_n as the mean degree of its partners (its direct neighbors in the bipartite network), where the mean is taken over the set of its partners. Here, the partner specialization of a focal plant species is the mean number of host plant species of its fungal symbionts (**Figure 2A**). The average of c_n over plant species of same bipartite degree recovers the standard bipartite degree correlation (sometimes termed connectivity correlation).

Projected degree (*l*): the projected degree l_n of a node n of a bipartite network is its degree in the corresponding projected network, that is, the number of nodes of the same set sharing at least one neighbor in the bipartite network (**Figure 2B**). Here, the projected degree of a focal plant species is the number of other plant species sharing fungal partners with it.

Projected network: 1-mode network built from a bipartite network, by considering only nodes of one set, and linking two nodes if they share at least one neighbor in the bipartite network (**Figure 2B**); the links of a projected network are also termed indirect links, mediated by nodes of the other set. A bipartite network is associated with two projected networks. Here, the plant projected network links ECM plants through their shared fungal partners (Supplementary Figure S10).

Projected weight (*s*): we defined the projected weight s_n of a focal node n as the total number of indirect, two-step connections to its neighbors in the projected network in its projected network (**Figure 2B**). Here, the projected weight of a focal plant species is the number of fungal species shared with other plants, where each fungal species is counted as many times as it indirectly links the focal species to another plant species. The projected weight of a focal plant species is in general different from its projected degree l_n, but related to its partner specialization c_n and its bipartite degree k_n according to $c_n = 1+(s_n/k_n)$.

network; and finally, when the ecological strategies of plants are considered, whether early- and late-successional species display different interaction patterns, in either the bipartite network or the projected plant network.

MATERIALS AND METHODS

Study Area

We assembled and analyzed a database on plant species and their associated ECM fungal species all over the Mediterranean island of Corsica (Conservatoire Botanique National de Corse, unpublished data; data are given in Supplementary Table S1, references in Supplementary References S2, detailed description of the methods in Supplementary Methods S3, and the workflow of data filtering in Supplementary Figure S4). The island covers 8681 km^2 of mountainous territory. It is the sole island in the Mediterranean basin presenting large surfaces of well-preserved native ECM forests (Quézel and Médail, 2003; Richard et al., 2005). The distribution and numbers of plant species and ECM fungal species are exceptionally well-known after over a century of intensive botanical and mycological surveys (Jeanmonod and Gamisans, 2007).

Plant Species Included in the Analysis

We constructed a network based on all known ECM plant species present in Corsica (Gamisans, 1991; Jeanmonod and Gamisans, 2007) excluding recently introduced species (*Eucalyptus globulus*, *Larix decidua*, *P. menziesii,* and *Populus nigra*). We also excluded mycoheterotrophic and mixotrophic plants (orchids and Pyroleae; Selosse and Roy, 2009) from the analysis because of the atypical physiology of their relationship with ECM fungi. Eleven additional plant species were excluded from the analysis because of their very low frequency (*Pinus pinea, Populus canescens*, *Pinus halepensis,* and *Quercus robur* subsp. *robur*) or because their ECM fungal communities are insufficiently documented (*Arbutus unedo*, *Fumana laevipes*, *Fumana thymifolia*, *Ostrya carpinifolia*, *Populus tremula*, *Quercus humilis* subsp. *Humilis,* and *Quercus petraea* subsp. *petraea*; cf. Supplementary Methods S3).

Salix species cover large areas in many locations of the island, where the different species co-occur and their local ECM communities have not been critically analyzed using molecular tools. The same is true for *Cistus* species. Therefore, we conservatively considered each of these genera as a single taxon in the analysis (*Salix* sp. on one hand and *Cistus* sp. one the other hand; cf. Supplementary References S2 and Supplementary Methods S3). The 16 resulting plant taxa (hereafter called plant species) included all the tree species dominating the forest stages of plant ecological successions in Corsica (Gamisans, 1991). They represented all forest ecosystems from sea level (sclerophyllous oak forests) to the upper altitudinal tree limit.

Fungal Species Included in the Analysis

For macromycetes, 411 species out of 610 ECM fungal species recorded in Corsica (67%, Supplementary Table S1; Supplementary Methods S3) were included in the network analysis. We retained only taxa for which were available (i) a well-resolved taxonomic treatment, and (ii) reliable data on host association within Corsica based on published records (Supplementary References S2).

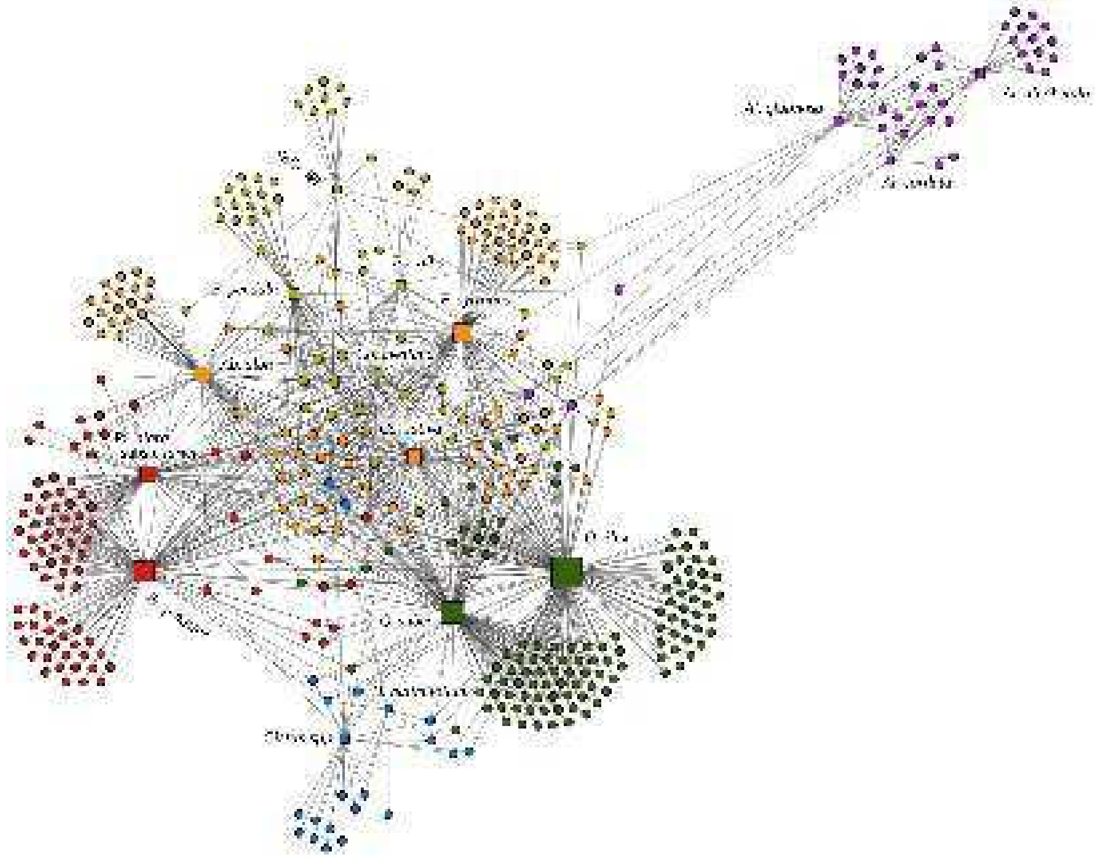


FIGURE 1 | Representation of the Corsican ectomycorrhizal (ECM) bipartite network based on the algorithm Atlas 2 under the software GEPHI (Bastian et al., 2009). Squares and circles represent interacting plant and fungal species, respectively. Links indicate plant–fungal interactions. The size of the squares is proportional to the degree of the plant species. Six modules based on Netcarto software (Guimerà and Amaral, 2005) are indicated by different colors. The following abbreviations indicate plant genera: Ab.: *Abies*; Al.: *Alnus*; B.: *Betula*; Ca.: *Castanea*; Co.: *Corylus*; F.: *Fagus*; H.: *Halimium*; Pi.: *Pinus*; Po.: *Populus*; Q.: *Quercus*.

Validation of the Interactions Included in the Analysis

In total, we defined 993 interactions (**Figure 1**) from 61 informative analyzed datasets extracted from either fruitbody surveys or DNA belowground studies (Supplementary References S2). This two sources of information are two complementary views of plant–fungal ECM links (Horton and Bruns, 2001). Briefly, we compiled a large set of publications (both peer-reviewed and 'gray literature'), books and records from expert field mycologists. The associations of the 411 selected fungal species with the 16 plant species were compiled as follows. To be validated, each association between plants and fungi was ascertained by molecular studies (Richard et al., 2004; Moreau et al., 2011; Rochet et al., 2011; Roy et al., 2013), or by validated observations within a monospecific forest/chaparral stand (one single ECM host) in Corsica, or by both methods. Molecular data were obtained from mycorrhizal sequencing in published studies. Only mycorrhizal root tips that were (i) taxonomically assigned to a given fungal species (blast) and (ii) related to a given plant host were included in the dataset. Herbarium collections (published or not; most came from the Lille University herbarium LIP, France) associated with field observations were systematically used to validate the taxonomic identity of fungal species.

Analysis of Bipartite and Projected Networks

We built a bipartite network including the 16 plant species and 411 ECM fungal species (**Figure 1**). The corresponding 16 × 411 binary matrix of association has value 1 at position (p, f) if fungal species f has been reported to be an ECM symbiont of plant species p, and 0 otherwise (Supplementary Table S1). Following network theory terminology, the number of ECM fungal species that are linked with a given plant species p is called its bipartite degree k_p (**Figure 2**). The number of plant species that are linked with a given fungal species f is called its bipartite degree k_f. Low k values characterize interaction-specialist species (*sensu* Öpik and Moora, 2012), while high k values characterize interaction-generalist species. Hereafter, plant specialization (generalism vs. specialism) will be used to refer to the realized biotic part of plant niche, and not to any theoretical niche of ECM plants in the Corsican region. The fungal species linked with the plant species p may also be linked with other plant species, and the number of plant species that are linked through one or more

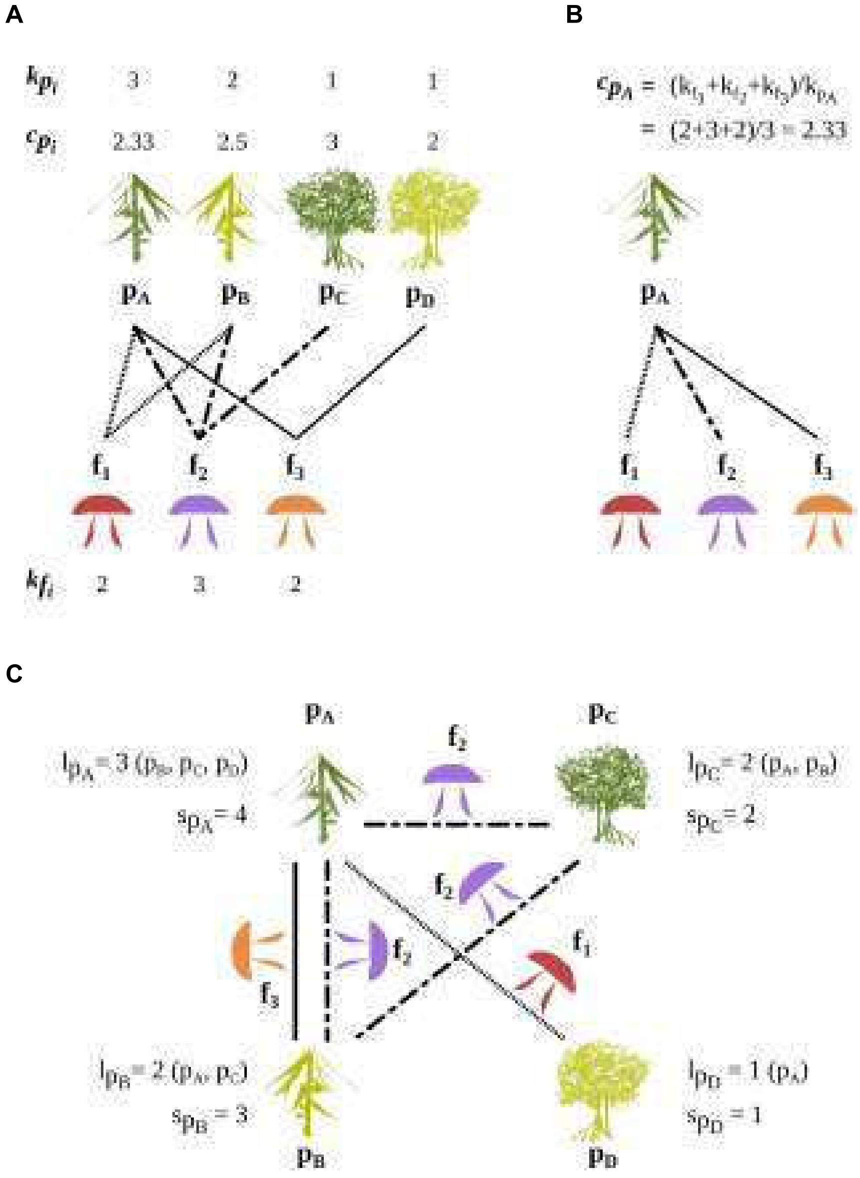


FIGURE 2 | A schematic bipartite network of four ECM plant species (p_A, p_B, p_C, p_D) and their three ECM fungal symbionts (f_1, f_2, f_3). The degree in the bipartite network is k_{p_i} for a plant species p_i and k_{f_j} for a fungal species f_j. The average degree of the fungal partners of a given plant species p_i is c_{p_i}. **(A)** Displays the degrees of plant and fungal species and the average degree of the fungal partners of each plant species. **(B)** Illustrates the detailed calculation of c_{p_A}.
(C) Illustrates the plant projected network composed of indirect (two-step) links through fungi, i.e., plant–plant links mediated by shared fungal symbionts. The number of plant species linked to a given plant species through s_{pi} fungi-mediated links is l_{p_i}. The numbers of indirect (two-step) links between a given plant species p_i and the other plant species is s_{pi}. In all panels, dotted, broken and continuous lines show plant–plant links made by the fungal species f_1, f_2, and f_3, respectively. The roles played by plants and fungi can be inverted to analyze the network from a fungal perspective.

of these shared fungal partners is called the projected degree of the plant species, l_p. These links constitute the projected network of plant species (or "plant projected network"). We likewise defined the projected degree of a fungal species, l_f, as the number of associated fungal species in the projected network, i.e., linked through plant species to the focal fungal species f (**Figure 2**).

A plant species can interact with another plant species *via* a single fungal species or *via* several ones. The number of fungal species linking two plant species is a measure of the strength of their association in the projected network. The total number of indirect (two-steps) links of the plant species p to other plant species is called its weight s_p in the plant projected network (links between plant species in **Figure 2C**). Likewise, the total number of plant-mediated links of a fungal species f to other fungal species is its weight s_f in the fungal projected network.

While l_p represents the number of plant species to which p is linked in the plant projected network, s_p represents the total number of links established *via* fungal species with these plant species. The contribution to s_p of a plant species is simply the number of ECM fungal species that it shares with p. Therefore, the ratio of s_p to l_p measures in an integrated way the redundancy of fungal species in establishing links between p and other plant species.

If the number of links of a given plant species to other plant species is selected for, then we may expect a trade-off for the plant between its specialization (value of k_p) and the specialization of the fungi (value of k_f) with which it associates. Indeed, a plant species associated with many specialized fungal species (high k_p and partners with low k_f) may be as well-linked (same s_p) with as many other plant species (same l_p) as a plant species associated with few generalist fungal species (low k_p and partners with high k_f). To explore this question, we introduced an additional quantity, c_p, measuring the average specialization of the fungal partners of a given plant species p. It is defined as the mean degree (in the bipartite network) of the fungal partners of the plant species, p, where the average runs over the set of these fungal partners. We then devised two null models to analyze the relationships between c_p and k_p (see below). A summary of the different network-related notions and their notation is given in the Glossary (**Box 1**).

Null Models

Null models were used to simulate random situations sharing some minimal constraints with the actual network by randomizing links among plant and fungi. Deviations of observed network statistics from the statistics measured in these random situations inform on whether species are more generalist or specialist than expected by chance, or whether they are associated with partners that are themselves more generalist or specialist than expected by chance, given the specified constraints.

First we devised a simple random model (null model 1) considering independently each pair of plant and fungal species and drawing a link with a probability equal to the density of links (actual number of links divided by the maximal number of links given the number of plant and fungal species) observed in the real network. As a consequence, the mean values of plant and fungal bipartite degrees are the same in this null model 1 and the real network.

The distributions and relationships between k_p and c_p values obtained with this null model were compared with the distribution and relationships obtained in another null model, in which the bipartite degree of each plant or fungal species is exactly the same as in the real network, but the links between species were randomized (null model 2). Both null models were generated using the *permat* functions within the R package Vegan with (model 2; method *quasiswap* of the function *permatswap*; Patefield, 1981) or without (model 1; function *permatfull*) preserving column and row sums (Oksanen et al., 2015). Using these two null models, it is possible to identify the correlations between k_p and c_p values which are simply due to the degree distributions in the dataset, i.e., to the distribution of specialism vs. generalism. Comparison of the data with null model 1 allowed detecting whether species bipartite degrees were distributed randomly within the network (Gotelli and Entsminger, 2003). We present our results on partner specialization as a scatter plot, keeping track of the plant species identity and the variance of their partner specialization (**Figure 3A**). Finally, observed values of c_p and k_p were compared with their statistics obtained in the null model 2 in order to detect whether the actual network differs from a random network in which levels of species specialization (values of k_p and k_f) were identical to those observed in the real data.

Given that the association matrix is highly asymmetric (16 plants * 411 fungi), our analysis focused on a plant perspective as more information (more potentially associated partners) is available for each plant, allowing a more powerful statistical analysis. The fungal-perspective analysis is presented in Supplementary Figure S5.

ECM Association Patterns and Ecological Strategy of Plant Hosts

Early- stage vs. late-stage status of each plant species was determined according to reference work on their ecology in Corsica (Jeanmonod and Gamisans, 2007; Rameau et al., 2008). Accordingly, all species belonging to the Salicaceae, Betulaceae, and Cistaceae were classified as early-stage plants, while all species belonging to the Pinaceae and Fagaceae were classified as late-stage plants. We compared the degrees of plant species in the bipartite network (k_p), their weights in the projected network (s_p) and average numbers of hosts of their fungal symbiotic species (c_p) in the sets of early-stage and late-stage plant species, using Mann–Whitney non-parametric tests. We measured modularity using the simulated annealing algorithm implemented in Netcarto (Guimerà and Amaral, 2005). This software also simulates random graphs with the same degree distribution as the original network to test for modularity significance (Guimerà et al., 2004).

RESULTS

Relationships between the Number of ECM Partners and their Specialization

Among the 16 plant species, association patterns varied from species-poor (low k_p values) fungal communities associated with Betulaceae (*Alnus, Betula, Corylus*, whose number of associated ECM fungal species ranged from 14 to 46) and Cistaceae (*Cistus* and *Halimium* with $k_p = 26$ and $k_p = 12$, respectively) to rich ECM fungal assemblages (high k_p values) associated with *Quercus suber* and *Quercus ilex* evergreen oaks (respectively 133 and 197 ECM linked fungal species; **Figure 3A**). There was no significant correlation between the bipartite degree k_p of a plant species and the interaction specialism c_p (average number of host species) of its associated fungal species (Spearman non-parametric rank correlation test, $p = 0.71$; **Figure 3A** green points).

The variances of the plant (k_p) and fungal (k_f) bipartite degrees were much higher in the real network than in the simple random model (null model 1; **Figure 3A** and Supplementary Figure S5 for k_f). We then constrained the null model so that the degree of each plant (k_p) and fungal species (k_p) is the same

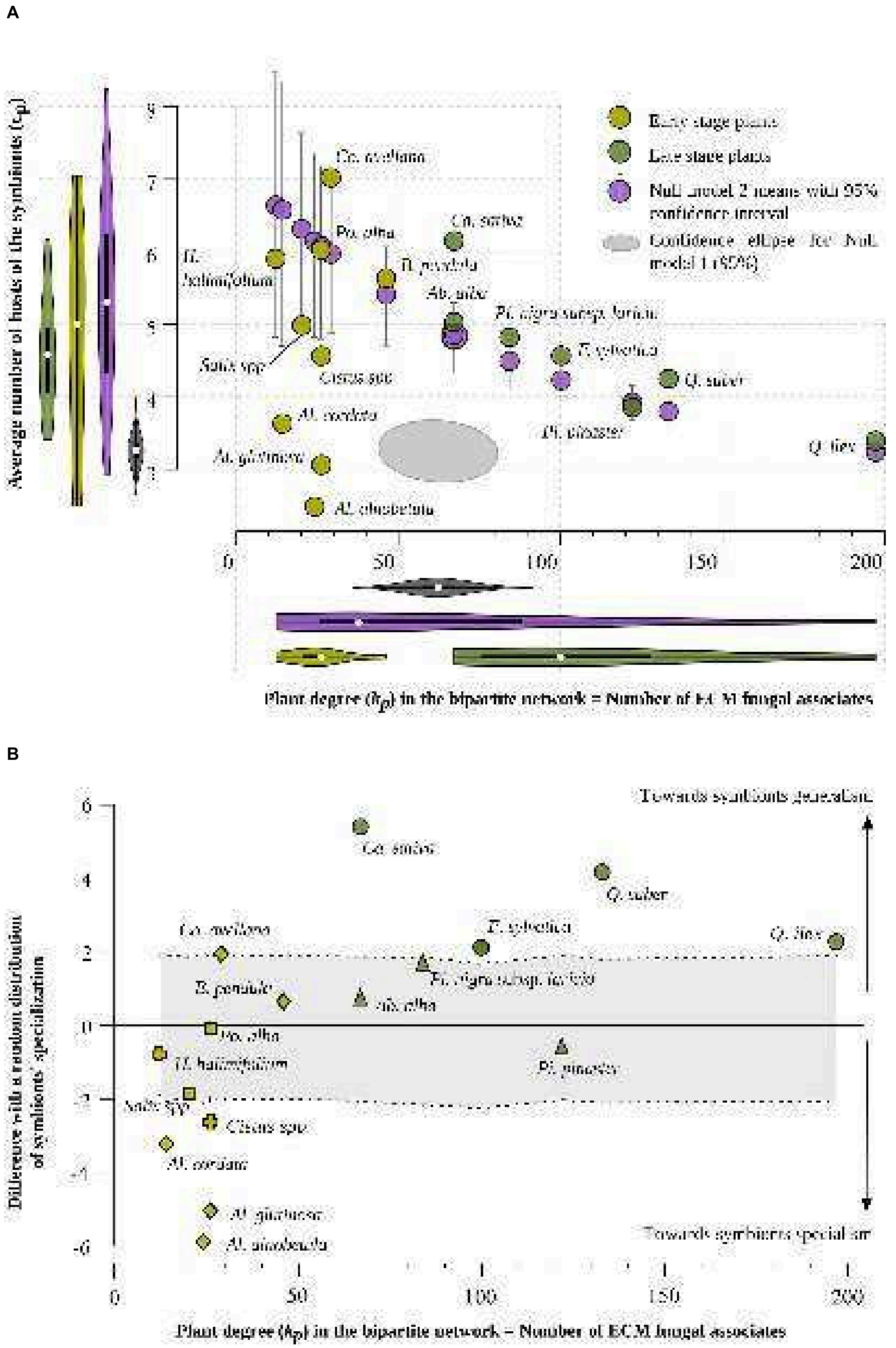


FIGURE 3 | Continued

FIGURE 3 | Continued

(A) Distribution of 16 ECM plant species according to their degree in the bipartite network, k_p, i.e., the number of their fungal partner species (X axis), and the average number of host species (i.e., average specialization) of their symbionts, c_p (Y axis). Light green and dark green dots correspond to early-stage and late-stage plant species, respectively. Gray ellipses display the 95% confidence intervals for null model 1. The null model 1 consists of a simple random model considering independently each pair of plant and fungal species and drawing a link with a probability equal to the density of links (actual number of links divided by the maximal number of links given the number of plant and fungal species) observed in the real network. Purple dots show positions of plant species in the null model 2. The null model 2 is based on a random distribution of the links in the bipartite network, at fixed bipartite degrees k_p or k_f for all partners. Bars indicate 95% confidence intervals. **(B)** Distribution of 16 ECM plant species according to their degree in the bipartite network, k_p, i.e., the number of their symbiont species (X axis) and the standardized deviation [standard effect size (SES)] of the average number of host species of their symbionts, c_p, with respect to its value in null model 2. A total number of 999 randomized networks were simulated to establish the null situation with which the actual c_p values were compared. For plant species located between the two dotted curves, the observed situation does not differ from a random distribution of their links, whereas a plant species located above the upper dotted line shows an excess of generalist species among its associated ECM fungal species, and a plant species situated below the lower dotted line shows a significant excess of specialist symbionts. The following abbreviations and signs are used to indicate plant genera and families: Ab.: *Abies*; Al.: *Alnus*; B.: *Betula*; Ca.: *Castanea*; Co.: *Corylus*; F.: *Fagus*; H.: *Halimium*; Pi.: *Pinus*; Po.: *Populus*; Q.: *Quercus*; circle: Fagaceae; triangle: Pinaceae; square: Salicaceae; cross: Cistaceae; diamond: Betulaceae.

as in the real network (null model 2, 999 permutations). The simulation revealed a strong negative correlation between plant degree (k_p) and mean interaction specialism of fungal partners (c_p) (**Figure 3A** purple points). Hence, k_p and k_f values impose a constraint on the structure of the network which *a priori* results in a strong negative correlation between number of associated fungal species and degree of specialization of these fungal species (**Figure 3A** purple points). In the real network, the absence of correlation between the bipartite degree k_p of a plant species and the average number of hosts c_p of its associated fungal species is unexpected given the distribution of k_p and k_f values.

Comparing the real network and the null model with constrained k_p and k_f values (null model 2) shows that the value of c_p deviates from the null model for more than half of the species (**Figure 3B**). Four plant species (*Cistus* sp., *Alnus glutinosa, Alnus alnobetula* subsp. *suaveolens,* and *Alnus cordata*) host significantly more specialist fungal species than would be expected by chance (given the distributions of degrees; p-values < 0.025 using the null model 2 for these species) while the four Fagaceae species (*Q. ilex*, *Q. suber, Fagus sylvatica,* and *Castanea sativa*) and *Corylus avellana* host significantly more generalist fungal species than expected by chance (**Figure 3B**; p-values < 0.025 using the null model 2 for these species).

Projected Networks of ECM Plants

Ten plant species are linked to all other plant species ($l_p = 15$; **Table 1**). Indeed, the number of plant species to which a plant species is linked in the projected network varies from 11 (*A. cordata* and *A. alnobetula*) to all other 15 species (*A. glutinosa* and all species belonging to Cistaceae, Pinaceae, or Fagaceae; **Table 1**). Behind the tendency of all plant species to saturate their projected network, there is wide variation in the number of ECM-mediated links (two-step links) that a given plant species makes with all other plant species (s_p ranging from 36 ECM-mediated links for *A. alnobetula* to 474 links for *Q. ilex*; Supplementary Figure S6). For instance, 12 ECM partners link *Halimium halimifolium* with all plant species (altogether mediating 59 two-step links), so that the neighborhood of *Halimium halimifolium* in the projected network includes all the plant species of the present survey. In contrast, *Betula pendula* symbiotic links do not saturate the plant–plant projected network even though its 46 associated ECM fungal species establish 214 fungi-mediated links.

Endemic alders, *A. cordata* and *A. alnobetula*, with $l_p = 11$ plant partners through $s_p = 37$ and 36 fungi-mediated links respectively, interact with slightly less plant species than the wide-ranging *A. glutinosa* ($l_p = 15$ through $s_p = 54$ links; **Table 1**). In our analysis, these two endemic alders establish no ECM fungi-mediated links with *Betula*, *Corylus*, *Populus,* or *Salix*.

There is wide variation in the number of ECM-mediated links (two-step links) that a given plant species makes with all other plant species (s_p values, taken as the weight of the plant species in the projected network), ranging from 36 ECM-mediated links for *A. alnobetula* to 474 links for *Q. ilex* (Supplementary Figure S6).

The redundancy l_p/s_p (ratio of the projected degree l_p to the number of comprised links s_p) ranges from $11/36 = 0.306$ for *A. alnobetula*, to $15/474 = 0.032$ for *Q. ilex* (**Table 1**). These results indicate a lower redundancy of the ECM-mediated links of *A. alnobetula* to other plant species as compared to *Q. ilex*.

ECM Associations along Ecological Succession

Early-stage vs. late-stage plant species displayed contrasted distributions of their numbers of associated fungal species (**Figure 3** and **Table 1**, with a maximal degree $k_p = 46$ for the set of early-stage species and a minimal degree $k_p = 67$ for the set of late-stage species). On average, early-stage plant species associated with five times fewer fungal species (mean $k_p = 24.78$ for early-stage vs. 110 for late stage plant species; $p < 10^{-4}$, Mann–Whitney non-parametric test; **Figure 4A**). They also present five times fewer fungi-mediated links to other plant species in the projected network (mean $s_p = 97.67$ vs. 365; Mann–Whitney $p < 10^{-4}$; **Table 1** and **Figure 4B**) and five times higher l_p/s_p ratios (mean ratio $= 0.38$ vs. 0.083; Mann–Whitney $p < 10^{-3}$). Nevertheless, their associated fungal species did not differ in their partner specialization c_p (average number of host species of its symbionts; mean $c_p = 4.83$ vs. 4.59; Mann–Whitney $p = 0.84$) from those associated with late-stage plant species (**Figures 3A** and **4C**).

We compared the sharing of ECM symbionts observed in the real system (i) between early- and late-stage plant species, (ii) among early-stage plant species, and (iii) among late-stage plant species, against the sharing calculated in the null model

TABLE 1 | Network properties of the 16 ECM plant taxa.

	H. halimifolium	***Al. cordata***	***Salix* sp.**	***Al. Alnobetula***	***Cistus* sp.**	***Al. glutinosa***	***Po. alba***	***Co. avellana***	***B. pendula***	***Ca. sativa***	***Ab. alba***	***Pi. nigra* subsp. *laricio***	***F. sylvatica***	***Pi. pinaster***	***Q. suber***	***Q. ilex***
k_p	12	14	20	24	26	26	26	29	46	67	67	84	100	122	133	197
c_p	5.92	3.64	5	2.5	4.58	3.08	6.04	7.03	5.65	6.16	5.04	4.83	4.58	3.87	4.26	3.41
l_p	15	11	13	11	15	15	13	13	13	15	15	15	15	15	15	15
s_p	59	37	80	36	93	54	131	175	214	346	271	322	358	350	434	474
n_{hs}	1	2	8	13	7	8	18	3	14	1	18	2	29	27	1	39

k_p: degree in the bipartite network, i.e., the number of their fungal partner species; c_p: average number of host species of their symbionts; l_p: degree of plant species in their projected network; s_p: total number of links of a plant species within its projected network; n_{hs}: number of hyper-specialists, i.e., strictly associated fungal taxa. See Glossary for more complete definitions of network metrics.

The following abbreviations were used to indicate plant genera: Ab.: Abies; Al.: Alnus; B.: Betula; Ca.: Castanea; Co.: Corylus; F.: Fagus; H.: Halimium; Pi.: Pinus; Po.: Populus; Q.: Quercus.

with constrained k_p and k_f values (null model 2). Fewer ECM fungal species (62) than expected by chance (108.52 ± 4.95 under null model 2) interact with both early- and late-stage plant species ($p < 10^{-3}$). More ECM fungal species (37) are exclusively associated with early-stage plant species than expected by chance (25.54 ± 4.21 under null model 2; $p < 10^{-3}$) given the number of partner species. On the contrary, the number of ECM fungal species (272) exclusively associated with late-stage plant species is close to the value expected by chance (277.21 ± 4.33 using null model 2; $p = 0.09$). These results show that during ecological successions, late-successional vegetation stages accumulate rich ECM communities comprised of specialist fungi that differ in composition compared to the communities associated with early-successional stages (Supplementary Figures S7–S9).

We further used the modularity of the whole network to characterize the sharing of ECM symbionts among early- and late-stage plant species. We detected six modules in a significantly modular system ($M = 0.458$, null model $= 0.362 \pm 0.002$). Three modules included only early-stage plant species (represented in light green, blue and purple in **Figure 1**) and the three others included the late-stage species (represented in orange, red and dark green in **Figure 1**).

Comparing *A. glutinosa* (early-stage) and *Q. ilex* (late-stage) illustrates the contrast between early- and late-stage plant species association patterns (**Figure 5**). Fungi-mediated interactions of *Q. ilex* are (i) quantitatively highly variable (from two links toward *A. cordata and A. alnobetula* to 131 links toward *Q. suber*) and (ii) qualitatively more numerous toward Fagaceae (*Q. suber*, *C. sativa,* and *F. sylvatica* accounted for 52.95% of the 474 indirect links of *Q. ilex*). All but two fungal species associating with *Q. suber* also associate with *Q. ilex* in Corsica (*Inocybe fibrosoides* is strictly associated with *Q. suber* and *Boletus pulverulentus* is strictly associated with *Q. suber* and *C. avellana*).

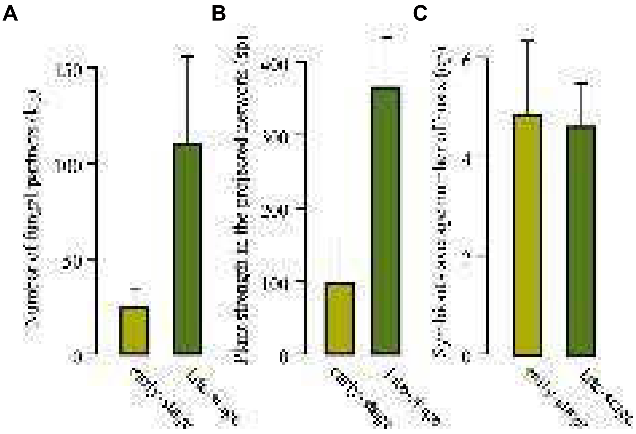


FIGURE 4 | (A) Comparison of the average plant bipartite degree (average of k_p), **(B)** average number of fungi-mediated links (average of s_p), and **(C)** average mean number of host species of fungal symbionts (average of c_p) over the set of early-stage plant species (light green) and the set of late-stage (dark green) plant species. Bars indicate standard errors.

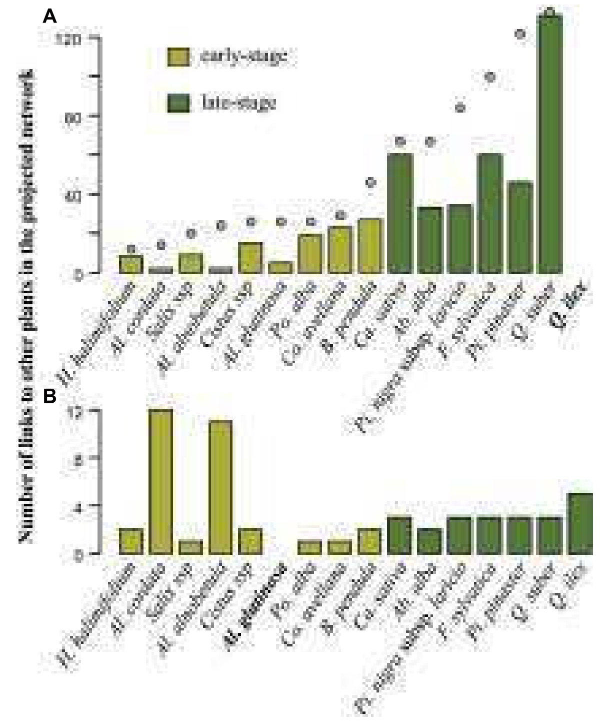


FIGURE 5 | Number of shared fungal species (in network terminology, number of fungi-mediated links) between (A) *Quercus ilex* or (B) *Alnus glutinosa* and other plant species (ordered at increasing number of fungal partners (k_p)). The color of the bars indicates early-stage (light green) and late-stage (dark green) plants species. **(A)** Gray dots indicate plant degrees (k_p) in the bipartite network (same scale on the vertical axis). The following abbreviations are used to indicate plant genera: Ab.: *Abies*; Al.: *Alnus*; B.: *Betula*; Ca.: *Castanea*; Co.: *Corylus*; F.: *Fagus*; H.: *Halimium*; Pi.: *Pinus*; Po.: *Populus*; Q.: *Quercus*.

Similarly, *A. glutinosa* presents a high variation in the number of fungi-mediated links toward other plant species, ranging from one (*C. avellana*, *P. alba,* and *Salix* sp.) to 12 (*A. cordata*). Both *A. glutinosa* and *Q. ilex* show higher numbers of fungi-mediated links toward plant species belonging to their own genus (*A. alnobetula* and *A. cordata* for *A. glutinosa*, and *Q. suber* for *Q. ilex*) and, for *Q. ilex*, to its family (*C. sativa* and *F. sylvatica* for *Q. ilex*; **Figures 1** and **5**).

DISCUSSION

In this paper we analyzed the main properties of the ECM bipartite network linking host plants and fungal species in Corsica and deciphered ECM network structure in an ecological perspective. We did not detect significant correlation between the specialization of plant species and the specialization of their symbionts across the whole plant dataset, but rather observed decoupled patterns of symbiont diversity (number of fungal partners associated with a given plant species) and the specialization of these symbionts (average number of plant partners of these symbionts). Our results show that early- and late-successional plant species share fewer fungal symbionts than expected by chance, with early-successional species presenting fewer links caused by fewer fungal partners than late-successional plant species, and no difference in average specialization of fungal symbionts of early- and late-successional plant species.

Relationship between the Number and the Specialization of ECM Symbionts

In our analysis, plant species hosting the richest ECM fungal assemblages associate with fungi whose host ranges are similar to those of plants with few fungal partners (**Figure 3A**). In other words, the analysis of the whole dataset shows no trade-off between plant specialization and the specialization of its symbionts. As a consequence, the number of fungal partners of a plant species (k_p) poorly reflects the number of plant species with which it has fungi-mediated indirect interactions (l_p).

Insights into the Hyper-diversity of Oak Symbionts

The positions of *Quercus* species in both **Figures 3A,B** confirm the well-known hyper-diversity of ECM communities of evergreen oaks (Richard et al., 2004; Morris et al., 2008). Here, we show that the high diversity of fungal symbionts dissimulates contrasted specialization degrees, with few hyper-generalists occurring among a disproportionately high number of interaction-specialist fungal species. Both oak species deviate from the null model by associating with fungi that are less specialized on average than expected by chance (**Figure 3B**). Interestingly, all but two fungal symbionts of *Q. suber* are also associates of *Q. ilex*. Only two of the 133 fungal symbionts of *Q. suber* are host-specific, while 39 species out of the 197 fungal associates of *Q. ilex* are host-specific (**Table 1**). This pattern may result from a mix of phylogenetic and ecological proximity. Indeed, the distribution and the ecological range (including soil chemical conditions and climatic requirements) of *Q. suber* in Corsica tend to be included within those of *Q. ilex* (Gamisans, 1991). This extensive sharing of fungal symbionts between the two oak species results in a low specialization of oak symbionts (low number of single-host fungal species). However, in these hyper-diverse communities, the symbionts are mostly oak specialists that establish few links to tree species belonging to other genera: 57 of the 131 fungal species associated with both oak species are only associated with oaks (Supplementary Figure S10).

Contrasted Structures of Bipartite and Plant Projected Networks

Our ECM network presents a modular structure (**Figure 1**) due to abundant plant–plant connections at genus (e.g., within *Alnus*, **Figure 5B**), family (e.g., within Fagaceae, **Figure 5A**; see also Supplementary Figure S7) and ecological (early- vs. late-stage, **Figure 4** and Supplementary Figure S8) levels. Our results complement those of previous studies that suggested modularity of below-ground mycorrhizal networks (Chagnon et al., 2012; Martos et al., 2012; Montesinos-Navarro et al., 2012).

The analysis of the structure of plant–plant interaction patterns (Supplementary Figure S10) through compatible fungal species reveals that generalism prevails in the plant projected network. Among the 16 species, 10 of them interact with all the other plant species and only two interact with fewer than 13 plant species (**Figure 3A**; **Table 1**). This tendency to saturate the projected network (Supplementary Figure S10) contrasts with the high heterogeneity in plant degree found in the bipartite network (**Figure 3A**). As a consequence, similar numbers of neighbors in the plant projected network hide highly variable numbers of symbionts (**Table 1**; **Figure 5**). The contrasted patterns observed for *A. glutinosa* and for *Q. ilex* illustrate this point. In our analysis, *A. glutinosa* saturates the plant–plant network based on only 54 fungal-mediated links whereas *Q. ilex* use 474 such links to do so (**Figure 3A**). This result points out the composite nature of *Alnus*-associated communities, which are comprised of (i) fungal symbionts belonging to *Alnus*-associated fungal lineages (e.g., *Alpova*, *Alnicola*), (ii) interaction specialists scattered across distant fungal lineages and (iii) a few generalist fungal species (Rochet et al., 2011; Roy et al., 2013).

Four species, including the three alder species and the *Cistus* sp. group, host more specialized symbionts on average in their projected network than expected by chance. Two of them, the endemic *A. alnobetula* subsp. *suaveolens* and *A. cordata*, displaying the lowest numbers of fungi-mediated links with other plant species in the whole dataset, share no fungal species with four other genera, *Betula*, *Corylus*, *Popula,* and *Salix*. This result may be surprising when we consider the convergent affinity of alders and the four latter genera for hygrophylic habitats in Corsica. These patterns may be partially explained by ecological requirements of *A. alnobetula* subsp. *suaveolens* and *A. cordata*. The first species highly dominates shrubby vegetation above the current altitudinal limit of forests, at high elevation (Jeanmonod and Gamisans, 2007). In these ecosystems, only endomycorrhizal trees (*Acer*, *Sorbus*) and scattered montane ECM species (*Pinus*, *Abies,* and *Fagus*), but no *Betula*, *Corylus*, *Populus* and *Salix*, remain in the landscape (Jeanmonod and Gamisans, 2007). The second *Alnus* species, *A. cordata*, displays (i) a low dependence on water compared to other alders, *Salix* and *Populus*, and (ii) an ability to establish under *Pinus*, *Abies*, *Fagus,* and *Quercus* during secondary successions (Gamisans, 1991; Jeanmonod and Gamisans, 2007). For these two endemic alders, their positions in the projected network indicate an unusual below-groundecology.

Below-ground Ecological Strategies of ECM Plants

In previously published literature, both the number of associated fungal species and the number of shared fungal species between plant species have been used for evaluating the potential of plants to interact with other plants through ECM fungal networks (Richard et al., 2005; Nara, 2006; Bingham and Simard, 2012). In our study, we did not hypothesize that the existence of a link between two plant species in the projected network induces any effect of one plant species on the local establishment of another. In this regard, our approach strongly differs from individual plant-centered studies investigating physical networks (Common Mycorrhizal Networks; e.g., Nara, 2006). The links we study here encompass a wide spectrum of plant–plant interactions, from the simple ability for a given plant to provide suitable habitats for other plant species through dispersed fungal propagules, to the possibility that roots of co-occurring species are inter-connected through shared mycelia.

A high plant degree (high number of fungal partners) has been hypothesized to increase physical networking and facilitate seedling establishment under pre-established trees in either conspecific (Dickie et al., 2005; Walker et al., 2005; Bingham and Simard, 2012) or mixed species populations (Amaranthus and Perry, 1989; Horton et al., 1999; Selosse et al., 2006) during secondary successions. The number of shared symbionts has been widely used as a proxy of the strength of the fungi-mediated association between pairs of host plants. Based on these ECM community overlaps, previous studies have suggested for instance facilitation of the establishment of oak forests in a Corsican succession process (*Quercus* – *Arbutus*; Richard et al., 2009), facilitation of coexistence of *Quercus* species (Walker et al., 2005), facilitation of presence of a species of *Helianthemum* at the edge of *Quercus* forests (Dickie et al., 2004) or the capacity of *Betula papyrifera* to constitute a nurse plant for *Pseudotsuga menziesii* (Simard et al., 1997; Simard and Durall, 2004; Bingham and Simard, 2012).

Beyond such fungi-mediated interactions between plant species, the ecological and evolutionary implications of the ability of an ECM plant species to interact with a high number of plant species remain undocumented. A saturated ECM projected network may increase fungal inoculum availability by maintaining compatible reservoirs on alternative hosts. This tendency to share symbionts with many other plants may enlarge the biotic component of the plant niche. Specifically, a high degree in the projected network may facilitate seedling establishment in vegetation of various stages and composition. Such plant-mediated facilitation mechanisms may have been particularly favored in Mediterranean ecosystems where summer drought drastically impacts tree recruitment (Bruno et al., 2003; Gómez-Aparicio et al., 2004). Further studies are required to ascertain the place of ECM inoculum-driven processes in facilitation mechanisms (Richard et al., 2009), which may potentially counterbalance local accumulation of pathogens on plant species (the so-called Janzen–Connell effect, already reported for Mediterranean tree species; Steinitz et al., 2011).

For fungal species, the ECM projected network provides a view of the pool of potentially interacting fungal species. As in plant-centered approaches, calculating the relative overlap in host plants between pairs of fungal species (for instance, using Jaccard distances) would provide fungal species attributes. Mediterranean forests are generally dominated by locally monospecific tree stands (Quézel and Médail, 2003). In this context, a high fungal degree (high number of hosts) may be important for broadening the range of forest types where a fungus can establish. Alternatively, constraining environmental conditions in Mediterranean ecosystems may act as primary abiotic filters selecting for both plant and fungal specialists. We note here that *Salix* and *Alnus* species are adapted to hydromorphic soils, where their host-specific fungal partners may exhibit adaptations to this specific abiotic environment.

In our study, ECM association patterns significantly differed depending on the ecological strategy of their plant host. On average, early-stage plants had five times fewer fungal associates than late-stage ones (**Figure 4**). Our data do not support the hypothesis that early-stage plants differ in the specialization of their symbionts from late successional species, but suggest that early-stage plants associate with significantly fewer ECM fungal species than late-stage plants. Additionally, long-lived late-successional plant species that dominate forest ecosystems for centuries allow a lasting fungal recruitment, which may entail the accumulation of pioneer ECM species. Interestingly, our dataset encompasses mostly species belonging to late-successional fungal genera (*Russula*, *Boletus*, *Amanita*, various "Aphyllophorales" including Bankeraceae and Hydnaceae; Last et al., 1983) that increase in relative abundance with tree aging (Horton and Bruns, 2001). Our results thus suggest that the cumulated effect of late-successional host-plant establishment (above-ground dynamics driven by plant ecological strategies) and host aging-related accumulation of late-successional stage fungal species (below-ground dynamics driven by fungal ecological strategies) lead to the hyper-diversity of ECM communities in mature forests, with no effect on the average specialization of the below-ground ECM diversity (Twieg et al., 2007).

CONCLUSION

To the best of our knowledge, our work documents for the first time the below-ground fungal counterpart of the ecological strategies of ECM host plants. Despite the unprecedented rhythm of research describing ECM communities worldwide, their role in plant community dynamics in general, and in host coexistence processes in particular, remains largely unexplored, mainly because most ECM fungi are not cultivable. We assembled a large qualitative dataset to propose a systemic view of the interactions established by the tremendous ECM fungal diversity at the scale of a large Mediterranean island. The ECM symbiosis shapes ecological and evolutionary-based interaction modules, and can help us understand the below-ground niche differences between early- and late-stage plants. The huge diversity of symbionts contributes to saturate plant–plant networks through highly variable numbers of fungi-mediated interactions between plants. Assembling quantitative data of below-ground plant–fungi interaction is crucial to grasp the ecological dimension of these contrasted patterns.

AUTHOR CONTRIBUTIONS

AT, FM, AL, FR originally formulated the idea, developed the methodology and performed statistical analyses. A-CM, J-MB, P-AM, FR generated data. AT, FM, P-AM, AL, FR wrote the initial manuscript. AT, FM, AL, A-CM, J-MB, M-AS, P-AM, and FR contributed to the final manuscript.

ACKNOWLEDGMENTS

This study was funded by the Conservatoire National Botanique de Corse. We are grateful to L. Hugot and J. Alesandri for helping us in assembling field data, and to Doyle McKey for checking the English. All experiments performed in this study comply with the current laws of France.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2015.00881

REFERENCES

Amaranthus, M. P., and Perry, D. A. (1989). Interaction effects of vegetation type and Pacific madrone soil inocula on survival, growth, and mycorrhiza formation of Douglas-fir. *Can. J. For. Res.* 19, 550–556. doi: 10.1139/x89-087

Bahram, M., Harend, H., and Tedersoo, L. (2014). Network perspectives of ectomycorrhizal associations. *Fungal Ecol.* 7, 70–77. doi: 10.1016/j.funeco.2013.10.003

Bascompte, J., and Jordano, P. (2007). Plant-animal mutualistic networks: the architecture of biodiversity. *Annu. Rev. Ecol. Evol. Syst.* 38, 567–593. doi: 10.1146/annurev.ecolsys.38.091206.095818

Bastian, M., Heymann, S., and Jacomy, M. (2009). Gephi: an open source software for exploring and manipulating networks. *ICWSM* 8, 361–362.

Bingham, M. A., and Simard, S. W. (2012). Mycorrhizal networks affect ectomycorrhizal fungal community similarity between conspecific trees and seedlings. *Mycorrhiza* 22, 317–326. doi: 10.1007/s00572-011-0406-y

Botnen, S., Unni, V., Carlsen, T., Eidesen, P. B., Davey, M. L., and Kauserud, H. (2014). Low host specificity of root-associated fungi at an Arctic site. *Mol. Ecol.* 23, 975–985. doi: 10.1111/mec.12646

Bruno, J. F., Stachowicz, J. J., and Bertness, M. D. (2003). Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.* 18, 119–125. doi: 10.1016/S0169-5347(02)00045-9

Bruns, T. D., Bidartondo, M. I., and Taylor, D. L. (2002). Host specificity in ectomycorrhizal communities: what do the exceptions tell us? *Integr. Comp. Biol.* 42, 352–359. doi: 10.1093/icb/42.2.352

Chagnon, P.-L., Bradley, R. L., and Klironomos, J. N. (2012). Using ecological network theory to evaluate the causes and consequences of arbuscular mycorrhizal community structure. *New Phytol.* 194, 307–312. doi: 10.1111/j.1469-8137.2011.04044.x

Chagnon, P.-L., Bradley, R. L., and Klironomos, J. N. (2014). Plant–fungal symbioses as ecological networks: the need to characterize more than just interaction patterns. *Fungal Ecol.* 12, 10–13. doi: 10.1016/j.funeco.2014.05.002

Dahlberg, A., Jonsson, L., and Nylund, J.-E. (1997). Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. *Can. J. Bot.* 75, 1323–1335. doi: 10.1139/b97-844

den Bakker, H. C., Zuccarello, G. C., Kuyper, T. W., and Noordeloos, M. E. (2004). Evolution and host specificity in the ectomycorrhizal genus *Leccinum*. *New Phytol.* 163, 201–215. doi: 10.1111/j.1469-8137.2004.01090.x

Dickie, I. A., Guza, R. C., Krazewski, S. E., and Reich, P. B. (2004). Shared ectomycorrhizal fungi between a herbaceous perennial (*Helianthemum bicknellii*) and oak (*Quercus*) seedlings. *New Phytol.* 164, 375–382. doi: 10.1111/j.1469-8137.2004.01177.x

Dickie, I. A., Schnitzer, S. A., Reich, P. B., and Hobbie, S. E. (2005). Spatially disjunct effects of co-occurring competition and facilitation. *Ecol. Lett.* 8, 1191–1200. doi: 10.1111/j.1461-0248.2005.00822.x

Gamisans, J. (1991). *La Végétation de la Corse*. Aix-en-Provence: Édisud.

Gómez-Aparicio, L., Zamora, R., Gómez, J. M., Hódar, J. A., Castro, J., and Baraza, E. (2004). Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. *Ecol. Appl.* 14, 1128–1138. doi: 10.1890/03-5084

Gotelli, N. J., and Entsminger, G. L. (2003). Swap algorithms in null model analysis. *Ecology* 84, 532–535. doi: 10.1890/0012-9658(2003)084[0532:SAINMA]2.0.CO;2

Guimerà, R., and Amaral, L. A. N. (2005). Functional cartography of complex metabolic networks. *Nature* 433, 895–900. doi: 10.1038/nature03288

Guimerà, R., Sales-Pardo, M., and Nunes Amaral, L. A. (2004). Modularity from fluctuations in random graphs and complex networks. *Phys. Rev.* 70:25101. doi: 10.1103/PhysRevE.70.025101

Hawksworth, D. L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycol. Res.* 105, 1422–1432. doi: 10.1017/S0953756201004725

Horton, T. R., and Bruns, T. D. (2001). The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Mol. Ecol.* 10, 1855–1871. doi: 10.1046/j.0962-1083.2001.01333.x

Horton, T. R., Bruns, T. D., and Parker, V. T. (1999). Ectomycorrhizal fungi associated with Arctostaphylos contribute to *Pseudotsuga menziesii* establishment. *Can. J. Bot.* 77, 93–102. doi: 10.1139/b98-208

Ives, A. R., and Godfray, H. C. J. (2006). Phylogenetic analysis of trophic associations. *Am. Nat.* 168, E1–E14. doi: 10.1086/505157

Jeanmonod, D., and Gamisans, J. (2007). *Flora Corsica*. Aix-en-Provence: EdiSud.

Krasnov, B. R., Fortuna, M. A., Mouillot, D., Khokhlova, I. S., Shenbrot, G. I., and Poulin, R. (2012). Phylogenetic signal in module composition and species connectivity in compartmentalized host-parasite networks. *Am. Nat.* 179, 501–511. doi: 10.1086/664612

Kretzer, A. M., Bidartondo, M. I., Grubisha, L. C., Spatafora, J. W., Szaro, T. M., and Bruns, T. D. (2000). Regional specialization of *Sarcodes sanguinea* (Ericaceae) on a single fungal symbiont from the *Rhizopogon ellenae* (Rhizopogonaceae) species complex. *Am. J. Bot.* 87, 1778. doi: 10.2307/2656828

Last, F. T., Mason, P. A., Wilson, J., and Deacon, J. W. (1983). Fine roots and sheathing mycorrhizas: their formation, function and dynamics. *Plant Soil* 71, 9–21. doi: 10.1007/BF02182637

Latapy, M., Magnien, C., and Vecchio, N. D. (2008). Basic notions for the analysis of large two-mode networks. *Soc. Netw.* 30, 31–48. doi: 10.1016/j.socnet.2007.04.006

Martos, F., Munoz, F., Pailler, T., Kottke, I., Gonneau, C., and Selosse, M.-A. (2012). The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. *Mol. Ecol.* 21, 5098–5109. doi: 10.1111/j.1365-294X.2012.05692.x

Molina, R., Massicotte, H. B., and Trappe, J. M. (1992). "Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications," in *Mycorrhizal Functioning: An Integrated Plant Fungal Process*, ed. M. F. Allen (London: Chapman and Hall), 357–423.

Montesinos-Navarro, A., Segarra-Moragues, J. G., Valiente-Banuet, A., and Verdú, M. (2012). The network structure of plant-arbuscular mycorrhizal fungi. *New Phytol.* 194, 536–547. doi: 10.1111/j.1469-8137.2011.04045.x

Moreau, P.-A., Rochet, J., Richard, F., Chassagne, F., Manzi, S., and Gardes, M. (2011). Taxonomy of Alnus-associated hypogeous species of *Alpova* and *Melanogaster* (Basidiomycota, Paxillaceae) in Europe. *Cryptogam. Mycol.* 32, 33–62. doi: 10.7872/crym.v32.iss1.2012.033

Morris, M. H., Smith, M. E., Rizzo, D. M., Rejmánek, M., and Bledsoe, C. S. (2008). Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (*Quercus* spp.) in a California woodland. *New Phytol.* 178, 167–176. doi: 10.1111/j.1469-8137.2007.02348.x

Nacher, J. C., and Akutsu, T. (2011). On the degree distribution of projected networks mapped from bipartite networks. *Physica A* 390, 4636–4651. doi: 10.1016/j.physa.2011.06.073

Nara, K. (2006). Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytol.* 169, 169–178. doi: 10.1111/j.1469-8137.2005.01545.x

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., et al. (2015). *R Package Version, 2–3–0.*

Öpik, M., and Moora, M. (2012). Missing nodes and links in mycorrhizal networks. *New Phytol.* 194, 304–306. doi: 10.1111/j.1469-8137.2012.04121.x

Patefield, W. M. (1981). Algorithm AS159. An efficient method of generating r x c tables with given row and column totals. *Appl. Stat.* 30, 91–97. doi: 10.2307/2346669

Quézel, P., and Médail, F. (2003). *Ecologie et Biogéographie des Forêts du Bassin Méditerranéen*. Paris: Elsevier.

Rameau, J.-C., Mansion, D., Dumé, G., and Albert, C. H. (2008). *Flore Forestière Française Tome 3*. Paris: Institut pour le développement forestier.

Richard, F., Millot, S., Gardes, M., and Selosse, M.-A. (2005). Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*. *New Phytol.* 166, 1011–1023. doi: 10.1111/j.1469-8137.2005.01382.x

Richard, F., Moreau, P.-A., Selosse, M.-A., and Gardes, M. (2004). Diversity and fruiting patterns of ectomycorrhizal and saprobic fungi in an old-growth Mediterranean forest dominated by *Quercus ilex* L. *Can. J. Bot.* 82, 1711–1729. doi: 10.1139/b04-128

Richard, F., Selosse, M.-A., and Gardes, M. (2009). Facilitated establishment of *Quercus ilex* in shrub-dominated communities within a Mediterranean ecosystem: do mycorrhizal partners matter? *FEMS Microbiol. Ecol.* 68, 14–24. doi: 10.1111/j.1574-6941.2009.00646.x

Rochet, J., Moreau, P.-A., Manzi, S., and Gardes, M. (2011). Comparative phylogenies and host specialization in the alder ectomycorrhizal fungi *Alnicola*, *Alpova* and *Lactarius* (Basidiomycota) in Europe. *BMC Evol. Biol.* 11:40. doi: 10.1186/1471-2148-11-40

Roy, M., Dubois, M.-P., Proffit, M., Vincenot, L., Desmarais, E., and Selosse, M.-A. (2008). Evidence from population genetics that the ectomycorrhizal basidiomycete *Laccaria amethystina* is an actual multihost symbiont. *Mol. Ecol.* 17, 2825–2838. doi: 10.1111/j.1365-294X.2008.03790.x

Roy, M., Rochet, J., Manzi, S., Jargeat, P., Gryta, H., Moreau, P.-A., et al. (2013). What determines Alnus-associated ectomycorrhizal community diversity and specificity? A comparison of host and habitat effects at a regional scale. *New Phytol.* 198, 1228–1238. doi: 10.1111/nph.12212

Selosse, M.-A., Richard, F., He, X., and Simard, S. W. (2006). Mycorrhizal networks: des liaisons dangereuses? *Trends Ecol. Evol.* 21, 621–628. doi: 10.1016/j.tree.2006.07.003

Selosse, M.-A., and Roy, M. (2009). Green plants that feed on fungi: facts and questions about mixotrophy. *Trends Plant Sci.* 14, 64–70. doi: 10.1016/j.tplants.2008.11.004

Selosse, M.-A., WEIss, M., Jany, J.-L., and Tillier, A. (2002). Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) LCM Rich. and neighbouring tree ectomycorrhizae. *Mol. Ecol.* 11, 1831–1844. doi: 10.1046/j.1365-294X.2002.01553.x

Simard, S. W., and Durall, D. M. (2004). Mycorrhizal networks: a review of their extent, function, and importance. *Can. J. Bot.* 82, 1140–1165. doi: 10.1139/b04-116

Simard, S. W., Perry, D. A., Jones, M. D., Myrold, D. D., Durall, D. M., and Molina, R. (1997). Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388, 579–582. doi: 10.1038/41557

Smith, J. E., Molina, R., Huso, M. M., Luoma, D. L., McKay, D., Castellano, M. A., et al. (2002). Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon U.S.A. *Can. J. Bot.* 80, 186–204. doi: 10.1139/b02-003

Smith, S. E., and Read, D. J. (2008). *Mycorrhizal Symbiosis*. New York, NY: Elsevier/Academic Press.

Steinitz, O., Troupin, D., Vendramin, G. G., and Nathan, R. (2011). Genetic evidence for a Janzen-Connell recruitment pattern in reproductive offspring of *Pinus halepensis* trees. *Mol. Ecol.* 20, 4152–4164. doi: 10.1111/j.1365-294X.2011.05203.x

Thébault, E., and Fontaine, C. (2010). Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science* 329, 853–856. doi: 10.1126/science.1188321

Twieg, B. D., Durall, D. M., and Simard, S. W. (2007). Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytol.* 176, 437–447. doi: 10.1111/j.1469-8137.2007.02173.x

van der Heijden, M. G. A., and Horton, T. R. (2009). Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *J. Ecol.* 97, 1139–1150. doi: 10.1111/j.1365-2745.2009.01570.x

Visser, S. (1995). Ectomycorrhizal fungal succession in jack pine stands following wildfire. *New Phytol.* 129, 389–401. doi: 10.1111/j.1469-8137.1995.tb04309.x

Walker, J. F., Miller, O. K., and Horton, J. L. (2005). Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the southern Appalachian Mountains. *Mol. Ecol.* 14, 829–838. doi: 10.1111/j.1365-294X.2005.02455.x

Wu, Q. (2000). Phylogenetic and biogeographic relationships of eastern Asian and eastern North American disjunct *Suillus* species (fungi) as inferred from nuclear sibosomal RNA ITS Sequences. *Mol. Phylogenet. Evol.* 17, 37–47. doi: 10.1006/mpev.2000.0812

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

4 Manuscrit C : "Why two plant species share as many ectomycorrhizal species at regional scale? A history of phylogeny, coexistence but not climate."

In a Nutshell

Rationale: As explained in the manuscript B, the potential sharing of ectomycorrhizal (ECM) fungal species among plants species may influence the physiology and ecology of ECM plants through both CMN (Common Mycorrhizal Networsk) and EMN (Ecological Mycorrhizal Networks). The aim of this letter is to investigate the determinism of fungal sharing among ECM plant species.

Using the data from manuscript B, we test three hypothesis concerning the number of fungal partners shared among plants. We hypothesize that: **(i)** coexisting plants tend to display more similar interaction niches than non-coexisting ones; **(ii)** climatic variables driving plant community assembly may also drive ECM association; In this case, plant species living under closed climate environment may present more similar partners than we may expected by chance; **(iii)** there is a phylogenetic conservatism in ECM associations, *i.e.* the most two plants are phylogenetically related, the most they share fungal partners.

Type of interaction: Ectomycorrhizal symbiosis at regional scale, mostly mutualistic

Ecological filters of fungal communities: Host phylogenetic relationship; Climatic variable endured by plant host; Level of ECM plant co-occurrence at regional scale

Main conclusions: Co-occurring plants species share more partners than expected by chance at the regional scale (*hyp. 1*). This pattern is not explained by the climatic domain shared by co-occurring plants (*hyp. 2*). Plant evolutionary history drives ECM associations at the regional scale (*hyp. 3*).

Why two plant species share as many ectomycorrhizal species at regional scale? A history of phylogeny, coexistence but not climatic environment.

Taudière A.[1, 2]***, Richard F*** [2]***., Lesne A.***[3, 4]***, Munoz F.***[5, 6]

1/ École Pratique des Hautes Études (EPHE) & Paris Research University, Paris, France; adrien.taudiere@cefe.cnrs.fr

2/ UMR 5175, CEFE – CNRS – Université de Montpellier, Montpellier, France

3/ CNRS, LPTMC UMR 7600, Université Pierre et Marie Curie-Paris 6, Sorbonne Universités, Paris, France

4/ CNRS, IGMM UMR 5535, Université de Montpellier, Montpellier, France

5/ UM2, UMR AMAP, Montpellier, France

6/ French Institute of Pondicherry, Pondicherry, India

Introduction

Ectomycorrhizal (ECM) symbiosis determines the functioning of many forest ecosystems, particularly in temperate and boreal biomes. Fungal partners explore soil and yield nutriment and water to the host in exchange of carbon (Smith & Read 2008). ECM fungal species are able to physically connect ECM plants through common mycorrhizal networks (CMN; reviewed in Simard *et al.* 2012), which allow carbon, nutrient transfer (Simard *et al.* 1997, Selosse *et al.* 2006) and stress signaling (Song *et al.* 2015). Moreover, ECM symbiosis is crucial during seed establishment and ecological succession. Beyond the possibility to make physical links among individual, sharing of ECM fungal species can mediate plant-plant positive interactions in stressful ecosystems by providing local symbiont inoculum to seedlings (Richard *et al.* 2009; Teste *et al.* 2009). The number and identity of ECM partners of a plant (interaction specialization) broadly varies at both local (e.g., Taschen *et al.* 2015) and global scale (e.g. den Bakker *et al.* 2004, Taudière *et al.* 2015), and thus represents various "interaction niches". Such variation in interaction specialization is expected to be related to the ecological amplitude of plants (ecological specialization) by determining their ability to establish ECM symbiosis and CMN in diverse environmental contexts (Molina & Horton 2015). However, how the architecture of ECM partnership relates to plant niche diversity and drives their community assembly dynamics still needs investigation. We here address whether plant coexisting in a same communities display similar or dissimilar fundamental interaction niches, i.e., whether they can share fungal partners.

In a given community, niche variation among coexisting plants is expected to reflect the assembly rules driving community dynamics (Weiher and Keddy 1995). We expect that these assembly rules also influence the similarity of interaction niches among coexisting ECM plants. Three basic processes can influence the similarity of interaction niches of ECM plants (Table 1). (i) If plants are in competition for the resources provided by ECM partners, we expect that coexisting plants display dissimilar interaction niches (Hyp. 1 of limiting similarity, Abrams 1983). In case of establishment facilitation *via* ECM, positive interaction conversely entails that coexisting plants should display similar interaction niches (Hyp. 1bis). (ii) Some local environmental variables can exert consistent filtering on plants species and concomitantly on fungal associates (Hyp. 2 of habitat filtering, Ackerly & Cornwell 2007). As ECM fungal species also respond differentially to environmental variables (e.g., Courty *et al.* 2016), coexisting plants may have more similar interaction niches due to the dual filtering of environment on plant and fungal species. (iii) We expect phylogenetic conservatism in ECM association, which means that related plants are more likely to share symbionts (Hyp. 3 of niche conservatism, Wiens *et al.* 2010). To test whether the degree of partners sharing among ECM plants at regional scale (i.e., the overlap of fundamental interaction niches of plant species) is related to host environment, evolutionary relationship or coexistence (Table 1), we studied the diversity of fundamental interaction niches among ECM plants in Corsica, France (993 interactions among 16 plants and 411 fungal taxa; more details in Taudiere *et al.* [2015]).

Hyp. 1: Coexisting plants tend to display more similar interaction niches than non-coexisting ones

The effect of ECM symbiosis on resource partitioning among plants is a critical but neglected aspect of plant community dynamics, which may strongly influence the outcome of plant-plant interactions (Selosse *et al.* 2006; Bever *et al.* 2010). The fundamental interaction niche with ECM fungal symbiont is a part of the fundamental plant niche related to resource use abilities, and it can therefore mediate competitive exclusion among plants. Moreover, ECM fungal species exhibit varying functional traits, including enzymatic activities (Courty *et al.* 2016) and soil exploration strategies (Agerer, 2001). We then expected that frequently co-occurring plant species should display more dissimilar interaction niches under the influence of limiting similarity (Hyp. 1). On the contrary, as seedlings establishment may be favored by the presence of ECM fungal inoculum, frequently co-occurring plants may harbor more similar ECM fungal diversity (facilitation, Hyp. 1bis). Montesinos-Navarro *et al.* (2012) previously proposed that shared endomycorrhizal symbionts can mediate plant-plant positive interactions in stressful ecosystems (also discussed in Valiente-Banuet & Verdu, 2013). We tested the hypotheses 1 and 1bis by performing a Mantel test (Mantel, 1967) between the Jaccard distances among plant communities and the matrix of the plant fungal partners (plants species × fungal symbionts). We found that coexisting plants tend to share more ECM fungal partners (Mantel test; 9 999 permutations; p-value = 0.0079).

Hyp. 2: Climatic filtering drives plant assembly, but does not relate to their ECM interaction niches

The second hypothesis relies on the idea that a ECM plant species can grow in a specific habitat and that its ECM fungal partners are also specialized to this habitat (ecological specificity *sensu* Molina & Horton, 2015). Some environmental variables, such as precipitation and temperature, are known to affect the distribution of both plants (e.g. Siefert *et al.* 2015) and ECM fungal species (e.g. Tedersoo *et al.* 2012). We therefore expect that plant species living in similar habitat share more ECM fungal partners than plants living in dissimilar habitats. We performed RLQ analysis (Dolédec 1996; Dray *et al.* 2014; see Courty *et al.* 2016 for an RLQ on ECM symbiosis) to decipher the statistical link between (i) a matrix of plant communities (SOPHY database; 1698 georeferences communities across Corsica island; de Ruffray *et al.* 1989), (ii) the climatic conditions in sampled locations (Worldclim data; Hijmans *et al.* 2004) and (iii) the corteges of ECM fungal partners of plants (data from Taudiere *et al.* 2015). This analysis somehow allow to infer the boundary of the fundamental climatic niche of ECM fungi through the distribution of their hosts.

Climatic variables drive plant community variation mostly along an altitudinal gradient (Fig. 2; p-value of null model 2 using 9 999 permutations in RLQ > 0.0001). However, climatic variables do not explain the potential fungal partners of plants (p-value of null model 4 in RLQ = 0.4828). Therefore, climatic habitat filtering drives plant community assembly but does not translate into distinct ECM interaction niches. Therefore, more similar interaction niches among coexisting plants is not explained by common climatic habitat filtering.

Hyp. 3: Phylogenetic conservatism in the ECM symbiosis influences interaction niches of coexisting plants

Phylogenetic conservatism in the ECM symbiosis is well known (e.g. Matheny *et al.* 2009). The phylogenetic scale of specificity range from intrageneric (e.g. *Tuber melanosporum* on *Quercus ilex* but not on *Q. coccifera*, Taschen *et al.* 2015; difference among *Gnetum* species, Tedersoo & Polme, 2012) to family level (e.g. *Leccinum spp* with Betulaceae/Salicaceae, den Bakker *et al.* 2004; *Suillus spp* on Pinaceae, Wu *et al.* 2000) and even higher taxonomic levels (Angiosperms *vs* Gymnosperms, Matheny *et al.* 2009). We thus hypothesized that plant phylogeny is related to ECM partner sharing. We addressed the extent of the relationship and the variation among plant clades by applying the method of Pavoine *et al.* (2010) to decompose the phylogenetic distance among plants in terms of ECM associations (Jaccard distance on the binary interaction matrix). The plant phylogeny was built using APG III (2006) and Timetree; Hedges *et al.* 2006). We used permutation scheme as in Taudiere *et al.* (2015) to compare the decomposition against the null hypothesis of no phylogenetic conservatism (999 permutations of the association matrix keeping constant the sums of rows and columns).

Overall, plants phylogenetically closer share more partners than expected by chance (Fig. 3; Spearman correlation test p-value = 0.0012, rho = 0.81). Plant species whose most common ancestor is older than 250 million years interact with more dissimilar ECM fungal partners than two plants choose at random (Fig. 3). On the contrary, plant species belonging to the same genus (Pinus, Quercus and Alnus) share far more partners than two plants at random. Even some young families (e.g. Facaceae) show the same trends. Two phylogenetic nodes are particularly interesting. The two Pinus species share far more fungal partners than expected by chance. On the contrary, a drastic shift in ECM fungal communities was apparent between Fagaceae and Betulaceae (node I3).

Despite the phylogenetic signal of interactions and the fact that coexisting plants tend to have more similar interaction niches, we did not find any correlation between phylogenetic distance and community distance (Mantel test; 9 999 permutations; p-value = 0.88).

Discussion and perspectives

Further analysis must test if individual locally share fungal species (realized interaction niches) or if it is only a regional pattern (fundamental interaction niches). Bogar & Kennedy (2012) showed in a bioassay that established *Alnus rhombifolia* mediate the ECM fungal community structure of *Betula occidentalis*, but further analysis with more species are needed to identify the scale of the pattern of ECM preferential association mediate by coexistence. Moreover, other environmental variables, in particular soil ones (e.g. N, P, organic matter, soil texture ...) may drive the fungal niche more than climatic ones.

References

Abrams P. **1983**. The Theory of Limiting Similarity. *Annual Review of Ecology and Systematics* **14**: 359–376.

Ackerly DD, Cornwell WK. **2007**. A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. *Ecology Letters* **10**: 135–145.

Agerer R. **2001**. Exploration types of ectomycorrhizae. *Mycorrhiza* **11**: 107–114.

den Bakker HC, Zuccarello GC, Kuyper TW, Noordeloos ME. **2004**. Evolution and host specificity in the ectomycorrhizal genus *Leccinum*. *New Phytologist* **163**: 201–215.

Bärlocher F, Boddy L. **2016**. Aquatic fungal ecology – How does it differ from terrestrial? *Fungal Ecology* **19**: 5–13.

Bever JD, Dickie IA, Facelli E, Facelli JM, Klironomos J, Moora M, Rillig MC, Stock WD, Tibbett M, Zobel M. **2010**. Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution* **25**: 468–478.

Bogar LM, Kennedy PG. **2013**. New wrinkles in an old paradigm: neighborhood effects can modify the structure and specificity of Alnus-associated ectomycorrhizal fungal communities. *FEMS Microbiology Ecology* **83**: 767–777.

Dolédec S, Chessel D, Braak CJF ter, Champely S. **1996**. Matching species traits to environmental variables: a new three-table ordination method. *Environmental and Ecological Statistics* **3**: 143–166.

Dray S, Choler P, Dolédec S, Peres-Neto PR, Thuiller W, Pavoine S, ter Braak CJ. **2014**. Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. *Ecology* **95**: 14–21.

Friedman J, Alm EJ. **2012**. Inferring Correlation Networks from Genomic Survey Data. *PLOS Comput Biol* **8**: e1002687.

Hedges SB, Dudley J, Kumar S. **2006**. TimeTree: a public knowledge-base of divergence times among organisms. *Bioinformatics* **22**: 2971–2972.

McIntire EJB, Fajardo A. **2014**. Facilitation as a ubiquitous driver of biodiversity. *New Phytologist* **201**: 403–416.

Molina R, Massicotte HB, Trappe JM. **1992**. Specificity phenomena in mycorrhizal symbioses: Community-ecological consequences and practical implications. *In:* Mycorrhizal functioning: an integrative plant-fungal process. London - New York: Allen M., 357–423.

Montesinos-Navarro A, Segarra-Moragues JG, Valiente-Banuet A, Verdú M. **2012**. The network structure of plant-arbuscular mycorrhizal fungi. *New Phytologist* **194**: 536–547.

Morueta-Holme N, Blonder B, Sandel B, McGill BJ, Peet RK, Ott JE, Violle C, Enquist BJ, Jørgensen PM, Svenning J-C. **2015**. A network approach for inferring species associations from co-occurrence data. *Ecography*

Pavoine S, Baguette M, Bonsall MB. **2010**. Decomposition of trait diversity among the nodes of a phylogenetic tree. *Ecological Monographs* **80**: 485–507.

Selosse M-A, Richard F, He X, Simard SW. **2006**. Mycorrhizal networks: des liaisons dangereuses? *Trends in Ecology & Evolution* **21**: 621–628.

Siefert A, Lesser MR, Fridley JD. **2015**. How do climate and dispersal traits limit ranges of tree species along latitudinal and elevational gradients? *Global Ecology and Biogeography*

Simard SW, Beiler KJ, Bingham MA, Deslippe JR, Philip LJ, Teste FP. **2012**. Mycorrhizal networks: Mechanisms, ecology and modelling. *Fungal Biology Reviews* **26**: 39–60.

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R. **1997**. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **388**: 579–582.

Smith SE, Read DJ. **2008**. *Mycorrhizal Symbiosis*. Academic Press.

Song YY, Simard SW, Carroll A, Mohn WW, Zeng RS. **2015**. Defoliation of interior Douglas-fir elicits carbon transfer and stress signalling to ponderosa pine neighbors through ectomycorrhizal networks. *Scientific Reports* **5**.

Taschen E, Sauve M, Taudiere A, Parlade J, Selosse M-A, Richard F. **2015**. Whose truffle is this? Distribution patterns of ectomycorrhizal fungal diversity in *Tuber melanosporum* brûlés developed in multi-host Mediterranean plant communities. *Environmental Microbiology* **17**: 2747–2761.

Taudiere A, Munoz F, Lesne A, Monnet A-C, Bellanger J-M, Selosse M-A, Moreau P-A, Richard F. **2015**. Beyond ectomycorrhizal bipartite networks: projected networks demonstrate contrasted patterns between early- and late-successional plants in Corsica. *Frontiers in Plant Science* **6**.

Tedersoo L, Bahram M, Toots M, DiéDhiou AG, Henkel TW, KjøLler R, Morris MH, Nara K, Nouhra E, Peay KG, *et al.* **2012**. Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology* **21**: 4160–4170.

Teste FP, Simard SW, Durall DM. **2009**. Role of mycorrhizal networks and tree proximity in ectomycorrhizal colonization of planted seedlings. *Fungal Ecology* **2**: 21–30.

The Angiosperm Phylogeny Group. **2009**. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**: 105–121.

Valiente-Banuet A, Verdú M. **2013**. Plant Facilitation and Phylogenetics. *Annual Review of Ecology, Evolution, and Systematics* **44**: 347–366.

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. **2015**. The importance of the microbiome of the plant holobiont. *New Phytologist* **206**: 1196–1206.

Weiher E, Keddy PA. **1995**. Assembly Rules, Null Models, and Trait Dispersion: New Questions from Old Patterns. *Oikos* **74**: 159–164.

Weiher E, Keddy P. **2001**. *Ecological Assembly Rules: Perspectives, Advances, Retreats.* Cambridge University Press.

Wiens JJ, Ackerly DD, Allen AP, Anacker BL, Buckley LB, Cornell HV, Damschen EI, Jonathan Davies T, Grytnes J-A, Harrison SP, *et al.* **2010**. Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters* **13**: 1310–1324.

Wu Q. **2000**. Phylogenetic and biogeographic relationships of eastern Asian and eastern north American disjunct *Suillus* species (fungi) as inferred from nuclear ribosomal RNA ITS sequences. *Molecular Phylogenetics and Evolution* **17**: 37–47.

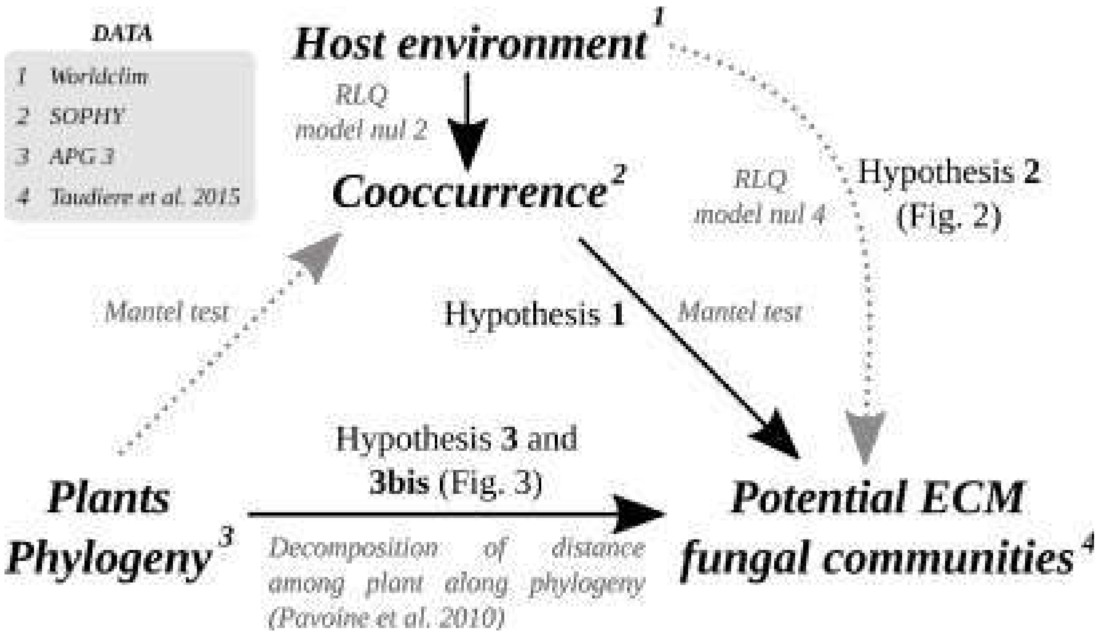


Figure 1: Schematic overview of hypothesis regarding the degree of partners sharing among ECM plants. Hypothesis are described in table 1. Numbers correspond to data used in the analysis.

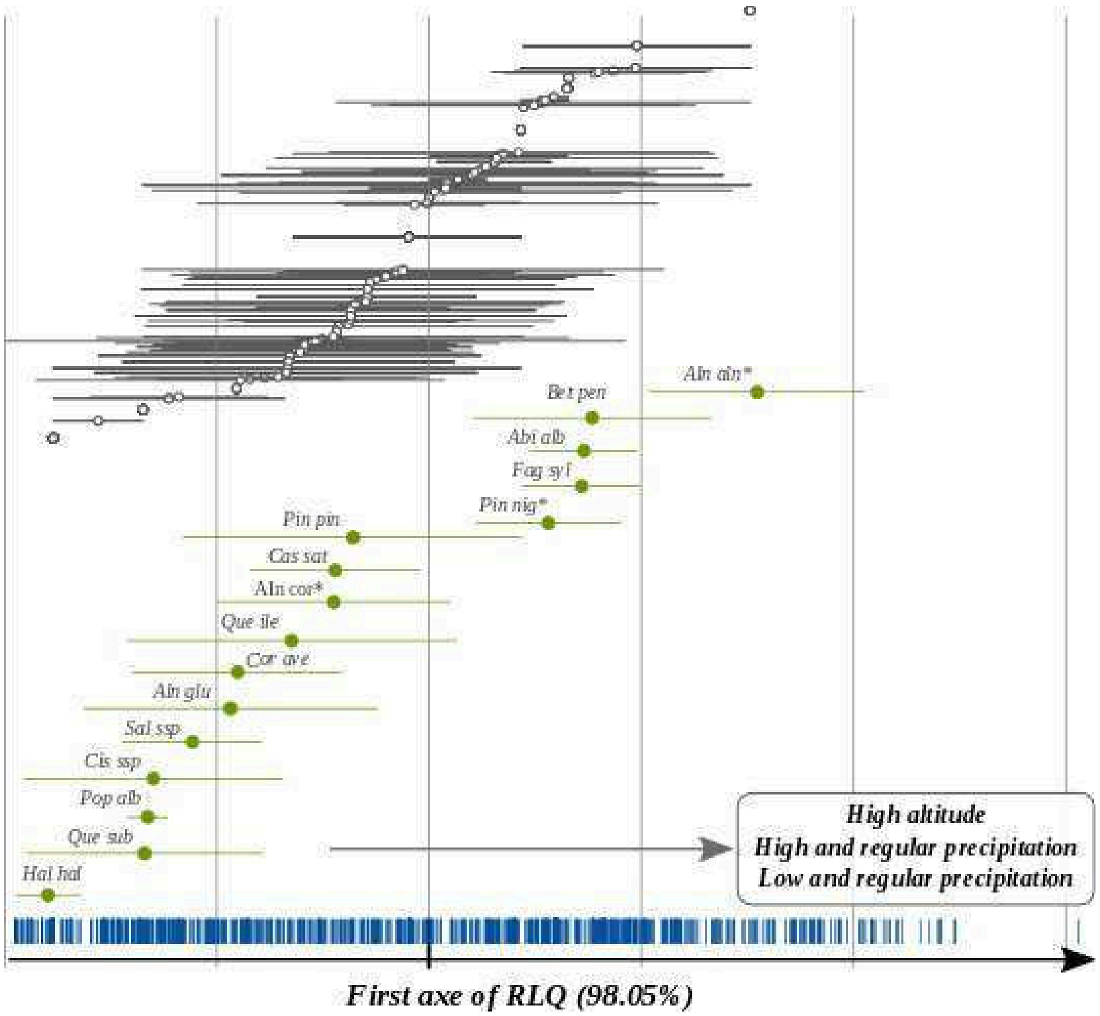


Fig. 2: Position of communities (blue bars), plants (green) and ECM fungal symbionts (grey) on the first axe of the RLQ analysis (98.05% of variation). Detailed results are available in figure S1. Plants names are abbreviated: *Abi alb*: *Abies alba*; *Aln aln*: *Alnus alnicola* subsp *suaveolens*; *Aln cor*: *Alnus cordata*; *Aln glu*: *Alnus glutinosa*; *Bet pen*: *Betula pendula*; *Cas sat*: *Castanea sativa*; *Cis spp: Cistus spp; Cor ave*: *Corylus avelana*; *Fag syl*: *Fagus sylvatica*; *Hal hal*: *Halimium halimifolium*; *Pin nig*: *Pinus nigra* subsp *laricio*; *Pin pin*: *Pinus pinaster*; *Pop alb*: *Populus alba*; *Que ile*: *Quercus ilex; Que sub*: *Quercus suber, Sal spp*: *Salix spp.*

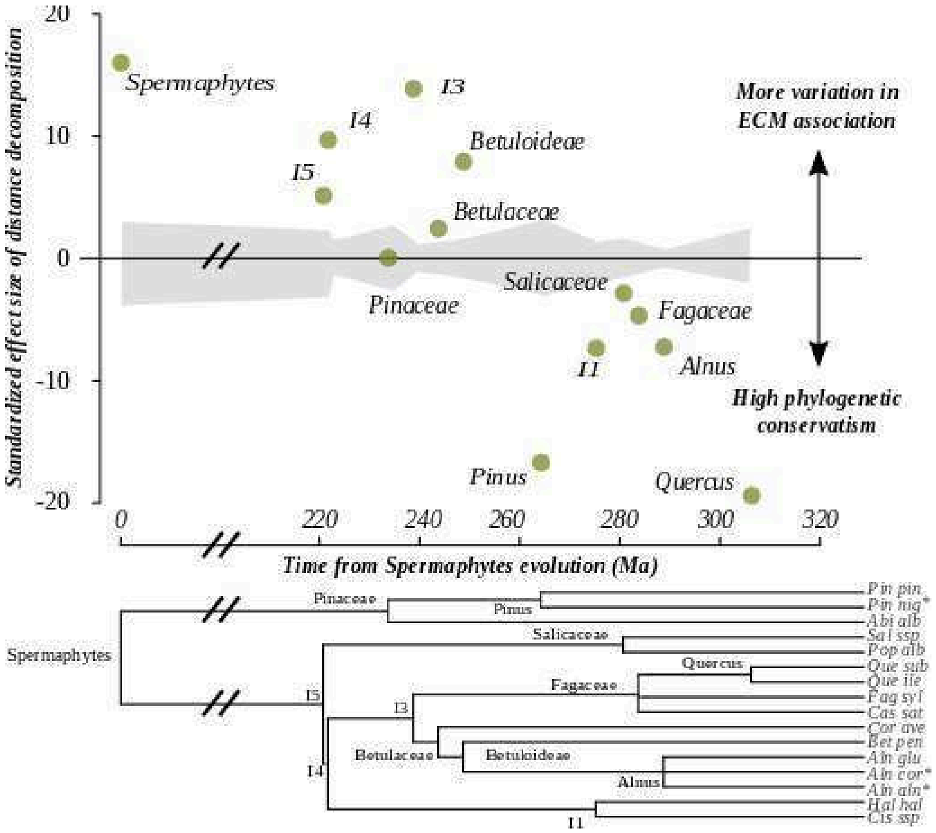


Fig. 3: Decomposition along the phylogeny of the distance among plants considering their fungal partners. Same abbreviations as figure 2. Gray shade indicate null model area with confidence interval equal to 0.025-0.975.

Table 1: Hypothesis invoked to explain the degree of partners sharing by ECM plants.

References: [1] Dray et al. 2014; [2] Pavoine et al. 2010; [3] Mantel, 1967

Hypothesis	Alternative hypothesis	Methods and *R functions*
1. The more two plants species coexist, the less they share ECM species - **limiting similarity**	1bis. The more two plants species coexist, the more they share ECM species – **facilitation**	Mantel [3] (bilateral) *mantel.test* (package ape)
2. The more two plants species live in similar abiotic habitat, the more they share ECM species - **environmental filtering of fungal species**	ECM fungal communities are independent of host environment	RLQ [1] (unilateral) *rlq* (package ade4)
3. The more two plants species are phylogenetically related, the more they share ECM species - **phylogenetic interaction conservatism**	ECM fungal communities are independent of host evolutionary history	Decomposition of distance along phylogeny [2] (unilateral)

Box 1: Glossary

- ***Common mycorrhizal network (CMN):*** Below-ground fungal mycelia which physically connect roots of different plant individuals.
- ***Ecological specialization:*** "The process by which an organism adapts to an increasingly narrow subset of its possible environments and persists in an increasingly narrow range of habitats." (Poisot *et al.* 2012)
- ***Fundamental niche:*** "The ensemble of abiotic environments in which a population can persist without external immigration, when not limited by habitat size or biotic interactions." (Poisot *et al.* 2012)
- ***Interaction niches:*** The part of the niche space that is dependent of interactions. In this paper, we focus on the area of ECM plant niches relying to their potential fungal partners (fundamental interaction niche).
- ***Interaction specialization:*** Tendency to interact with few or lot of partners. In the case of the ECM interaction, fungal interaction specialization is often called host-specificity of the fungal species in mycological literature.
- ***Limiting similarity:*** "The limiting similarity approach looks to see whether pairs of species, that are judged to be similar in niche, co-occur less often that one would expect on a random basis." (Weiher & Keddy 2001)
- ***Niche conservatism***: "The retention of niche-related ecological traits over time." (Wiens *et al.* 2010)
- ***Realized niche:*** "Limitations to, or extension of, the fundamental niche resulting from species interactions, chance events and history." (Poisot *et al.* 2012)

Supplementary materials:

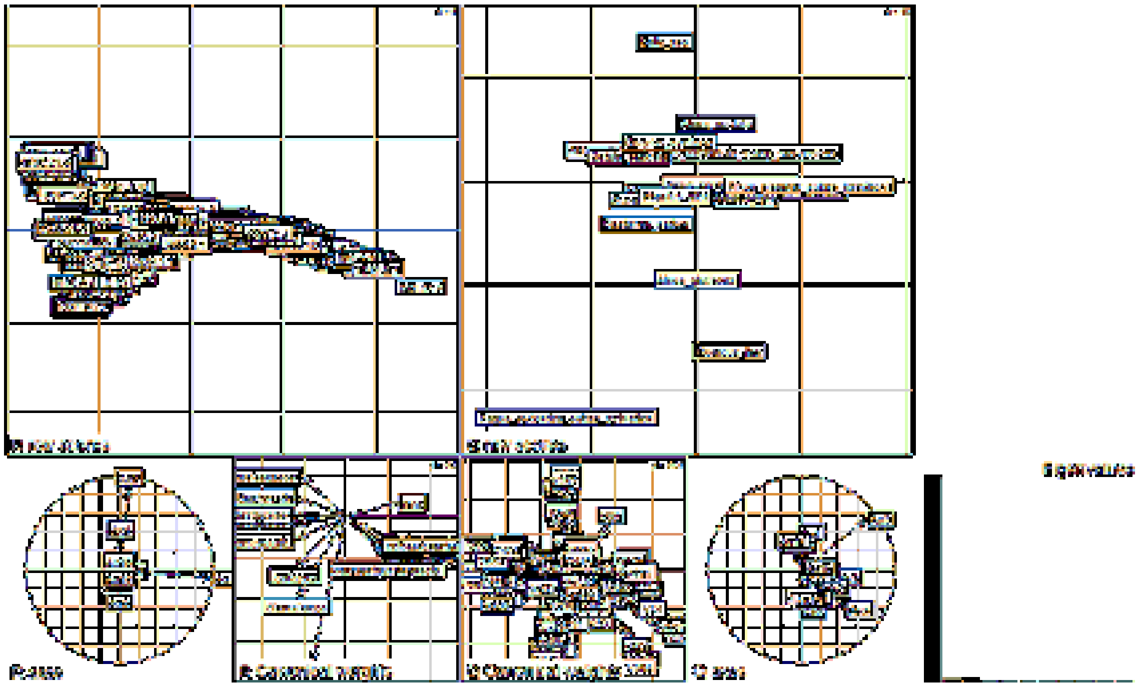
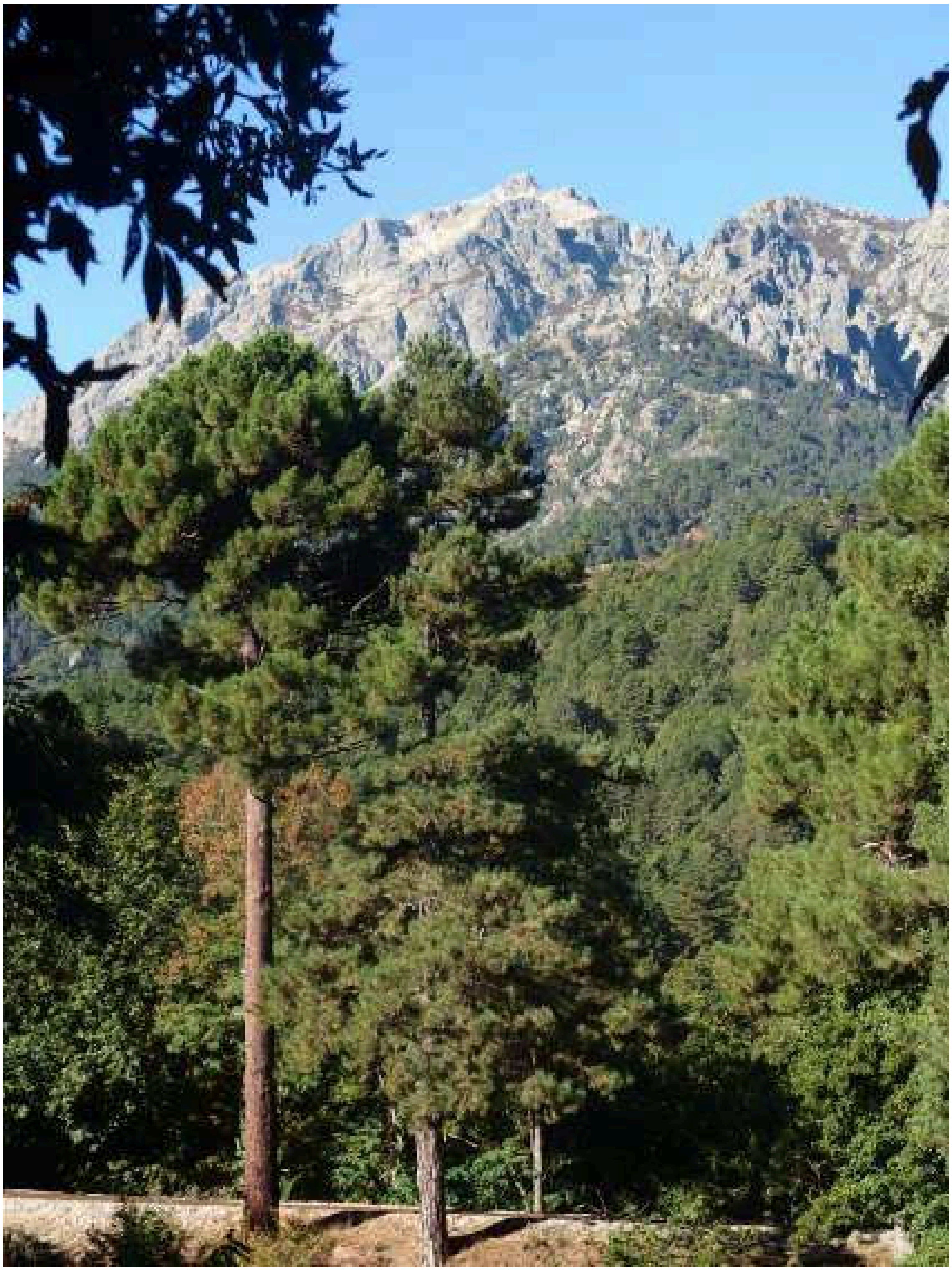


Fig. S1: Plot of RLQ results. V1 to V411 correspond to fungal taxa. Climatic variables are detailed in the worldclim site (http://worldclim.org/).

5 "Effect of host taxonomy and coexistence on ectomycorrhizal fungal communities" (Projet de manuscrit D)



Coexistence d'un pin maritime et d'un pin laricio devant le Monte d'Oro.

IN A NUTSHELL

This is a work in progress. See section works progress and draft D in appendix ⚙.

Rationale: In the manuscripts B and C, we explore the ectomycorrhizal interactions network in the ecological domain of *Pinus nigra* subsp. *laricio*, i.e. in supramediterranean and montaeouse belts of Corsica. In this work, interactions between ECM hosts and their symbionts are defined as the potential for a plant species p to interact with a fungal species f somewhere in Corsica and sometimes. Under this scenario, interacting species at the scale of Corsica define *potentiality* of interaction at local scale (*akin* fundamental niche). The realized ECM interactions (realized interaction niche) among individuals at local scale depends on the ECM network at the scale of Corsica (fundamental interaction niche), but may differ due to local characteristics and drift.

Here, we characterize the ECM communities of three co-occurring tree species (*Fagus sylvatica*, *Pinus pinaster* and *Pinus nigra* subsp *laricio*) at three different scales (Fig. III.1): (i) regional scale, and EMN of Corsica (*cf.* manuscript B), (ii) forest patch (1/4 ha), and the potential for the realized ECM interaction (CMN) based on the inoculum present in the soil surrounding tree roots and (iii) intermingled root fragments, and the CMN among alive root tips at a given time. The second and third scales of analysis were compared between contexts of monospecific and mixed forests (*Fagus sylvatica* – *Pinus nigra* and *Pinus nigra* – *Pinus pinaster*). In mixed forests, we sampled roots of co-occurring species in the same soil cors in order to document the effect of plant coexistence on ECM root tip fungal communities at fine scale (Number of root tips indicated in the table III.1). In this thesis, we only introduce preliminary results concerning the second and third scales of analysis.

Type of interaction: Local ectomycorrhizal symbiosis, mostly mutualistic

Ecological filters of fungal communities: Host at the local scale; Difference between local available inoculum and active ECM root tips; Effect of species coexistence on association patterns and networking among tree individuals.

Main results:

- *Soil ECM $alpha$-diversity is not affected by ECM vegetation composition*

At the forest patch, ECM fungal diversity indicators are identical whatever the forest tree composition (Fig. III.2; adjusted p-values > 0.05 using Tuckey test). In particular, mixed forests display the same ECM diversity than pure stands.

- *ECM vegetation drives soil ECM community composition*

Soil ECM communities are shaped by sites and host identity ((Fig. III.3; Table III.2). Sites and interactions between sites and host also drive significant shifts in ECM soil communities (PerManova; Table III.2). Moreover, soil ECM fungal assemblages of mixed *Fagus-Pinus* forests are intermediary between fungal communities of pure *Fagus* and pure *Pinus* forests.

Main conclusions: Coexistence of ECM plant species in mixed forests affects the composition but not the diversity of soil ECM fungal communities.

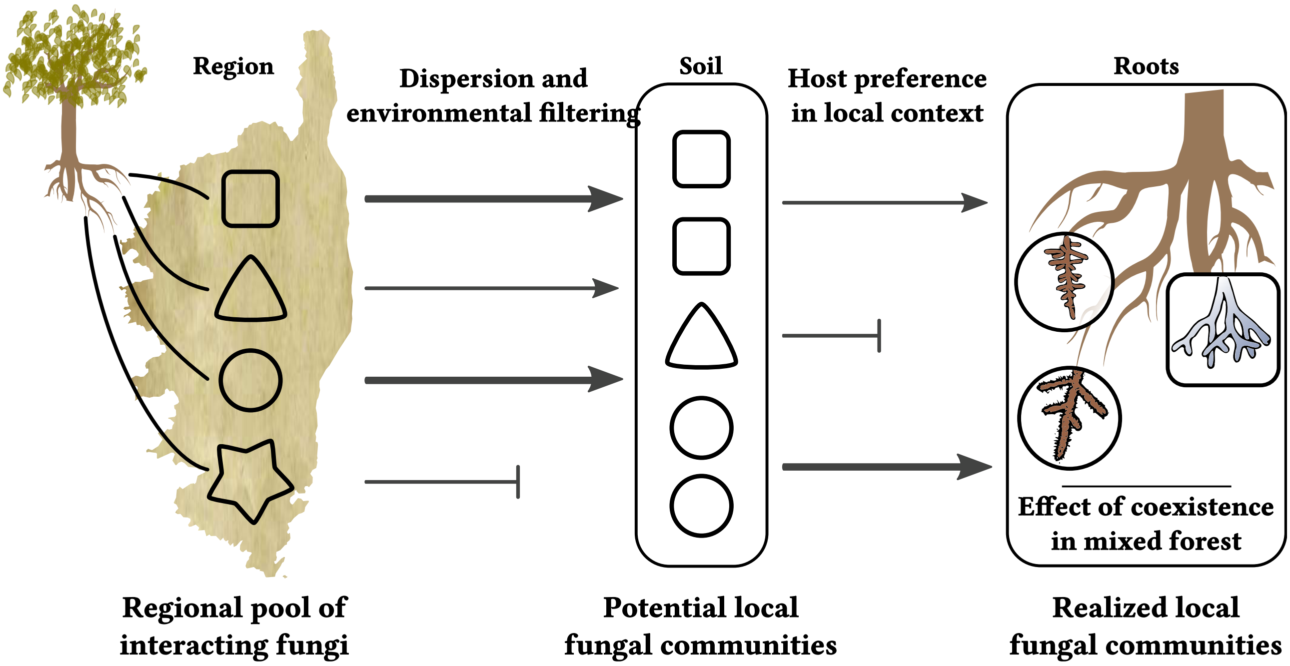


Figure III.1 – Schematic view of fungal communities interacting with plant at different scales: Shapes indicate fungal species. Arrow width are proportional to the relative abundance of each species pass through ecological filters (see Koide *et al.* 2011 for a discussion on those ecological filters). Note that in this study the network as regional pool depict qualitative interactions whereas soil and root tip composition is quantitative (or semi-quantitative; see discussion) thanks to the molecular abundances.

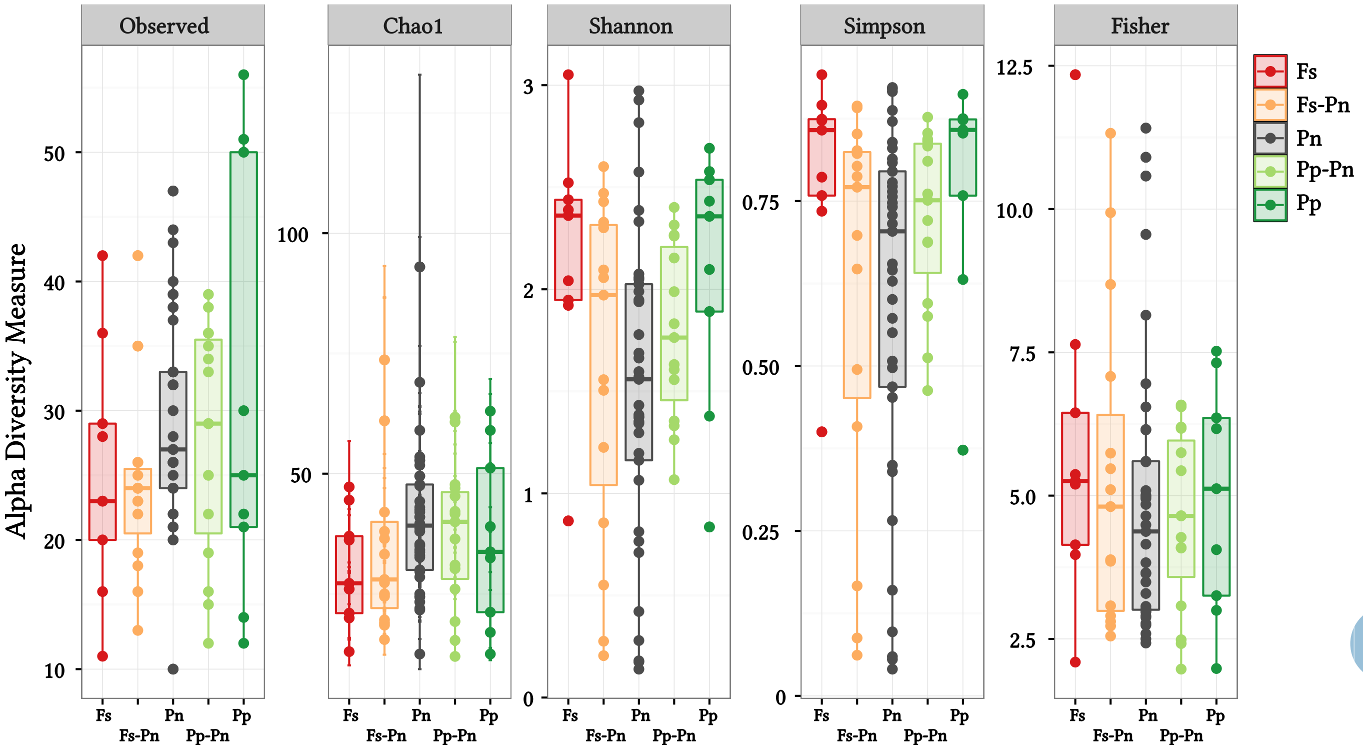


Figure III.2 – Diversity of ECM taxa in soil regarding forest composition: Abbreviations : Fs: *Fagus sylvatica*; Pn: *Pinus nigra* subsp. *laricio*; Pp: *Pinus pinaster*.

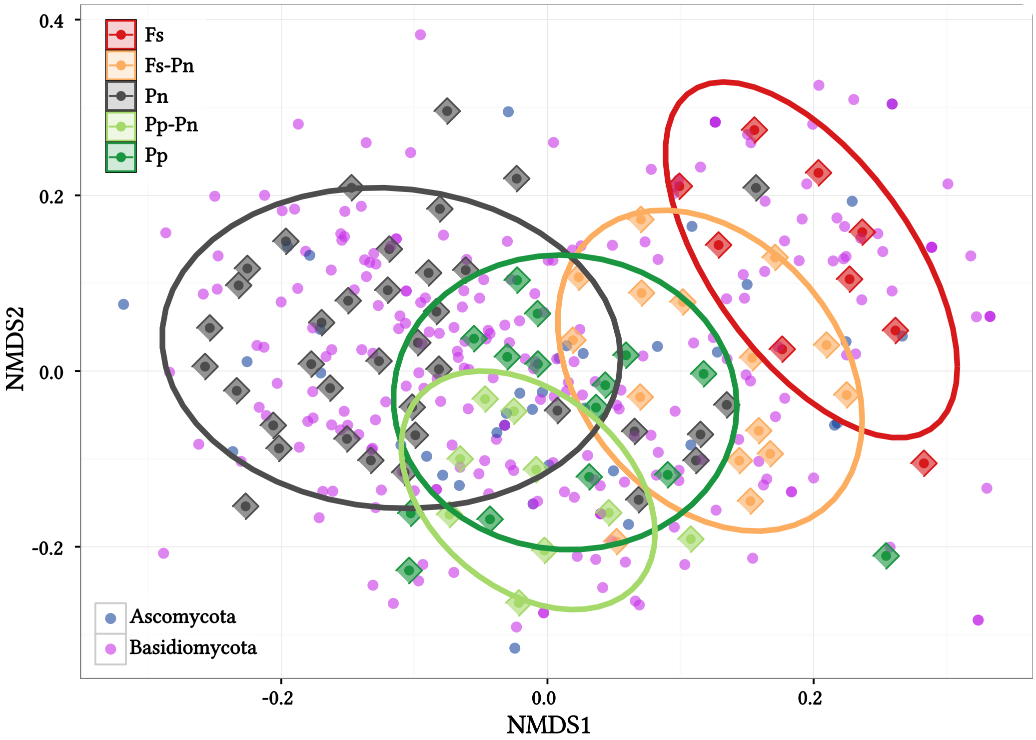


Figure III.3 – Non metric multidimensional scaling (Bray distances) showing the difference in ECM fungal composition in soil in function of surrounding host: Stress is equal to 29.5%. Abbreviations : Fs: *Fagus sylvatica*; Pn: *Pinus nigra* subsp. *laricio*; Pp: *Pinus pinaster*.

Table III.1 – Number of sites, plots, soil cores and samples for each modalities.

ECM tree species	Sites	Plots	Cores	Samples	Mycorrhizas
F. sylvatica	3	9	45	9	1800
P. nigra subsp. *laricio*	4	12	60	43	8600
P. pinaster	3	9	45	9	1800
F. sylvatica – P. nigra	3	3	15	15	∼4 500
P. pinaster – P. nigra	3	3	15	15	∼4 500

Table III.2 – Decomposition of soil ECM fungal communities in function of host and sites: PerManova analysis (Anderson 2001) using 9 999 permutations.

Variables	Df	Sums Of Sqs	MeanSqs	F.Model	R2	Pr(>F)
Host	4	3.430	0.85748	2.0514	0.08888	0.0001
Sites	5	3.280	0.65598	1.5693	0.08499	0.0001
Host × Sites	6	3.039	0.50654	1.2118	0.07875	0.0034
Residuals	69	28.842	0.41800		0.74738	
Total	84	38.591			1.0000	

6 Manuscrit E : "Using ectomycorrhizal bipartite networks for the conservation of fungal diversity: an indices-based approach"

IN A NUTSHELL

Rationale: As compared to that of plants, the conservation of fungal diversity meets difficulties to become operational. In particular, our poor knowledge of the high diversity of ectomycorrhizal (ECM) fungi contrasts with the importance of their ecological role in forests.

In this letter, we propose a framework of indicators to measure conservation values of ECM trees regarding their place and their role in ECM networks in Corsica, i.e. under a systemic perspective. Systemic conservation values of ECM trees in Corsica were calculated, and compared to classical plant-centered indicators.

Type of interaction: Ectomycorrhizal symbiosis, mostly mutualistic

Main conclusions: At the tree species level, classical conservation metrics such as rarity and phylogenetic originality are negatively correlated with systemic conservation values. On the other hand, current European programs of conservation take into account the most important tree species regarding their role in ECM networks and ecosystem functioning based on this symbiosis. Network metrics allow implementing plant-centered indicators to protect ECM fungi and ecosystems functions relying on ECM symbiosis.

Using ectomycorrhizal bipartite networks for the conservation of fungal diversity: an indices-based approach

Adrien Taudiere[1, 2]*, Annick Lesne[3, 4] Christopher Carcaillet[1, 5], François Munoz[6, 7], Laetitia Hugot[8] and Franck Richard[2]*

1/ École Pratique des Hautes Études (EPHE) & Paris Research University, Paris, France; adrien.taudiere@cefe.cnrs.fr

2/ UMR 5175, CEFE – CNRS – Université de Montpellier, Montpellier, France

3/ CNRS, LPTMC UMR 7600, Université Pierre et Marie Curie-Paris 6, Sorbonne Universités, Paris, France

4/ CNRS, IGMM UMR 5535, Université de Montpellier, Montpellier, France

5/ Laboratoire d'Écologie des Hydrosystèmes Naturels et Anthropisés (UMR5023 CNRS), Université Lyon 1, 69622 Villeurbanne, France; christopher.carcaillet@ephe.sorbonne.fr

6/ UM2, UMR AMAP, Montpellier, France

7/ French Institute of Pondicherry, Pondicherry, India

8/ Conservatoire Botanique de Corse, Route d'Aleria, 20250 Corte ; Laetitia.Hugot@oec.fr

** Corresponding authors:* ***adrien.taudiere@cefe.cnrs.fr; franck.richard@cefe.cnrs.fr***

Integrating the conservation of interactions in management to protect more than species

The emergency to protect biodiversity and the ecosystems functions and services provided to human societies is no longer contested. However, the extraordinary diversity of species forming most biocoenoses—in particular when considering poorly-known biota such as fungi (Hawksworth 2001)—raises important questions and challenges for conservation ecologists about the best way to define conservation strategies and prioritize efforts which may take into account as many species as possible.

If species-centered approaches have been successfully used for many organisms, including animals and plants (e.g. Habitat Directive 92/43, Bird Directive 79/409, National Red Lists), the conservation of species rich and insufficiently described lineages accommodates little with this approach. For fungi, species-based approaches are particularly complex to implement, because knowing the distribution of their vegetative individuals (mycelia) is currently out of reach. Moreover, the taxonomy within many fungal groups is in process of being further clarified (Money 2013), including the cases of macromycetes among the most conspicuous ones (e.g. morels, Loizides et al. 2015). More, and from a practical perspective, species-based approach are somehow unrealistic when considering the small number of field mycologists that are currently in charge of species data recording (e.g. regional datasets, atlas) in public institutes devoted to biodiversity conservation, as compared to that of botanists.

To tackle this issue in a context of urgent need of tools for landscape stakeholders and of unprecedented threaten on fungi (see Heilmann-Clausen and Christensen 2005 for saproxylic fungi), alternative conservation approaches transcending species level can be thought, including those which consider phylogeny as possible proxy of taxa (e.g. Maherali and Klironomos 2007 for vesiculo-arbuscular mycorrhizal fungi).

Similarly, the recent application of network theory to biological systems (Tylianakis et al. 2010; Valiente-Banuet et al. 2014) offers promising perspective for systemic conservation strategies aiming at considering more than species. Conserving biological interactions—the functional backbone of life (Nicholls 2006) is a prerequisite to protect biodiversity (Soulé 2003). In particular, mutualistic interaction networks (Bascompte and Jordano 2007) offers promising perspective for plant-based indirect conservation strategies for fungi. Here we propose to investigate the potential for ectomycorrhizal (ECM) interaction network-derived indices to build integrative conservation strategies for both parts (fungi vs. plant) of the association in the case study of Corsica Mediterranean Island.

Macromycetes conservation and the example of ectomycorrhizal (ECM) symbiosis

As compared to fungi, plants received years of theoretical researches devoted to define functional (life-history traits), demographic (distribution patterns and dynamics) and evolutionary (e.g. phylogenetic originality; Pavoine et al. 2005; Veron et al. 2015) bases of the current priorities and guidelines of conservation. These researches allowed the development of an arsenal of practical tools (e.g. *ex situ* collections, botanical conservatories, protected areas and populations) for enlightening decision makers and managers. For instance, the Natura 2000 network of protected areas has been established at the end of a distribution-based prioritizing process using natural range, population dynamics and abundance of species across Europe (Council Directive 92 /43 /EEC).

Our knowledge of the ecology of the 1.5-3 M of fungal species (Hawksworth 2012) reflects neither their importance for ecosystem functioning (Bardgett and van der Putten 2014), nor their potential as indicator of ecosystem functioning (Heilmann-Clausen et al. 2015), nor the wide range of services they provide to other organisms, including humans (Yun and Hall 2004; Łukasz et al. 2012). The absence of fungi in conservation planning reflects in its current use, the restriction of the term mycology to a science devoted to study the organismal biology of fungi (Rambold et al. 2013). The reasons of the insufficient recognition of mycology as a major field of life science are not only biological, but also historical. Thus, fungi suffered until recently of their ancient classification as lower plants and the general suspicion about fungi in English-speaking world (Heilmann-Clausen et al. 2015). More, as compared to plants, usable mycological knowledge for conservation (e.g. distribution, population dynamics) increases slowly because of the difficulty to identify fungal species and to delimit individuals in the field, even for the most conspicuous ones (the so-called macromycetes, which produce macroscopic sexual apparati). Consequently, the operational toolkit to achieve fungal conservation remains limited as compared to plants, despite the existence of considerable datasets generated by ambitious research in Northern Europe (Dahlberg et al. 2010; Heilmann-Claussen et al. 2015) and promising first attempts in developing species-centered approaches for fungi (e.g. IUCN red list for Fungi; Dahlberg and Mueller 2011).

As for other fungi, mycologists face difficulties to achieve full appreciation of the role of ECM fungi in socio-ecosystems (van der Linde et al. 2012). ECM symbiosis is a major funcional component of soil structure (Rillig and Mummey 2006), plant community dynamics (van der Heijden et al. 2015), biochemical cycles (Courty et al. 2010; Averill et al. 2014). ECM fungi are involved in intra- and interspecific plant facilitation processes (van der Heijden and Horton, 2009; Richard et al. 2009) by transferring carbon through common mycorrhizal network (CMNs; Simard et al. 1997) or by providing inoculum sources for seedlings (Taudiere et al. 2015). Thereby ECM symbiosis is of primary importance in primary (Nara 2006) and secondary plant succession (Richard et al. 2009) for both inter (e.g. Dickie et al. 2004) and intra-specific interactions (e.g. Kranabetter et al. 2009).

From a conservation perspective, these indispensable symbionts of major temperate and boreal tree species share most disadvantages of fungi, including hyper-diverse patterns at both local (e.g. Bahram et al; 2011) and global scale (Tedersoo et al. 2010) and in insufficient taxonomic knowledge of some of their major lineages (e.g. Thelephoraceae, Sebacinaceae, Cortinariaceae, Inocybaceae).

Systemic indicator to jointly conserve ECM fungi, plants and their interactions

Now, it is well recognized that plants can't be considered as standalone entities. They form inextricable collective entities with micro-organisms (the holobiont; Vandenkoornhuyse et al. 2015). So, how to protect photobionts if we don't care about their mycobiont, an essential part of their extended phenotype? Under this perspective, most temperate, boreal and some tropical trees depend on their mutualistic interactions with ECM fungi. In this interaction, heterotroph mycetes obtain carbon from their host and provide root protection, stress resistance, water supply and nutrient uptake, in particular for nitrogen and phosphorus to the autotroph (Selosse et al. 2006).

Fungal Kingdom has been convincingly presented as a “source of novel tools and approaches for conservation of megadiverse organism groups” (Heilmann-Clausen et al. 2015). Here we propose a method to jointly consider ECM plants, fungi and interaction in a systematic view of the conservation of fungal diversity. Specifically, we successively i) define a set of six network-centered metrics usable as complementary traits at the plant species level in conservation policies, ii) illustrate their use in the case study of Corsica Island, iii) test their complementarity with classical plant-centered metrics in their efficiency to take into account the fungal biodiversity in Corsica and iv) question the compatibility of a network-based approach with the ongoing conservation policy elaborated from the Habitat Directive.

ECM interaction networks as system models for fungal conservation

Toward systemic indicators in conservation biology

Network analysis of ecological interactions recently generated a lot of interest (Girvan and Newman 2002; Bascompte and Jordano 2007; Barabasi 2016). In biological sciences, analytical approaches were developed to study the stability and resilience within networked ecosystems (e.g. Thébault and Fontaine 2010; de Vries et al. 2013; Zou et al. 2016) as well as co-extinction scenarii (e.g. Montoya et al. 2006; Pocock et al. 2012). As a consequence, an increasingly high number of scientists suggest considering ecological interactions as central component of biodiversity to evaluate and conserve in planning (e.g. Tylianakis et al. 2010; Valiente-Banuet et al. 2014). However, we still lack metrics to use as appropriate indicators of the conservation status of networked biological systems, with predictive values on their long-term maintenance. We here propose a set of conservation indicators (Table 1) including “classical indicators” (rarity, endemism, functional/phylogenetic originality) and “systemic indicators” aiming at protecting plant's partners and interactions network.

ECM fungi as a powerful networked system model

One fundamental property of ECM symbiosis is the highly variable level of specificity from both sides (fungus *vs* plant) within the interaction. First, the number of partners varies greatly among ECM plant species (Roy et al. 2013; Botnen et al. 2014; Taudiere et al. 2015). As a consequence, protecting a plant species which feed a high diversity of fungi (interaction specialist) is a way to indirectly maintain favorable biotic conditions for ECM fungal diversity, including symbionts strictly associated to this host. Reflexively, ECM fungal species display host specificity or preference (Molina et al. 1992; den Bakker et al. 2004). Thus, narrow host range fungi (interaction specialist) present on a given location are highly dependent on the persistence of their host in the landscape.

We here develop six network-centered indices (Table 1) as an attempt to include interaction between ECM plants and fungi in the development of integrative conservation planning. The proposed indices are calculated at the plant species level, in order to provide a set of usable traits that could be added by botanists in systemic conservation planning. In this approach, we assume that i) the analyzed interaction networks are modular (Taudière et al. 2015), as most mutualistic networks analyzed so far (Bascompte and Jordano, 2007), and ii) plant-fungal interaction links are of same strength. Using binary matrix of plant-fungi association, we propose two indices based on the number of fungal partners (plant degree k_p, defining the fungal attractiveness of a plant toward ECM fungal diversity; Glossary and Table 1)and the specificity ($1/c_p$,, defining the dependency of ECM fungal diversity toward a focal plant species; Glossary and Table 1 of fungal partners to a plant species to appreciate the values of ECM plant species (Table 1). Using projected networks, we further propose two metrics that take into account the ability of plants to indirectly link pairs of fungal species in unique way in the interaction network ($1/re_p$, measuring the specificity of plant-mediated fungus-fungus links; Glossary and Table 1), but also the ability of plants to create links toward other plant species through shared fungal species (l_p; defining the indirect among-plant connectedness; Glossary and Table 1. Last, we propose two network architecture-derived indices to consider the ability of plants to consolidate ECM ecological networks within (z_p, measuring the ability of a plant to interconnect the components of its module) and among network modules (z_p and P_p measuring the ability of a plant to connect the modules forming the network).

With a surface of 8 748 km^2, Corsica is the fourth largest Island in Mediterranean basin after Sicily, Sardinia and Cyprus. This French island is located within one of the three main biodiversity hotspots of plant endemism in the world (Myers et al. 2000). This mountainous and geologically complex island concentrates plant diversity, endemism and conservation stakes at the European level. For vascular plants, 32% of the 240 endemics (*sensu lato*) are threatened (Médail and Verlaque 1997), and 16 are listed in Annexes II and IV of the habitat directive (CEE 92/43), including ECM trees such as the endemic Corsican pine (*Pinus nigra* subsp. *laricio* (Poir.) Maire).

In Corsica, the state of knowledge of ECM plant-fungi interaction is exceptional, particularly regarding other large Mediterranean islands (Taudière et al. 2015). Fully referenced database compiling the current knowledge on vascular plant distribution patterns (Jeanmonod et Gamisans 2007; CBNC, 2016) in one hand and macrofungal diversity (CBNC, 2012) on the other hand, have been produced at the scale of the island. From this corpus, a binary matrix of ECM plant-fungi association has been assembled and analyzed to decipher the temporal distribution of ecological links during secondary successions (Fig. 1; Taudière et al. 2015). Here we apply the developed method to the same matrix to compare the 16 ECM vascular plants present in Corsica regarding the dependency of the 411 macrofungal species they support through 993 interaction links (Fig. 2). The comparison reveals contrasted patterns among plant species (Fig. 2). Species in the Fagaceae (in particular *Quercus ilex* L.; Fig. 3) emerge as key species in the interaction networks despite a low conservation value regarding the plant-centered indicators (Fig. 2). Contrastingly, species in the Pineaceae and the Betulaceae (in particular the Italian alder *Alnus cordata* [Loisel] Duby; Fig. 3) show more balanced patterns between network-related traits and species-centered conservation stakes (Fig. 2). In the island, 9 of the 16 ECM plant species are concerned by the annex II of the European directive 92/43 (Fig. 3). These 9 species feed 394 (95.9%) ECM fungi, and are responsible of 928 (93.5%) plant-fungal links in the network. In a plant-centered approach, the habitat Directive satisfactory cover conservation stakes, except for *Abies Alba*. When considering the value of the same set of plants regarding their role in the ECM interaction networks, most important ones (*e.g. Quercus ilex, Pinus pinaster* Aiton) benefit of protection (Fig. 3), *Fagus sylvaticus* L. apart (a species combining high k_p, z_p, P_p, l_p scores; Fig. 2 d). Among the three endemic plant species, *Pinus laricio* support low conservation stakes regarding ECM networks, while the two endemic *Alnus* species harbor a low number of ECM species (low k_p scores) but those fungal species are original (high $1/c_p$ scores), particularly in the case of *Alnus alnobetula* subsp. *suaveolens* (Req.) Lambinon & Kerguelen (odorant alder; Fig. 2 and Fig. 3).

This brief comparison shows the discrepancy between hierarchy of ECM plants in Corsica in a network-based and a plant species-based perspectives (Spearman ranked test: rho = -0.70; p-value = 0.0025). In other words, plant species with high intrinsic conservation value (*Pinus* *laricio*; *Salix spp; Populus spp*) do not match with those of high interest for a systemic and functional conservation of ECM fungi and interactions.

From the case study to a transferable method: limits and opportunities

The presented method is an attempt to integrate a major soil biota to the conservation of terrestrial ecosystems. The proposed toolbox could be substantially improved by further conceptual developments currently out of reach, including the weight of interactions from a plant perspective, but also by the addition of neglected fungal lineages in conservation strategies, such as resupinate families (e.g. Thelephoraceae). Indeed, fungi are sensitive to other environmental parameters than the availability of their related hosts (Lilleskov et al. 2011). When transferred to a continental context, the method developed in delimited insular area faces classical issues of conservation policies and planning, including the definition of homogeneous areas/units, which may here correspond to delineated networked entities. Unfortunately, the discrepancy between the geographies of soil biota and plant species at various scales (from local to continental) and their potentially differential response to the current climate change make this step complex. .

Assembling interaction matrices represents considerable compilation efforts and needs a time-consuming data validation step. This work could be synergistically achieved during regional red list defining processes, and could be extended to other fungal guilds which show high host specificities (e.g. saprobic fungi including wood decayers; Zhou and Hide 2001). In this scenario, mycologists may contribute to plant conservation, and participle to unify plant-based and fungi-based initiatives. It seems simpler in the case of ECM fungi as the mutualistic nature of the ECM interaction imposes that the protection of mutualist fungi *via* their hosts obligatory benefits to plants.

References

Averill C, Turner BL, Finzi AC. **2014**. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature* **505**: 543–545.

Bahram M, Põlme S, Kõljalg U, Tedersoo L. **2011**. A single European aspen (Populus tremula) tree individual may potentially harbour dozens of Cenococcum geophilum ITS genotypes and hundreds of species of ectomycorrhizal fungi. *FEMS Microbiology Ecology* **75**: 313–320.

den Bakker HC, Zuccarello GC, Kuyper TW, Noordeloos ME. **2004**. Evolution and host specificity in the ectomycorrhizal genus *Leccinum*. *New Phytologist* **163**: 201–215.

Barabási A-L. **2016**. *Network Science*. UK: Cambridge University Press.

Bardgett RD, van der Putten WH. **2014**. Belowground biodiversity and ecosystem functioning. *Nature* **515**: 505–511.

Bascompte J, Jordano P. 2007. Plant-animal mutualistic networks: The architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics* **38**: 567–593.

Botnen S, Vik U, Carlsen T, Eidesen PB, Davey ML, Kauserud H. 2014. Low host specificity of root-associated fungi at an Arctic site. *Molecular Ecology* **23**: 975–985.

Bruns TD, Bidartondo MI, Taylor DL. 2002. Host specificity in ectomycorrhizal communities: what do the exceptions tell us? *Integrative and Comparative Biology* **42**: 352–359.

Courty P-E, Buée M, Diedhiou AG, Frey-Klett P, Le Tacon F, Rineau F, Turpault M-P, Uroz S, Garbaye J. 2010. The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. *Soil Biology and Biochemistry* **42**: 679–698.

Dahlberg A, Genney DR, Heilmann-Clausen J. 2010. Developing a comprehensive strategy for fungal conservation in Europe: Current status and future needs. *Fungal Ecology* **3**: 50–64.

Dahlberg A, Mueller GM. 2011. Applying IUCN red-listing criteria for assessing and reporting on the conservation status of fungal species. *Fungal Ecology* **4**: 147–162.

Dickie IA, Guza RC, Krazewski SE, Reich PB. 2004. Shared ectomycorrhizal fungi between a herbaceous perennial (*Helianthemum bicknellii*) and oak (*Quercus*) seedlings. *New Phytologist* **164**: 375–382.

Girvan M, Newman ME. 2002. Community structure in social and biological networks. *Proceedings of the National Academy of Sciences* **99**: 7821–7826.

Guimerà R, Amaral LAN. 2005. Cartography of complex networks: modules and universal roles. *Journal of Statistical Mechanics: Theory and Experiment* **2005**: 1–13.

Hawksworth DL. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological research* **105**: 1422–1432.

Hawksworth DL. 2012. Global species numbers of fungi: are tropical studies and molecular approaches contributing to a more robust estimate? *Biodiversity and Conservation* **21**: 2425–2433.

van der Heijden MGA, Horton TR. 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology* **97**: 1139–1150.

van der Heijden MGA, Martin FM, Selosse M-A, Sanders IR. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* **205**: 1406–1423.

Heilmann-Clausen J, Barron ES, Boddy L, Dahlberg A, Griffith GW, Nordén J, Ovaskainen O, Perini C, Senn-Irlet B, Halme P. 2015. A fungal perspective on conservation biology. *Conservation Biology* **29**: 61–68.

Heilmann-Clausen J, Christensen M. 2005. Wood-inhabiting macrofungi in Danish beech-forests – conflicting diversity patterns and their implications in a conservation perspective. *Biological Conservation* **122**: 633–642.

Jeanmonod D, Gamisans J. 2007. *Flora Corsica.* Aix-en-Provence, France: EdiSud.

Kranabetter JM, Durall DM, MacKenzie WH. 2009. Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest. *Mycorrhiza* **19**: 99–111.

Latapy M, Magnien C, Vecchio ND. 2008. Basic notions for the analysis of large two-mode networks. *Social Networks* **30**: 31–48.

Lilleskov EA, Hobbie EA, Horton TR. 2011. Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecology* **4**: 174–183.

van der Linde S, Holden E, Parkin PI, Alexander IJ, Anderson IC. 2012. Now you see it, now you don't: The challenge of detecting, monitoring and conserving ectomycorrhizal fungi. *Fungal Ecology* **5**: 633–640.

Loizides M, Bellanger J-M, Clowez P, Richard F, Moreau P-A. 2016. Combined phylogenetic and morphological studies of true morels (Pezizales, Ascomycota) in Cyprus reveal significant diversity, including *Morchella arbutiphila* and *M. disparilis* spp. nov. *Mycological Progress* **15**: 1–28.

Łuczaj Ł, Pieroni A, Tardío J, Pardo-de-Santayana M, Sõukand R, Svanberg I, Kalle R. **2012**. Wild food plant use in 21st century Europe: the disappearance of old traditions and the search for new cuisines involving wild edibles. *Acta Societatis Botanicorum Poloniae* **81**: 359–370.

Maherali H, Klironomos JN. **2007**. Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* **316**: 1746–1748.

Médail F, Verlaque R. **1997**. Ecological characteristics and rarity of endemic plants from southeast France and Corsica: Implications for biodiversity conservation. *Biological Conservation* **80**: 269–281.

Molina R, Massicotte HB, Trappe JM. **1992**. Specificity phenomena in mycorrhizal symbioses: Community-ecological consequences and practical implications. Mycorrhizal functioning: an integrative plant-fungal process. London - New York: Allen M., 357–423.

Money NP. **2013**. Against the naming of fungi. *Fungal biology* **117**: 463–465.

Montoya JM, Pimm SL, Solé RV. **2006**. Ecological networks and their fragility. *Nature* **442**: 259–264.

Moreau P-A, Rochet J, Richard F, Chassagne F, Manzi S, Gardes M. **2011**. Taxonomy of *Alnus*-associated hypogeous species of *Alpova* and *Melanogaster* (Basidiomycota, Paxillaceae) in Europe. *Cryptogamie, Mycologie* **32**: 33–62.

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J. **2000**. Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–858.

Nara K. **2006**. Pioneer dwarf willow may facilitate tree succession by providing late colonizers with compatible ectomycorrhizal fungi in a primary successional volcanic desert. *New Phytologist* **171**: 187–198.

Newman ME. **2006**. Modularity and community structure in networks. *Proceedings of the National Academy of Sciences* **103**: 8577–8582.

Nicholls H. **2006**. Restoring Nature's Backbone. *PLoS Biology* **4**: e202.

Pavoine S, Ollier S, Dufour A-B. **2005**. Is the originality of a species measurable? *Ecology Letters* **8**: 579–586.

Pocock MJO, Evans DM, Memmott J. **2012**. The robustness and restoration of a network of ecological networks. *Science* **335**: 973–977.

Rambold G, Stadler M, Begerow D. **2013**. Mycology should be recognized as a field in biology at eye level with other major disciplines–a memorandum. *Mycological progress* **12**: 455–463.

Richard F, Selosse M-A, Gardes M. **2009**. Facilitated establishment of *Quercus ilex* in shrub-dominated communities within a Mediterranean ecosystem: do mycorrhizal partners matter? *FEMS Microbiology Ecology* **68**: 14–24.

Rillig MC, Mummey DL. **2006**. Mycorrhizas and soil structure. *New Phytologist* **171**: 41–53.

Roy M, Rochet J, Manzi S, Jargeat P, Gryta H, Moreau P-A, Gardes M. **2013**. What determines *Alnus*-associated ectomycorrhizal community diversity and specificity? A comparison of host and habitat effects at a regional scale. *New Phytologist* **198**: 1228–1238.

Selosse M-A, Richard F, He X, Simard SW. **2006**. Mycorrhizal networks: des liaisons dangereuses? *Trends in Ecology & Evolution* **21**: 621–628.

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R. **1997**. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **388**: 579–582.

Soulé ME, Estes JA, Berger J, Del Rio CM. **2003**. Ecological effectiveness: conservation goals for interactive species. *Conservation Biology* **17**: 1238–1250.

Taudiere A, Munoz F, Lesne A, Monnet A-C, Bellanger J-M, Selosse M-A, Moreau P-A, Richard F. **2015**. Beyond ectomycorrhizal bipartite networks: projected networks demonstrate contrasted patterns between early- and late-successional plants in Corsica. *Frontiers in Plant Science* **6**.

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Kõljalg U. **2008**. Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phytologist* **180**: 479–490.

Tedersoo L, May TW, Smith ME. 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* **20**: 217–263.

Thébault E, Fontaine C. 2010. Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science* **329**: 853–856.

Tylianakis JM, Laliberté E, Nielsen A, Bascompte J. 2010. Conservation of species interaction networks. *Biological Conservation* **143**: 2270–2279.

Valiente-Banuet A, Aizen MA, Alcántara JM, Arroyo J, Cocucci A, Galetti M, García MB, García D, Gómez JM, Jordano P, et al. 2014. Beyond species loss: the extinction of ecological interactions in a changing world. *Functional Ecology* ***29****: 299–307.*

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. 2015. The importance of the microbiome of the plant holobiont. *New Phytologist* **206**: 1196–1206.

Veron S, Davies TJ, Cadotte MW, Clergeau P, Pavoine S. 2015. Predicting loss of evolutionary history: Where are we? *Biological Reviews*

de Vries FT, Thebault E, Liiri M, Birkhofer K, Tsiafouli MA, Bjornlund L, Bracht Jorgensen H, Brady MV, Christensen S, de Ruiter PC, *et al.* 2013. Soil food web properties explain ecosystem services across European land use systems. *Proceedings of the National Academy of Sciences* **110**: 14296–14301.

Yun W, Hall IR. 2004. Edible ectomycorrhizal mushrooms: challenges and achievements. *Canadian Journal of Botany* **82**: 1063–1073.

Zhou D, Hide KD. 2001. Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. *Mycological Research* **105**: 1449–1457.

Zou K, Thébault E, Lacroix G, Barot S. 2016. Interactions between the green and brown food web determine ecosystem functioning. *Functional Ecology*

Glossary

Host preference/specificity: For a species living in its host, host specificity refers to the number of species partners (Bruns et al. 2002). Host preference refers to the tendency to prefer some species than other without strict specificity (Tedersoo et al. 2008).

Module: In network terminology, a module is a subset of nodes (e.g. plant and fungal species) highly inter-connected them and poorly connected to other subsets of same nature (Newman 2006). In the case of ecological networks, modularity may reflect ecological and evolutionary processes (Taudiere et al. 2015).

Ectomycorrhizal (ECM) symbiosis: Mutualistic symbiosis involving mostly ligneous plants and fungal organisms, and dominatingtemperate and boreal ecosystens. The plant host feeds its associated fungi and obtains mineral nutrition, water uptake and root protection from its fungal partners (Selosse et al. 2006).

Phylogenetic originality: This metrics proposed by Pavoine and collaborators (2005) measures the evolutionary distinctiveness of a species in a given area (function *originality* in the R package *ade4* [Dray and Dufour 2004]). It quantifies how few relatives a species has and how phylogenically distant they are (Veron et al. 2015).

Table 1: List of ecological indicators for a combined conservation of plants, fungi and their interactions. Operability levels correspond to the degree of availability of data concerning ECM symbiosis (from + to ++++) with the special case of Corsica within parentheses, when different. For ecological interactions, knowledge is often available, including in the “gray literature”, but this knowledge is scattered and we lack database synthesizing this knowledge. 1 Taudiere et al. 2015; 2 Latapy et al. 2008; 3 Guimera and Amaral 2005; 4 Alpova corsicus (Moreau et al. 2011)

Class of indicator	**Indicator**	**Conservation objective**	**Rationale**	**Origins of indicators**	**Network formulation**	**Operational status in Corsica**
Species centered (from plant or fungus perspectives)	Rarity	Rarity	Rare species are more endangered	Distribution database and Ecological knowledge	Number of occurrences of a plant species *p* or a fungal species *f*	Plants ++++ Macromycetes + (++)
	Endemism	Endemism	Endemic species are more endangered and sometimes supported original ecosystems	Distribution database	Restricted natural geographical range of a plant species *p* or a fungal species *f*	Plants ++++ (240 species) Macromycetes + (1 species) [4]
	Phylogenetic or functional originality	Phylogenetic or functional originality	Original species may play unique role in community and deliver unique function for ecosystems	Phylogenetic tree / traits matrix	Evolutionary distinctiveness of a species in a given area	Plants +++ Macromycetes ++
Network centered, from a plant perspective	Fungal attractiveness	Integrated conservation of Fungi	A plant species feeding numerous fungal species is a tank for fungal taxonomic diversity	Interaction database for the plant *p*	Number of partners of a plant p (k_p) [1]	Scattered knowledge +++ Database ++ (+++)
	Interaction originality		A plant species feeding fungi which interact with few other plants may be crucial in the survival of these specialist fungal species	Networks interactions matrix	Inverse of the mean number of partners of associated fungal species of a plant p ($1/c_p$) [1]	Scattered knowledge ++ (+++) Database + (+++)
	Indirect among-fungi connectedness	Conservation of original fungal communities	As a lot of fungal species show host preference, the taxonomy of a plant drives fungal community. Thus, plant species that harbor unique paired of fungal species are the potential place for original interactions among fungal species. On the contrary, the extinction of plant species making only redundant links among fungal species may be of less importance in a conservation	Projected network for fungi	Inverse of the redundancy of links formed by a plant p in the fungal projected network ($1/re_p$) [2]	
	Indirect among-plant connectedness	Conservation of indirect interactions among plants	As plant succession and seedlings establishment depends on ECM fungal species, plants sharing ECM fungal symbiont with numerous other plants species may be implicate in indirect facilitation of those numerous plants species	Projected network for plants	Number of plants sharing at least one fungal species in common with plant p (l_p) [1]	
	Within-module connectedness	Conservation of plants structuring the network	Plant species that structure their module may be of primary importance in the network structure and consequently its functioning	Network architecture	Within-module degree of plant p (z_p) [3]	
	Among-module connectedness		Plant species linking modules may be of primary importance in the network structure and consequently its functioning	Network architecture	Participation coefficient of plant p (P_p) [3]	

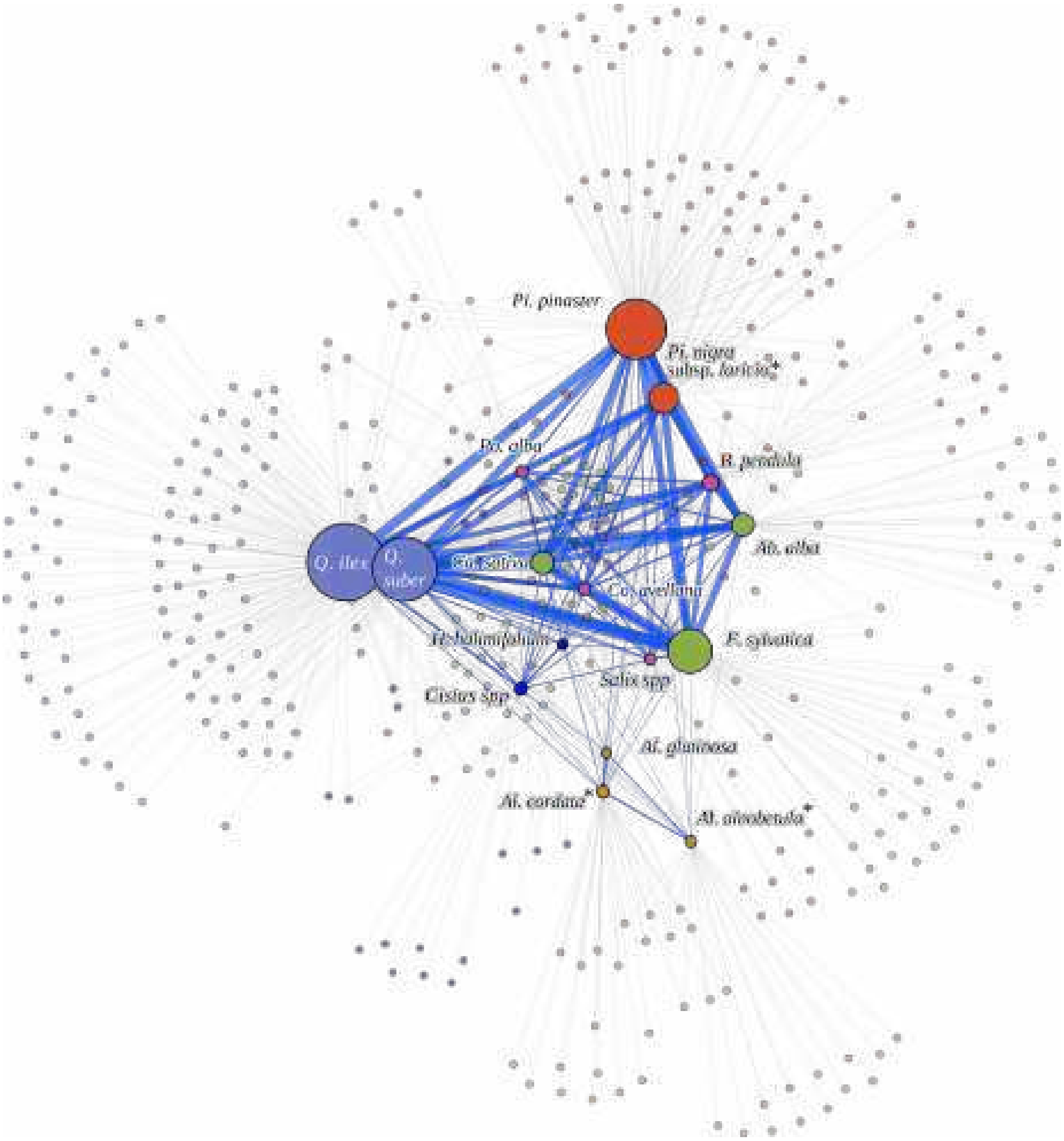


Figure 1: ECM interaction networks at the scale of Corsica Island. Plants genus abbreviation are Ab.: *Abies*; Al.: *Alnus*; B.: *Betula*; Ca.: *Castanea*; Co.: *Corylus*; F.: *Fagus*; H.: *Halimium*; Pi.: *Pinus*; Po.: *Populus*; Q.: *Quercus*. Gray edges links plants and fungi. Blue edges represent the indirect links among plant through sharing of fungal partners, forming the plant projected network. Node size is proportional to the number of partners and node with the same color belong to the same module.

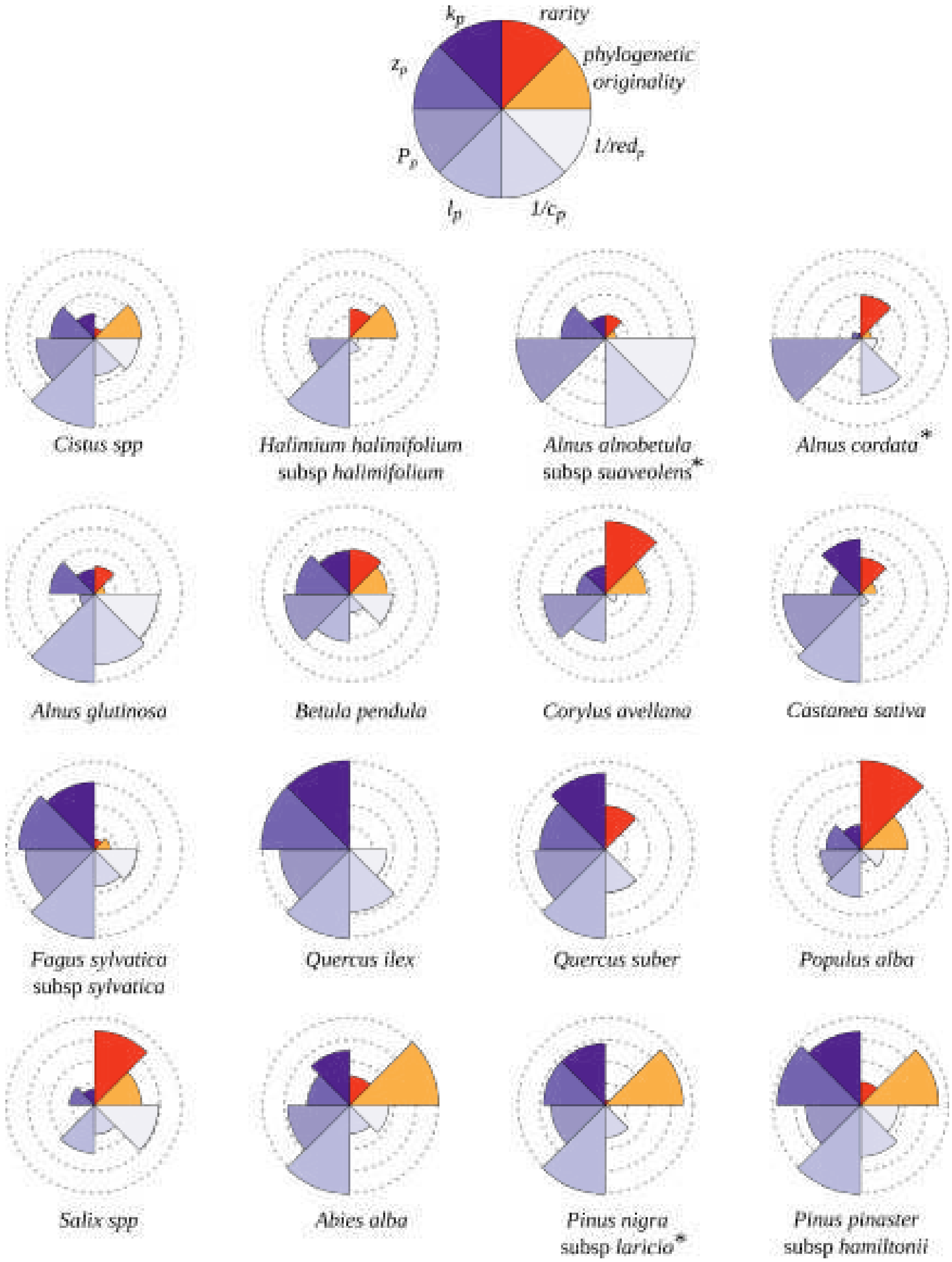


Figure 2: Caracterisation of the 16 ECM plant taxa present in Corsica regarding their place in the ECM network (blue sectors) and their value in plant-centered conservation approaches (orange sectors). Values are centered and scaled. Endemic plants are followed by an asterisk. See Table 1 for the legend of indicator.

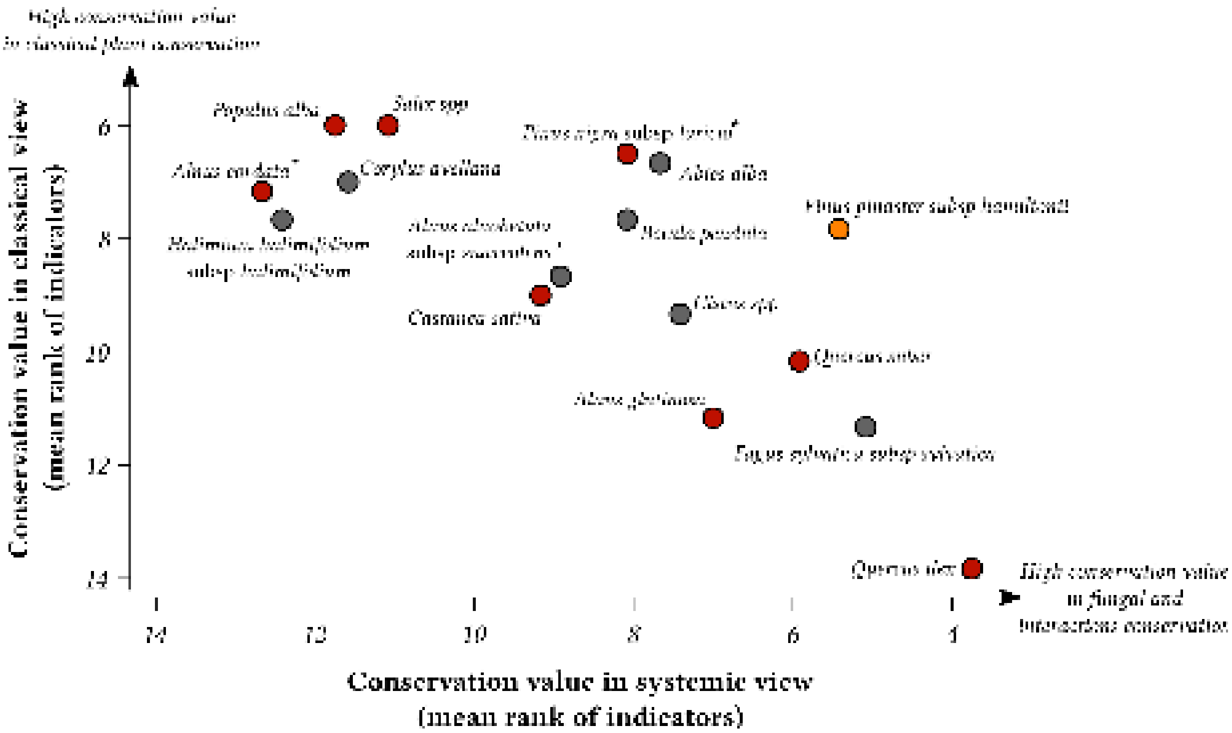


Figure 3: Conservation values of the 16 ECM plant taxa present in Corsica Island using plat-based and network-based indices.. Conservation values for a plant species *p* were computed using the mean rank of species *p* in classical (endemism, rarity and phylogenetic originality) or systemic indicators (see Table 1 and fig. 2 for details). Red and orange circles represent respectively proprietary and of communitarian interest for Europe.

Chapitre IV

Effet du feu sur les communautés de champignons ectomycorhiziens

> « *There is fire on the mountain / And nobody seems to be on the run / Oh there is fire on the mountain top / And no one is running* »
>
> Ayo, *Fire On The Mountain*, 2007

« Trace » du feu de Rospa-sorba (2000)

1 Synopsis

Ce chapitre s'intéressent aux effets du feu sur les communautés de champignons ECM à travers deux manuscrits. Tout d'abord, nous avons effectué une revue de la littérature quasi-exhaustive des publications qui documentent un effet du feu sur la symbiose ECM. Nous avons sélectionné 73 publications qui vérifiaient nos critères d'inclusion dans la revue (résultats originaux concernant l'effet de feu de taille significative sur une composante de la symbiose). Ce travail a permis de décrire la géographie relative au corpus de connaissances disponibles, et de comparer les effets de diverses modalités de feu (notamment dirigé vs accidentel) sur les communautés ECM. En complément de cette revue de la littérature, nous avons mis en place un protocole d'échantillonnage des sols et des apex ECM dans des forêts de pins laricio de Corse ayant été le siège d'histoires de feu différentes. Les analyses de cet échantillonnage sont en cours en raison de délais importants dans l'analyse Miseq des échantillons, réalisée au Max Planck Institute for Molecular Biomedecine (RNA Biology Laboratory).

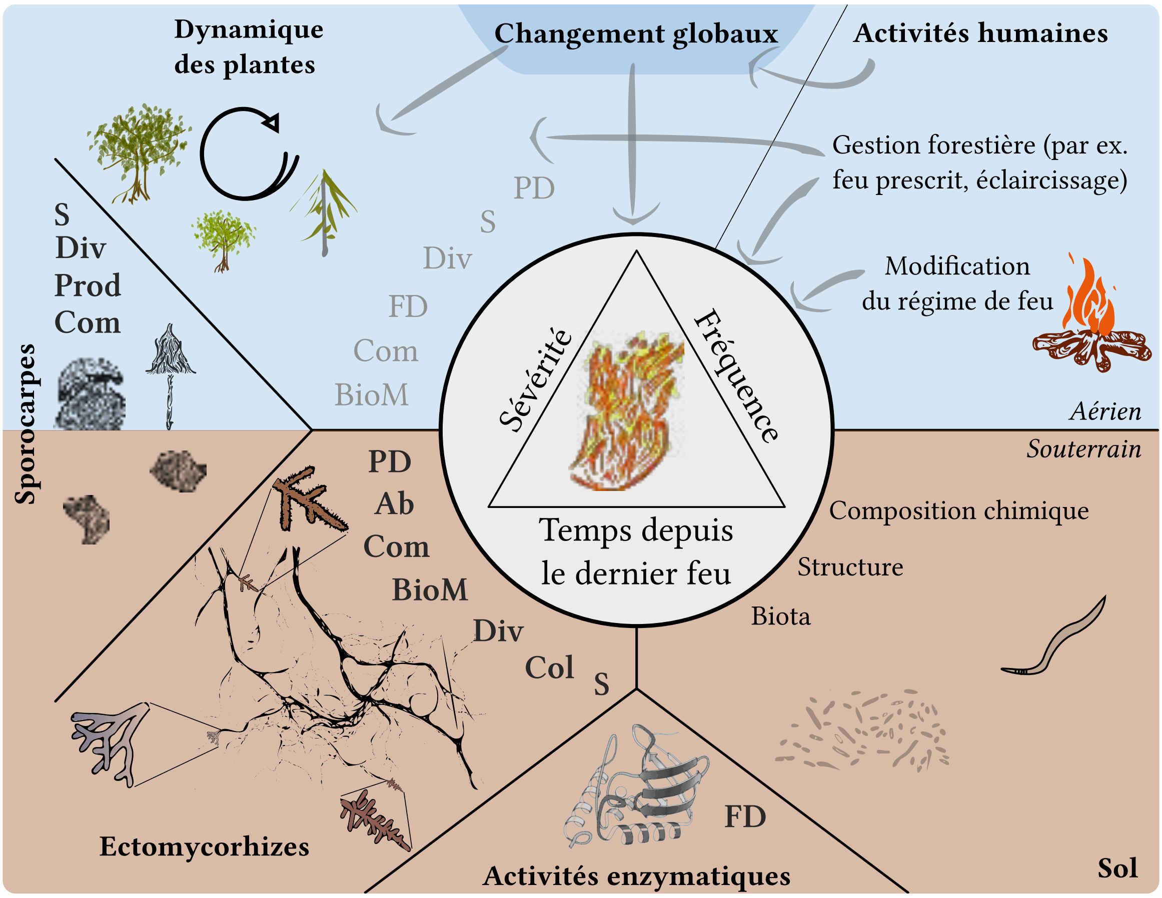


Figure IV.1 – Schéma des effets potentiels du feu sur la symbiose ECM : Cette figure résume les différentes composantes de la symbiose qui sont affectées par la sévérité du feu, sa fréquence et le temps écoulé depuis le dernier feu (voir manuscrit F). **Ab** : Abondances (par ex. nombre d'apex ou longueur des hyphes) ; **BioM** : Biomasse ; **Col** : taux de colonisation des racines ; **Com** : Composition et structure des communautés d'apex ou de sporophores ; **Div** : Diversité taxinomique (par ex. indice de Simpson) ; **FD** : Diversité fonctionnelle (activités enzymatiques) ; **PD** : Diversité phylogénétique ; **Prod** : Production de sporophores (par ex. poids sec par unité de surface) ; **S** : Richesse spécifique.

2 Manuscrit F : "Review on fire effects on ectomycorrhizal symbiosis, an unachieved work for a scalding topic"

IN A NUTSHELL

Rationale: Fire and ectomycorrhizal (ECM) symbiosis are crucial drivers of the dynamics of millions of hectares of nemoroboreal forests. The direct or indirect impacts of fire on ectomycorrhizal community dynamics elicits vegetation responses during decades. Deciphering the effects of fire on ECM interactions is particularly timely regarding the consensual development of soil-impacting technics such as prescribed burning. We review most of the literature on the effect of fire on ectomycorrhizal (ECM) interactions using a method derived from meta-analysis. In total, we report, classify and discuss the result of 72 publications including three previous reviews on close topics.

Type of interaction: Ectomycorrhizal symbiosis, mostly mutualistic

Ecological filters of fungal communities: Wildfire and prescribed burning; Direct (*e.g.*, death of mycelium) or indirect effects (changes in soil chemical composition and vegetation cover)

Main conclusions: Despite its massive socio-economic consequences, fire in ECM ecosystems is understudied and its effects on ECM soil biota are still poorly understood. Moreover, research on this important topic is geographically fragmentary regarding the distribution of ECM forests worldwide, and taxonomically restricted. Both wildfire and prescribed burning undoubtedly drive a shift in the composition of above- and below-ground ECM fungal communities. Fire effect on ECM fungal diversity is more controversial, with almost half of the reviewed studies reporting non significant effect. To summarize, fire shapes ECM fungal communities and their dynamics for decades in various ways which need to be explored.

Review on fire effects on ectomycorrhizal symbiosis, an unachieved work for a scalding topic

Adrien Taudière [1, 2*], Franck Richard [2] & Christopher Carcaillet [3]

1/ École Pratique des Hautes Études (EPHE) & Paris Research University, Paris, France; adrien.taudiere@cefe.cnrs.fr

2/ UMR 5175, CEFE – CNRS – Université de Montpellier, Montpellier, France

3/ Laboratoire d'Écologie des Hydrosystèmes Naturels et Anthropisés (UMR5023 CNRS), Université Lyon 1, 69622 Villeurbanne, France; christopher.carcaillet@ephe.sorbonne.fr

* Corresponding authors: adrien.taudiere@cefe.cnrs.fr

Abstract: Millions of hectares of ectomycorrhizal (ECM) forests provide most of the wood resource in the northern hemisphere. These fire-prone ecosystems concentrate an astonishing diversity of mutualistic soil fungi that are pivotal for seedling establishment, tree growth and forest functioning and dynamics.

Here, we review the effects of fire in these forests using a systematic screening of the literature. We reveal that a low number of field studies (73) directly address this issue, in a restricted geographic area that partially represents the geography of ECM biodiversity stakes and fire risk. The analyzed literature consensually reports long-term shifts in the composition of ECM fungal communities after fire. Contrastingly, the effects of fire on fungal diversity and richness at the local scale continue to be debated among researchers, and need to be documented further using adequate experimental device to limit the effects of some identified methodological biases. Furthermore, our analysis emphasizes the urgent need to carefully consider the effects of prescribed burning belowground. This is an important conclusion because this widely implemented and efficient management tool to prevent wildfires may impact ECM soil communities in the same way as uncontrolled events.

Our analysis finally highlights the need of refining the concept of ECM post-fire fungi, by taking advantage of promising tools, such as next-generation sequencing and quantitative PCR applied to mycelia and spores, to integrate the vegetative traits of fungi in integrative definitions.

Keywords: ectomycorrhizal symbiosis; wildfire; fungal ecology; disturbance; prescribed burning; forest management

Highlights

- Ectomycorrhizal (ECM) fungi are major drivers of forest growth and dynamics
- The geography of studies partially reflects ECM biodiversity stakes regarding fire
- Fires drives shifts in the composition of ECM communities
- The deleterious effect of fire on the richness of ECM communities is not consensual

I) Introduction

Ectomycorrhizal (ECM) symbiosis occurs in only 3% of the plant species on the Earth, but thousands of fungal species provide access to belowground resources for ECM autotroph organisms (Smith and Read 2008). Otherwise, fire is the main terrestrial disturbance (Bond and Keeley 2005) and has likely since hundreds of million years (Scott and Glasspool 2006; Bowman 2009; Bond 2015). Despite the low number of plant species involved, fungal ECM associations transcend plant strategies with respect to their response to fire.

Fire is a global phenomenon that affects population dynamics, community structure and biodiversity, and has consequences for biogeochemical cycles, landscape properties and climate dynamics (Johnson 1992; Whelan 1995; Bond and van Wilgen 1996). Current ongoing climate change should alter fire risk at the local-to-global scale, with a general increases of this risk (Moritz et al. 2012; Bedia et al. 2015), although some regions may experience a decrease due to complex passive or active land-use effects (Pausas and Fernandez-Munoz 2012; Fréjaville and Curt 2015) and the spatial heterogeneity of climate dynamics (Girardin et al. 2009). Globally, this is likely to result in altered biogeochemical cycles (e.g. Abbott et al. 2016) and threats to biodiversity (e.g. MacKey et al. 2012; Moritz et al. 2014). Thus, more comprehensive and functional studies are needed to improve understanding of the biota–fire relationships and trajectories likely to be associated with current global scale changes. Among these studies, better knowledge of ECM symbiosis in relation to fire is crucial. Fire-induced ecological strategies of ECM plants extend from the post-fire favored species (pyrophilous; e.g. Cistaceae, Myrtaceae *pro parte*, Pinaceae *pro parte*) to the sensitive pyrofugals (e.g. *Fagus sylvatica* in Europe; *Pinus cembra* in Siberia and European mountains; *Thuja* spp. in North America). As a consequence, ECM symbioses represent an illuminating model for understanding the ability of fire to shape the distribution of soil biota, and for highlighting the biology and the ecology of fungi and symbiotic relationships regarding fire disturbance.

During the last decade, four reviews (Fig. 1) successively addressed: (i) the consequences of fire on ECM fungal diversity in boreal forests, with a special focus on Fennoscandian ecosystems (Dahlberg 2002); (ii) the post-fire response of global soil fungal diversity (Cairney and Bastias 2007) with a focus on Australian ecosystems (McMullan-Fischer et al. 2011); and (iii) the specific response of ECM fungal communities in *Pinus* dominated forests (Karst et al. 2014). Here we aim at extending these analyses by delivering the first exhaustive global review addressing the response of ECM fungi to fire, i.e. without restrictions regarding the taxonomy or biogeography of hosts.

The ectomycorrhizal obligatory symbiosis is found in the belowground part of most terrestrial biomes, and currently dominates the boreal, temperate, and Mediterranean forests, in both sclerophyllous and deciduous vegetation (Smith and Read 2008). The around 6 000 species of known ECM fungi are mostly uncultivable organisms, dependent on their host for carbon and vitamins, while they provide water, nutrients and root protection for autotrophs (Smith and Read 2008). Studies have shown that numerous fungal ECM species are sensitive – in terms of occurrence, distribution, abundance, colonization rate - to abiotic shifts, including nitrogen variation in soil through natural (Kranabetter et al. 2009) or anthropic processes (Lilleskov et al. 2004), and climatic conditions that affect both their vegetative and reproductive structures (Shahin et al. 2013; Boddy et al. 2014). These organisms also respond to biotic signals from their host under stressed conditions (Karst et al. 2014) or after disturbance (e.g. clear cutting; Jones et al. 2003), and from interactions between ECM fungal species (Courty et al. 2016).

Whereas fire is of growing concern for stakeholders and an inexhaustible subject for researchers, with its continuing social relevance (Fig. S1), we demonstrate in the first section of the present review that studies ecologically linking ECM and fire are geographically restricted (Fig. 2) despite a gentle rise in publication rate (Fig. 1).

If fire directly affects ECM tree and shrub communities, there are consequent changes to the temporal dynamics of plant communities (e.g. Blarquez and Carcaillet 2010), biogeochemical cycles and ecosystem processes (e.g. Wardle et al. 2003), and favorable conditions for secondary succession are created (Whelan 1995). In soil, host signals (stress or death) and fire induced-abiotic shifts drive post-fire dynamics of the ECM fungal community. These widely described patterns, essentially *in situ* studied, is the theme of the second section of this review.

Recurrent fires impose selection pressures on organisms, creating plants with physiological adaptations (e.g. Sala et al. 2001) and a wide variety of life history traits, such as cone serotiny (e.g. Habrouk et a. 1999) and thick bark (e.g. Jackson et al. 1999; Keeley et al. 2011; Frejaville et al. 2013) or adapted regeneration strategies (e.g. Armstrong 2011). By analogy, ECM fungi exhibit a wide spectrum of functional diversity, with underlying vegetative (Agerer 2001 for a review), enzymatic (Courty et al. 2010) and reproductive attributes (e.g. production of resistant propagules such as sclerotia; Smith et al. 2015) that have been related to their ability to respond to fire at the species level. The synthesis of this body of knowledge is the central goal of the third section of our review.

Ongoing climate change will increase fire frequency and severity during the 21st century in most regions of the Earth (Pechony and Shindell 2010; Flannigan et al. 2013; Moritz et al. 2014), despite regional-scale heterogeneity of fire responses to global warming (Girardin et al. 2009). As a consequence, managing fire risk, including using prescribed burning as a tool, has become timely in many contexts and opens up new fields of study. It is, therefore, time to highlight potential future issues concerning ECM-fire ecology; these are covered in the four-last section of this review.

(II) Recent and spatially clustered research

We found a total of 182 publications that potentially examine the effect of fire on ECM symbioses. The bulk of publications (171) was found by searching the Web of Science™ ("All Databases") using the term "fire AND ectomycorrh*"in the topic the 11 April 2016. Additional publications were located using Google Scholar (two additional publications) and references found in the publications initially identified (9 publications). Of these 182 publications, only 73 bring some insight on the effect of fire on ECM symbiosis (publication list in Table S1; flowchart of the publication selection in Fig. 3). For each publication, we characterized the biome, the geographical coordinates, the origin of fires (wildfire *vs* prescribed burning), the fire modalities tested (e.g. fire frequency), the type of analysis (e.g. diversity or community analyses), the ECM host(s), the chemical and morphological analyses performed, and the identification methods used for taxonomic assignment; we then summarized the most relevant results (Table 1; see Table S2 for complete data and supplementary methods S1 for details).

Most of the research on the fire effect on ECM symbiosis has been carried out since 2000, and half of these studies were published after 2005 (Fig. 1), indicating an apparent increase in this issue. However, the ratio between the number of publications dealing with fire impact on ECM symbiosis and the total number of publications about fire (0.35 ±0.19 %) or about ECM interactions (1.09 ±0.53 %) was constant from 1991 to 2015 (Fig. S2). Thus, even if the publication numbers on the fire effect on ECM symbiosis increase apparently, the relative importance of the subject regarding ECM and fire literature is stable.

Studies were mostly located in temperate (35 pub.) and Mediterranean (20) ecosystems, with some in boreal (10) and few in tropical (2) (Fig. 2, S3). More than one third of the publications report on studies located in the USA (26) and only 14 are located in the southern hemisphere, including 10 in Australia. Three intensively studied areas, namely the west coast of the USA (11), southwestern Europe (12) and, to a lesser extent, southeastern Australia (5), accounted all together for 40% of all the publications excluding the four reviews (Fig. 2). This geographic distribution of field studies reflects more the location of ECM research groups around the world (Fig. S4) than the geography of ECM forests. Thus, there are extensive publication-free areas (e.g. Southern and Eastern parts of the Mediterranean basin, tropical Americas, most of Asia), and there is a discrepancy between the geography of fires and that of the research on the effect of fire on ECM forests (Fig. 2). Only two publications (Brundrett et al. 1996, Akema et al. 2009) reported studies located in tropical forests, and only one (Akema et al. 2009) focused on the Dipterocarpaceae, despite the great ecological (Peay et al. 2015) and economic importance (Kettle et al. 2012) of this ECM plant family.

In addition to geographical clustering, the majority of research dealing with the fire impact on ECM symbiosis focused on *Pinus*-dominated ecosystems (Fig. 4, 44 pub. including 5 bioassay pub., 60% of total pub. number) in the contexts of both prescribed burning (27 studies) and wildfires (12 studies), and to a lesser extent on *Eucalyptus* spp. (10 pub. of which 7 covered prescribed burning and the remaining three, wildfire, 15% of total pub. number).

When crossing-referencing fire risk and research locations, our analysis reveals concentrations of documented case studies in three main regions of the world, with most attention paid to two tree genera. Our study underlines the absence of research in the eastern Mediterranean basin, in Africa (e.g. about the *Cedrus atlantica* ECM symbiosis in northeastern Africa), and in Central America (e.g. forests of endemic oaks and firs). To a lesser extent, this overview reveals a lack of research in tropical biodiversity hotspots where ECM tree species are also present (e.g. white-sand forest in South America [Roy et al. 2016]; Dipterocarpaceae forests in Indonesia [Akema et al. 2009]; members of Caesalpinoideae such as *Gilbertiodendron* spp. in Africa [Bâ et al. 2012]). The *Gilbertiodendron* spp. may even be favored by human-induced fire in Africa (Tovar et al. 2014), the history of which remains largely unknown on this continent, but which would have started during the African Iron Age, sometime between 4000 and 2000 yrs ago according to model-based simulations (Archibald et al. 2012) and charcoal-based fire reconstruction (Marlon et al. 2013). It would certainly have had strong ecological effects on ecosystems (Bayon et al. 2012). Tovar and coauthors (2014) do shed light on the ecology of ECM tropical trees in these poorly described ecosystems (Bâ et al. 2012), which are severely impacted by human activities and fire regime (e.g. *Coccoloba uvifera* in Antilles; Séne et al. 2015).

(III) Fire alters hyper-diverse ECM fungal assemblages

(a) Weak statistical support for the effect of fire on ECM fungal communities

The way researchers analyze the response of ECM fungal communities to fire varies widely between studies (Fig. 4, Table 1 and 2). In total, five types of descriptors were used to study the response of ECM fungi to fire, namely: 1) the abundance, measured as the number of hyphal tips or the hyphal length; 2) the biomass, estimated as either the amount of mycelium or the dry weight of fruiting bodies; 3) the root colonization, based on the number of root tips or percentages of root with ECM tips; 4) the taxonomic (e.g. Simpson's index), phylogenetic or functional diversity (catabolic enzyme profiling); and 5) the community composition or structure. The gathered body of work includes a minority of studies (26 out of 69, i.e. 38%) based on the direct sequencing of biological material (Fig. 4d), while three quarters primarily take a morphological approach to diversity, examining either ECM fruiting bodies or root tips (morphotyping).

Interestingly, 25% of the [*community parameter* × *publications*] combinations (numbers in Tables 1 and 2) failed to find statistical support for the fire effect on ECM fungal community descriptors. There are many potential causes for this lack of significance, including case studies with no clear evidence of fire on the measured parameter (real biological patterns in a robust dataset; e.g. Robinson et al. 2008); but also studies with methodological limitations (e.g. only morphotype identification; de Roman and de Miguel 2005) or low statistical power due to the limited number of samples (e.g. n = 9 for three modalities in Kennedy et al. 2015) or species (Longo et al. 2011). In the last study, the low richness (i.e. species number; in total there were 23 morphotypes, 11 morphotypes per sample on average) limited the chances of detecting a significant effect of fire on richness, but the high number of ECM root tips analyzed (22 449) strongly increased the chance of spotting a significant effect on species abundances. Accordingly, they found a fire effect on diversity, i.e. taking into account species abundances, but no effect on richness. Finally, richness is an intrinsically 'degraded' information of community composition: it is possible to calculate richness from community composition, but the same richness can hide very different community compositions (Wilsey et al. 2005). Therefore, detecting a shift in richness requires large numbers of samples and/or fungal assemblages that are highly sensitive to fire. One possible way to solve this issue is to use abundance-based diversity metrics such as Chao (Chao et al. 2004) or the Hill index (Hill 1973), which have not yet been used to assess the fire effect on ECM fungal communities. Beyond statistical significance (p-value), future studies need to assess the effect size of a fire on ECM interactions (e.g. percentage of variation in root colonization or fungal biomass, proportion of variance explained in community variation using PERMANOVA; Anderson 2001) to understand the magnitude of the fire effect in relation to other driving factors (e.g. dispersion, limiting similarity, habitat filtering) that shape ECM symbioses dynamics.

(b) A lack of consensuses about the effect on fungal richness and diversity

In all, 20 publications out of 69 (29%) examined the effect of fire on fungal richness, including studies of 13 wildfires and seven prescribed burnings (Table 1, Fig. 5). For this widely documented parameter of ECM fungal communities, no consensus emerged about the effect of wildfire, with an equal number (6 pub. each) recording negative and neutral effects but, interestingly, no positive response (Table 1, Fig. 5; one publication report variation with no clear direction). Moreover, the aboveground parts (sporocarps) of ECM fungal communities exhibit negative responses to fire in terms of richness (6 out of 8 studies), while belowground mycelia mostly (5 out of 7) showed no fire effect. In conclusion, wildfires may primarily cause a decline in reproductive emergence of some ECM fungal species, with a weaker vegetative dimension of this impact. From nine publications documenting change in ECM fungal diversity due to fire, seven were carried out in wildfire contexts. In terms of richness, wildfires negatively affect ECM fungal diversity in half of the studies (4 pub.), whilst there was no detectable effect in others (3, Fig. 5).

In contrast, studies pertaining to prescribed burnings tend to reveal a more consistent (6 out of 7 pub.) negative impact on ECM fungal community richness. These impacts appear consistently belowground (using either soil sequencing [2 pub.] or root tip morphotyping [4 pub.]), and may be complemented by (i) studies extending over a longer period and (ii) aboveground investigations questioning the effect of the extensively used practice of burning on ECM fungal diversity, including the reproduction of edible post-fire mushrooms. Providing such a comprehensive overview may allow stakeholders to use this management tool with better knowledge of its impacts on ECM symbiosis, this mostly invisible part of forest biodiversity and functioning (Richard et al. 2005).

(c) Fires drive marked shifts in ECM fungal community composition

While richness is a poorly informative proxy of fire impact on ECM fungal communities, this review underlines the fact that fire does drive marked changes in local (i.e. stand-level) fungal assemblages. Indeed, the most striking feature of this review is the strong fire effect on the composition of ECM fungal communities both on the reproductive (fruiting bodies) and vegetative (belowground root tips) *apparatus* of ECM fungi. In all, most publications (21 out of 26) reported a significant compositional response of ECM fungal communities to fire at the local scale (Tables 1 and 2). As expected based on the physics of wildfires, all publications documenting soil depth, frequency and/or severity show a greater fire impact on soil surface horizons compared to deeper horizons (Table S3), along with cumulative effects with frequency and severity of the event (Table 2).

Our searches identified studies conducted in a wide variety of fire contexts (Fig. 4e-f): wildfire (44 pub.), prescribed burning (21) and bioassay (14 pub. including 5 limited to *ex-situ* bioassays). With respect to wildfire, the most frequently used sampling designs are space for time substitution (10 “chronosequences”), and one-shot post-fire approaches (10 “time-elapsed”). For prescribed burning, the majority of results are based on either repeated sampling (7 “before/after”) or comparisons of sites which undergone different frequency of fire (6 “frequency” pub.).

The few studies using controlled experimental designs (Fig. 4b), with *ex-situ* set ups (5 "bioassay") or both *in-* and *ex-situ* (1 "prescribed burning + bioassay" and 5 "wildfire + bioassay") allow the mechanisms of the observed changes in ECM fungal communities to be deciphered with respect to the complexity of fire disturbance (e.g. heat, chemical changes, physical and/or microclimatic shifts, etc). For instance, the experimental addition of ash was used in bioassays, with contrasted results regarding the measured "community parameter" (4 pub., Table S3). While Mahmood et al. (2003) found a positive effect of ash on fungal biomass, Grogan et al. (2000) found no effect on richness, diversity or on community composition, and Peay et al. (2009) found a significant effect on community composition when "ashes" were added in combination with a heat treatment. These contrasting outcomes also reveal marked differences in experimental designs. For instance from removing "ash" from wildfire stands (Grogan et al. 2000) to introducing "ash" from a prescribed burning area into experimental pots (Peay et al. 2009). Unfortunately, in these ash-based studies, the term "ash" does not distinguish pure ash (mineral particles) from small charcoal fragments (charred organic matter), which have strong ecosystem effects (Zackrisson et al. 1996) because of polyphenol adsorption and affecting the nitrogen cycle through microbial activity (Hättenschwiler and Vitousek 2000; DeLuca et al. 2006). Furthermore, ash and the charcoal-controlled nitrogen cycle have a confounding effect on the soil by modifying its pH in relation to time since the last fire (Keeley et al. 1998), and thus affecting fungal ecology.

(d) Fire modifies root colonization

Numerous studies illustrated decreasing root colonization by ECM fungi (i.e. the proportion of short roots associated with ECM fungi) after wildfire and prescribed burning (Table 1). Unsurprisingly, increasing fire severity augments this negative effect on the vegetative component of ECM symbiosis (e.g. Launonen et al. 1999). However, Palfner et al. (2008) found that 2 year-old *Nothofagus alpina* seedlings that established after fire tended to harbor more root tips in the deepest soil horizons and higher root biomass than seedlings of the same age established in undisturbed soils. Post-fire *Nothofagus alpina* still formed ectomycorrhizas below 25 cm depth whereas fine roots of control seedlings did not reach that depth. More studies are needed to confirm whether there is increasing ECM colonization at depth, induced as a positive consequence of fire affecting water distribution down the soil profile (Palfner et al. 2008), possibly caused by combustion of hydrophobic soil organic matter or volatile organic compounds.

(e) Fire shapes ECM fungal biota over a long period

Sixteen studies examined the temporal trends exhibited by ECM fungal communities after fire, 10 using wildfire chronosequences and six using monitoring mostly following wildfire (Fig. S5). Kipfer et al. (2011) found that ECM fungal richness decreases shortly after fire and stabilizes 15-18 years after the event. This is probably due to the fact that complete soil recolonization by fungi takes several years, i.e. the "resilience time". Treseder et al. (2004) found that the roots ECM colonization takes up to 15 years to return to pre-fire levels. Moreover, Holden et al. (2013) provided evidence that fungal hyphae need at least 24 years to recover their pre-fire length (Holden et al. 2013). Diversity indices show the same trends more than 10 years after fire both in boreal (15-20 years; LeDuc et al. 2013) and in temperate ecosystems (>26 years; Twieg et al. 2007). An interesting perspective for further research is exploring the mecanisms that delay the recovery of ECM fungal communities after fire across a wide range of fire regime and environmental conditions.

Because the resilience time can be very long, empirical or experimental studies that measure the community composition within a time period <20 years might conclude that there is a lack of resilience in ECM fungal communities exposed to a fire event (Robinson et al. 2008; Twieg et al. 2007; Visser 1995; Kipfer et al. 2011). For instance, community composition was still different from that of unburned sites five years after burning of *Eucalyptus diversicolor* forests (Robinson et al. 2008) and 18 years after burning in *Pinus sylvestris* stands (Kipfer et al. 2011). Finally, two studies found restoration of community composition 41 years (Visser 1995) and 65 years (Twieg et al. 2007) after wildfire.

This rather long resilience delay of ECM fungal communities may be the result of (i) the low dispersion ability of some ECM fungal species (see Peay and Bruns 2014), (ii) the prolonged effect of fire on soil physical and chemical characteristics (Certini et al. 2005) or (iii) the prolonged effect of fire on other partners of ECM fungal organisms including microbiota dynamics (e.g. the mycorrhiza helper bacteria; Frey-Klett 2007) and host physiology (Franklin et al. 2014). These three hypotheses could be tested by measuring the temporal patterns of microbiota, plant physiology and soil characteristics across a distance gradient from unburned areas in a single ecological system.

(IV) ECM fungal species respond differently to fire according to their ecological strategies

Post-disturbance changes in community composition and structure are driven by the ecological strategies of species (for example ruderal species including the pyrophylic; Warcup 1990; Martin-Pinto et al. 2006; Buscardo et al. 2011). Some fungal species are considered to be fire-dependent, i.e. dependent upon fire and/or the immediate post-fire conditions to complete their life cycle, and secure their long-term survival through sexual reproduction. For instance, in Swedish boreal forests, 40 macrofungal species, mostly saprotrophs, are considered fire-dependent (Dahlberg 2002). For ECM fungal diversity, Visser (1995) proposed classifying fungal fruiting behavior into three groups: early-stage (e.g. *Coltricia perennis* and *Thelephora* spp.), multi-stage (e.g. *Suillus brevipes* and *Cenococcum geophilum*) and late-stage fungi (e.g. *Cortinarius* spp., *Russula* spp. and *Suillus tomentosus*). The following section summarizes the response diversities of ECM fungal communities to fire, in terms of spore bank assemblages.

(a) Contrasting strategies based on spore bank composition

After disturbance, ECM fungi colonize the roots of surviving trees or establishing seedlings from sexual spores, sclerotia (asexual resistant propagules), or remnant mycelia already established on surviving trees (e.g. after clear cutting; Jones et al. 2003). The influence of fire on spore germination still remains poorly studied (but see Glassman et al. 2015). With respect to sclerotia, only one study (Torres and Honrubia 1997) has documented a positive wildfire effect on the formation of these vegetative propagules. On the other hand, the importance of the establishment of new individual fungi from spore germination after fire has been well illustrated in the specific case of *Suillus pungens* population genetics (Bruns et al. 2002a). This case, and the case of the hypogeous Basidiomycete *Rhizopogon*, illustrate (i) the spore longevity of these early-stage fungi, as dormant propagules in soils, (ii) the capacity of spores to stay receptive to tree roots and form ECM on newly established seedlings and (iii) that the role of the spore bank may be central during the temporal sequence of post-fire ECM colonization (Glassman et al. 2015).

(b) Contrasting vegetative responses among species

In the context of fire, contrasting responses among co-occurring species forming ECM fungal communities have been widely documented. Among the positive responses, it has been observed that the Basidiomycetes *Thelephora terrestris* and *Suillus brevipes* increased in abundance during a post-fire succession in the *Pinus banksiana* Canadian boreal forest (LeDuc et al. 2013), as did *Descolea antarctica* on seedling roots of *Nothofagus alpina* in Patagonia (Palfner et al. 2008). From a functional perspective, Clemmensen and coauthors (2015) recently illustrated the dominance of cord-forming ECM fungi (aggregations of hyphae, e.g. in *Cortinarius* spp. and *Suillus* spp.; Boddy 1993) in early-successional stages after fire, and the role of these fungi in the mobilization of nitrogen and carbon from sequestrated forms in boreal soils. Finally, bioassays using seedlings grown in heated soil indicate that spores of some species are heat resistant (Peay et al. 2009) and belong to species favored by fire in terms of relative abundances (e.g. *Rhizopogon* spp.; Baar et al. 1999; Izzo et al. 2006). At the other extreme of ecological strategies, late-stage ECM fungal species show negative responses to fire. For instance, LeDuc et al. (2013) identified fire-sensitive species in the *Cortinarius* and *Russula* genera, recovering 19 years after the fire. These few fire-sensitive species, all macrofungi belonging to dominant genera aboveground in the studied systems, require further research involving more robust sampling designs (larger sample sizes, molecular techniques, etc.) to examine the ECM fungal diversity that is less responsive to fire.

(c) The functional enigma of massive post-fire fruiting

Post-fire fungi are classically defined as species which massively fruit within the first years after fire. Documented cases mostly concern saprobic macromycetes such as morels (*Morchella* spp.), *Gyromitra*, *Sphaerosporella*, and *Tricharina* (Miller et al. 1998; Richard et al. 2015; Larson et al. 2016) whose ephemeral fruitbodies cover forest soil immediately after fires. Contrastingly, only very few post-fire ECM fungi fulfill this requirements, e.g. the basidiomycete *Hebeloma antracophilum*, and we still know little about the effect of fire on their biological cycle in soil. Other lineages of post-fire Ascomycetes are likely to be putatively mycorrhizal, such as the Pezizale fungus *Geopyxis carbonaria*. This species massively fruits, only once, a few months after fire (Vrålstad et al. 1998), and remains vegetatively cryptic in the periods between fires. The ECM symbiosis offers an ideal model for conceptualizing the notion of post-fire fungi using both reproductive (fruitbody abundance) and vegetative (soil mycelia and ECM root tip dynamics) responses of ECM fungal species.

(V) Future direction

a) Mixed methods and knowledge

The development of next generation sequencing (NGS) techniques opens up the possibility of new research perspectives on fungal biology and ecology (e.g. Buée 2009; Öpik 2009). This technology, as yet, is not an efficient way to assess precisely the abundance of molecular taxa within ECM fungal communities (Nguyen 2015). However, its ability to access to the vegetative components of assemblages, combined with its efficiency in detecting rare species, is a strength when examining the effect of fire on the composition of hyper-diverse ECM fungal communities (Glassman et al. 2015), but also a promising tool to integrate the vegetative response of species to fire in an integrative and functional definition of post fire ECM fungi.

Applying NGS methods to vegetative or sexual propagules of ECM fungi is a promising way to understand the mechanisms underlying recolonization by ECM fungi after fire, and to test the importance of spore inflow (*vs.* recolonization via remnant soil mycelia) in determining the composition of early stage fungal communities. In particular, this tool may provide valuable information about the ecology of ECM fungal communities, by estimating the importance of wind- *vs.* animal-dispersal processes (Claridge 1992) by using molecular analysis to compare spore traps (e.g. Peay and Bruns 2014), feces (endozoochory; Valentini et al. 2009) and the external surfaces of potential dispersers (ectozoochory, Lilleskov and Bruns, 2005). In addition, the unprecedented sensitivity of NGS methods is a technological opportunity to track post-fire ECM fungal species in unburned ecosystems, and should solve the mystery of their cryptic biology over the years, often decades, between fires.

b) Changes in ECM fungal strategies in response to fire

One fundamental issue that remains for community ecologists is to understand the mechanisms involved in and primarily driving the composition shift of ECM fungal communities affected by fire. Two non-exclusive forces may govern the temporal dynamics of ECM fungal diversity in soil after disturbance.

First, fire induces marked habitat shifts by directly modifying chemical and physical characteristics of the soil organic layer (Certini et al. 2005) that primarily select for fungal species with post-fire adapted niche requirements. A functional trait-based approach (for instance examining the nutritional status of post-fire species; Courty et al. 2005; Artz et al. 2009) applied to a compatible database (e.g. UNITE; Kõljalg et al. 2013; DEEMY; Agerer and Rambold 2004) could help to evaluate the influence of environmental filtering on the functional diversity of ECM fungal assemblages after fire. Phylogenetic conservatism of traits (Mouquet et al. 2012) could also be tested to provide insights into the changes of functions associated with post-fire ECM fungi.

Second, fire induces plant community shifts and then indirectly affects the taxonomic composition of post-fire ECM fungal assemblies via obligate interactions with a restricted range of hosts (specificities; Bruns et al. 2002b). However, early- and late- successional plant species differ in their ability to accumulate ECM fungal diversity (e.g. Taudière et al. 2015). Thus, one may hypothesize that narrow host-range fungal species are primarily specific to ecological contexts that favor their host with respect to the abiotic niche. Diachronic analyses deciphering the temporal patterns of ECM fungal communities in relation to their specificity to post-fire hosts are needed to provide insights into the role of specificity in the response of ecosystems to fire.

c) Forest management and conservation

One of the most striking results of the present review is the consensus that there is a negative effect of prescribed burning on aboveground ECM fungal richness (i.e. reproductive structures), with no consequences on the belowground (mostly vegetative) communities. However, the range of studies is limited, and further work is needed. The literature-reported negative fire impact on ECM fungal fruiting contrasts with the massive and positive sexual response of many post-fire saprotrophs (e.g. *Morchella* spp.; Dahlberg 2002; Richard et al. 2015). Hence, if only the aboveground component of ECM fungal communities was taken into account, fire would be considered a negative process for ECM fungal communities. Nonetheless, a more central role of fire emerges when combining both quantitative and qualitative dimensions of these communities. The documented shift in ECM fungal composition, both below- and aboveground, highlights the need to upscale studies of the impact of fire on ECM fungal community diversity to the landscape scale.

From a functional perspective, the path is now clear: there is a need to investigate the indirect effects of fire (including prescribed burnings and wildfires) from a forestry perspective. In particular, further research could address whether fire related-composition shifts in ECM fungal communities alter forest productivity, for instance seedling nutrient uptake and growth, or timber yield. Overall, there is a need to build bridges between ecosphysiologists, dendrochronologists and ECM fungal community ecologists to develop integrated research for the benefit of stakeholders and forest managers.

References

Abbott BW, Jones JB, Schuur EAG, et al (2016) Biomass offsets little or none of permafrost carbon release from soils, streams, and wildfire: an expert assessment. Environ Res Lett 11:34014. doi: 10.1088/1748-9326/11/3/034014

Agerer R (2001) Exploration types of ectomycorrhizae. Mycorrhiza 11:107–114. doi: 10.1007/s005720100108

Agerer R, Rambold G (2004) DEEMY: An information system for characterization and determination of ectomycorrhizae. München, Germany. [WWW Document]. Available at: http://www.deemy.de/.

Akema T, Nurhiftisni I, Suciatmih, Simbolon H (2009) The impact of the 1998 forest fire on ectomycorrhizae of Dipterocarp trees and their recovery in tropical rain forests of east Kalimantan, Indonesia. Japan Agricultural Research Quarterly 43:137–143.

Anderson MJ. (2001) A new method for non-parametric multivariate analysis of variance. Austral ecology 26(1):32–46.Archibald S, Staver AC, Levin SA (2012) Evolution of human-driven fire regimes in Africa. PNAS 109:847–852. doi: 10.1073/pnas.1118648109

Armstrong G (2011) Evidence for the equal resilience of *Triodia spp.* (Poaceae), from different functional groups, to frequent fire dating back to the late Pleistocene. Heredity 107:558–564. doi: 10.1038/hdy.2011.42

Artz RRE, Reid E, Anderson IC, et al (2009) Long term repeated prescribed burning increases evenness in the basidiomycete laccase gene pool in forest soils. FEMS Microbiol Ecol 67:397–410. doi: 10.1111/j.1574-6941.2009.00650.x

Bâ AM, Duponnois R, Moyersoen B, Diédhiou AG (2012) Ectomycorrhizal symbiosis of tropical African trees. Mycorrhiza 22:1–29. doi: 10.1007/s00572-011-0415-x

Baar J, Horton TR, Kretzer AM, Bruns TD (1999) Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. New Phytol 143:409–418. doi: 10.1046/j.1469-8137.1999.00452.x

Bayon G, Dennielou B, Etoubleau J, et al (2012) Intensifying weathering and land use in Iron Age central Africa. Science 335:1219–1222. doi: 10.1126/science.1215400

Bedia J, Herrera S, Gutiérrez JM, et al (2015) Global patterns in the sensitivity of burned area to fire-weather: Implications for climate change. Agric For Meteorol 214–215:369–379. doi: 10.1016/j.agrformet.2015.09.002

Blarquez O, Carcaillet C (2010) Fire, fuel composition and resilience threshold in subalpine ecosystem. PLOS ONE 5:e12480. doi: 10.1371/journal.pone.0012480

Boddy L (1993) Saprotrophic cord-forming fungi: warfare strategies and other ecological aspects. Mycol Res 97:641–655. doi: 10.1016/S0953-7562(09)80141-X

Boddy L, Büntgen U, Egli S, et al (2014) Climate variation effects on fungal fruiting. Fungal Ecol 10:20–33. doi: 10.1016/j.funeco.2013.10.006

Bond WJ (2015) Fires in the Cenozoic: a late flowering of flammable ecosystems. Front Plant Sci. doi: 10.3389/fpls.2014.00749

Bond WJ, Keeley JE (2005) Fire as a global “herbivore”: the ecology and evolution of flammable ecosystems. Trends Ecol Evol 20:387–394. doi: 10.1016/j.tree.2005.04.025

Bond WJ, Wilgen BW (1996) Fire and Plants. Chapman & Hall, Dordrecht

Bowman DMJS, Balch JK, Artaxo P, et al (2009) Fire in the Earth System. Science 324:481–484. doi: 10.1126/science.1163886

Brundrett, N. Ashwath, Jasper (1996) Mycorrhizas in the Kakadu region of tropical Australia: II. Propagules of mycorrhizal fungi in disturbed habitats. Plant Soil 184:173–184.

Bruns T, Tan J, Bidartondo M, et al (2002a) Survival of *Suillus pungens* and *Amanita francheti* ectomycorrhizal genets was rare or absent after a stand-replacing wildfire. New Phytol 155:517–523. doi: 10.1046/j.1469-8137.2002.00468.x

Bruns TD, Bidartondo MI, Taylor DL (2002b) Host specificity in ectomycorrhizal communities: what do the exceptions tell us? Integrative and Comparative Biology 42:352–359. doi: 10.1093/icb/42.2.352

Buée M, Reich M, Murat C, et al (2009) 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol 184:449–456. doi: 10.1111/j.1469-8137.2009.03003.x

Buscardo E, Freitas H, Pereira JS, Angelis P (2011) Common environmental factors explain both ectomycorrhizal species diversity and pine regeneration variability in a post-fire Mediterranean forest. Mycorrhiza 21:549–558. doi: 10.1007/s00572-011-0363-5

Buscardo E, Rodriguez-Echeverria S, Martin MP, et al (2010) Impact of wildfire return interval on the ectomycorrhizal resistant propagules communities of a Mediterranean open forest. Fungal Biol 114:628–636. doi: 10.1016/j.funbio.2010.05.004

Cairney JWG, Bastias BA (2007) Influences of fire on forest soil fungal communities. Can J For Res 37:207–215. doi: 10.1139/X06-190

Certini G (2005) Effects of fire on properties of forest soils: a review. Oecologia 143:1–10. doi: 10.1007/s00442-004-1788-8

Chao A, Chazdon RL, Colwell RK, Shen T-J (2004) A new statistical approach for assessing similarity of species composition with incidence and abundance data: A new statistical approach for assessing similarity. Ecol Lett 8:148–159. doi: 10.1111/j.1461-0248.2004.00707.x

Claridge A (1992) Is the relathionship among mycophagous marsupials, mycorrhizal fungi and plants dependent on fire? Aust J Ecol 17:223–225. doi: 10.1111/j.1442-9993.1992.tb00801.x

Clemmensen KE, Finlay RD, Dahlberg A, et al (2015) Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. New Phytol 205:1525–1536. doi: 10.1111/nph.13208

Courty P-E, Buée M, Diedhiou AG, et al (2010) The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biol Biochem 42:679–698. doi: 10.1016/j.soilbio.2009.12.006

Courty P-E, Munoz F, Selosse M-A, et al (2016) Into the functional ecology of ectomycorrhizal communities: environmental filtering of enzymatic activities. J Ecol. doi: 10.1111/1365-2745.12633

Courty P-E, Pritsch K, Schloter M, et al (2005) Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests: Methods. New Phytol 167:309–319. doi: 10.1111/j.1469-8137.2005.01401.x

Dahlberg A (2002) Effects of fire on ectomycorrhizal fungi in fennoscandian boreal forests. Silva Fenn 36:69–80.

de Roman M, de Miguel AM (2005) Post-fire, seasonal and annual dynamics of the ectomycorrhizal community in a *Quercus ilex* L. forest over a 3-year period. Mycorrhiza 15:471–482. doi: 10.1007/s00572-005-0353-6

DeLuca TH, MacKenzie MD, Gundale MJ, Holben WE (2006) Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. Soil Sci Soc Am J 70:448. doi: 10.2136/sssaj2005.0096

Flannigan M, Cantin AS, de Groot WJ, et al (2013) Global wildland fire season severity in the 21st century. For Ecol Manage 294:54–61. doi: 10.1016/j.foreco.2012.10.022

Franklin O, Näsholm T, Högberg P, Högberg MN (2014) Forests trapped in nitrogen limitation – an ecological market perspective on ectomycorrhizal symbiosis. New Phytol 203:657–666. doi: 10.1111/nph.12840

Fréjaville T, Curt T (2015) Spatiotemporal patterns of changes in fire regime and climate: defining the pyroclimates of south-eastern France (Mediterranean Basin). Climatic Change 129:239–251. doi: 10.1007/s10584-015-1332-3

Frejaville T, Curt T, Carcaillet C (2013) Bark flammability as a fire-response trait for subalpine trees. Front Plant Sci. doi: 10.3389/fpls.2013.00466

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36. doi: 10.1111/j.1469-8137.2007.02191.x

Girardin MP, Ali AA, Carcaillet C, et al (2009) Heterogeneous response of circumboreal wildfire risk to climate change since the early 1900s. Glob Chang Biol 15:2751–2769. doi: 10.1111/j.1365-2486.2009.01869.x

Glassman SI, Levine CR, DiRocco AM, et al (2015) Ectomycorrhizal fungal spore bank recovery after a severe forest fire: some like it hot. ISME J. doi: 10.1038/ismej.2015.182

Grogan P, Baar J, Bruns TD (2000) Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. J Ecol 88:1051–1062. doi: 10.1046/j.1365-2745.2000.00511.x

Habrouk A, Retana J, Espelta JM (1999) Role of heat tolerance and cone protection of seeds in the response of three pine species to wildfires. Plant Ecol 145:91–99.

Hättenschwiler S, Vitousek PM (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends Ecol Evol 15:238–243. doi: 10.1016/S0169-5347(00)01861-9

Hill MO (1973) Diversity and evenness: A unifying notation and its consequences. Ecology 54:427–432. doi: 10.2307/1934352

Holden SR, Gutierrez A, Treseder KK (2013) Changes in soil fungal communities, extracellular enzyme activities, and litter decomposition across a fire chronosequence in Alaskan boreal forests. Ecosystems 16:34–46. doi: 10.1007/s10021-012-9594-3

Izzo A, Canright M, Bruns TD (2006) The effects of heat treatments on ectomycorrhizal resistant propagules and their ability to colonize bioassay seedlings. Mycol Res 110:196–202. doi: 10.1016/j.mycres.2005.08.010

Jackson JF, Adams DC, Jackson UB (1999) Allometry of Constitutive Defense: A Model and a Comparative Test with Tree Bark and Fire Regime. Am Nat 153:614–632. doi: 10.1086/303201

Johnson EA (1992) Fire and Vegetation Dynamics Studies from the North American Boreal Forest. Cambridge University Press, Cambridge

Jones MD, Durall DM, Cairney JWG (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytol 157:399–422. doi: 10.1046/j.1469-8137.2003.00698.x

Karst J, Randall MJ, Gehring CA (2014) Consequences for ectomycorrhizal fungi of the selective loss or gain of pine across landscapes. Botany 92:855–865. doi: 10.1139/cjb-2014-0063

Keeley JE, Fotheringham CJ (1998) Mechanism of smoke-induced seed germination in a post-fire chaparral annual. J Ecol 86:27–36. doi: 10.1046/j.1365-2745.1998.00230.x

Keeley JE, Pausas JG, Rundel PW, et al (2011) Fire as an evolutionary pressure shaping plant traits. Trends Plant Sci 16:406–411. doi: 10.1016/j.tplants.2011.04.002

Kennedy NM, Robertson SJ, Green DS, et al (2015) Site properties have a stronger influence than fire severity on ectomycorrhizal fungi and associated N-cycling bacteria in regenerating post-beetle-killed lodgepole pine forests. Folia Microbiologica 60:399–410. doi: 10.1007/s12223-014-0374-7

Kettle CJ, Maycock CR, Burslem D (2012) New directions in Dipterocarp biology and conservation: A synthesis. Biotropica 44:658–660. doi: 10.1111/j.1744-7429.2012.00912.x

Kipfer T, Moser B, Egli S, et al (2011) Ectomycorrhiza succession patterns in *Pinus sylvestris* forests after stand-replacing fire in the Central Alps. Oecologia 167:219–228. doi: 10.1007/s00442-011-1981-5

Kõljalg U, Nilsson RH, Abarenkov K, et al (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277. doi: 10.1111/mec.12481

Kranabetter JM, Durall DM, MacKenzie WH (2009) Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest. Mycorrhiza 19:99–111. doi: 10.1007/s00572-008-0208-z

Larson, A.J., Cansler, C.A., Cowdery, S.G., Hiebert, S., Furniss, T.J., Swanson, M.E., Lutz, J.A., 2016. Post-fire morel (*Morchella*) mushroom abundance, spatial structure, and harvest sustainability. Forest Ecology and Management 377, 16–25. doi:10.1016/j.foreco.2016.06.038

Launonen TM, Ashton DH, Keane PJ (1999) The effect of regeneration burns on the growth, nutrient acquisition and mycorrhizae of *Eucalyptus regnans* F. Muell. (mountain ash) seedlings. Plant Soil 210:273–283. doi: 10.1023/A:1004609912315

LeDuc SD, Lilleskov EA, Horton TR, Rothstein DE (2013) Ectomycorrhizal fungal succession coincides with shifts in organic nitrogen availability and canopy closure in post-wildfire jack pine forests. Oecologia 172:257–269. doi: 10.1007/s00442-012-2471-0

Lilleskov EA, Bruns TD (2005) Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. Mycologia 97:762–769. doi: 10.3852/mycologia.97.4.762

Lilleskov EA, Bruns TD, Horton TR, et al (2004) Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. FEMS Microbiol Ecol 49:319–332. doi: 10.1016/j.femsec.2004.04.004

Longo M, Urcelay C, Nouhra E (2011) Long term effects of fire on ectomycorrhizas and soil properties in *Nothofagus pumilio* forests in Argentina. For Ecol Manage 262:348–354. doi: 10.1016/j.foreco.2011.03.041

Mackey B, Berry S, Hugh S, et al (2012) Ecosystem greenspots: identifying potential drought, fire, and climate-change micro-refuges. Ecol Appl 22:1852–1864. doi: 10.1890/11-1479.1

Mahmood S, Finlay RD, Fransson A-M, Wallander H (2003) Effects of hardened wood ash on microbial activity, plant growth and nutrient uptake by ectomycorrhizal spruce seedlings. FEMS Microbiol Ecol 43:121–131. doi: 10.1111/j.1574-6941.2003.tb01051.x

Marlon JR, Bartlein PJ, Daniau A-L, et al (2013) Global biomass burning: a synthesis and review of Holocene paleofire records and their controls. Quat Sci Rev 65:5–25. doi: 10.1016/j.quascirev.2012.11.029

Martin-Pinto P, Vaquerizo W, Penalver F, et al (2006) Early effects of a wildfire on the diversity and production of fungal communities in Mediterranean vegetation types dominated by *Cistus ladanifer* and *Pinus pinaster* in Spain. For Ecol Manage 225:296–305. doi: 10.1016/j.foreco.2006.01.006

McMullan-Fisher SJM, May TW, Robinson RM, et al (2011) Fungi and fire in Australian ecosystems: a review of current knowledge, management implications and future directions. Aust J Bot 59:70–90. doi: 10.1071/BT10059

Miller SL, McClean TM, Stanton NL, Williams SE (1998) Mycorrhization, physiognomy, and first-year survivability of conifer seedlings following natural fire in Grand Teton National Park. Can J For Res 28:115–122. doi: 10.1139/cjfr-28-1-115

Moritz MA, Batllori E, Bradstock RA, et al (2014) Learning to coexist with wildfire. Nature 515:58–66. doi: 10.1038/nature13946

Moritz MA, Parisien M-A, Batllori E, et al (2012) Climate change and disruptions to global fire activity. Ecosphere 3:art49. doi: 10.1890/ES11-00345.1

Mouquet N, Devictor V, Meynard CN, et al (2012) Ecophylogenetics: advances and perspectives. Biol Rev 87:769–785. doi: 10.1111/j.1469-185X.2012.00224.x

Nguyen NH, Smith D, Peay K, Kennedy P (2014) Parsing ecological signal from noise in next generation amplicon sequencing.

Olson DM, Dinerstein E, Wikramanayake ED, et al (2001) Terrestrial Ecoregions of the World: A New Map of Life on Earth A new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. BioScience 51:933–938.

Öpik M, Metsis M, Daniell TJ, et al (2009) Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New Phytol 184:424–437. doi: 10.1111/j.1469-8137.2009.02920.x

Palfner G, Ines Canseco M, Casanova-Katny A (2008) Post-fire seedlings of *Nothofagus alpina* in Southern Chile show strong dominance of a single ectomycorrhizal fungus and a vertical shift in root architecture. Plant Soil 313:237–250. doi: 10.1007/s11104-008-9697-y

Pausas JG, Fernández-Muñoz S (2012) Fire regime changes in the western Mediterranean basin: from fuel-limited to drought-driven fire regime. Climatic Change 110:215–226. doi: 10.1007/s10584-011-0060-6

Pausas JG, Ribeiro E (2013) The global fire–productivity relationship. Glob Ecol Biogeogr 22:728–736. doi: 10.1111/geb.12043

Peay KG, Bruns TD (2014) Spore dispersal of basidiomycete fungi at the landscape scale is driven by stochastic and deterministic processes and generates variability in plant-fungal interactions. New Phytol 204:180–191. doi: 10.1111/nph.12906

Peay KG, Garbelotto M, Bruns TD (2009) Spore heat resistance plays an important role in disturbance-mediated assemblage shift of ectomycorrhizal fungi colonizing *Pinus muricata* seedlings. J Ecol 97:537–547. doi: 10.1111/j.1365-2745.2009.01489.x

Peay KG, Russo SE, McGuire KL, et al (2015) Lack of host specificity leads to independent assortment of dipterocarps and ectomycorrhizal fungi across a soil fertility gradient. Ecol Lett 18:807–816. doi: 10.1111/ele.12459

Pechony O, Shindell DT (2010) Driving forces of global wildfires over the past millennium and the forthcoming century. PNAS 107:19167–19170.

Richard F, Bellanger J-M, Clowez P, et al (2015) True morels (*Morchella*, Pezizales) of Europe and North America: evolutionary relationships inferred from multilocus data and a unified taxonomy. Mycologia 107:359–382. doi: 10.3852/14-166

Richard F, Millot S, Gardes M, Selosse M-A (2005) Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*. New Phytol 166:1011–1023. doi: 10.1111/j.1469-8137.2005.01382.x

Roy M, Schimann H, Braga-Neto R, Da Silva RAE, Duque J, Frame D, Wartchow F, Neves MA (2016). Diversity and distribution of ectomycorrhizal fungi from amazonian lowland white-sand forests in Brazil and French Guiana. Biotropica 48:90–100. doi:10.1111/btp.12297Robinson RM, Mellican AE, Smith RH (2008) Epigeous macrofungal succession in the first five years following a wildfire in karri (*Eucalyptus diversicolor*) regrowth forest in Western Australia. Austral Ecol 33:807–820. doi: 10.1111/j.1442-9993.2008.01853.x

Sala A, Carey EV, Keane RE, Callaway RM (2001) Water use by whitebark pine and subalpine fir: potential consequences of fire exclusion in the northern Rocky Mountains. Tree Physiol 21:717–725.

Scott AC, Glasspool IJ (2006) The diversification of Paleozoic fire systems and fluctuations in atmospheric oxygen concentration. PNAS 103:10861–10865.

Séne S, Avril R, Chaintreuil C, et al (2015) Ectomycorrhizal fungal communities of *Coccoloba uvifera* (L.) L. mature trees and seedlings in the neotropical coastal forests of Guadeloupe (Lesser Antilles). Mycorrhiza 25:547–559. doi: 10.1007/s00572-015-0633-8

Shahin O, Paul NM-S, Rambal S, et al (2013) Ectomycorrhizal fungal diversity in *Quercus ilex* Mediterranean woodlands: variation among sites and over soil depth profiles in hyphal exploration types, species richness and community composition. Symbiosis 61:1–12. doi: 10.1007/s13199-013-0252-0

Smith ME, Henkel TW, Rollins JA (2015) How many fungi make sclerotia? Fungal Ecol 13:211–220. doi: 10.1016/j.funeco.2014.08.010

Smith SE, Read DJ (2008) Mycorrhizal Symbiosis, 3rd edn. Academic Press

Taudière A, Munoz F, Lesne A, et al (2015) Beyond ectomycorrhizal bipartite networks: projected networks demonstrate contrasted patterns between early- and late-successional plants in Corsica. Front Plant Sci. doi: 10.3389/fpls.2015.00881

Torres P, Honrubia M (1997) Changes and effects of a natural fire on ectomycorrhizal inoculum potential of soil in a *Pinus halepensis* forest. For Ecol Manage 96:189–196. doi: 10.1016/S0378-1127(97)00058-3

Tovar C, Breman E, Brncic T, et al (2014) Influence of 1100 years of burning on the central African rainforest. Ecography no-no. doi: 10.1111/ecog.00697

Treseder KK, Mack MC, Cross A (2004) Relationships among fires, fungi, and soil dynamics in Alaskan Boreal Forests. Ecol Appl 14:1826–1838. doi: 10.1890/03-5133

Twieg BD, Durall DM, Simard SW (2007) Ectomycorrhizal fungal succession in mixed temperate forests. New Phytol 176:437–447. doi: 10.1111/j.1469-8137.2007.02173.x

Valentini A, Pompanon F, Taberlet P (2009) DNA barcoding for ecologists. Trends Ecol Evol 24:110–117. doi: 10.1016/j.tree.2008.09.011

Visser S (1995) Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytol 129:389–401. doi: 10.1111/j.1469-8137.1995.tb04309.x

Vrålstad T, Holst-Jensen A, Schumacher T (1998) The postfire discomycete *Geopyxis carbonaria* (Ascomycota) is a biotrophic root associate with Norway spruce (*Picea abies*) in nature. Mol Ecol 7:609–616.

Warcup J (1990) Occurrence of ectomycorrhizal and saprophytic discomycetes after a wild fire in a eucalypt forest. Mycol Res 94:1065–1069.

Wardle DA, Hörnberg G, Zackrisson O, et al (2003) Long-term effects of wildfire on ecosystem properties across an island area gradient. Science 300:972–975.

Whelan RJ (1995) The Ecology of Fire. Cambridge University Press

Wilsey BJ, Chalcraft DR, Bowles CM, Willig MR (2005) Relationships among indices suggest that richness is an incomplete surrogate for grassland biodiversity. Ecology 86:1178–1184.

Zackrisson O, Nilsson M-C, Wardle DA (1996) Key ecological function of charcoal from wildfire in the boreal forest. Oikos 77:10–19. doi: 10.2307/3545580

Table 1: Qualitative effect of fire on different components of biodiversity. Numbers correspond to the publication reporting the effect of interest (publication list in Table S1). Publications highlighted in bold used molecular techniques and an asterisk (*) indicates publications using Next-Generation Sequencing (NGS). +, -, = and ~ denote respectively significant positive effect, significant negative effect, no significant effect and significant change due to fire (i.e. change without clear and univocal direction). **Ab:** Abundances (e.g. Tip numbers or hyphal Length); **BioM**: Fungal Biomass; **Col**: Colonization of roots (e.g. root tip numbers, % of root with ECM); **Com**: Community composition and/or structure (e.g. DCA, CCA, PERMANOVA, NMS, anosim) using DNA analysis of root tips (**Com**) or sporocarp survey (**Sporoc. Com**); **Div**: Taxonomic Diversity (e.g. Simpson index); **FD**: Functional diversity (Enzymatic activities); **PD**: fungal Phylogenetic diversity; **Prod**: Production of sporocarps (e.g. dry weight); **S**: Species richness.

	Fungal component	+	-	=	~
Wildfire	Ab		70	**33**	**12**, **19***, 22, 46, 51, 65, 72
	BioM		1		
	Col	50, 51	22, 42, 57, 59, 70	**67**	
	Com			22, **33**	**6**, **12**, **14***,**19***, **24**, **32**, 51, **67**, 71
	Div		**6**, **33**, 46, **67**	**13**, 22, 42	
	FD			**32**	**19***
	Sporoc. Com				1, 58, 71, 72
	Prod		23, 46, 48	**33**, 58	18, 31
	S		1, 39, 46, 48, 65, 72	**6**, **13**, 22, **33**, 42, 58	**14***
Prescribed burning	Ab		**17**, **21**, 49		**10**
	BioM		25, 27, **61**, **63**	**4**	
	Col		9, 73	38	
	Com			**62**	**3,7**
	Div		**21**, 27		
	PD	**4**			
	Prod		49	52	
	S		**17**, **21**, 27, 49, **60**, **61**	**3**	

Table 2: Qualitative effect of severity, frequency and times since fire on different components of biodiversity (same legend as for Table 1).

	Fungal component	+	-	=	~
Severity	Ab		**21**	70	
	BioM		1		
	Col		40, 73	70	
	Com			**28**, **35**	40
	Div		**6**, **21**	**28**, **35**, 56	
	S		1, **21**	**28**, **35**, **56**	
Frequency	Ab		68		
	Com				**3**, **7**, **8***, **13**, **15**
	Div		**15**, 68		
	S		**8***, **15**, 68		
Chrono-sequence	Ab	**29***, **64***			**69**
	BioM			27	
	Col	22		55, 71	66
	Com				26, **29***, **37**, **41**, **57**, **69**, 71
	Div	**41**	27		26, **69**
	S	**37**, **41**, 71		27, **29***	26, **69**

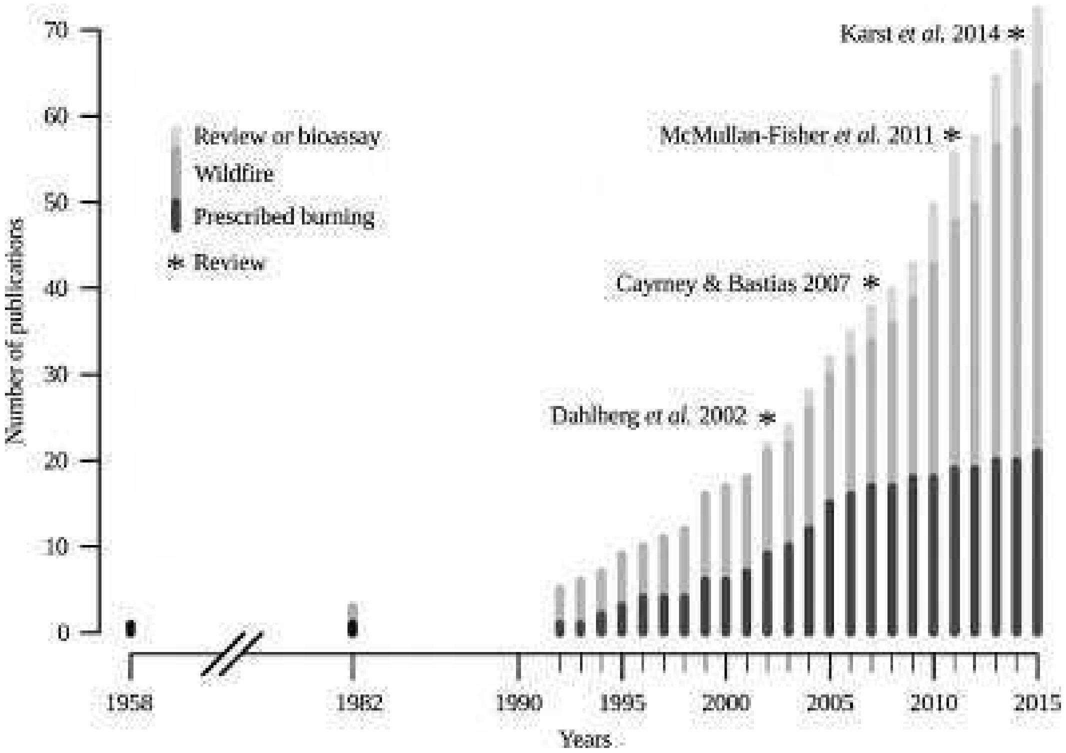


Fig. 1: Timeline of publications included in the review (total=73) dealing with prescribed burning (dark blue), wildfire (grey) or bioassay/review (light grey). Stars indicate the four reviews dealing, at least in part, with the effect of fire on ectomycorrhizal interactions.

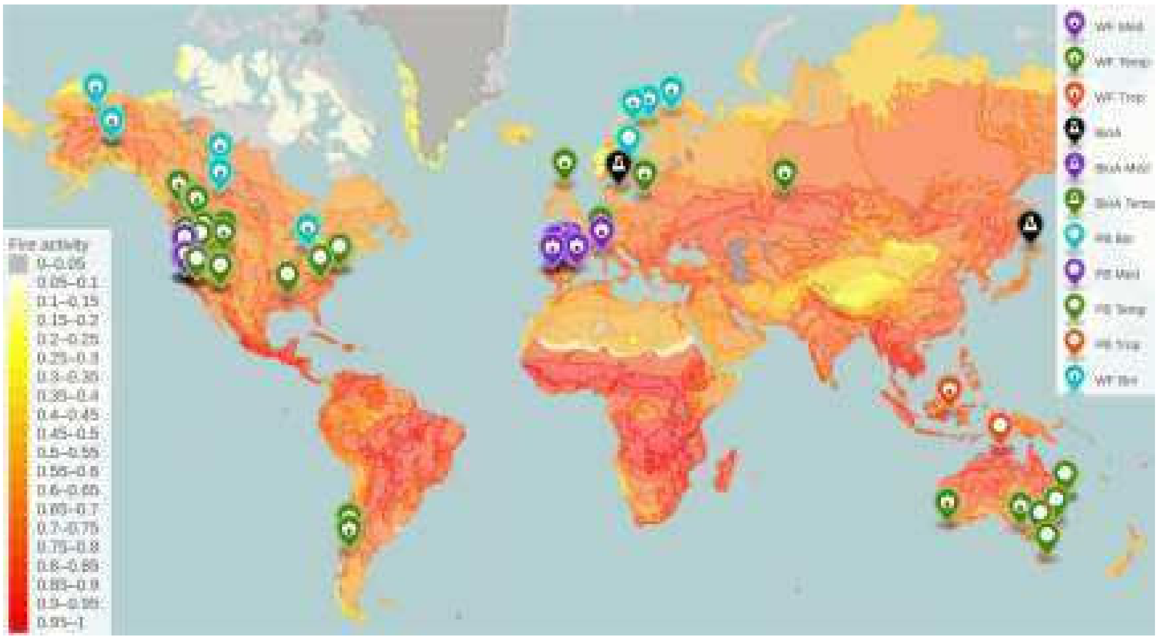


Fig. 2: Geographical distribution of the areas studied in the published data on the effect of fire on ectomycorrhizal interactions. Marker colors correspond to biomes (blue: boreal; purple: Mediterranean; green: temperate; orange: tropical; black for bioassay-based studies). Marker icons correspond to the type of fire (fire icon for wildfire, Erlenmeyer flasks icon for bioassay and circles for prescribed burning). Terrestrial ecoregions follow the World Wildlife Fund classification (WWF; Olson et al. 2001; Fig. S3). Global fire activity information data by ecoregion are from Pausas and Ribeiro (2013) using FIRMS (Fire Information for Resource Management System, NASA from January 2001 to December 2009; see figure S3 for publication locations and ecoregions only) where the fire activity index was defined as the logarithm of the average number of fire incidences per region, divided by the region area. The interactive version of this map is available online (https://github.com/adrientaudiere/ReviewFireEcmMap).

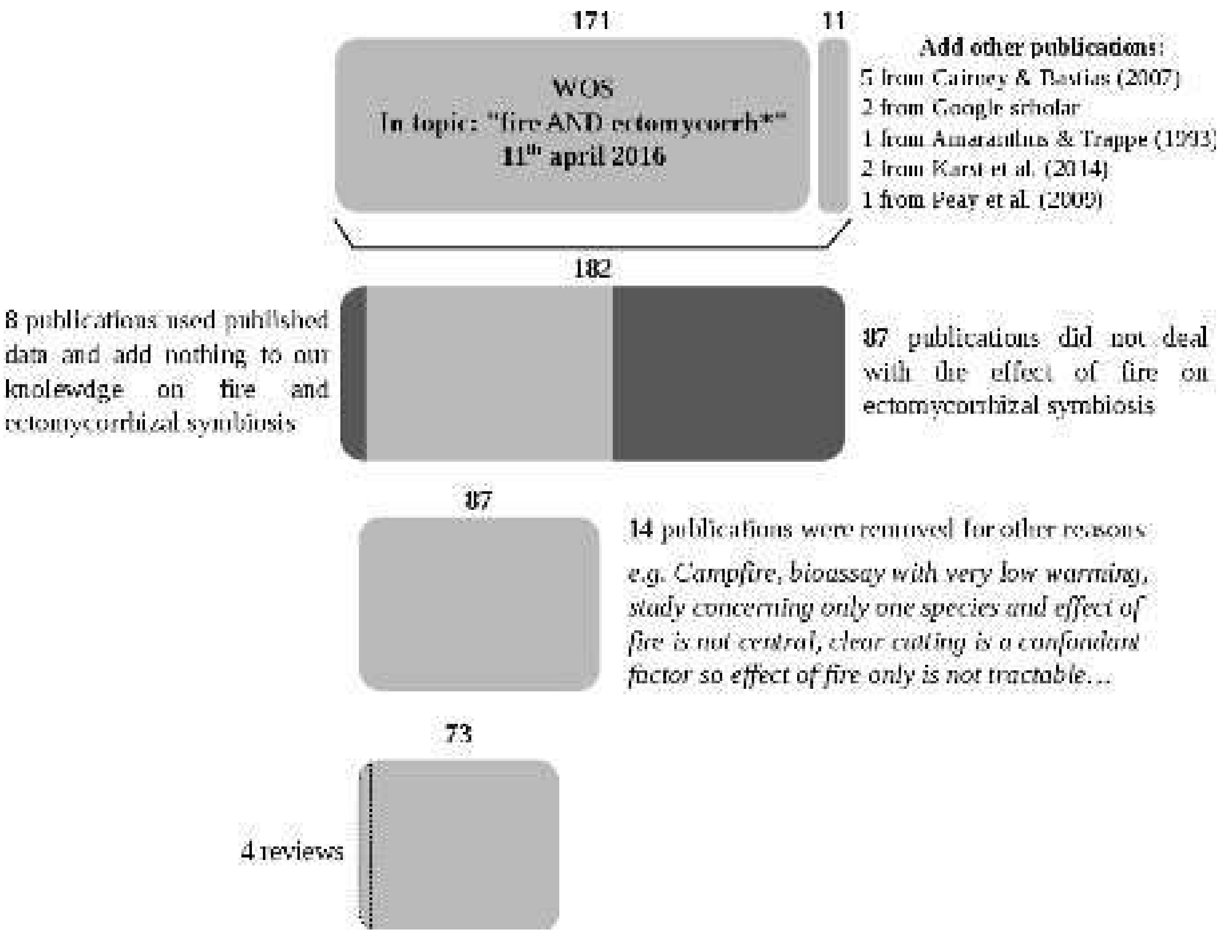
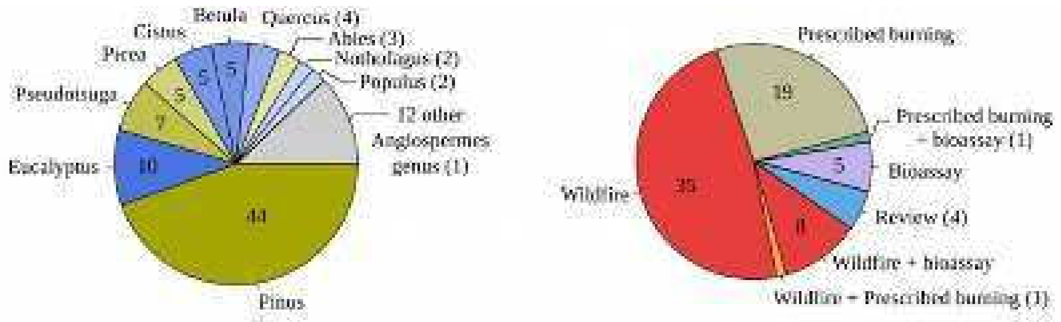
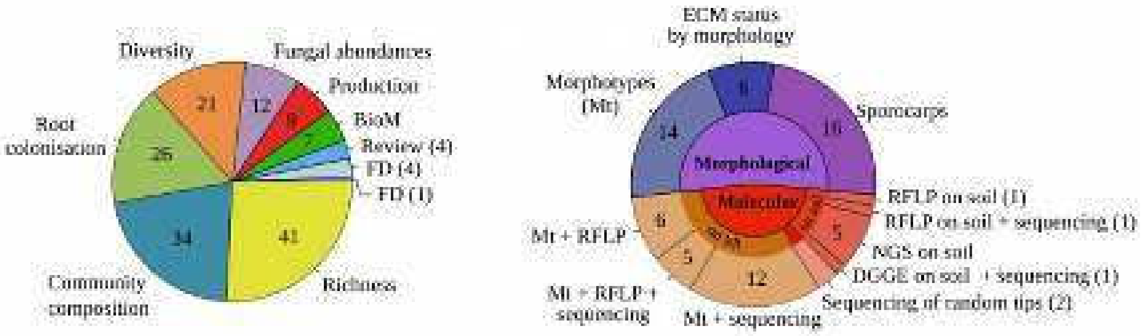


Fig. 3: Flowchart of selection of the 73 publications included in the review.



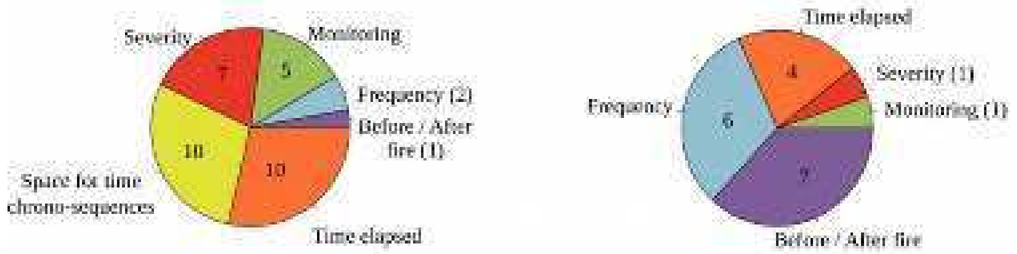
(a) Host genus

(b) Types of fire



(c) Analysis

(d) Identification methods



(e) Wildfire modalities

(f) Prescribed burning modalities

Fig. 4: Distribution of plant host genus (a), types of fire (b), types of analysis (c), identification methods for fungal taxonomy assignment (d) and fire modalities (e-f) in the literature. See supplementary methods for details. **FD**: Functional Diversity; **PD**: Phylogenetic Diversity; **BioM**: Biomass; **Production**: Sporocarp production; **Mt:** Morphotype; **RFLP**: Restriction Fragment Length Polymorphism; **NGS**: Next-Generation Sequencing; **DGGE**: denaturing gradient gel electrophoresis.

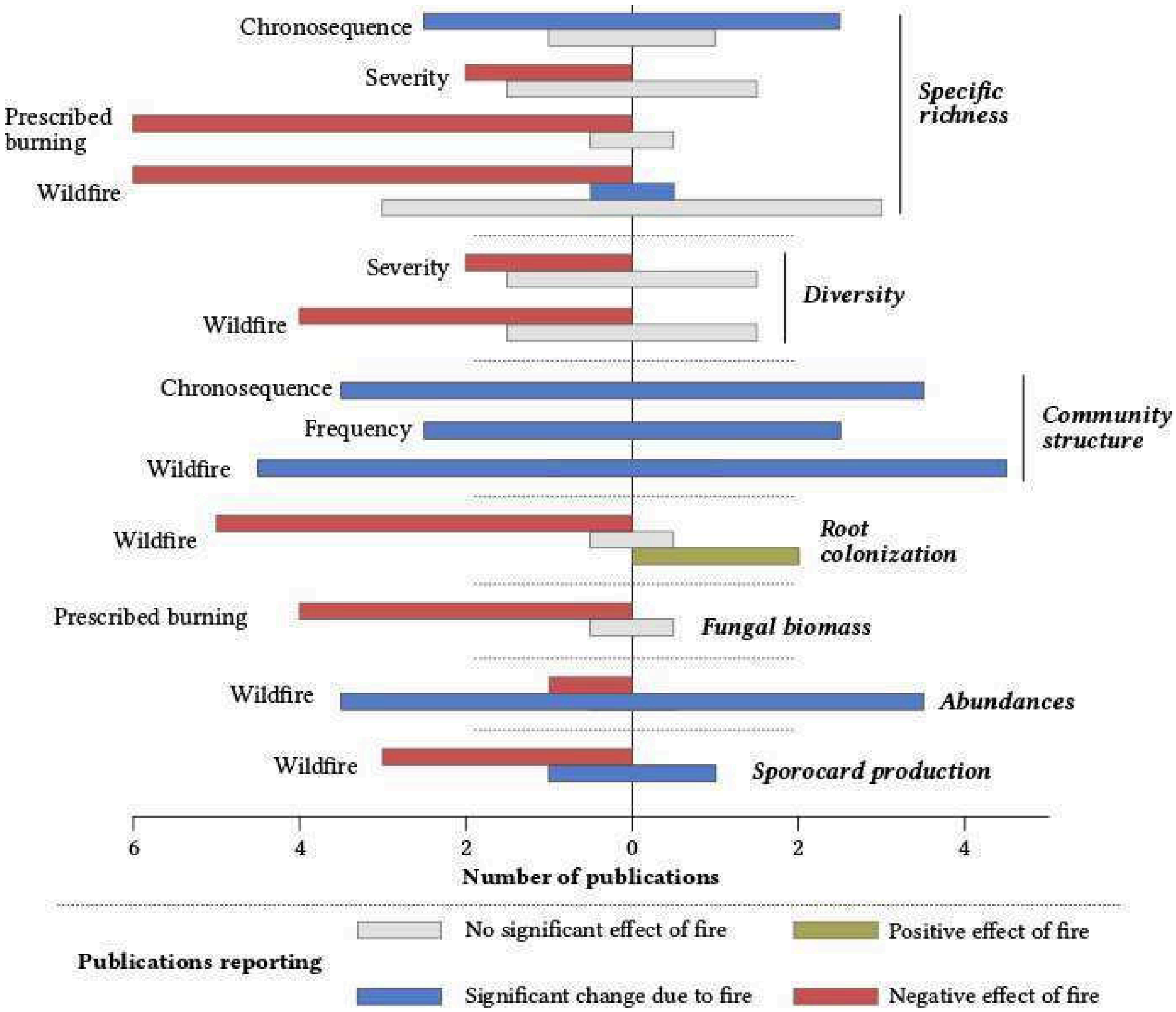


Fig. 5: Number of published results (based on Table 1 and 2) treating the effect of fire on ECM fungi. Only cases documented in more than 4 publications are shown.

3 "Effect of fire on ECM fungal communities in soil and roots of *Pinus nigra* subsp. *laricio* (Corsica, France)" (Projet de manuscrit G)

Vue d'une zone parcourue par un feu au niveau du col de Bavelle.

In a Nutshell

This is a work in progress. See section works progress and draft G in appendix ⚙.

Rationale: Fire affects multiple facets of ECM symbiosis including species richness, diversity, colonization rate, fruitbody production and composition of ECM fungal communities (manuscript F). However, the response of ECM communities to the interaction between fire severity, time since fire and soil depth was never assessed. Moreover, previous works studied either active ECM fungi (e.g. using fruitbody surveys or root tip sequencing) or the combination of latent and active communities of ECM fungi (e.g. soil sequencing).

In this study, we described the ECM fungal communities from Corsican pine forests which have undergone various fire histories. We explored both fire severity (crown and destructive fire *vs* surface fire *vs* unburnt forests) and time elapsed since the last fire (6, 14, 21, 34 and 44 years after fire). We sampled both soil and ECM root tips in organic and mineral soil layers, and sequenced all samples using the Illumina technology.

Type of interaction: Ectomycorrhizal symbiosis, mostly mutualistic

Ecological filters of fungal communities: Wildfire in interaction with soil depth

Main results:

◕ *Surface fires increase soil ECM fungal diversity*

Surface fires significantly promote higher ECM fungal diversity when compared to both unburnt and crown fire stands (Fig. IV.2a). Moreover, ECM fungal diversity is higher in mineral than in organic layer, while species richness doesn't differ between soil horizons (Fig. IV.2b).

◕ *Wildfire severity shapes soil ECM fungal communities across soil profiles*

Both fire severity and site drive ECM fungal community composition (PerManova; Table IV.1, Fig. IV.3 and IV.4). On the contrary, soil depth and its interaction with fire severity don't affect ECM fungal communities (PerManova; Table IV.1, Fig. IV.5). Crown fire (high severity) results in greater change in soil ECM communities than surface fire (low severity) in both mineral and organic layers (Fig. IV.3).

◕ *Wildfire affects soil OTU molecular abundance*

Main OTUs with abundance shift in response to fire occurrence and sevzerity (Fig. IV.4 and IV.5). In particular, OTUs belonging to Russulales (e.g. *Lactifluus* and *Russula*) and Agaricales (e.g. *Inocybe*) are more abundant in the absence of fire. On the other hand, two OTUs belonging in *Rhizopogon* and *Geopora* genera, as well as one in the *Suillus* genus, are favored by fire (Fig. IV.4 and IV.5). Interestingly, *Geopora sp* (Ascomycetes) is favored by destructive fire but not by surface fire.

Main conclusions: After 15 years, the signature of wildfires on soil ECM fungal communities is still detectable, even in forests that undergone surface fire. Both mineral and organic layers are impacted in the same way. Fire negatively impacts numerous ECM OTUs but promotes a few of them.

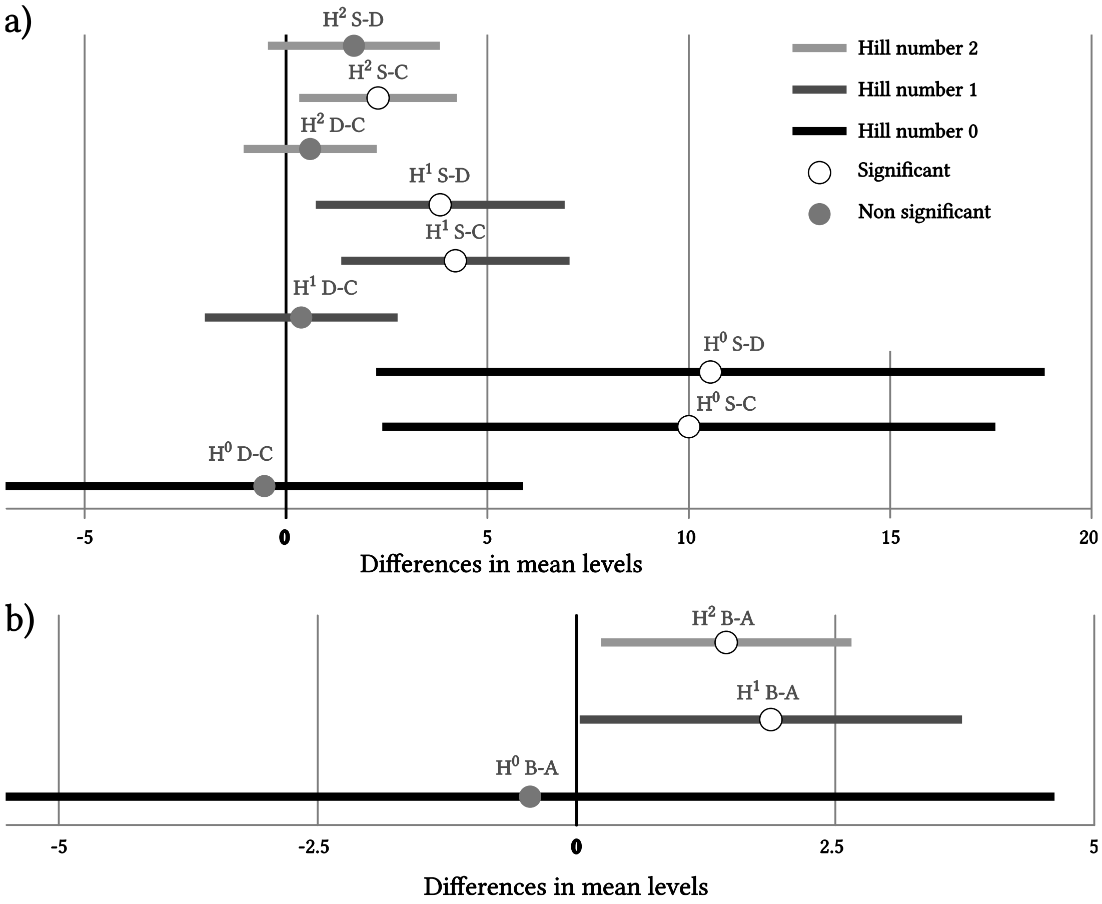


Figure IV.2 – Effect of (a) fire severity (C: control; S: surface fire; D: destructive crown fire) and (b) depth (A: organic layer and B: mineral layer) on ECM fungal diversity in soil: X-axes indicate the difference in mean levels and confidence interval using Tukey honest significant difference on linear models involving Hill numbers (H^0, H^1 et H^2) in function of sample size (square root of the number of sequence) and either fire severity (panel **a**) or depth (panel **b**).

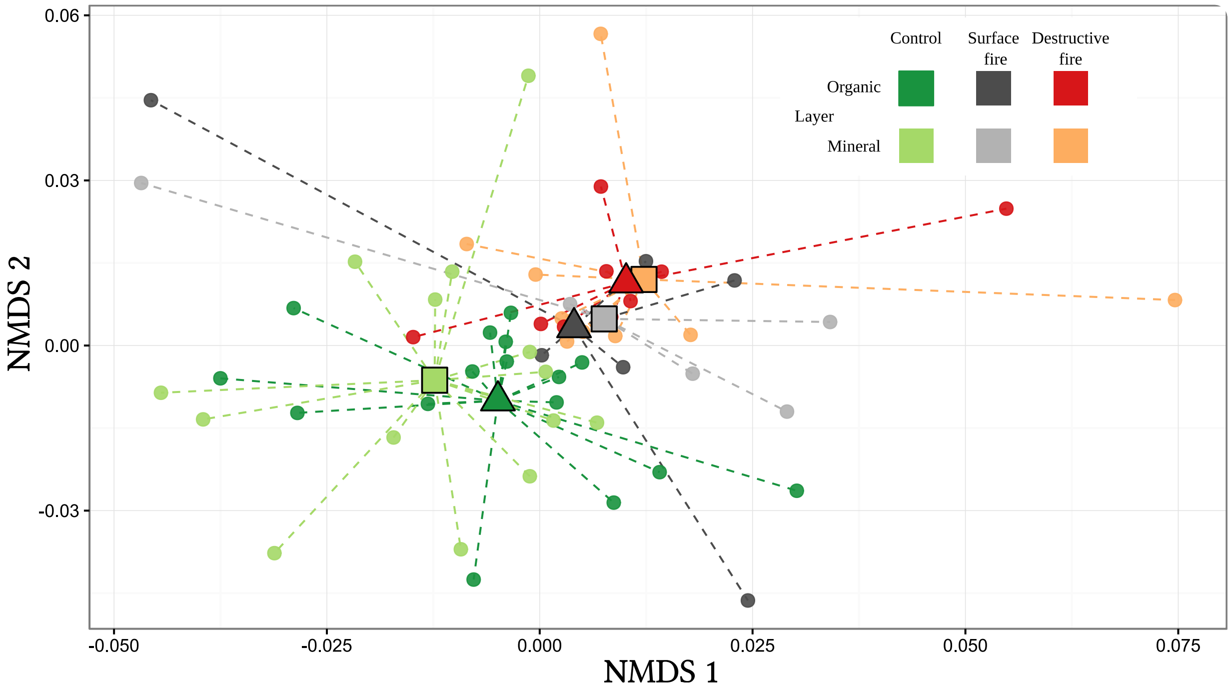


Figure IV.3 – Non metric multidimensional scaling (Gower distances) of ECM fungal composition in soil regarding fire severity and depth.

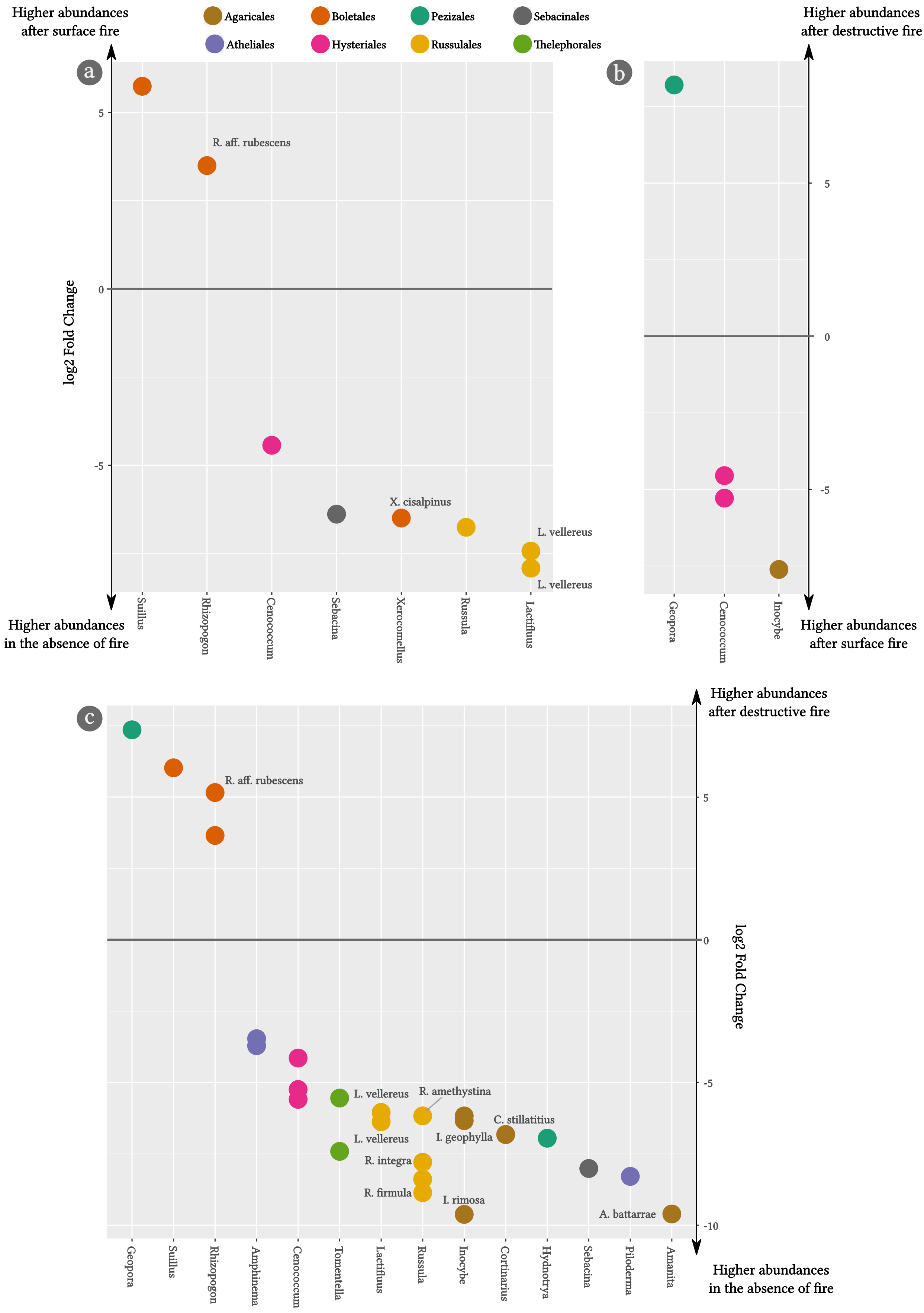


Figure IV.4 – Change in molecular abundance of ECM OTUs present in soil in function of fire severity: Each point is an ECM OTU whose abundance significantly shift regarding fire severity (*Deseq2* R package). Panel (**a**) compare abundance after surface fire and without fire. Panel (**b**) compare abundance after destructive fire and surface fire. Panel (**c**) compare abundance after destructive fire and without fire. Colors indicate fungal Order. Note the scale of y-axis are different for each panel.

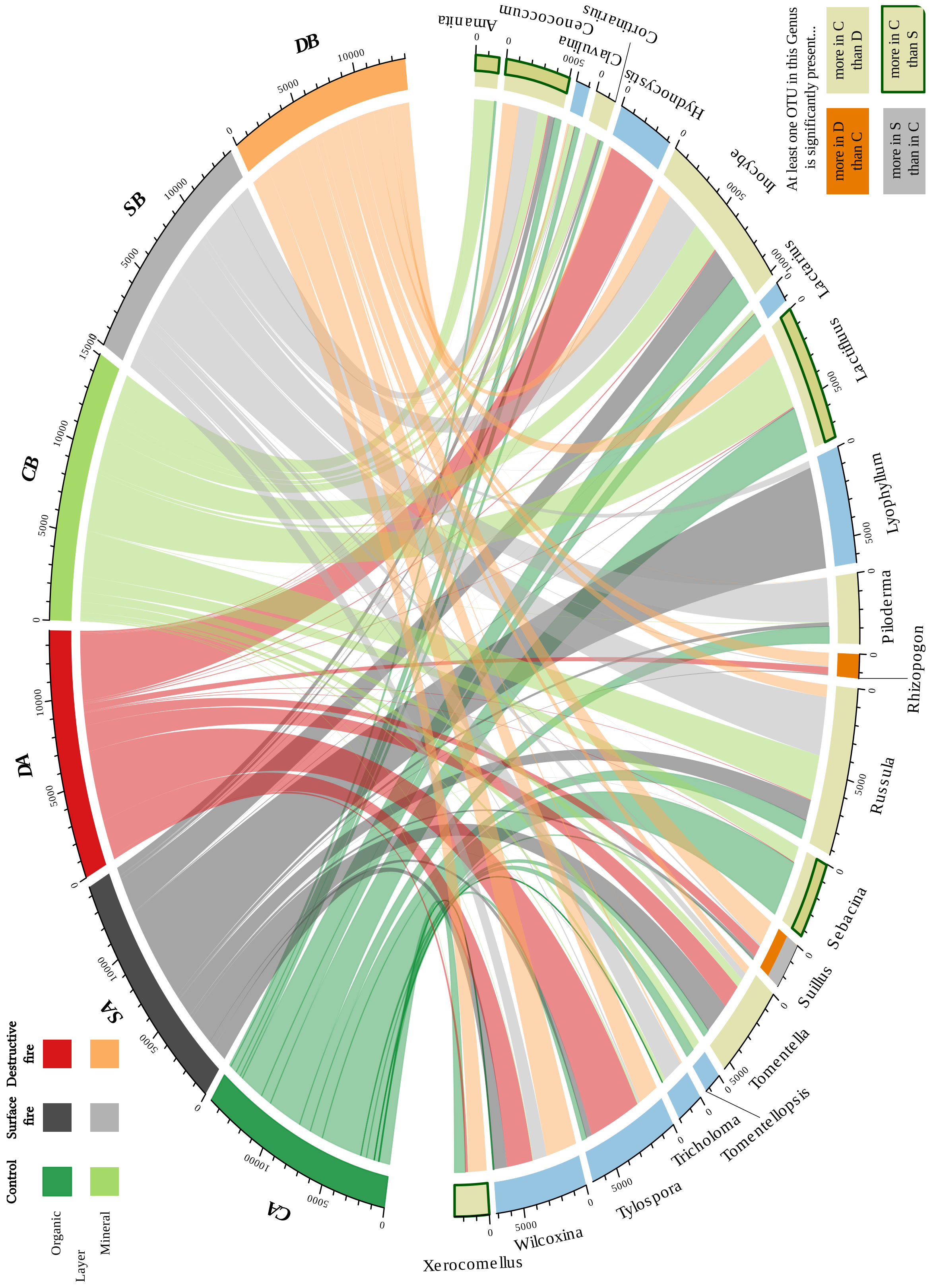


Figure IV.5 – Distribution of the molecular abundances of ECM fungal Genus in function of fire severity and depth: All modalities were first rarefy at the same sequences numbers. Only fungal genus representing more than 1% of the total number of sequences are represented. Modalities and ribbons colors are the same as figure IV.2. Fungal genus with at least one OTU that differ in molecular abundance (*DESeq2* package; *cf.* Fig. IV.4) among different fire severity are colored (color scheme in the left down corner). Genus in blue do not contain any OTU showing significant preference regarding fire severity.

Tableau IV.1 – Decomposition of soil ECM fungal communities in function of fire severity and depth : PerManova analysis (Anderson 2001) using 9 999 permutations.

Variables	**Df**	**Sums Of Sqs**	**MeanSqs**	**F.Model**	**R2**	**Pr(>F)**
Sites	2	1.6097	0.80485	1.88789	0.05994	0.0001
Fire severity	2	2.0577	1.02886	2.41334	0.07662	0.0001
Depth	1	0.4019	0.40187	0.94266	0.01496	0.5736
Fire severity × Depth	2	0.6183	0.30913	0.72511	0.02302	0.9929
Residuals	52	22.1687	0.42632		0.82546	
Total	59	26.8563			1.00000	

Chapitre V

Structures des communautés de champignons endophytiques des aiguilles de pin laricio

> « *C'est une grande épreuve mais c'est aussi une belle aventure.* »
>
> Franck Richard, *Des racines et des ailes*, 28/01/2015

Canopé de pin laricio (Pinus nigra subsp. laricio) dans la vallée du Verghello

1 Manuscrit H : "Finding fungi in a needle stack: contrasted α- and β-diversity of endophytic Ascomycetes in Mountainous pine forests"

IN A NUTSHELL

Rationale: Like other plants, pines accumulate functionally obligate communities of endophytic fungi in their leaf. The spatial distribution and the process underlying community assembly patterns of foliar endophytic fungi (FEF) are poorly known despite its importance for plant growth and health. Here we document the communities of FEF associated with the Corsican pine across the natural geographical range of this species using NGS.

Type of interaction: Endophytism, interaction outcome for both partners is poorly known and range from parasitism to mutualism

Ecological filters of fungal communities: Environment filter through host age and elevation in the tree; Geographical filter (sites)

Main conclusions: Spatial location (valleys) was the main driver of both diversity and composition of FEF communities before tree age, whereas light exposure doesn't affect FEF communities significantly.

Finding fungi in a needle stack: contrasted α- and β-diversity of endophytic Ascomycetes in Mountainous pine forests

Adrien Taudière [12*], Jean-Michel Bellanger [1], Sebastian Leidel [3], Leatitia Hugot [4], Pierre-Arthur Moreau [5], Franck Richard [1]

Affiliation and Address:

1/ UMR 5175 CEFE – CNRS - Université de Montpellier - Université Paul Valéry Montpellier - EPHE - INSERM, Campus CNRS, 1919 Route de Mende, F-34293 Montpellier, France

2/ École Pratique des Hautes Études (EPHE) & Paris Research University, Paris, France

3/ Max Planck Research Group for RNA Biology, Max Planck Institute for Molecular Biomedicine, Von-Esmarch-Strasse 54, 48149 Muenster, Germany

4/ Conservatoire Botanique National de Corse. Route d'Aleria. 20250 Corte, France.

6/ Département de Botanique, Faculté des Sciences Pharmaceutiques et Biologiques, Université Lille - Nord de France B. P. 83, 59006 Lille Cedex, France.

* Corresponding author: adrien.taudiere@cefe.cnrs.fr; franck.richard@cefe.cnrs.fr

Abstract:

In their leaves, plant accumulate functionally obligate communities of endophytic fungi. As compared to other compartments of the plant holobiont, the spatial distribution and the process underlying community assembly patterns of foliar endophyte fungi (FEF) are poorly known. Here we document the communities of FEF associated with the Corsican pine (*Pinus nigra* subsp. *laricio*) in needles collected across the natural geographical range of this species. Within the described regional pattern, we examine the role of tree age and light exposure as local scale-drivers of community structure and composition. Deep sequencing identified 8 243 608 Ascomycota ITS-2 sequences clustered into 642 FEF OTUs. Regardless of the used bioinformatics pipeline, site was the main driver of both diversity and composition of FEF communities across Corsica Island. Tree age slightly affected FEF community composition, whereas light exposure didn't drive any diversity and composition shift. This study provides the first regional picture of FEF diversity and reveals the complementarity of Corsica micro-regions in supporting distinct biodiversity and conservation stakes.

Keyword: foliar endophyte; fungi; community ecology; Illumina; *Cyclaneusma minus*

Research highlights:

- Plants host highly diversified micro-organisms in their tissues
- We sequenced foliar endophytic fungi (FEF) of *Pinus nigra* ssp. *laricio* using MiSeq
- We found 8 243 608 Ascomycota ITS-2 sequences clustered into 642 FEF OTUs
- FEF richness and diversity are not affected by microclimate (shade vs light)
- FEF composition is affected by microclimate and not by tree age
- Similar results are obtained using various bioinformatics pipelines

Introduction

Plants are complex constructions mixing micro and macro-organism genotypes and phenotypes (Partida-Martinez and Heil 2011; Vandenkoornhuyse et al. 2015). In forest canopies, leaves of the approximate 3 trillion trees on earth not only support the entire food web in forest ecosystems (Crowther et al. 2015), but also represent a keystone place for interactions between autotrophs and living components of their microbiomes. Phyllosphere microbiota — the community of all microorganisms living at the surface (epiphytes) or inside leaves (endophytes) — is mostly composed of Fungi and Bacteria (Christian et al. 2015; Hardoim et al. 2015). These micro-organisms are involved in plant growth, survival and ecology (Porras-Alfaro and Bayman 2011, Friesen et al. 2011). From a functional perspective, the phyllosphere microbiome mediates functional traits (Friesen et al. 2011) and is considered as an essential component of plant extended phenotype (Kembel et al. 2014).

Endophytic microorganisms *largo sensu* encompass mutualistic (endophytes *stricto sensu*), pathogen and commensal species, mostly belonging to Bacteria or Fungi phylum (Hardoim et al., 2015). At the species level, many fungal endophytes are of unknown trophic status, and all cases coexist on tree leaves, from truly mutualists to parasites. Foliar endophytic fungi (FEF) depends on numerous abiotic and biotic parameters (Sieber 2007). Some FEF may also act as “pioneer” decomposers (Yuan and Chen, 2014), benefiting from early dispersal and substrate colonization. This strategy, as other fungal symbioses (e.g. mycorrhizal), may have derived from free-living lifestyles, as confirmed by phylogenetic analyses that revealed numerous shifts between necrotrophic and endophytic lineages (Delaye et al. 2013). In the present research, FEF are defined by their habitat , not by their functional attributes (Hardoim et al. 2015).

It has been shown that the functioning of the plant holobiont depends on FEF composition (Hardoim et al. 2015, Vandenkoornhuyse et al. 2015). For autotrophs, fungal endophytism supposedly provides protection against various pathogens, abiotic stress such as drought, but also against herbivory (Porras-Alfaro and Bayman, 2011; Christian et al. 2015). For example, the fungal endophyte *Colletotrichum tropicale* reduces the defoliation of *Cucumis sativus by* leaf-cutting ants (Estrada et al. 2013). These positive interactions between plants and FEF properties have prompted a wide range of clinical tests by pharmacists (e.g. for antitumoral, antibacterial, fungicide abilities; reviewed in Kaul et al. 2012, and Mishra et al. 2014) and ambitious industrial developments (e.g. for fuel production and insecticides properties; Strobel 2014; Mousa & Raizada, 2013). For ecological sciences, endophytes are promising model organisms to test ecological concepts (Meyer and Leveau 2012), but they also represent a considerable, albeit still largely neglected microbiota directly involved in ecosystem functioning (Friesen et al. 2011; Yuan and Chen 2014; Hardoim et al. 2015).

In community ecology, the scale of composition variation is often used to disentangle assembly processes among macro-organisms (e.g. Münkemüller et al. 2014, Taudière & Violle 2016). For filamentous fungi, identifying the factors governing the co-occurrence of species and the determinants of spatial distribution of highly structured and patchy communities is a central issue across lifestyles, including vesiculo-arbuscular mycorrhizal symbionts (e.g. Wolfe et al. 2007, Mummey and Rillig 2008, Dumbrell et al. 2010), ectomycorrhizal symbionts (e.g. Lilleskov et al. 2004, Peay et al. 2007), grass endophytes (Higgins et al., 2014), and woody leaf endophytes (Arnold et al. 2003, U'Ren et al. 2012). For endophyte micromycetes, and using isolate culturing or DNA sequencing, previous publications provided insights into the determinants and the scale of FEF community variation (Table 1). FEF communities show spatial structuration at the tree individual scale: FEF species assemblages i) are more similar among spatially-closed leaves within the same beech (*Fagus sylvatica*) individual than among distant ones (Cordier et al. 2012), ii) differ in composition across gradients of elevation (e.g. within *Fagus sylvatica*; Siddique and Unterseher 2016) and iii) vary in abundance across gradients of age of leaves (e.g. within *Pinus sylvestris*; Millberg et al. 2015), old needles being more colonized than young ones (e.g. within *Pinus sylvestris*; Helander et al. 1994). At the inter-individual scale, FEF communities also show structuration patterns, both across genotypes within species (*Populus balsamifera*, Balint et al. 2013 and 2015) and across species (Kembel and Mueller 2014).

Understanding community assembly processes of FEF is crucial to more accurately estimate the importance of FEF in plants biology and ecology. However, combined assessment of between- and among- trees structure of FEF community has never been performed, and the factors governing composition shifts among FEF communities are still poorly understood. Here, we aim at exploring the diversity of endophytic ascomycetes present within leaves of the endemic tree *Pinus nigra* subsp *laricio* (the so-called Corsican pine), whatever their effect on plant fitness (endophytes *largo sensu*). Corsican Pine is a priority species in its natural range according to the Habitat Directive CEE 92/43. We describe the structure and the composition of FEF communities at three complementary and nested scales of interaction with their host, from deep shade leaves to fully exposed canopies, from young saplings to multisecular individuals, and across the natural biogeographical range of the Corsican pine in the island (Figure 1). To reach our goal, a nested sampling design was applied in three highly preserved (old-growth stands) sites to provide a comprehensive overview of FEF biodiversity associated with this emblematic tree species of the Mediterranean basin.

Material and methods

Sampling procedure

In Corsica Island, *Pinus nigra* subsp. *laricio* covers 25 164 ha (Inventaire Forestier National, 2006) of vegetation established on acidic soils and dominating landscapes from 900 to 1 800 m in elevation (Gamisans & Marzocchi, 1996). Across the Island, this highly long-lived tree species persists in few well conserved (old-growth) stands where long-term natural dynamic processes shaped uneven aged populations (Gamisans & Marzocchi, 1996; Norstedt et al. 2001). In these forests, scattered and multisecular individuals dominate a dense understory made of various cohorts of pines, from deep shade seedlings to canopy mature trees.

In May 2013, in three *Pinus nigra* subsp. *laricio* old-growth stands (Asco *vs.* Verghello *vs.* Bavella forest; Figure 1), the following sampling procedure was carried out. Nine individuals were selected (three > 250 yrs.-old, the so-called "ancient"; three 70-130 yrs.-old, the so-called "mature"; three 10-15 yrs.-old, the so-called "young"; Figure 1), at a minimum distance of 50 m from each other. For each of the 27 selected trees, 1 yr.old needles were collected in three contrasted microclimatic conditions (full shade lower branches, the so-called "low"; middle height branches, the so-called "intermediate"; full light canopy branches, the so-called "high"). In total, 81 needle samples have thus been collected for analysis.

Sample preparation, DNA extraction and Illumina sequencing

Collected needles were transferred to the CEFE laboratory, and prepared for further DNA analysis. In the majority of recent studies on FEF, the leaf surface has been sterilized before DNA extraction or isolation methods (Table 1) but some studies mixed epiphytic and endophytic organisms (see Rastogi et al. 2013 for a review on phyllosphere microbiota).

Bioinformatic pipeline

The detailed workflow from raw sequences to OTU tables and taxonomy is available in figure S1 and the associated script in supplementary materials S2. In short, R1 and R2 sequences were merged using Qiime (*join_paired_ends.py* command; Caporaso et al. 2010), quality filtered using sickle (*single se* command, quality threshold -q set to 33), demultiplexed (through a script provided by Balint et al. 2014) and dereplicated. FastQC (Andrews 2010) quality profiles before and after quality filtering are available in supplementary figures S3 and S4. Chimeras where removed using Usearch (*uchime_ref* command; Edgar et al. 2011) with Unite (Kõljalg et al. 2013) as reference database (dynamic v.7 release of 01-08-2015, which includes numerous endophytic taxa since the effort of Nilsson et al. 2014). Non fungal ITS sequences were discarded using ITSx (Bengtsson-Palme et al. 2013). Moreover, ITSx allows selecting solely sequences encompassing ITS-2 region. From the 26 911 635 unmerged sequences we obtained, 468 871 corresponded to unique fungal sequences, encompassing ITS2 (Figure 1, S1) of length 196.4 ± 6.9 bp. ITSx found a lot of unexpected “Tracheophyta” sequences (231 009 unique sequences), some of which were undoubtedly assigned to fungal sequences present in Unite database. The analysis was then rerun after merging ITSx-based Tracheophyta and fungal sequences (Table 2, SM 10) without significantly altering our conclusions.

We used five different clustering methods (Qiime open reference, Qiime closed reference, Uparse discarding unique sequences; Uparse including unique sequences and Swarm; Caporaso et al. 2010; Edgar et al. 2013; Mahé 2015) to define OTUs. Here are presented the results of Uparse (without unique sequences) for its highly robust and conservative clustering method (Edgar 2013). All the analyses performed using the four other methods are presented as supplementary materials (SM 9, 12-14) and yielded results consistent with the presented analysis. Every OTU-representative sequence was taxonomically assigned in Unite using RDP classifier through the *assign_taxonomy.py* Qiime function (bootstrap threshold 80% following recommendation of Claesson et al. 2009). Comparison among the four taxonomic assignation methods (RDP, Blast, Mothur and Uclust) is presented as supplementary material (SM 5). Then, a OTU table was assembled using the function *-usearch_global* (Usearch). Finally, OTUs were assigned to one functional guild (e.g. lichenized) and/or trophic mode (e.g. saprotrophe) when possible, using FUNGuild (Nguyen et al. 2015).

Ecological analyses were then performed under R 3.2.4 (R Core Team 2016) mainly through the Phyloseq package 1.12.2 (McMurdie 2013). In the subsequent analysis, we only took into account samples with more than 20 000 sequences (i.e. 72 out of the 81 samples) and OTUs represented by more than five sequences (following Brown et al. 2015). We provide a dynamic report script (SM 9) to rerun all the analyses using different parameters of filtering, namely the minimum number of sequences per sample, the minimum number of samples per OTU and the minimum number of sequences per OTU (need the knitr package; Xie 2015).

Statistical analyses of endophyte diversity

Local diversity was assessed by Hill number (Hill, 1973) as in Balint and collaborators (2015): ^{q}H with q = 0 (Species richness), q = 1 (exponential of Shannon's entropy index) and q = 2 (inverse of Simpson's concentration index). ^{1}H, and even more ^{2}H, measures evenness by weighting more abundant taxa than rare ones. Hill numbers were calculated using R package vegan 2.3-4 (Oksanen et al. 2013). The effect of explanatory variables on Hill numbers was evaluated using linear models and ANOVA. Balint et al. (2015) method was applied to controls for difference in sequence number among samples: square roots of the number of reads were the first predictors of diversity in used linear models. Tukey HSD tests were used to compare Hill numbers among pairs of sampling modalities (i.e. site, cohort of tree age, needle location regarding light exposure). OTUs accumulations curves were computed using the function *rarefy* from the package vegan (R script is available in appendix SM 9). For accumulation curves in figure 4, the dataset including singletons was used (Uparse 1) to avoid the false tendency to reach a plateau due to the artefactual absence of the rarest OTUs.

Beta-diversity among samples was assessed by performing a NMDS on Bray distance. All multivariate methods (NMDS, PCoA and RDA; see Paliy and Shankar [2016] for a review of multivariate methods in microbial ecology), whatever the distance computation used (Bray *vs* Gower distance), gave comparable classification (Appendix S9). We partitionned the variance between explanatory variables (Site, Age, Elevation and all their interactions) with a PerManova (Anderson, 2001). Finally, differences in abundance distribution of OTUs among samples and variables were tested using the DESq2 package (v. 1.8.2; Love et al. 2014).

Results

Outputs of bioinformatics pipelines

Keeping identical filtering parameters (i.e. number of sequences per sample >= 20 000 and number of sequences per OTU >= 5), we found between 251 and 9 325 OTUs depending of the clustering methods (Table 2). The most conservative de novo clustering method (Uparse clustering without unique sequences and after removing sequences assigned putatively to "Tracheophyta" by ITSx) revealed 662 OTUs. The results being congruent across clustering methods (SM S9 – S14), we hereafter only report the detailed results for this conservative *de novo* clustering method. Out of the 662 OTUs, 20 (21 986 sequences, 3%) were discarded because either they were present only in samples with less than 20 000 sequences (8 OTUs; 21 948 sequences) or they were represented by less than 5 reads (12 OTUs; 38 sequences). A total of 8 243 608 sequences representing these 642 OTUs were assigned to sample. Taxonomic distribution of sequences and OTUs is available online using Krona widget (Ondov, 2011; S5).

Taxonomic assignment of sequences

The quality of assignment, as measured by the bootstrap from RDP classifier, was not correlated with the number of sequences in the OTUs (Spearman correlation test: rho = 0.019; p-value = 0.628, Figure S16). Moreover, considering the most precise taxonomic rank given with confidence by RDP to OTUs (bootstrap value > 80%), only the Class rank presented a statistically lower number of sequences than other taxonomic ranks (anova: t-value: -3.23; p-value = 0.0013, Fig. S17). Therefore, taxonomic knowledge of fungal endophyte of *Pinus nigra* subsp. *laricio* seems not biased towards molecular abundancy of fungal species. In other words, our knowledge of molecularly abundant Ascomycota endophytes is still limited.

The rich community of FEF (642 OTUs, Figure 2a and 3) was dominated by Dothideomycetes, in number of OTUs (26.9% of all OTUs) and in number of sequences (37.8% of all sequences) and Leotiomycetes (39.7% of all sequences and 10.9% of all OTUs). The most represented OTU (2 226 714 sequences, 27%, Figure 2a) was taxonomically assigned to *Cyclaneusma minus* (Leotiomycetes) and was also the only OTU represented in all the 72 samples. This taxon was more represented than the most abundant Orders, namely Capnodiales (16.7%) and Pleosporales (14.6%).

The majority of abundant OTUs (i.e. the 183 OTUs represent by more than 1 000 sequences, Figure 2b) were assigned to Dothideomycetes (61), and in a lesser extent to Leotiomycetes

(24), Lecanoromycetes (17), Eurotiomycetes (16), Sordariomycetes (4) and Tremellomycetes (1). The 60 remaining OTUs couldn't be assigned at the Class rank. Despite the high sequencing depth (in all, 8 000 000 of usable sequences), rarefied species accumulation curves clearly supported unsaturated communities, for all tested factors (Figure 4b), consistent with previous studies (Zimmerman and Vitousek 2012; Yang et al. 2016).

Functional assignment of fungal OTUs

The functional assignment of OTUs could be realized only for those taxonomically assigned to genera of known and homogeneous ecological strategy in the FUNGuild database. In all, 29% of OTUs, accounting for 18% of sequences, could be functionally assigned (Fig. S21). The large majority of these OTUs were classified as saprotrophic with unknown ecology (15.6% of OTUs accounting for 12% of sequences), plant parasites (7.1% of OTUs accounting for 4.7% of sequences) or lichenized fungi (3.8% of OTUs accounting for 1.1 % of sequences).

Diversity of fungal endophytes across sites, age of trees and light exposure

The number of endophytic fungal OTUs per sample averaged 107.5 ± 37.3 (observed richness, Fig 4b) and 114.6 ± 39.9 (Chao richness). In comparison, the mean observed richness per *Pinus nigra* subsp. *laricio* individual (*i.e.* merging the three samples of a given tree) averaged 170.9 ± 43.4 (data not shown). Neither site, nor host age, nor sampling elevation within the tree affected OTUs richness (Figure 3, Hill number with $q = 0$; Table 3). However, Simpson relative diversity (Hill number 1) was higher at Asco and Verghello than at Bavella, and Shannon relative diversity (Hill number 2) was higher at Asco than at Verghello than at Bavella (Tuckey HSD test: $p.adj < 0.05$, see appendix S9 for more details).

Consequently, a sample of 30 needles harvested on a square meter range is sufficient to estimate the endophytic diversity of a tree, whatever its age and whatever the location in the tree.

A low beta-diversity at all taxonomic levels

The number of shared OTUs among samples was high for the three tested variables (Figure 4a, 5, 6 and 8). In all, 242 OTUs out of 642 (37.7 %, Table4c) were present at all sites. This proportion increased up to 68.9 % when considering only OTUs found in three samples or more, *i.e.* when removing OTUs that couldn't be present at our three sites. Moreover, even after removing from the analysis all OTUs represented by only one sequence (Figure 4a), the proportion of OTUs present at only one site remained modest (44.5 %, Table4c), especially when taking into account OTUs present in at least 3 samples (8.5 %).

The low among-site variation in the composition of fungal communities is mostly structured by sites (13.8 % of variance; data not shown), and in a lesser extent by site*age interaction (9 % of variance) and by host age (3.9 % of variance; Table 4). The analysis of differential distribution of OTUs among sites (R package DESeq2) highlighted the taxonomic diversity of OTUs responsible for these among-site differences in community composition (Figure 7).

Discussion

Is Cyclaneusma minus a pathogen?

Cyclaneusma minus often dominates pine needle endosphere (Sieber 2007). Despite its putative pathogenicity on *Pinus spp* (Watt et al. 2012), we found that *C. minus* is present in all samples and account for more than 27% of the total number of sequences. This contradiction may be explained either (i) by the massive fructification of *C. minus* when the leaves are old and sick without being responsible of this senescence; (ii) or by a latent pathogenecity of *C. minus* that appears under certain conditions (Sieber 2007; Hardoim et al. 2015).

A high cosmopolitan diversity

Despite our very conservative bioinformatic pipeline, we found more OTUs than all previous studies except Kembel and Mueller (2014) who sampled 57 plant species in tropical forest. When using the same clustering methods (Uclust) and filtering parameters than Bàlint et al. (2015), Bullington et al. (2015), Siddique and Unterseher (2015), and Kemler et al. (2013), we found more OTUs than all these studies, respectively: 2 825 *vs* 2 022, 4 150 *vs* 1 358, 3 214 *vs* 414, and 4 150 *vs* 2 415.

Even with more than eight million sequences (at least twice as the number found in previous studies; Tab 1) and 72 samples (~2 160 needles), the present work could not detect all the diversity of Ascomycota FEF (Fig 4b). This is a remarkable finding when considering the studied material (a unique host species), the low beta diversity observed, and the known properties of the used marker (ITS alone is supposed to underestimate OTU richness; Gazis et al. 2016). Each of the 27 sampled tree sheltered 26.6% ± 6.7% of the total gamma diversity (170.9 OTUs ± 43.4 on a total of 642). In other words, in the studied system, very young and multisecular individuals support similar conservation stakes. This striking result questions the processes underlying these patterns. In particular, the mechanisms explaining the early accumulation of FEF diversity on young pine individuals questions the role of old trees in old-growth stands, as potential reservoirs of fungal inoculum. Complementary assessments of the structure of FEF communities in even-aged stands would provide information about the role of the demographic structure of tree populations on the structuration of FEF communities.

FEF communities are only sparsely affected by host age

Host age explains about 4% of FEF composition with and without taking into account OTU molecular abundances (Table 2). Even if PerManova detects host age as a significant driver of FEF communities, spatial location explains far more variance with (13.6%) and without molecular abundances (8.9%). Our results contrast with previous studies. For instance, Oono et al. (2015) found a more diverse community in *Pinus taeda* seedlings (2 years-old) than in adult trees, using both culture and culture-independent methods. Moreover, they found a very low proportion of shared OTUs between seedlings and adults (14.4%). On the contrary, our results using deep sequencing reveal that host age is not a significant driver of FEF communities (80% of OTUs present in more than 3 samples [351] were present in young, mature and old trees [282; Fig. 4a]).

Previous works, often based on culture methods, have suggested that FEF may be legated mostly via horizontal transfers (Arnold 2007, Rodriguez et al. 2009; Hardoim et al. 2015). Our results support an alternative scenario for the transmission from old to young trees, throughthe maternal transmission of microbiome to offspring. Even if more work is required to formally demonstrate this hypothesis, such vertical heredity of FEF may explain the observed high similarity among host microbiomes of Laricio pines regardless of host age (this study), as well as the high fungal diversity observed in pine seedlings (Oono et al. 2015) and in young trees (this study).

FEF, new candidates to revisit the Baas-Becking hypothesis?

The “everything is everywhere: *but* the environment selects” (Baas-Becking 1934; de Wit and Bouvier, 2006) hypothesis is still debated (O'Malley 2007). Thus, contrasted patterns of fungal species distribution have been described. On the one hand, some studies found structured biogeographical patterns in micro-organisms (reviewed in Martiny et al. 2006). On the other hand, cosmopolitan distribution of micro-organisms have been reported (*e.g.* root-associated fungi, Queloz et al. 2011; soil Ascomycota, Green et al. 2004). In our case, the observed difference in FEF composition among the micro-region of Corsica may be explained by dispersal filtering (*everything is NOT everywhere*) but also by different plant environments that shape their FEF communities (*environment select*). Our results are a necessary first step to test these two hypotheses.

In addition to their high diversity, the low intra-individual and inter-cohort turnover make FEF an ideal guild to test the Baas-Becking hypothesis. Indeed, because a few tree needles are sufficient to describe the diversity at the local scale, one may now design a dedicated sampling spanning numerous sites across geographical and environmental gradients. Zimmerman and Vitousek (2012) already showed that strong environmental gradients can structure FEF composition in leaves of an endemic tree in Hawaii (*Metrosideros polymorpha*). More work is needed to gain insights in these issues, but we believe the present study may inspire new experimental designs and tools to address some of them.

Acknowledgement:

This study was funded by the Conservatoire Botanique National de Corse. We are grateful to the Parc Naturel Regional de Corse for their logistical help during needle sampling. All experiments performed in this study comply with the current laws of France.

References:

Abdelfattah A, Li Destri Nicosia MG, Cacciola SO, Droby S, Schena L. 2015. Metabarcoding analysis of fungal diversity in the phyllosphere and carposphere of olive (*Olea europaea*). *PLoS ONE* **10**: e0131069.

Abhijeet Singh YM. 2014. Understanding the Biodiversity and Biological Applications of Endophytic Fungi: A Review. *Journal of Microbial & Biochemical Technology* **s8**.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral ecology* **26**: 32–46.

Andrews S, others. 2010. FastQC: A quality control tool for high throughput sequence data. *Reference Source*.

Arfi Y, Buée M, Marchand C, Levasseur A, Record E. 2012. Multiple markers pyrosequencing reveals highly diverse and host-specific fungal communities on the mangrove trees *Avicennia marina* and *Rhizophora stylosa*. *FEMS Microbiology Ecology* **79**: 433–444.

Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA. 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences* **100**: 15649–15654.

Baas Becking LGM. 1934. *Geobiologie of inleiding tot de milieukunde*. Den Haag: W.P. Van Stockum & Zoon.

Bálint M, Bartha L, O'Hara RB, Olson MS, Otte J, Pfenninger M, Robertson AL, Tiffin P, Schmitt I. 2015. Relocation, high-latitude warming and host genetic identity shape the foliar fungal microbiome of poplars. *Molecular Ecology* **24**: 235–248.

Bálint M, Schmidt P-A, Sharma R, Thines M, Schmitt I. 2014. An Illumina metabarcoding pipeline for fungi. *Ecology and Evolution* **4**: 2642–2653.

Bálint M, Tiffin P, Hallström B, O'Hara RB, Olson MS, Fankhauser JD, Piepenbring M, Schmitt I. 2013. Host genotype shapes the foliar fungal microbiome of balsam poplar (*Populus balsamifera*). *PLOS ONE* **8**: e53987.

Bastian M, Heymann S, Jacomy M, others. 2009. Gephi: an open source software for exploring and manipulating networks. *ICWSM* **8**: 361–362.

Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, Godhe A, De Wit P, Sánchez-García M, Ebersberger I, de Sousa F, *et al.* 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other Eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution* **4**: 914–919.

Brown SP, Veach AM, Rigdon-Huss AR, Grond K, Lickteig SK, Lothamer K, Oliver AK, Jumpponen A. 2015. Scraping the bottom of the barrel: are rare high throughput sequences artifacts? *Fungal Ecology* **13**: 221–225.

Bullington LS, Larkin BG. 2015. Using direct amplification and next-generation sequencing technology to explore foliar endophyte communities in experimentally inoculated western white pines. *Fungal Ecology* **17**: 170–178.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, *et al.* 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**: 335–336.

Christian N, Whitaker BK, Clay K. 2015. Microbiomes: unifying animal and plant systems through the lens of community ecology theory. *Frontiers in Microbiology* **6**.

Claesson MJ, O'Sullivan O, Wang Q, Nikkilä J, Marchesi JR, Smidt H, Vos WM de, Ross RP, O'Toole PW. 2009. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PLOS ONE* **4**: e6669.

Clemente JC, Ursell LK, Parfrey LW, Knight R. 2012. The impact of the gut microbiota on human health: An integrative view. *Cell* **148**: 1258–1270.

Coince A, Cordier T, Lengellé J, Defossez E, Vacher C, Robin C, Buée M, Marçais B. 2014. Leaf and root-associated fungal assemblages do not follow similar elevational diversity patterns. *PLoS ONE* **9**: e100668.

Cordier T, Robin C, Capdevielle X, Desprez-Loustau M-L, Vacher C. 2012. Spatial variability of phyllosphere fungal assemblages: Genetic distance predominates over geographic distance in a European beech stand (*Fagus sylvatica*). *Fungal Ecology* **5**: 509–520.

Crowther TW, Glick HB, Covey KR, Bettigole C, Maynard DS, Thomas SM, Smith JR, Hintler G, Duguid MC, Amatulli G, *et al.* 2015. Mapping tree density at a global scale. *Nature* **525**: 201–205.

Delaye L, García-Guzmán G, Heil M. 2013. Endophytes versus biotrophic and necrotrophic pathogens—are fungal lifestyles evolutionarily stable traits? *Fungal Diversity* **60**: 125–135.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. **2010**. Idiosyncrasy and overdominance in the structure of natural communities of arbuscular mycorrhizal fungi: is there a role for stochastic processes? *Journal of Ecology* **98**: 419–428.

Edgar RC. **2010**. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460–2461.

Edgar RC. **2013**. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**: 996–998.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. **2011**. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.

Estrada C, Wcislo WT, Van Bael SA. **2013**. Symbiotic fungi alter plant chemistry that discourages leaf-cutting ants. *New Phytologist* **198**: 241–251.

Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E. **2011**. Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and Systematics* **42**: 23–46.

Gazis R, Kuo A, Riley R, LaButti K, Lipzen A, Lin J, Amirebrahimi M, Hesse CN, Spatafora JW, Henrissat B, *et al.* **2016**. The genome of Xylona heveae provides a window into fungal endophytism. *Fungal Biology* **120**: 26–42.

Green JL, Holmes AJ, Westoby M, Oliver I, Briscoe D, Dangerfield M, Gillings M, Beattie AJ. **2004**. Spatial scaling of microbial eukaryote diversity. *Nature* **432**: 747–750.

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A. **2015**. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews* **79**: 293–320.

Helander ML, Sieber TN, Petrini O, Neuvonen S. **1994**. Endophytic fungi in Scots pine needles: spatial variation and consequences of simulated acid rain. *Canadian Journal of Botany* **72**: 1108–1113.

Higgins KL, Arnold AE, Coley PD, Kursar TA. **2014**. Communities of fungal endophytes in tropical forest grasses: highly diverse host- and habitat generalists characterized by strong spatial structure. *Fungal Ecology* **8**: 1–11.

Hinchliff CE, Smith SA, Allman JF, Burleigh JG, Chaudhary R, Coghill LM, Crandall KA, Deng J, Drew BT, Gazis R, *et al.* **2015**. Synthesis of phylogeny and taxonomy into a comprehensive tree of life. *Proceedings of the National Academy of Sciences* **112**: 12764–12769.

Jacomy M, Venturini T, Heymann S, Bastian M. **2014**. ForceAtlas2, a Continuous Graph Layout Algorithm for Handy Network Visualization Designed for the Gephi Software. *PLOS ONE* **9**: e98679.

Jumpponen A, Jones KL. **2009**. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. *New Phytologist* **184**: 438–448.

Kaul S, Gupta S, Ahmed M, Dhar MK. **2012**. Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. *Phytochemistry Reviews* **11**: 487–505.

Kembel SW, O'Connor TK, Arnold HK, Hubbell SP, Wright SJ, Green JL. **2014**. Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proceedings of the National Academy of Sciences* **111**: 13715–13720.

Kemler M, Garnas J, Wingfield MJ, Gryzenhout M, Pillay K-A, Slippers B. **2013**. Ion Torrent PGM as tool for fungal community analysis: a case study of endophytes in *Eucalyptus grandis* reveals high taxonomic diversity. *PLOS ONE* **8**: e81718.

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, *et al.* **2013**. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* **22**: 5271–5277.

Lilleskov EA, Bruns TD, Horton TR, Taylor DL, Grogan P. **2004**. Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. *FEMS Microbiology Ecology* **49**: 319–332.

Love MI, Huber W, Anders S. **2014**. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**: 550.

Mahé F, Rognes T, Quince C, de Vargas C, Dunthorn M. **2015**. Swarm v2: highly-scalable and high-resolution amplicon clustering. *PeerJ* **3**.

Martiny JBH, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, *et al.* **2006**. Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology* **4**: 102–112.

McMurdie PJ, Holmes S. **2013**. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data (M Watson, Ed.). *PLoS ONE* **8**: e61217.

Meyer KM, Leveau JHJ. 2012. Microbiology of the phyllosphere: a playground for testing ecological concepts. *Oecologia* **168**: 621–629.

Millberg H, Boberg J, Stenlid J. 2015. Changes in fungal community of Scots pine (*Pinus sylvestris*) needles along a latitudinal gradient in Sweden. *Fungal Ecology* **17**: 126–139.

Mousa WK, Raizada MN. 2013. The Diversity of Anti-Microbial Secondary Metabolites Produced by Fungal Endophytes: An Interdisciplinary Perspective. *Frontiers in Microbiology* **4**.

Mummey DL, Rillig MC. 2008. Spatial characterization of arbuscular mycorrhizal fungal molecular diversity at the submetre scale in a temperate grassland. *FEMS Microbiology Ecology* **64**: 260–270.

Münkemüller T, Gallien L, Lavergne S, Renaud J, Roquet C, Abdulhak S, Dullinger S, Garraud L, Guisan A, Lenoir J, *et al.* 2014. Scale decisions can reverse conclusions on community assembly processes. *Global Ecology and Biogeography* **23**: 620–632.

Nguyen D, Boberg J, Ihrmark K, Stenström E, Stenlid J. 2016. Do foliar fungal communities of Norway spruce shift along a tree species diversity gradient in mature European forests? *Fungal Ecology* **23**: 97–108.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2015. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*.

Nilsson RH, Hyde KD, Pawłowska J, Ryberg M, Tedersoo L, Aas AB, Alias SA, Alves A, Anderson CL, Antonelli A, *et al.* 2014. Improving ITS sequence data for identification of plant pathogenic fungi. *Fungal Diversity* **67**: 11–19.

O'Malley MA. 2007. The nineteenth century roots of'everything is everywhere'. *Nature Reviews Microbiology* **5**: 647–651.

Ondov BD, Bergman NH, Phillippy AM. 2011. Interactive metagenomic visualization in a Web browser. *BMC bioinformatics* **12**: 385.

Oono R, Lefèvre E, Simha A, Lutzoni F. 2015. A comparison of the community diversity of foliar fungal endophytes between seedling and adult loblolly pines (*Pinus taeda*). *Fungal Biology* **119**: 917–928.

Oono R, Lutzoni F, Arnold AE, Kaye L, U'Ren JM, May G, Carbone I. 2014. Genetic variation in horizontally transmitted fungal endophytes of pine needles reveals population structure in cryptic species. *American Journal of Botany* **101**: 1362–1374.

Paliy O, Shankar V. 2016. Application of multivariate statistical techniques in microbial ecology. *Molecular Ecology* **25**: 1032–1057.

Partida-Martinez LPP, Heil M. 2011. The microbe-free plant: fact or artifact? *Frontiers in Plant Science* **2**.

Peay KG, Bruns TD, Kennedy PG, Bergemann SE, Garbelotto M. 2007. A strong species-area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. *Ecology Letters* **10**: 470–480.

Porras-Alfaro A, Bayman P. 2011. Hidden fungi, emergent properties: endophytes and microbiomes. *Annual Review of Phytopathology* **49**: 291–315.

Queloz V, Sieber TN, Holdenrieder O, McDonald BA, Grünig CR. 2011. No biogeographical pattern for a root-associated fungal species complex. *Global Ecology and Biogeography* **20**: 160–169.

Rameau J-C, Mansion D, Dumé G, Gauberville C. 2008. *Flore forestière française Tome 3*. Paris: Institut pour le développement forestier.

Rastogi G, Coaker GL, Leveau JHJ. 2013. New insights into the structure and function of phyllosphere microbiota through high-throughput molecular approaches. *FEMS Microbiology Letters* **348**: 1–10.

Rodriguez RJ, White Jr JF, Arnold AE, Redman RS. 2009. Fungal endophytes: diversity and functional roles. *New Phytologist* **182**: 314–330.

Siddique AB, Unterseher M. 2016. A cost-effective and efficient strategy for Illumina sequencing of fungal communities: A case study of beech endophytes identified elevation as main explanatory factor for diversity and community composition. *Fungal Ecology* **20**: 175–185.

Sieber TN. 2007. Endophytic fungi in forest trees: are they mutualists? *Fungal Biology Reviews* **21**: 75–89.

Strobel G. 2014. The story of mycodiesel. *Current Opinion in Microbiology* **19**: 52–58.

Taudiere A, Violle C. 2016. cati: an R package using functional traits to detect and quantify multi-level community assembly processes. *Ecography* **39**: 699–708.

U'Ren JM, Riddle JM, Monacell JT, Carbone I, Miadlikowska J, Arnold AE. 2014. Tissue storage and primer selection influence pyrosequencing-based inferences of diversity and community composition of endolichenic and endophytic fungi. *Molecular Ecology Resources* **14**: 1032–1048.

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. **2015**. The importance of the microbiome of the plant holobiont. *New Phytologist* **206**: 1196–1206.

de Wit R, Bouvier T. **2006**. 'Everything is everywhere, but, the environment selects'; What did Baas Becking and Beijerinck really say? *Environmental Microbiology* **8**: 755–758.

Wolfe BE, Mummey DL, Rillig MC, Klironomos JN. **2007**. Small-scale spatial heterogeneity of arbuscular mycorrhizal fungal abundance and community composition in a wetland plant community. *Mycorrhiza* **17**: 175–183.

Yang T, Weisenhorn P, Gilbert JA, Ni Y, Sun R, Shi Y, Chu H. **2016**. Carbon constrains fungal endophyte assemblages along the timberline. *Environmental Microbiology* **18**: 2455–2469.

Yuan Z, Chen L. **2014**. The Role of Endophytic Fungal Individuals and Communities in the Decomposition of Pinus massoniana Needle Litter. *PLOS ONE* **9**: e105911.

Zhang T, Yao Y-F. **2015**. Endophytic fungal communities associated with vascular plants in the high arctic zone are highly diverse and host-plant specific. *PLOS ONE* **10**: e0130051.

Zimmerman NB, Vitousek PM. **2012**. Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. *Proceedings of the National Academy of Sciences* **109**: 13022–13027.

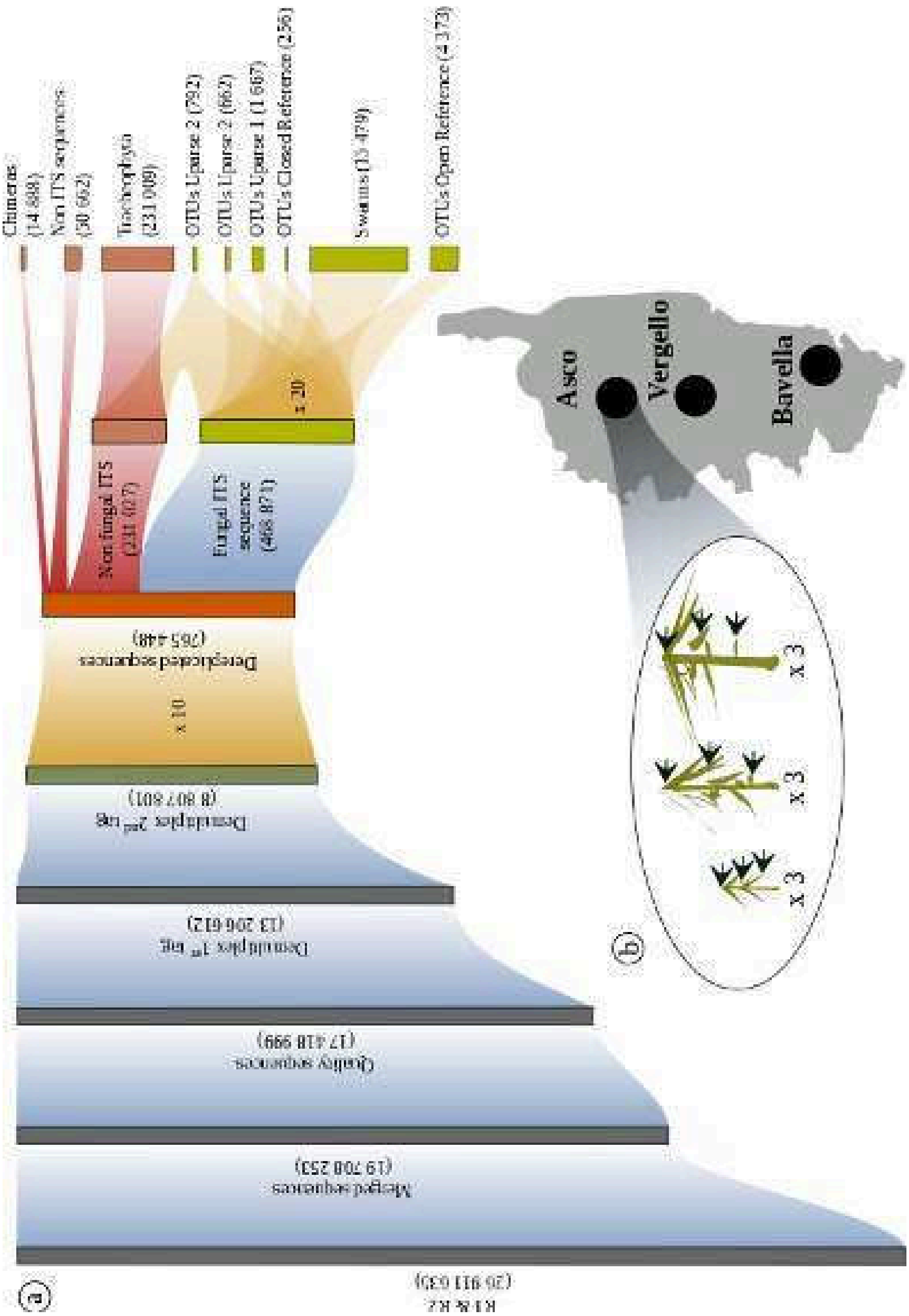


Figure 1: (a) Number of sequences/OTUs along the bioinformatic pipeline. Blue, red and yellow ribbons indicate analyzed sequences, discarded sequences and clustering stage, respectively. Detailed pipeline is available in figure S1. “Uparse 1” retains unique sequences whereas “Uparse 2” discards all unique sequences before OTU clustering. **(b) Schematic overview of the sampling scheme**: at each site, needles were collected at three tree heights on three young, three mature and three old individual trees.

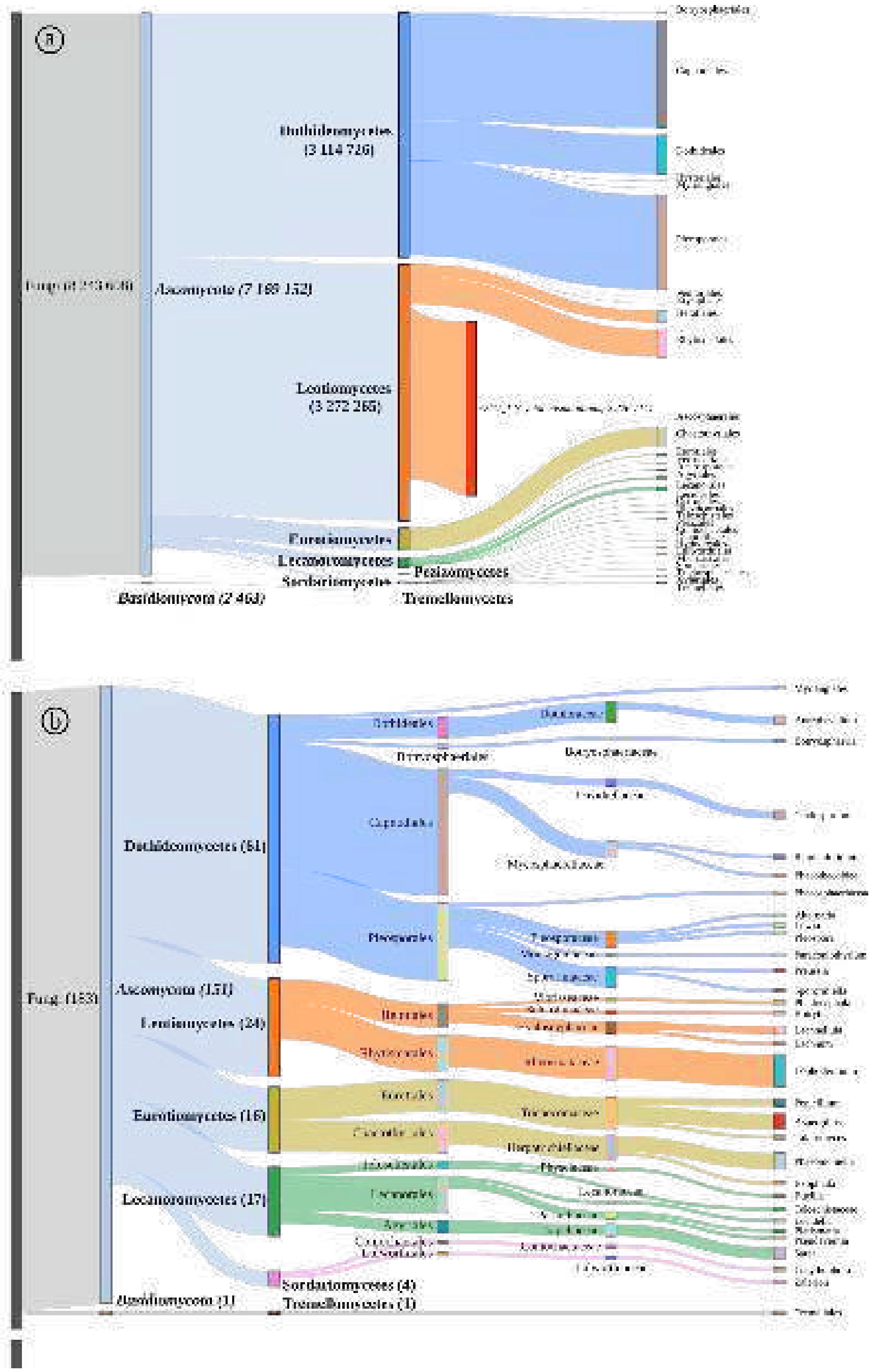


Figure 2: (a) Distribution of molecular abundances (in number of sequences) according to the taxonomy of OTUs. (b) Taxonomic distribution of OTUs represented by at least 1000 sequences.

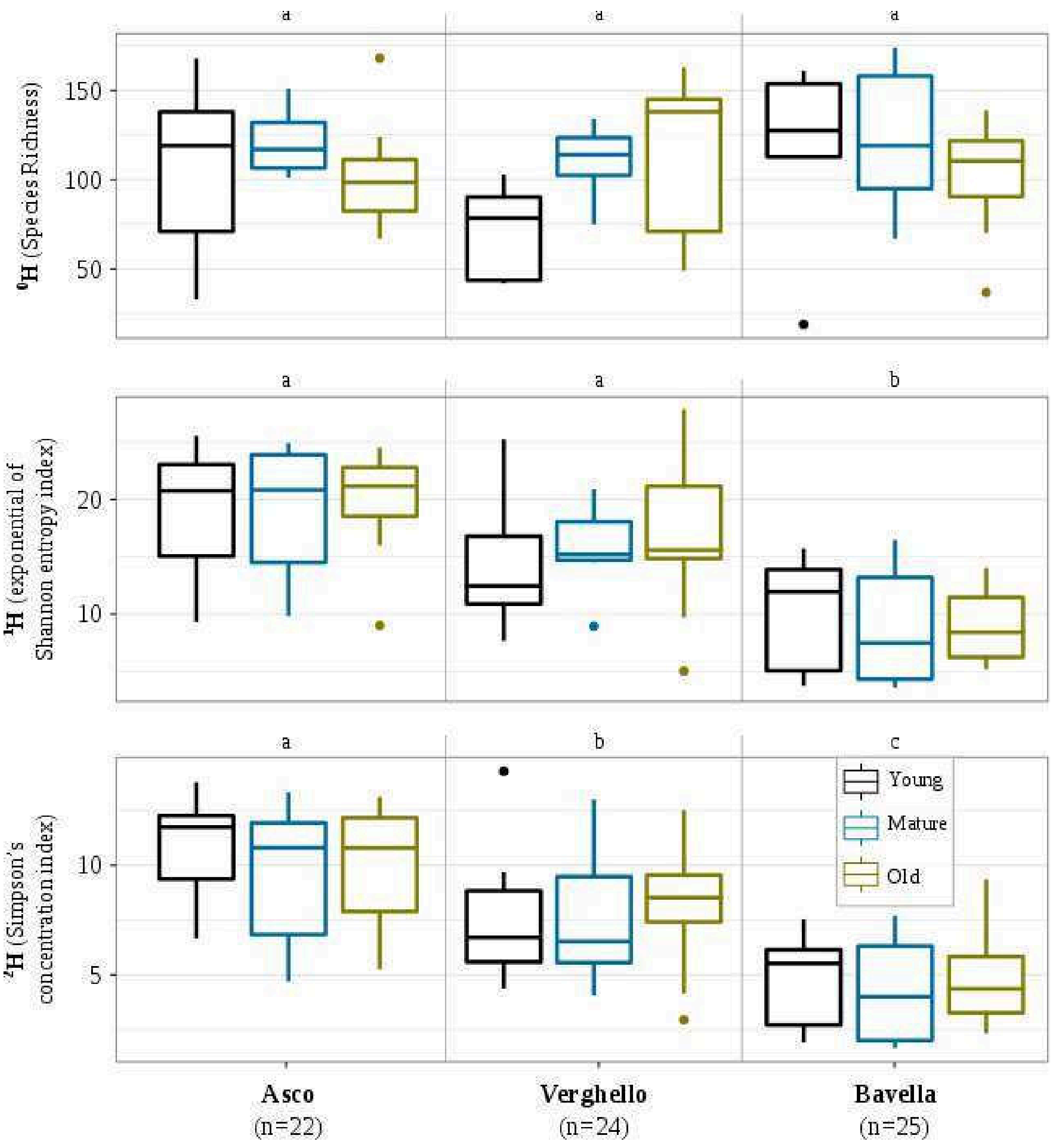


Figure 3: Effect of sites and tree age on alpha diversity, measured by Hill numbers. ^{0}H (species richness), ^{1}H (exponential of Shannon's entropy index), ^{2}H (inverse of Simpson's concentration index). Black, blue and green indicate the endophytic communities on young, mature and old trees, respectively. The significance of the differences was assessed by a linear model (Table 3). Letters show the results of Tuckey HSD tests among sites after accounting for difference in sampling size (p-adjust < 0.05). Neither host age nor elevation shows significant differences by Tuckey HSD tests for the three Hill numbers.

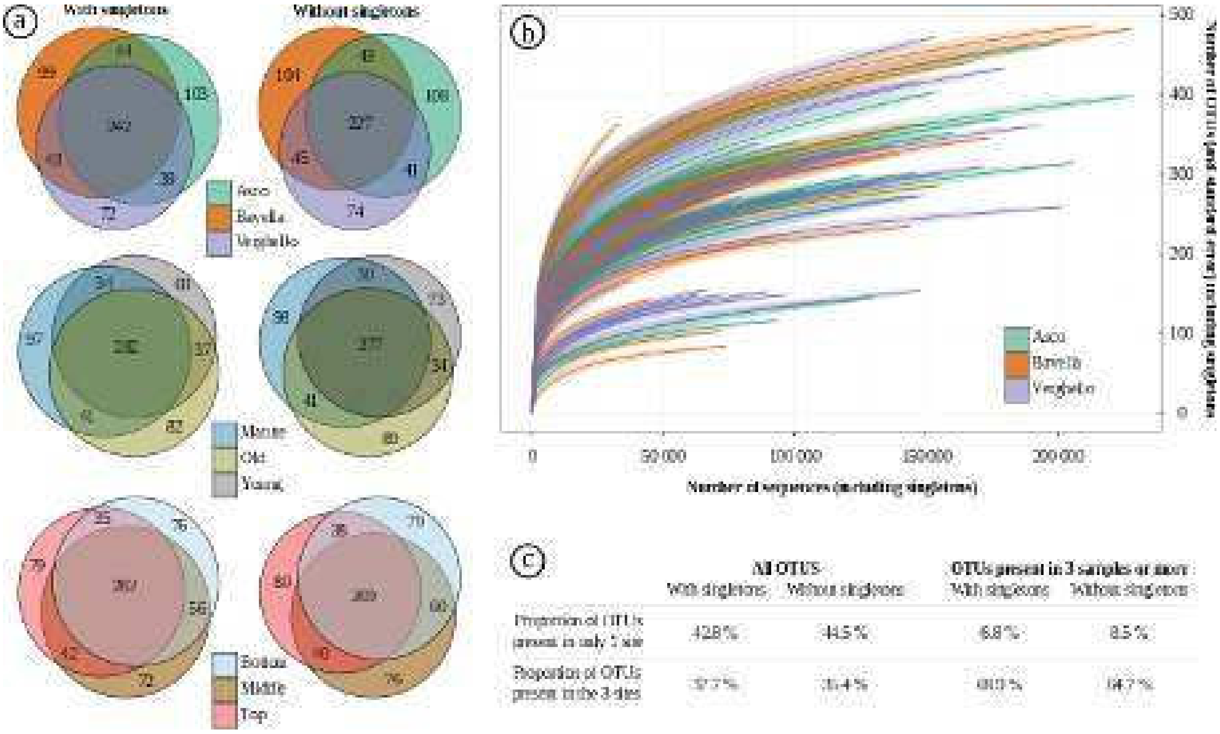


Figure 4: (a) Venn diagrams of shared OTUs among sample variables. The number of OTUs shared among sites, trees of different age, and light exposure are indicated with (left) or without (right) sample singletons (*i.e.* removing OTUs when represented by only one sequence in the sample). Centers and areas of circles were computed using the package venneuler. **(b) OTU accumulation curves of samples in function of number of sequences**. Accumulation curves represent the dataset with singletons (*Uparse 1*). Colors indicate sites. Shaded areas indicate the double of estimate standard errors computed using vegan. **(c) Proportions of site-specific and site-ubiquitous OTUs.** Proportions are estimated with and without sample singletons (*i.e.* removing or not OTUs when represented by only one sequence in the sample) and including or not OTUs represented in less than 3 samples.

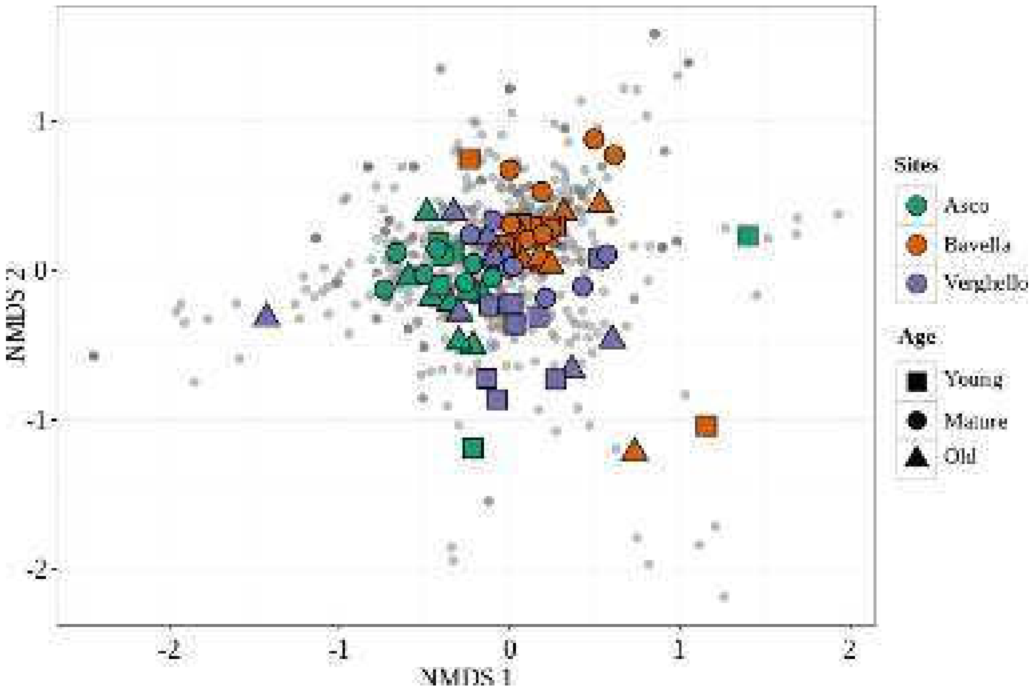


Figure 5: Effect of sites and tree age on beta diversity. Projection of the non-metric multidimensional scaling (stress = 0.21) of the OTU Table. Grey points represent OTUs, colors illustrate sites and symbols illustrate tree age. The significance of these effects were assessed by a PerManova (Table 3). See appendix S9 for alternative multivariate analysis.

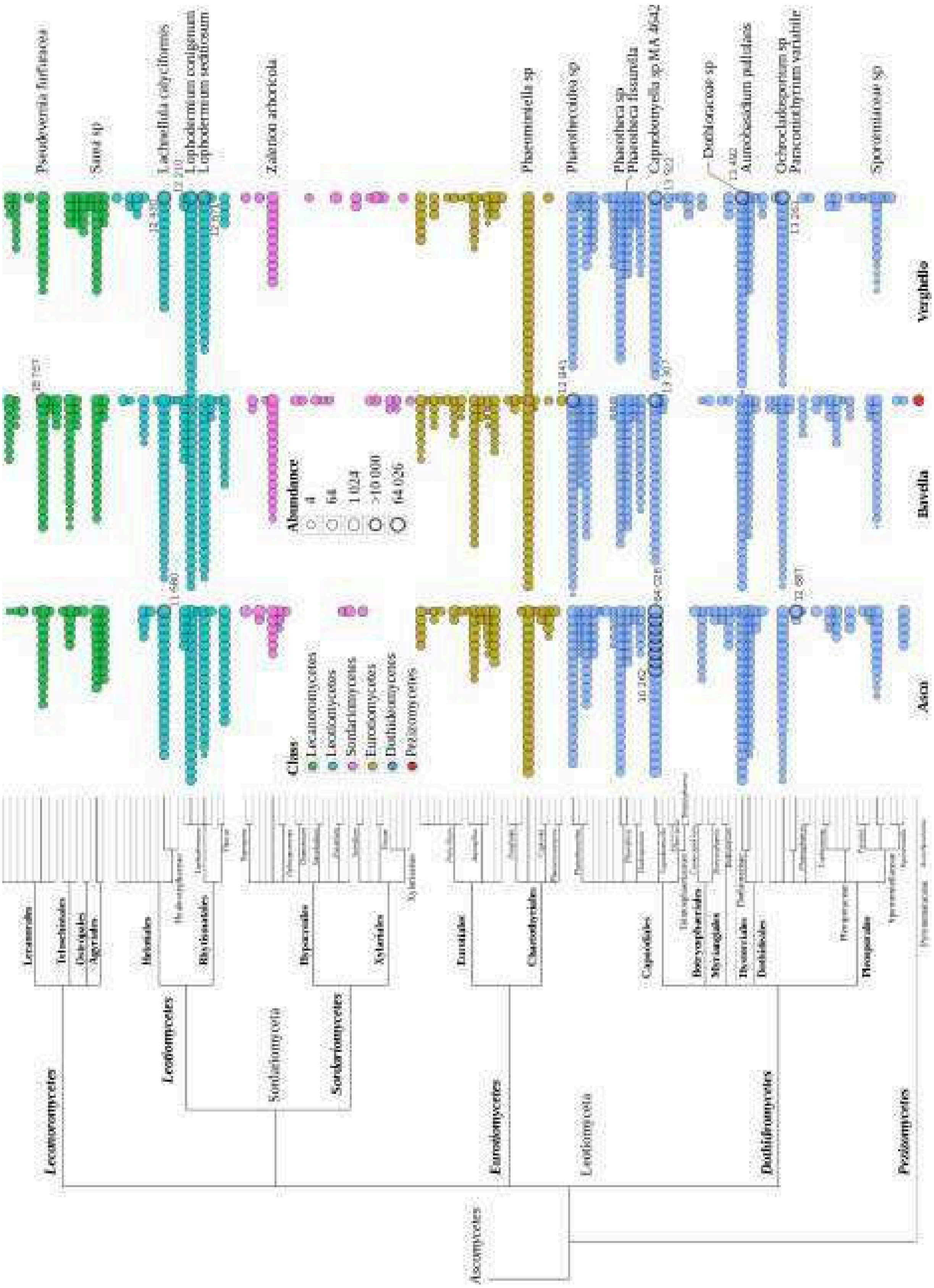


Figure 6: Distribution of molecular abundances (in number of sequences) of OTUs at the three study sites and across the phylogeny of Ascomycota. Each line corresponds to one or several OTUs having the

same species name. Only OTUs taxonomically assigned to species are presented. Species with more than 10 000 sequences are named on the right of the figure. Molecular abundance in each sample is represented by the diameter of the circle and it also determines the order of samples. "Abundance" values greater than 10 000 sequences in a sample are indicated. Colors indicate fungal Classes. The molecular abundance distribution of each OTU is presented for each site (Asco vs. Bavella vs. Verghello). The phylogeny was build using taxonomy of the Unite database (Kõljalg et al. 2013) and the Open Tree of Life (Hinchliff et al. 2015). Therefore, branch length is not informative.

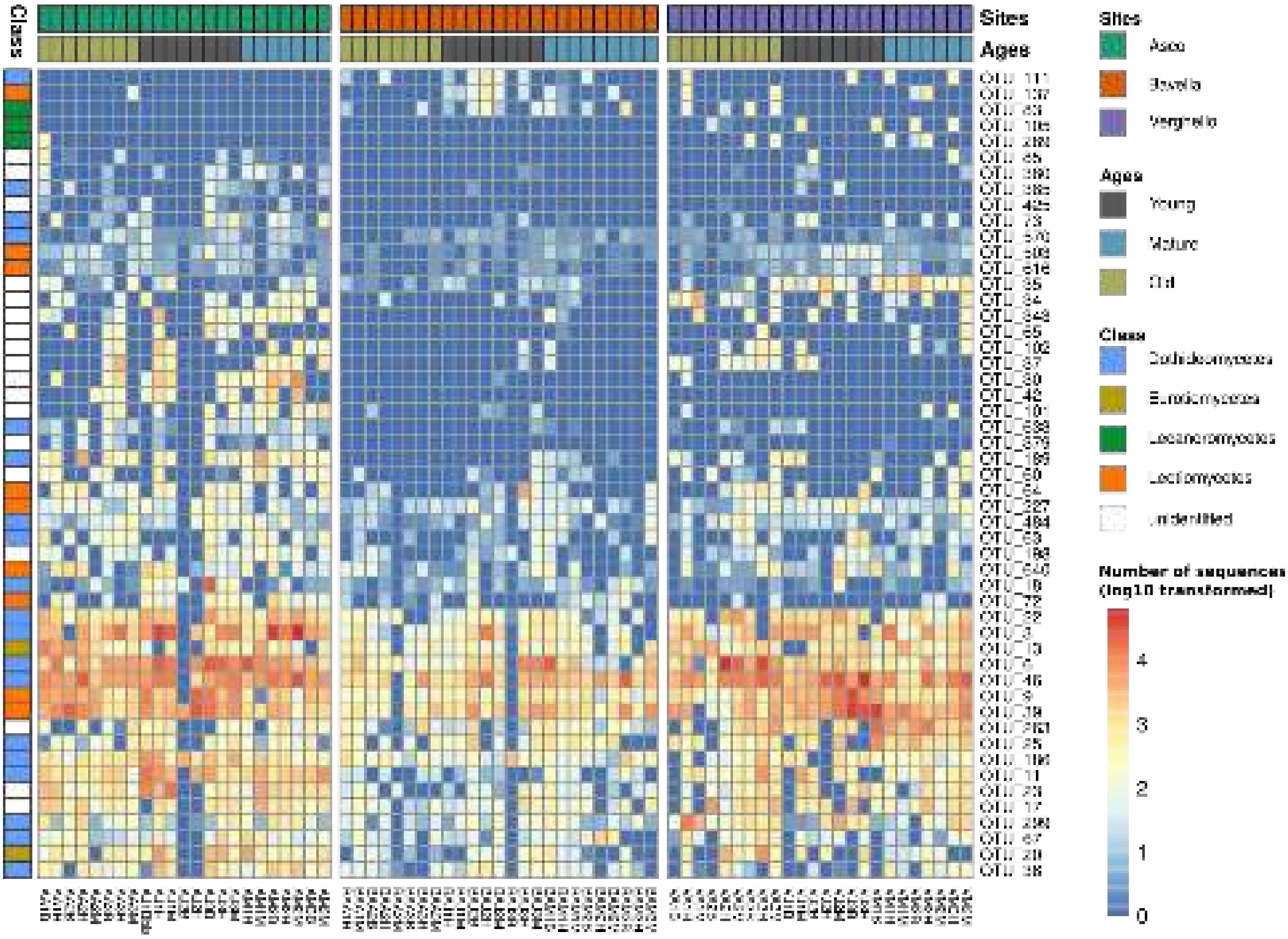


Figure 7: Molecular abundance (number of sequences) distribution of OTUs showing significant differences across sites. Columns indicate samples. Lines correspond to OTUs detected by the DESq2 method to be significantly different in abundance among sites. OTUs are ranked in function of their similarity of distribution among samples. The molecular abundance distribution of each OTU is split by sites (Asco, Bavella and Verghello).

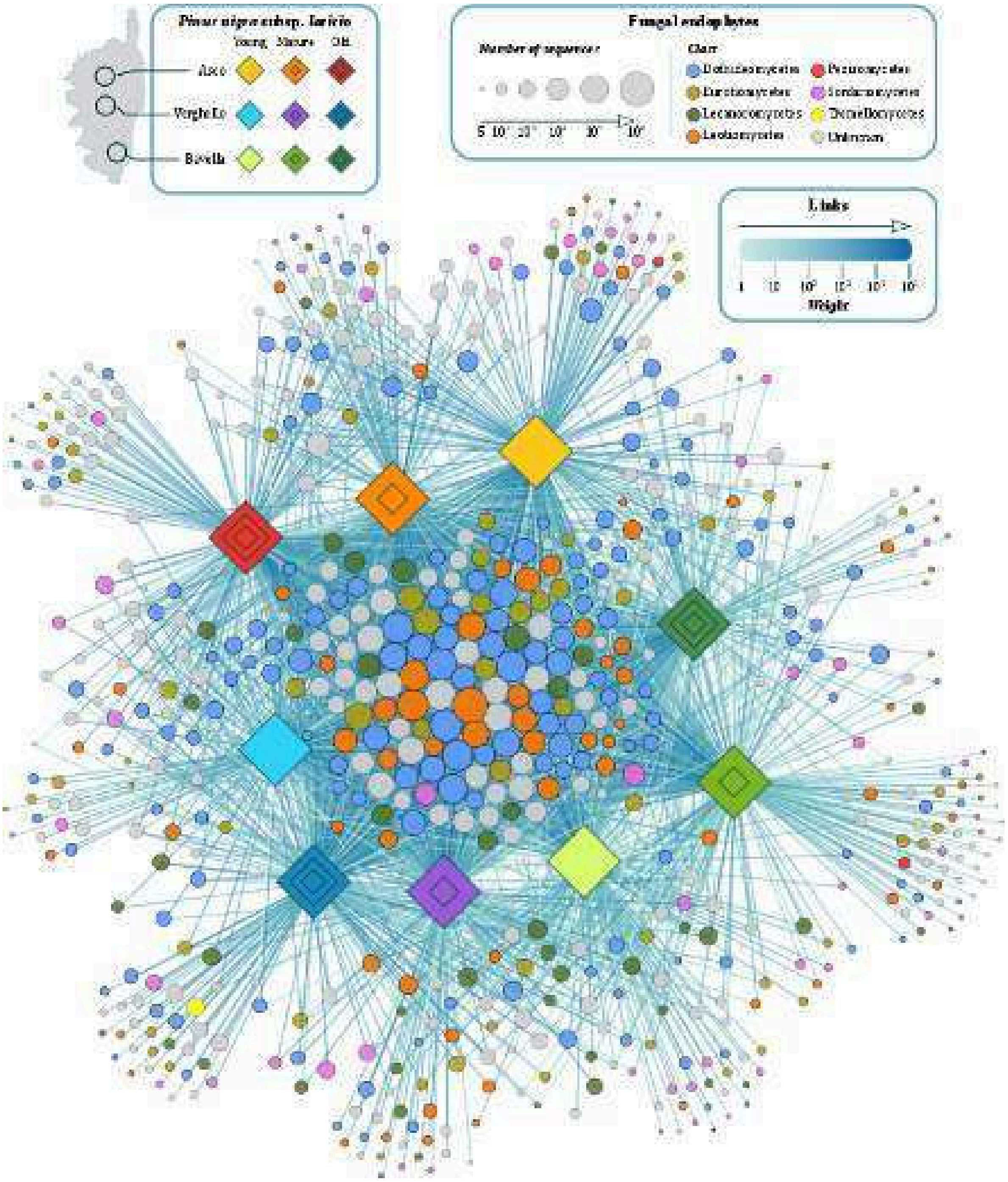


Figure 8: Interaction network between fungal endophytic OTUs and *Pinus nigra* subsp. *laricio* cohort (age × site). The positions of nodes were obtained using the force Atlas 2 algorithms (Jacomy et al. 2014) in the Gephi software (Bastian et al. 2009). Diamonds represent pine individuals of the same age (defined by the number of nested diamonds) at the same site (defined by the color). Circles represent fungal OTUs. Size and color of circles indicate, respectively, the number of sequences (molecular abundances) and the Class the OTU. Links color (intensity of blue) is proportional to the number of sequences in OTUs for each pine cohort (link weight).

Table 1: Recent publications on tree leaf endophytic fungi (extended table is presented in supplementary Table S18). See Sieber (2007) for a review of older works. The number of OTUs was found using 97 % identity clustering except if another value is indicated, and this number is singleton-free for NGS (Next Generation Sequencing: 454, Ion torrent and Illumina). Moreover, some studies also discarded rare OTUs, *i.e.* OTUs with a number of sequences (*min nb seq*) below an arbitrary threshold. Note that NGS studies used different softwares and/or parameters to cluster OTUs from raw sequences (see Větrovský & Baldrian, 2013 for a discussion concerning 454). Tested determinant list the factors tested for their effect either on fungal diversity (α-diversity; *e.g.* Richness or Simpson diversity) or composition (β-diversity; *e.g.* using PerManova). = indicates the absence of statistically significant effect, + indicates a significant but weak effect and ++ indicates a significant and strong effect.

	References	**Methods**	**Nb of sequences**	**Host species**	**Number of OTUs**	**Dominant Taxa**	**Tested determinant (explanatory factor)**
Without sterilization of leaf surfaces	Abdelfattah et al. 2015*	454	19 719	*Olea Europae*	**124** (Blast)	Dothideomycetes; Aureobasidium; Devriesia; Pseudocercospora	Different plant organs α(+) β(++)
	Coince et al. 2014	454	149 946	*Fagus sylvatica*	**1 457** (Uclust)	Cryptococcus; Taphrina	Region α(=) β(++)
							PH α(=) β(+)
							Mean temperature α(=) β(++)
	Cordier et al. 2012*	454	96 130	*Fagus sylvatica*	**1 604** (Uclust)	*Lalaria inositophila*; Taphrinales; Tremellales	Spatial scales (from tree to leaves) β(++)
	Jumpponen and Jones, 2009 *	454	18 020	*Quercus macrocarpa*	**360** (95%, CAP3)	*Microsphaeropsis sp; Alternaria sp*	Urban *vs* rural stands α(++) β(++)
	Millberg et al. 2015*	454	75 855	*Pinus sylvestris*	**598** (98 %; SCATA)	Artoniomycetes; Dothideomycetes; Leothiomycetes	Latitude α(=) β(+)
							Natural *vs* plantation stand α(+) β(++)
							Needle age α(=) β(+)
							Needle health α(++) β(++)

With sterilization of leaf surfaces	Arfi et al. 2011	454	209 544	*Avicennia marina and Rhizophora stylosa*	**2 877** (98%; CD-HIT-EST)	Dothideomycetes; Lecanoromycetes; Sordariomycetes	Host Species α(+) β(++) Immersed *vs* emerged β(++)
	Bálint et al. 2015	Illumina	1 759 757	*Populus balsamifera*	**2 022** (Uclust, *min nb seq* = 10)	Dothideomycetes ; Mycosphaerella	Warming experiment α(+) β(++) Latitude α(++) β(+) Host genetics α(+) β(+)
	Bullington and Larkin, 2015	Illumina	2 540 729	*Pinus monticola*	**1 358** (Uclust)	Dothideomycetes; Davidiella	None
	Kembel and Mueller, 2014	Illumina	4 131 371	57 species	**11 848** (Uclust)	Sordariomycetes; Dothideomycetes; Eurotiomycetes	Host Species β(++) Leaf functional traits β(+) Host phylogenetic relatedness β(=)
	Oono et al. 2015	Isolate & Sanger	491	*Pinus taeda*	**118** (99 %)	Dothideomycetes; Leothiomycetes	Adults *vs* seedlings α(++) β(++)
	Siddique and Unterseher, 2016	Illumina	820 441	*Fagus sylvatica*	**414** (Uclust, *min nb seq* = 5)	Helotiales; Capnodiales; Pleosporales	Altitude α(++) β(++) Vital *vs* senescent leaves α(=) β(+)
	U'ren et al. 2014	454	33 310	*Pinus leiophylla*	**19** (95 %; Mothur)	Dothideomycetes; Sordariomycetes	None
	Zimmerman and Vitousek, 2012	454	665 155	*Metrosideros polymorpha*	**2 552** (95%; Esprit)	Dothideomycetes	Rainfall α(=) β(++) Elevation α(=) β(++)
	This study	Illumina	~8 200 000 *cf* table 2	*Pinus nigra* subsp. *laricio*	662 (Uparse) 4 150 (Uclust) *cf* table 2	Dothideomycetes; *Cyclaneusma minus*	Sites α(+) β(++) Host ages α(=) β(+) Elevation α(=) β(=)

Table 2: Number of OTUs and associated sequences obtained by various clustering algorithms: Uclust (Edgar, 2010) using Qiime (Caporaso et al. 2010) with (Open reference) or without (Closed reference) de novo clustering; Swarm (Mahé et al. 2014) and Uparse (Edgar et al., 2013) with (-minsize 1) or without (-minsize 2) conserving singletons; Uparse applied to all ITS2 sequences (*i.e.* without removing potential Tracheophyta by ITSx ; Bengtson et al. 2013; see Fig. S1 for details). Final filtering discards samples with less than 20 000 sequences and OTUs represented by less than 5 sequences in the 72 remaining samples. Numbers in bold indicate presented values in this section results.

		With singletons		**Without singletons**		**Final filtering**	
		OTUs	*Sequences*	*OTUs*	*Sequences*	*OTUs*	*Sequences*
Qiime	*Open reference*	4 373	8 398 038	4 150	8 397 815	3 382	8 373 567
	Closed reference	256	6 473 782	251	6 473 777	233	6 460 489
Swarm	*-d 1*	15 479	8 419 809	9 325	8 413 655	6 064	8 382 948
Uparse	*-minsize 1*	1 667	8 335 341	1 477	8 335 151	1 302	8 312 594
	-minsize 2	**-**	**-**	662	8 265 594	**642**	**8 243 608**
	-minsize 2 without ITSx removal	-	-	792	8 441 951	789	8 420 192

Table 3: ANOVA results of the linear model testing for the effect of the square root of the number of reads, the site, the host age and the elevation on Hill numbers: ^{0}H (species richness), ^{1}H (exponential of Shannon's entropy index), ^{2}H (inverse of Simpson's concentration index). P-value inferior to 0.05 are in bold. For illustration of sites and host ages effects, see figure 3.

		^{0}H : species Richness		**^{1}H**		**^{2}H**	
		Estimate ± sd	P-value	Estimate ± sd	P-value	Estimate ± sd	P-value
	(Intercept)	51.53 ± 22.98	**0.0285**	12.22 ± 3.39	**0.0006**	7.04 ± 1.78	**0.0002**
	sqrt(readNb)	0.18 ± 0.06	**0.0029**	0.02 ± 0.01	0.0569	0.01 ± 0.004	0.1459
Sites	*Bavella*	8.1 ± 10.31	0.4348	-9.56 ± 1.52	**<0.0001**	-5.39 ± 0.8	**<0.0001**
	Verghello	-10.86 ± 10.34	0.2976	-3.55 ± 1.53	**0.0233**	-2.23 ± 0.8	**0.0071**
Age	*Old*	-9.72 ± 10.18	0.3432	0.87 ± 1.5	0.5636	0.65 ± 0.79	0.4132
	Young	-21.92 ± 10.48	**0.0404**	-0.48 ± 1.55	0.7556	0.46 ± 0.81	0.5706
Elevation	*Middle*	13.4 ± 10.39	0.202	1.88 ± 1.53	0.2251	0.6 ± 0.81	0.4606
	Top	5.74 ± 10.26	0.578	1.34 ± 1.51	0.3794	0.57 ± 0.8	0.4771

Table 4: Result of PerManova testing the effect of sites, host age, elevation and all interactions on distance among samples in terms of OTU composition. P-value lower than 0.05 are shown in bold. Results were similar using only OTUs present in more than 30 samples and using binary OTU Table (*i.e.* not taking into account the number of sequences; appendix SM 9).

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Sites	2	1.9928	0.99639	5.7795	0.13599	**0.0001**
Age	2	0.5816	0.29082	1.6869	0.03969	**0.0164**
Elevation	2	0.4931	0.24653	1.4300	0.03365	0.0637
Sites:Age	4	1.3357	0.33394	1.9370	0.09115	**0.0003**
Sites:Elevation	4	0.6550	0.16375	0.9498	0.04470	0.5740
Age:Elevation	4	0.6390	0.15975	0.9266	0.04360	0.6303
Sites:Age:Elevation	8	1.1991	0.14988	0.8694	0.08182	0.8295
Residuals	45	7.7580	0.17240		0.52940	
Total	71	14.6543			1.00000	

Supplementary materials:

FIG S1 (pdf): Bioinformatics pipeline

SM S2 (pdf): UNIX scripts for bioinformatics pipeline

FIG S3 (html): FastQC result of sequences quality before quality filtering. Available at: https://github.com/adrientaudiere/FEF_paper/

FIG S4 (html): FastQC result of sequences quality after quality filtering. Available at: https://github.com/adrientaudiere/FEF_paper/

FIG S5 (html): Comparison of four taxonomic assignation methods (RDP, Blast, Mothur and Uclust). We build this figure using Krona (Ondov et al. 2011). Available at: https://github.com/adrientaudiere/FEF_paper/

FIG S6 (html): Distribution of sequences and OTUs number in the fungal taxonomy for the Uparse results (without unique sequences). We build this figure using Krona (Ondov et al. 2011). Available at: https://github.com/adrientaudiere/FEF_paper/

FIG S7 (html): Distribution of sequences and OTUs number in the fungal taxonomy for the Swarm results. We build this figure using Krona (Ondov et al. 2011). Available at: https://github.com/adrientaudiere/FEF_paper/

FIG S8 (html): Comparison of OTUs distribution in the fungal taxonomy for the Uparse results (without unique sequences) and the Swarm results. We build this figure using Krona (Ondov et al. 2011). Available at: https://github.com/adrientaudiere/FEF_paper/

SM S9 (pdf and associated rnw): Ecological analysis of the Uparse dataset discarding unique sequences in the Bio-informatic pipeline. Available at: https://github.com/adrientaudiere/FEF_paper/

SM S10 (pdf and associated rnw): Ecological analysis of the Uparse dataset discarding unique sequences in the Bio-informatic pipeline but keeping sequences putatively assigned to “Tracheophyta” by ITSx. Available at: https://github.com/adrientaudiere/FEF_paper/

SM S11 (pdf and associated rnw): Ecological analysis of the Uparse dataset allowing unique sequences in the Bio-informatic pipeline. Available at: https://github.com/adrientaudiere/FEF_paper/

SM S12 (pdf and associated rnw): Ecological analysis of the Swarm dataset. Available at: https://github.com/adrientaudiere/FEF_paper/

SM S13 (pdf and associated rnw): Ecological analysis of the Open Ref dataset. Available at: https://github.com/adrientaudiere/FEF_paper/

SM S14 (pdf and associated rnw): Ecological analysis of the Closed Ref dataset. Available at: https://github.com/adrientaudiere/FEF_paper/

FIG S15 (doc): Distribution of sequences and OTUs number in the different Order among sites and host ages.

FIG S16 (doc): Assignation quality by RDP classifier measured by bootstrap values in relation to the number of sequences by OTUs.

FIG S17 (doc): Number of sequences by OTUs (molecular Abundances) in function of the more precise taxonomic rank assigned with confidence by RDP classifier (bootstrap value > 80%).

TAB S18 (doc): Extend version of table 1

FIG S19 (doc): Proportion of singletons (mean ± standard error on 99 permutations) in function of the number of sequences.

TAB S20 (doc): Result of PerManova including tree individual

FIG S21 (doc): Distribution of OTUs (left panel) and sequences (right panel) across putative ecology

Chapitre VI

Effet des perturbations sur les communautés de champignons saprotrophes

> « *The world depends on fungi, because they are major players in the cycling of materials and energy around the world.* »
>
> Edward Osborne Wilson, *An Interview With E.O. Wilson, the Father of the Encyclopedia of Life* (*The New York Times*), 23 oct. 2008

1 Synopsis

Dans ce chapitre, nous présentons deux manuscrits documentant les effet des perturbations sur les communautés de champignons saprotrophes (manuscrit I et projet de manuscrit J). Les deux manuscrits diffèrent tant du point de vue des organismes étudiés (champignons saproxyliques des forêts de chênes verts *vs* champignons saprotrophiques édaphiques des forêts de pins laricio) que des méthodes d'échantillonnages (relevé de sporophores *vs* séquençage à haut débit [NGS] du sol) et des perturbations étudiées (chablis *vs* feu).

Une réunion de mycologue en Corse avec la société britannique de mycologie a permis la découverte de sporophores de *Xylobolus subpileatus* dans la forêt du Fango (Corse, France). Cette forêt ancienne, comportant des peuplements surannés marqués par la présence de nombreuses trouées naturelles (Panaıotis et coll. 1997), est connue pour sa richesse sur le plan mycologique (Richard et coll. 2009). Le chêne vert y côtoie la bruyère arborescente, le filaire et l'arbousier. Nous avons pu caractériser (i) les dynamiques temporelles de fructifications de champignons saprotrophes et ECM, et (ii) la cinétique d'émergence des fructifications de *Xylobolus subpileatus* en fonction de l'âge des trouées naturelles, et donc du temps de séjour du chablis au sol. Par ailleurs, l'échantillonnage de la diversité fongique du sol des forêts supraméditerranéennes et montagnardes de Corse a permis de caractériser de nombreux champignons appartenant à d'autres guildes que celle des champignons ECM. Ainsi, nous avons identifié dans les sols des forêts de pins laricio les espèces suivantes :

- *Cryptococcus terreus* (Tremelales) est un champignon de type levure vivant dans le sol (Berger 2016) ;
- *Oidiodendron chlamydosporicum* (Eurotiomycetes) est un champignon du sol capable de former des endomycorhizes éricoïdes (Lacourt et coll. 2001) ;
- *Mortierella humilis* (Mortierellales) est un champignon décomposeur ayant de fortes capacités cellulolytiques (Varnaitė et coll. 2008) ;
- *Geomyces auratus* (Leotiomycetes) est un champignon présent dans le sol en particulier dans les zones froides (sols boreaux ; Minnis et Lindner 2013). Ce genre est bien connu des écologues depuis que l'espèce *Pseudogymnoascus destructans* (anciennement *Geomyces destructans*) a été identifiée comme l'agent impliqué dans le syndrome du nez-blanc chez plusieurs espèces de chauves souris nord-américaines (Lorch et coll. 2011), pathologie qui entraîne une mortalité importante (Carte de l'expansion du phénomène) ;
- *Penicillium restrictum* (Eurotiales) est un champignon ubiquiste principalement présent dans le sol. Cette espèce pourrait favoriser les végétaux en les protégeant des champignons antagonistes par production de composés antifongiques mais également par mycoparasitisme direct (Nicoletti et De Stefano 2012).

Nous avons sélectionné les OTUs assignées à des espèces connues comme des saprotrophes pour analyser l'effet du feu sur les communautés de cette guilde particulièrement importante dans les processus de décompositions (projet de manuscrit J).

2 Manuscrit I : "*Xylobolus subpileatus*, a hyper-specialized basidiomycete functionally linked to old canopy gaps"

IN A NUTSHELL

Rationale: Documenting the ecological successions occurring in forest canopy gaps is an efficient way to characterize the autoecology of organisms and to provide insights into the ecological processes governing the temporal dynamics of species within communities. We here studied the ecology of *Xylobolus subpileatus* in Corsica, where the species was recently discovered during a field work with the British Mycological Society. We used a synchonic analysis to explore the relation between the age of natural canopy gaps and the presence of *Xylobolus* fructifications on logs of different ligneous species.

Type of interaction: Saproxylic fungi, commensal interactions *prima facie* but may be considered as mutualistic at large temporal scale because saproxylic fungi are crucial for ecosystem functioning and therefore, for plants.

Ecological filters of fungal communities: Mosaic of natural gaps in forest; Time of residence on the ground; Size and taxonomy of tree logs

Main conclusions: *Xylobolus subpileatus* is restricted to old (more than seven years) logs of *Quercus ilex* (holm oak). This high ecological specialization may constrain the occurrence pattern of *Xylobolus subpileatus* despite its worldwide distribution. In contrast, old gaps harbor less ectomycorrhizal and saprobic fungal diversity above-ground (sporocarps). The dynamic component of treefall in forest ecosystems is the living place for rare and possibly functionally original species such as *Xylobolus subpileatus*, which is threaten by past and current forest management practices.

Xylobolus subpileatus, a hyper-specialized basidiomycete functionally linked to old canopy gaps

A. Taudiere [1,2], J.-M. Bellanger [1], P.-A. Moreau [3], C. Carcaillet [2,4], A. Christophe [1], T. Læssøe [5], C. Panaïotis [6]J.-M. and F. Richard [1]

Affiliation and Address:

1/ UMR 5175 CEFE – CNRS - Université de Montpellier - Université Paul Valéry Montpellier - EPHE - INSERM, Campus CNRS, 1919 Route de Mende, F-34293 Montpellier, France

2/ École Pratique des Hautes Études (EPHE) and Paris Research University, Paris, France

3/ Département de Botanique, Faculté des Sciences Pharmaceutiques et Biologiques, Université Lille - Nord de France B. P. 83, 59006 Lille Cedex, France.

4/ Laboratoire d'Écologie des Hydrosystèmes Naturels et Anthropisés (UMR5023 CNRS), Université Lyon 1, 69622 Villeurbanne, France

5/ Natural History Museum of Denmark/Department of Biology, Universitetsparken 15, København Ø, Denmark.

6/ Office de l’Environnement de la Corse, Conservatoire Botanique National de Corse. Avenue Jean Nicoli

**** Corresponding authors: adrien.taudiere@cefe.cnrs.fr; franck.richard@cefe.cnrs.fr***
Tel: +33 (0)4.67.61.32.62 Fax: +33 (0)4.67.61.41.21.38

ABSTRACT

Documenting the ecological successions occurring in forest canopy gaps is an efficient way to characterize the autoecology of organisms, and to provide insights into the ecological processes governing the temporal dynamics of species within communities. In this study, we analyzed the fruiting patterns of a rare but widely distributed, saproxylic, stereoid macromycete, *Xylobolus subpileatus*, during natural canopy gap ageing, to document its ecological requirements regarding the specificity and the temporality of the substrate-fungus interaction. In one of the last *Quercus ilex* old-growth forests, (Corsica island, western Mediterranean basin) we systematically recorded and molecularly analyzed *X. subpileatus* basidiomes in 86 dendrochronologically dated natural canopy gaps representing a 45 year-long sequence of residence time of tree logs on the forest floor. *Xylobolus subpileatus* exclusively fruited on *Q ilex* logs, and never on woody debris of other species, i.e. *Arbutus unedo*, *Erica arborea*, *Phillyrea latifolia* and *Fraxinus ornus* , when natural forest dynamics processes provide a wide variety of co-occurring woody substrates to decay. The study evidenced that the probability of fruiting of *X. subpileatus* positively correlates with the age of canopy gaps to reach its maximum at the very end of the decaying process. (ca 40 years after treefall) Contrastingly, in the canopy gaps, the communities of litter saprobic and ectomycorrhizal macromycetes showed a negative correlation between the age of gaps and, both the abundance and the richness of fruitbodies for these two guilds.

Our results emphasize the high ecological specialization of *X. subpileatus* regarding both the biotic and abiotic dimensions of its ecological niche. This work finally highlights the imperative need to preserve the last patches of old-growth Mediterranean forests to secure the persistence of endangered and functionally original macromycetes whose presence is highly dependent on complete decaying processes.

Keywords: *Xylobolus*, *Quercus ilex*, old-growth standMediterranean forest, coarse woody debris, natural canopy gaps, conservation

INTRODUCTION

Identifying the factors that determine the tremendous concentration of biological diversity in old-growth forests is a pivotal question for conservation ecologists, but also an inspiration for forest managers to implement integrated management in cultivated ecosystems. During the past decades, this issue particularly interested mycologists, who widely documented the diversity and the specificity of fungal guilds characterizing old-growth forests (e.g. Smith et al. 2002 and Junninen et al. 2006 for ectomycorrhizal and wood decaying fungi, respectively), but also addressed the overall issue of historical legacies of human practices on the diversity of fungal communities in forests of the Northern hemisphere. In this regard, recent showedstudies both short- (e.g. logging; see Lonsdale et al. 2008 and Paillet et al. 2010 for reviews) and long-term (e.g. millenarian past land used; Dupouey et al. 2002) anthropic disturbances alter the fungal communities dynamics.

Whatever the considered ecological guild, old-growth forests accommodate particularly rich fungal biotas in temperate and boreal biomes (plant mutualists, decayers). From a belowground perspective, these ecosystems are dominated by long-lived ectomycorrhizal (ECM) tree species (e.g. Fagaceae, Pinaceae) that accumulate species rich (Goodman and Trofymow 1998) and dynamic (Izzo et al. 2005) ECM fungal communities, which tend to differ in composition as compared to those in managed ecosystems (Smith et al. 2002). Furthermore, because they accumulate large-diameter woody debris in all stages of decay, old-growth forests are considered as unique in the amount of suitable habitats for rich communities of wood-inhabiting fungi (Bader et al. 1995; Brazee et al. 2014), including infrequent (Stokland and Larsson 2011) or particularly demanding species in terms of ecological requirements (Norstedt et al. 2001; Pentillä et al. 2004).

In old-growth forests, biotic (e.g. insect outbreaks, root pathogenic fungi, dwarf mistletoe; Worrall et al. 2005) and abiotic (e.g., wind and snow; Mc Carthy et al. 2001) disturbances asynchronously provoke canopy openings of various sizes, by causing the fall of single or groups of trees (review: Mc Carthy et al. 2001). As a consequence, the physiognomy of forest ecosystems driven by canopy gap dynamics is typically made of complex mosaics of small-scale patches of vegetation that are in different stages of response to disturbance (Worrall et al. 2005; Oldeman 2012). From its creation to its complete closure, canopy gap is the place of marked shifts in abiotic conditions (light availability, temperature and moisture) These changes, taken together with the existence of contrasted life-strategies of fungi adapted to the different stages of wood decomposition, drive succession of organisms within gaps, from early- to late-successional species (Jönsson et al. 2008). For mycorrhizal fungal guilds, gap-driven dynamics have been poorly investigated so far. Contrastingly, the succession of saproxylic fungi on fallen logs haves been accurately characterized, mainly in boreal and temperate forests. During the decaying process, composition shifts occur within these communities, from early- to late-successional assemblages successively dominated by ascomycete and basidiomycete species (e.g. Lindblad 1998; Rajala et al. 2012), with succession scenarios partially determined by log size and architecture, but also by the identity of the primary fungal agent causing treefall (Heilmann-Clausen and Christensen 2003).

The wood-inhabiting stereoid basidiomycetebo *Xylobolus subpileatus* (Fig. 1a) is a widely distributed macromycete based on 113 records (including 70 in Costa Rica) from 14 countries from all continents but Africa (GBIF, 12 july 2016). This fungus, as well as its sister species *X. frustulatus*, is rare all over Europe and has been proposed by mycologists to be considered as endangered in Austria and Bulgaria, and critically endangered in the Czech Republic (Kotlaba 1986; Papp 2011). Although knowledgethe of this rare species is still fragmentary, evidence of its ecological specialization hass been documented. First, *X. subpileatus* has been recorded only on dead oak wood, with marked preference for old individuals (Long 1915; Parmasto 2001). Second, *X. subpileatus* selectively hydrolyses cell lignin, provoking characteristic heart-rotting honeycomb-like patterns of decay (Fig. 1b), where medullary ray parenchyma and earlywood- vessels are not readily decayed and remain between pockets of degraded material (Blanchette 1984). The extreme ecological specialization of *X. subpileatus* and its consequences on its fine-scale spatiotemporal distribution in forest ecosystems has never been investigated.

In the Mediterranean basin, primary vegetation represents less than 5% of its original extent (Myers et al. 2000). In this area, old-growth forests are extremely rare because of millennia of intensive and uninterrupted land-uses of forest (Rackham and Grove 2001; Quezel and Médail 2003). In the mountainous island of Corsica (Fig. 2a), human activities since the Age metal as (ca. 5 000 years) have dramatically reduced both the quality and quantity of natural forests (Panaïotis et al. 1997, 1998) and likely the fire occurrences (Leys et al. 2014), which is the main large disturbance in Mediterranean forestsHowever, only few small and quasi-inaccessible patches of old-growth forests persist, where both the long-term continuity of forest cover (Land registers, État Major; Conservatoire National Botanique de Corse) and the absence of logging at the time scale of the complete sylvogenetic cycle (Panaïotis et al. 1998) are attested by historical maps and textual archives on forests. The most emblematic one, the *Fango* Man and Biosphere Reserve forest (Fig. 2b), has been continuously monitored for macromycetes since 1999 (Richard et al. 2004). One strength of this exceptionally well-preserved *Quercus ilex* old-growth stand is the possibility to accurately dating forest canopy gaps using *Phillyrea latifolia*, an understory shrub used as markers of disturbance date using their capacity to spontaneously re-sprout immediately after the opening (Panaïoitis et al. 1995). In this study, we took advantage of the discovery of *X. subpileatus* in the *Fango* permanent device during the British Mycological Society field trip in Corsica (2013) to test for the ecological requirement of this species in a privileged context where a large variety of usable woody substrates co-occur, from various species of various ages and dimensions remaining on the forest floor until their complete decay.

Using a synchronic analysis on 86 natural canopy gaps of known age, we described the fine-scale distribution of *X. subpileatus* basidiomes on the basis of (i) the identity of the plant forming the colonized log, (ii) the dimension of considered fragment and (iii) the age of the corresponding canopy gap (time since treefall). We predicted that the ecological specialization of *X. subpileatus* induces a preferential fruiting toward *Q. ilex*, on the largest logs and in the oldest canopy gaps. We finally compared the temporal patterns of *X. subpileatus* fruiting with the fruiting patterns of ECM and saprobic fungal communities in the same gaps using the dataset in Richard et al. (2004). We predicted that the abundance and the richness of ECM and saprobic fruitbodies in gaps decrease during their ageing, while *X. subpileatus* basidiomes show an opposite pattern. From a forest perspective, we aimed at testing the role of forest gaps, until the very end of their recovery process, in providing suitable habitat for highly specialized and rare organisms in natural-driven ecosystems.

MATERIAL AND METHODS

Study site

The study site is situated in the *Fango* forest (42°20′N; 8°49′E), in the northwestern part of the island of Corsica (Fig. 2). The *Fango* valley has been a Man and Biosphere (MAB) reserve since 1973, as it contains rare stands of old-growth *Quercus ilex* (holm oak) forests (Panaïotis et al. 1995; Quézel and Médail 2003). This forest covers a 4 318-ha area on Hercynian granite with enclaves of volcanic rhyolites. Soils are alocrisols (Richard et al. 2009) with mull humus overlying (i) a thick organic layer with a slightly acidic pH ranging from 5.7 to 6.4 and a C:N ratio ranging from 24 to 28 and, (ii) a poorly fissured rhyolitic bedrock (Richard et al. 2009).

The climate is subhumid with a mean annual rainfall of 750 mm and an average annual temperature of 14.6℃ at 192 m above sea level. (asl) Temperatures range from 3.5℃ (mean January minima) to 29.9℃ (mean July maxima).

Characteristics of the *Q. ilex* old growth stand

The study has been carried out within a district of about 1 500 ha, named *Perticato*, extending from 90 to 1 619 m asl (*Capu a u Ghjallichiccia*; Fig. 2b*)*, and where a permanent plot has been designed in 1994 and devoted to the study of forest dynamics (Panaïotis et al. 1995). The vegetation at the study site is a complex mosaic of tall shrubby patches dominated by evergreen Mediterranean species (*macchia*) and of old- growth forest stands, mainly located in the lower parts of secondary valleys. In forest patches, large *Q. ilex* trees dominate a 7-m-high, species poor and dense understory layer that persists under the oak canopy. This understory is composed of macchia species, i.e. *Phillyrea latifolia*, *Erica arborea*, *Arbutus unedo* and scattered individuals of *Fraxinus ornus*, *Cistus salviifolius* and *C. monspeliensis* (Richard et al. 2009).

Textual forest archives unambiguously attest that the district of Perticato has not been exploited for wood and livestock (cows and goats) since 1827 (Panaïotis et al. 1995). For this reason, the long-term expression of forest dynamics generated a high density of natural canopy gaps of about 100 m^2 each, which occur when old *Q. ilex* stems (170 ±46 years) break and fall down (Panaïotis et al. 1995, 1997).

Dating of canopy gaps

Canopy gaps are small-scale disturbances involving either a part of a single tree (hereafter *partial* canopy gaps), or the entire canopy of a single tree (hereafter *single* canopy gaps), or several treefalls (hereafter *multiple* canopy gaps; Mc Carthy et al. 2001). Our study included falls of either trees (*Q. ilex*) or large shrub individuals (*Phillyrea latifolia*, *Erica arborea*, *Arbutus unedo* and *Fraxinus ornus*; Fig. 2c).

The residence time of tree stems on the ground, *i.e.* the dating of fall events, was estimated using the synchronic method developed by Panaïotis and coauthors (1995) based on the high sprouting speed and rate of *Phillyrea latifolia*, which produces new aerial and basal stem sproutsduring the first spring after canopy opening. In each sampled canopy gap, we then accurately dated the age of *Phillyrea latifolia* stem sprouts to assess the date of the tree fall event (Panaïotis et al. 1995 and Fig. 1c-e). In 11 gaps, the dynamics of *P. latifolia* re-sprouting attested a succession of canopy opening events and made uncertain the assignation of the different parts of fragmented logs to a given event. All analyses were carried out with and without these gaps (Table S1 and Fig. S3). As the results are consistent with and without taking into account these gaps, we finally included these gaps into the presented analysis, and their age was reduced to the date of the most recent event recorded (conservative dating regarding the observed dynamics of *X. subpileatus*).

Sampling of *Xylobolus subpileatus* basidiomes

The survey was conducted in April 2015 in a 25-ha old growth stand where a 6 400-m^2 (160 m × 40 m) permanent transect was established in 1994 (Panaïotis et al. 1997; Fig. 1c-e and Fig. 2). In the present study, all canopy gaps present in the 25-ha area were visited, dated using the method by Panaïotis et al. (1995), and the presence of basidiomes of *Xylobolus subpileatus* in dated gaps was systematically investigated. In each gap where *X. subpileatus* was suspected to be present, basidiomes were collected and stored for subsequent microscopic description and molecular analyses.

In addition, in all canopy gaps located into the 6 400-m^2 area covered by the permanent transect, supplementary measurements were performed to refine the ecological requirements of *X. subpileatus*. On each log colonized by the fungus, the surface covered by *X. subpileatus* basidiomes was measured, and the dimension (length, diameter at each tip, and volume) of the colonized stem was estimated.

Taxonomic assignment of *X. subpileatus* basidiomes

In order to confirm the taxonomic identity and homogeneity of the collected fungal material, 16 voucher collections assigned to *Xylobolus subpileatus* were randomly selected out of 22 colonized logs, microscopically analyzed, and compared to five reference collections assigned to genus *Xylobolus* by expert field mycologists (T. Læssøeæø, Denmark; G. Trichies, France; J.-P. Vidonne, France). Two voucher collections of the phylogenetically related species *Stereum hirsutum* were also sampled for comparative purposes. All the samples are kept in the personal herbarium at the CEFE (Montpellier, France) and one representative collection of the Corsican *X. subpileatus* is deposited at the LIP herbarium (Faculté des sciences pharmaceutiques et biologiques, Université Lille 2, France), under the codeLIP0401115.

All collections were then molecularly analyzed. Briefly, DNA was extracted and ITS locus was amplified from dried specimens as described in Loizides et al. (2016). PCR amplification and sequencing were performed with the primers ITS1F and ITS4 or ITS4B (Gardes and Bruns 1993). Validated sequences were deposited in GenBank under the accession numbers listed in Table 1. The analyzed dataset includes these sequences as well as a subset of publically available ITS sequences of *Xylobolus spp* (*i.e.* *X. subpileatus, X. frustulatus, X. apricans* and *X. annosus*) and other Stereaceae in GenBank, selected by BLAST. Phylogenetic analyses were all performed online at www.phylogeny.lirmm.fr (Dereeper et al. 2008) and on the CIPRES Science Gateway, as described in Loizides et al. (2016). The tree depicted in figure S1 corresponds to the 50% majority-rule consensus phylogram resulting from the Bayesian Inference (BI) of phylogeny but a Maximum Likelihood (ML) analysis has been conducted as well. Branch supports have been assessed using the Bayesian posterior probabilities (BPP, as percentages) and the Shimodaira–Hasegawa version of the approximate likelihood-ratio test (SH-aLRT), respectively. When statistically significant, *i.e.* when BPP ≥95% and SH-aLRT >0.8 (Bellanger et al. 2015), and except for the *X. subpileatus* subclade which displays both supports, these values have been omitted and the branch thickened on the tree.

Analysis of fruiting patterns in canopy gaps

In order to compare the temporal dynamics of *X. subpileatus* and *S. hirsutum* occurrence with the general fruiting patterns of fungal communities in canopy gaps, we re-analyzed the dataset presented in Richard et al. (2004). In this study performed at the same site, basidiomes of soil macromycetes, including decayers and ectomycorrhizal fungi, had been mapped at a 0.1-m accuracy during the period 1999-2003 in the 6 400 m^2 permanent transect, and their spatial distribution was analyzed regarding their distance to canopy gaps (Richard et al. 2004).

Here we used this dataset to describe the temporal dynamics of basidiome production and fungal species richness in ten dated canopy gaps during the period extending from treefall event (canopy gap creation) and complete log decomposition (end of the decaying process). Briefly, basidiome abundances and species richness were calculated in a circle of 5 m in radius centered on the fallen tree trunk in the 6 400-m^2 permanent transect gaps where *X. subpileatus* basidiomes area were assessed.

Statistical analyses

The effect of the age of canopy gaps on the presence of *X. subpileatus* and *S. hirsutum* basidiomes was tested using a General Linear Model (function *glm*, R-Develoment Core Team, 2016). Confidence intervals were calculated using the function *confint.glm* from the MASS R packages (Venables and Ripley, 2002). Correlation between gap ages and basidiomes richnesses and numbers were tested using Pearson correlation test.

RESULTS

Taxonomic assignment of analyzed basidiomes

The phylogenetic analysis of our ITS sequence dataset indicates that, as currently defined, *Xylobolus* is polyphyletic, with three distinct lineages nested within Stereaceae (marked by asterisks in Fig. S1): *X. apricans*, *X. spectabilis*, and a weakly supported clade encompassing almost all the other *Xylobolus* sequences, including those of our two reference collections of *X. frustulatus*, type of the genus. The latter lineage is labeled "*Xylobolus*" in Fig. S1 because a taxonomic revision of the genus, clearly beyond the scope of the present work, would be necessary to stabilize names, species limits and phylogenetic boundaries in *Xylobolus*. Nevertheless, all the sixteen suspected *Xylobolus* samples collected in Corsica, nest within that lineage, as a strongly supported monophyletic subclade so far not represented in public databases. Collections in this clade are 100% ITS identical, and include LIP0401115, a sample collected in the same area during the 2013 annual foray of the British Mycological Society, and taxonomically assigned to *X. subpileatus* by T. Læssøeæø. Because this species apparentlydisplays a wide distribution and as no morpho-anatomical features observed in Corsican collections contradict the original diagnosis, we thus apply this binomial to the subclade, at least provisionally, even though no sequence from the North American type material is currently available to support our interpretation. The analysis also attested the identity of two collections of *Stereum hirsutum* (Fig. S1, AT44 and AT67), a common and widespread species with broad ecological and morphological ranges, often confused with other Stereaceae species.

Characteristics of analyzed canopy gaps

In all, we sampled 86 canopy gaps within a forest patch of 16 ha in area (Fig. 2c). Our sampling included 4, 48 and 34 multiple, single and partial multiple canopy gaps, respectively (Table S2). The analysis included a large majority of *Q. ilex* gaps (n=65, 81.3%) occurring in dense forest patches. The analysis also included canopy openings in *macchia* patches, and mostly logs of *Arbutus unedo* (11, 13.8%) and *Erica arborea* (8, 10.0%), with very few individuals of *Phillyrea latifolia* (1) and *Fraxinus ornus* (1).

The date of treefall could be estimated in 80 (93%) canopy gaps. Our dataset included openings ranging from 1 (tree fallen in 2014) to 45 years old (in 1970)in age, with 14.6 years on average (Fig. 1c-e and Fig. S2). The temporal distribution of treefall events showed two main periods of gap creation corresponding to [1-5] and [30-40] years-old logs, and a massive and brief episode that created many partial and single gaps 12 years before our sampling, due to a late spring snow event in 2003 (Fig. S2).

Distribution of *Xylobolus subpileatus* on plant species

Basidiomes of *X. subpileatus* (Fig. 1a) were detected in 22/80 dated canopy gaps. In all, the species was recorded in 37.3% of the total number of dated *Q. ilex* gaps and was never detected on logs belonging to any other plant species. The typical honeycomb-like decomposition pattern of decayed wood was frequently observed in the vicinity of basidiomes (Fig. 1b). *X. subpileatus* fruited in gaps from 7- to 40-years old (mean: 23.5 ± 10.5; Fig. 3a). Thefruiting probability significantly increased with gap ageing, gaining 7% of its value per year of log residence on the ground ($P<0.05$ by one-way ANOVA; Table 2), and ranged from 18.5 to 83.4% in seven- and 40-years old gaps, respectively (Fig. 3a).

The analysis of the fine-scale distribution of basidiomes on *Q. ilex* logs revealed the presence of the fungus on logs of diameters from 15 to 80 cm (median: 27 cm), of length from 1.25 to 14 m (median: 5.91 m), and of volume from 0.02 to 2.36 m^3 (median: 0.21 m^3; Table S2). The log volume was not significantly correlated with the occurence of *X. subpileatus* basidiomes ($p = 0.394$ using glm; Fig. 4). The surface covered by *X. subpileatus* basidiomes varied from 0.13 to 1.01 m^2 (median: 0.2 m^2; Fig. 4). The fungus covered from 0.24 to 18.25% (median: 7%) of the log surface (Table S2). The colonization of *X. subpileatus* has contrasted patterns before and after 20 years since treefall (Fig. 4, Table 2).

By comparison, basidiomes of *Stereum hirsutum* were recorded in only 6 canopy gaps and only on relatively young (mean: 8.3 ±3.1 years) *Q. ilex* logs (Fig. 3b). In contrast with *X. subpileatus*, the probability of *S. hirsutum* basidiome occurrence decreased with gap ageing (Fig. 3b). However, this correlation was not significant due to the low number of records of this species (Table 2).

Dynamics of ectomycorrhizal and saprobic basidiomes abundance and diversity within gaps

In our analysis, the abundance of both ectomycorrhizal and saprobic basidiomes (dataset: *cf.* Richard et al. 2004) in canopy openings significantly decreased with the gap age (Fig. 5, all $p < 0.05$ using Pearson correlation test). Relating to the patterns of occurrence of *X. subpileatus*, the production of basidiomes decreased from the youngest canopy gaps colonized by *X. subpileatus* (with 41.4 and 29.8 basidiomes per 100 m^2 for ECM and saprobic macromycetes in 7-years old gaps, respectively; Fig. 5) to the average residence time of colonized logs (with 18 and 0.4 basidiomes per 100 m^2 for ECM and saprobic macromycetes in 23-years old gaps, respectively; Fig. 5a).

The temporal dynamics of species richness showed similar patterns for the two guilds. Ectomycorrhizal species richness markedly decreased during gap ageing. Relating to the patterns of occurrence of *X. subpileatus*, species richness decreased from the youngest canopy gaps colonized by *X. subpileatus* (with 20.9 and 7.3 species per 100 m^2 for ECM and saprobic macromycetes in 7-years old gaps, respectively; Fig. 5b) to the average time of residence of colonized logs (with 9.6 and 0.4 species per 100 m^2 for ECM and saprobic macromycetes in 23-years old gaps, respectively; Fig. 5b).

DISCUSSION

In this study, we used a unique experimental design to characterize the ecology of *X. subpileatus* along a 40 years long sequence of forest canopy gap dynamics. We demonstrated that in the context of abundant and diversified substrates to decay, this species exclusively fruited on oak logs of various dimensions. Using the dating of canopy openings, we evidenced that the fruiting of *X. subpileatus* was tightly correlated to the age of gaps. Indeed, basidiomes were found in 171%1 of canopy gaps less than 20 years old, whereas 67% of gaps from 20-45 years old contained logs colonized by this species. At the very end of the decaying process (40-years old canopy gaps), *X subpileatus* basidiomes were almost systematically present (84% of sampled logs). The following discussion will end by a management and conservation perspective of this rare fungus species based on the new ecological progress provided by the present study.

X. subpileatus preferentially reproduces in closed canopy gaps

Our analysis of the temporal distribution of *X. subpileatus* basidiomes provided evidence of the latefruiting of *X. subpileatus* on fallen logs. Fruitbodies were never detected on logs within the seven years following treefall, and the probability of presence increased by 7% per year of residence on the ground until the complete decaying of the substrate (Fig. 3a). At its sexual stage, the strong affinity of *X. subpileatus* for highly decayed woody debris brings some insight about the ecological niche of this rare species. The increasing of its fruiting occurrence during gap ageing maythus be a response to both biotic and abiotic shifts that simultaneously take place at ground level in old-growth forests patches (Oldeman 2012). In terms of ecological strategies, *X. subpileatus* fruiting occurrence may reflect niche specialization toward highly decayed logs and/or exceptional competitive abilities.

First, the temporal pattern of *X. subpileatus* fruiting may indicate its affinity for deep shade environments. Indeed, from the time ofthe treefall to the complete canopy recovery by adjacent trees and understory shrubs, the amount of light entering into the gap progressively decreases and induces changes in the microclimatic conditions (moisture, temperature, daily variability, etc.) for leaving organisms on the logs (Messier et al. 1999; Mc Carthy et al. 2001). Studies showed that sun exposure affects composition of saproxylic fungal communities in temperate forests, with species fruiting in fully exposed large gaps, while others require closed, multilayered openings (Heilmann-Clausen 2005). In the context of the dry and fluctuating Mediterranean climate, sun exposure in new gaps and dry microclimatic conditions may contribute to differing the fruiting of *X. subpileatus* on logs.

Second, the temporal distribution of *X. subpileatus* basidiomes would amiror high degree of functional specialization towards lignin to complete its biological cycle (Blanchette 1984). Indeed, during decaying process, physical and chemical properties of wood gradually change. In particular, water and lignin contents increase with loss of cellulose and with decrease in density (Rajala et al. 2012), as a result of a functional succession of saproxylic species on the log. During this process, early dominant soft-rot ascomycetes are progressively replaced by white- and brown-rot species of basidiomycetes (Rayner and Boddy 1988). Regarding catabolic abilities of the corresponding species, this species succession reflects the turnover from cellulose decayers to organisms that are efficient in degrading polyphenols, including lignin (Daniel and Nilsson, 1998). From this perspective, the very late dominance of *X. subpileatus* fructifications on *Q. ilex* logs may primarily be a positive response to the emergence of highly concentrated substrates in lignin in canopy gaps at the end of the decaying process. Then, it is likely that lignin concentration acts as a chemical filter for many saproxylic fungi but not for *X. subpileatus* (Otjen and Blanchette 1984), and as a driver of the dynamics of ecological succession on logs.

Third, the dynamics of *X. subpileatus* sexual reproduction echo it highly competitive strategy regarding ecological succession (Grime et al. 1977, Last et al. 1987). Indeed, interspecific fungal interactions become more complex during the wood decaying process. Based on a large corpus of basidiome records in temperate and boreal forests, but also using the sequencing of mycelia in decaying wood, it has been established that species richness increases during the decaying process (Kubartová et al. 2012), and interspecific fungal interactions accumulate within (*i.e.* among saproxylic species) and among (*i.e.* between saproxylic and ectomycorrhizal species) guilds of fungi (Rajala et al. 2012; Ottoson et al. 2015). In other words, in late phases of decay, white-rot fungi not only outcompete among themselves, but also with rich communities of ectomycorrhizal delignifying species, that become the most dominant life-strategy in wood. In our studied system, it is likely that the temporal pattern of *X subpileatus* occurrence in gaps reflects its high competitive abilities with particularly rich fungal communities (Richard et al. 2004). The use of next-generation sequencing methods in this exceptional set of dated gaps should provide complementary insight into the ecological niche of this species, but also into that of ectomycorrhizal communities in Mediterranean ecosystems. More, further studies investigating the inter individual genetic of *X. subpileatus* at the local scale may usefully complete this panorama by adding information concerning the place of sexual reproduction in structuring populations.

Implication for the conservation of fungal diversity

In this study, we compared the temporal fruiting pattern of *X. subpileatus* to (i) that of the related species *Stereum hirsutum* (Fig. 3) and (ii) those of the global ECM and saprobic fruiting communities of macromycetes in the same study site (Fig. 5; Richard et al. 2004).

We found evidence for contrasteding requirements of *X. subpileatus* and *S. hirsutum* regarding the age of canopy gaps enabling their fruiting. The two species never co-occurred in the sampling design. However, our dataset does not provide evidence of strict niche segregation pattern regarding the gap age: indeed, if *S. hirsutum* was not detected in gaps >12 yrs old, *X. subpileatus* was fruiting yet in 7 yrs old openings, allowing a 5yrs long theoretical overlap. Before concluding about possible niche-differentiation mechanism between these two close relatives, additional work is needed to accurately describe the occupancy pattern of their respective mycelia in logs since treefall to the end of decaying process.

From a conservation perspective, and when considering basidiome occurrence only, our finding highlights the complementarity of canopy gaps of uneven age in our study site in maintaining populations of these two species. Further, our results support the exceptional capacity of old-growth forests to allow the expression of contrasted ecology at the very fine scale due to natural dynamics of gaps (Fournier et al. 2012) Indeed, in old-growth forests more than anywhere, the stochasticity of disturbance regimes governs the temporal distribution of treefall hazards, and allows the persistence of all stages of wood decay at the scale of forest patches, in turn allowing a continuous beginning of new ecological successions (Wilson 1999).

The probability of occurrence of *X. subpileatus* basidiomes on the one hand, and the fruiting patterns of both litter saprobic and ectomycorrhizal macromycetes on the other hand, show inverted patterns during gap closure (Fig. 5). Comparable case studies are lacking, particularly in the Mediterranean area, mostly because equivalent experimental devices are scarce (Quézel and Médail 2003). For the most documented succession of wood-inhabiting fungi, early peaks of species richness were observed in temperate and boreal forests, a few years after treefall (Lindhe et al. 2004), but no clear pattern emerges from the available reports when considering the entire sequence of wood decaying (e.g. Berglund et al. 2005). However, opposite patterns of species richness and occurrence of red-listed species of wood-inhabiting fungi have been reported from studies comparing contrasted management history in northern European forests (e.g. Heilmann-Clausen and Christensen 2005). Our results provide supplementary evidence, in the Mediterranean bioclimatic region, that species richness is an unsatisfactory proxy of the presence of the most threatened elements of fungal diversity, in the same guild (Heilmann-Clausen and Christensen 2005), or in different ones (Fig. 5).

One chief implication of our research is the possibility to accurately date *X. subpileatus* fruiting regarding the entire sylvogenetic cycle. A fine-scale analysis of the patch dynamics (Panaïotiset al. 1997, 1998) showed that natural treefall occur for oak individuals of 170 ±46 years in age. In this context, *X. subpileatus* shows a maximum of occurrence 40 years after canopy openings, *i.e.* about (170+40 yrs) 210 years since the beginning of the long period without human intervention on the forest stand. In the Mediterranean basin, such temporal continuity of naturalness is particularly unlikely to occur because of the ancient and particularly high anthropic pressure in the region, and we may predict that potentially suitable habitats for *X. subpileatus* are limited to very few locations (Rackham and Grove 2001; Quezel and Medail 2003) where the species could be sought within multi-secular old-growth forest.

From a management perspective, the ecological requirements of *X. subpileatus* highlight the high value of the remaining old-growth *Q. ilex* forests canopy gaps for macromycete conservation. The conservation of this functionally original species requires the maintenance of large woody debris in context of fire risk and socio-political demand to limit their abundance in forest ecosystems and across landscapes. Indeed, maintaining favorable habitats for this demanding species requires (i) keeping intact forest cover during centuries and (ii) allowing the accumulation and the residence on forest soil of large woody debris for decades, in forest ecosystems which are highly sensitive to fire risk (Sacchelli et al. 2013), since millennia (Carcaillet et al. 1997). Historically, before the modern era and during most of the postglacial in the Corsican mountain, the mean fire interval is estimated to ca. 80 years (Leys et al. 2014), which is definitely shorter to the time required for the occurrence of *X. subpileatus*. Although, this unique quantitative fire reconstruction in Corsica does not directly concern *Q. ilex* forest but mixed *Pinus nigra-Fagus sylvatica* forest, it indicates that the conservation of any old-growth forest is needed for the conservation of biodiversity linked to time since the last disturbance. Combined altogether, ecological and management constraints impose Mediterranean old-growth forests as key ecosystems for the conservation of forest biodiversity, because they provide unique habitat and resources for some of the rarest organisms.

Conclusion

Because they are among the most threatened organisms in Europe, saproxylic fungi have fueled an intense debate about how they should be conserved. In the forest Mediterranean island of Corsica, saproxylic biota constitute a major conservation problem, not only because they depend on the most important forestry product, i.e. dead wood, but also because their habitat worries forest managers regarding fire risk.

We here demonstrate that the fruiting conditions of *Xylobolus subpileatus* are subordinate to forests highly preserved from human exploitation, where large woody debris take decades to be mineralized by long and complex chains of organisms, at the very end of that the optimized conditions for this rare species occur. In these old-growth stands, only large *Quercus ilex* logs are suitable habitats for this late-successional species, because they last long enough on forest floor to enable the accomplishment of a complete composition turnover from cellulose to lignin decaying fungi. Therefore, preserving old-growth stands and their natural dynamics from fire risk management practices is essential to secure the regional persistence of this endangered and functionally original species whose current distribution is dependent on few hectares of preserved forest patches.

Author Contributions

AT, FR originally formulated the idea, developed the methodology and performed statistical analysis. AT, FR and AC generated data. J-MB and P-AM performed phylogenetic analyses. FR and AT wrote the initial manuscript. All authors contributed to the final version of the manuscript.

Acknowledgments

This study is dedicated to the memory of Justin Smith, who accompanied the discovery survey in summer 2013 and died from sudden arrhythmic death syndrome, on the 3rd of March 2014. We sincerely thank Basile Zorabiniu for its precious help during field survey. This study was funded by the Observatoire de REcherche Méditerranéen de l'Environnement (OSU OREME, UMS3282 CNRS and UMS223 IRD.) All the experiments performed in this study comply with the current French regulation.

References

Bader, P., Jansson, S., and Jonsson, B.G. 1995. Wood-inhabiting fungi and substratum decline in selectively logged boreal spruce forests. Biological Conservation 72(3): 355–362. doi:10.1016/0006-3207(94)00029-P.

Bellanger, J.-M., Moreau, P.-A., Corriol, G., Bidaud, A., Chalange, R., Dudova, Z., and Richard, F. 2015. Plunging hands into the mushroom jar: a phylogenetic framework for Lyophyllaceae (Agaricales, Basidiomycota). Genetica 143(2): 169–194. doi:10.1007/s10709-015-9823-8.

Berglund, H., Edman, M., and Ericson, L. 2005. Temporal variation of wood-fungi diversity in boreal old-growth forests: implications for monitoring. Ecological Applications 15(3): 970–982. doi:10.1890/04-0628.

Blanchette, R.A. 1984. Screening wood decayed by white rot fungi for preferential lignin degradation. Applied and Environmental Microbiology 48(3): 647–653.

Brazee, N.J., Lindner, D.L., D'Amato, A.W., Fraver, S., Forrester, J.A., and Mladenoff, D.J. 2014. Disturbance and diversity of wood-inhabiting fungi: effects of canopy gaps and downed woody debris. Biodiversity and Conservation 23(9): 2155–2172. doi:10.1007/s10531-014-0710-x.

Carcaillet, C., Barakat, H.N., Panaïotis, C., and Loisel, R. 1997. Fire and late-Holocene expansion of Quercus ilex and Pinus pinaster on Corsica. Journal of Vegetation Science 8(1): 85–94. doi:10.2307/3237246.

Daniel, G., and Nilsson, T. 1998. Developments in the study of soft rot and bacterial decay. *In* Forest products biotechnology, Taylor & Francis Ltd. A. Bruce & J.W. Palfreyman, London - Bristol. pp. 37–62.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.-F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.-M., and Gascuel, O. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36(Web Server issue): W465-469. doi:10.1093/nar/gkn180.

Dupouey, J.-L., Dambrine, E., Laffite, J.-D., and Moares, C. 2002. Irreversible impact of past land use on forest soils and biodiversity. Ecology 83(11): 2978–2984. doi:10.1890/0012-9658(2002)083[2978:IIOPLU]2.0.CO;2.

Fournier, T.P., Brossier, B., Gauthier, O., and Carcaillet, C. 2012. Post-Fire Dynamics and Spatial Heterogeneity of Woody Debris Associated with *Pinus halepensis* in an Oak—Pine Mediterranean Ecosystem. Ecoscience 19(4): 356–363. doi:10.2980/19-4-3502.

Gardes, M., and Bruns, T.D. 1993. ITS primers with enhanced specificity for Basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology 2(2): 113–118. doi:10.1111/j.1365-294X.1993.tb00005.x.

Goodman, D.M., and Trofymow, J.A. 1998. Comparison of communities of ectomycorrhizal fungi in old-growth and mature stands of Douglas-fir on southern Vancouver Island. Northwest Science 72: 91–93.

Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. The American Naturalist 111(982): 1169. doi:10.1086/283244.

Heilmann-Clausen, J. 2005. Diversity of saproxylic fungi on decaying beech wood in protected forests in the county of Halland. *In* Länsstyrelsen Halland. Örjan Fritz.

Heilmann-Clausen, J., and Christensen, M. 2003. Fungal diversity on decaying beech logs–implications for sustainable forestry. Biodiversity & Conservation 12(5): 953–973.

Heilmann-Clausen, J., and Christensen, M. 2005. Wood-inhabiting macrofungi in Danish beech-forests – conflicting diversity patterns and their implications in a conservation perspective. Biological Conservation 122(4): 633–642. doi:10.1016/j.biocon.2004.10.001.

Izzo, A., Agbowo, J., and Bruns, T.D. 2005. Detection of plot-level changes in ectomycorrhizal communities across years in an old-growth mixed-conifer forest. New Phytologist 166(2): 619–630. doi:10.1111/j.1469-8137.2005.01354.x.

Jönsson, M.T., Edman, M., and Jonsson, B.G. 2008. Colonization and extinction patterns of wood-decaying fungi in a boreal old-growth *Picea abies* forest. Journal of Ecology 96(5): 1065–1075. doi:10.1111/j.1365-2745.2008.01411.x.

Junninen, K., Similä, M., Kouki, J., and Kotiranta, H. 2006. Assemblages of wood-inhabiting fungi along the gradients of succession and naturalness in boreal pine-dominated forests in Fennoscandia. Ecography 29(1): 75–83. doi:10.1111/j.2005.0906-7590.04358.x.

Kotlaba, F. 1986. Stereum frustulatum–pevník rozpraskanỳ, jeho ekologie a zeměpisné rozšíření v Československu. Česká Mykol 40(3): 129–141.

Kubartová, A., Ottosson, E., Dahlberg, A., and Stenlid, J. 2012. Patterns of fungal communities among and within decaying logs, revealed by 454 sequencing. Molecular Ecology 21(18): 4514–4532. doi:10.1111/j.1365-294X.2012.05723.x.

Last, F.T., Dighton, J., and Mason, P.A. 1987. Successions of sheathing mycorrhizal fungi. Trends in Ecology & Evolution 2(6): 157–161. doi:10.1016/0169-5347(87)90066-8.

Leys, B., Finsinger, W., and Carcaillet, C. 2014. Historical range of fire frequency is not the Achilles' heel of the Corsican black pine ecosystem. Journal of Ecology 102(2): 381–395. doi:10.1111/1365-2745.12207.

Lindblad, I. 1998. Wood-inhabiting fungi on fallen logs of Norway spruce: relations to forest management and substrate quality. Nordic Journal of Botany 18(2): 243–255. doi:10.1111/j.1756-1051.1998.tb01877.x.

Lindhe, A., Åsenblad, N., and Toresson, H.-G. 2004. Cut logs and high stumps of spruce, birch, aspen and oak – nine years of saproxylic fungi succession. Biological Conservation 119(4): 443–454. doi:10.1016/j.biocon.2004.01.005.

Loizides, M., Bellanger, J.-M., Clowez, P., Richard, F., and Moreau, P.-A. 2016. Combined phylogenetic and morphological studies of true morels (Pezizales, Ascomycota) in Cyprus reveal significant diversity, including *Morchella arbutiphila* and *M. disparilis* spp. nov. Mycological Progress 15(4): 1–28. doi:10.1007/s11557-016-1180-1.

Long, W.H. 1915. A honeycomb heart-rot of oaks caused by *Stereum subpileatum*. Journal of Agricultural Research 5(10): 421–430.

Lonsdale, D., Pautasso, M., and Holdenrieder, O. 2008. Wood-decaying fungi in the forest: conservation needs and management options. European Journal of Forest Research 127(1): 1–22. doi:10.1007/s10342-007-0182-6.

McCarthy, J. 2001. Gap dynamics of forest trees: A review with particular attention to boreal forests. Environmental Reviews 9(1): 1–59. doi:10.1139/er-9-1-1.

Messier, C., Doucet, R., Ruel, J.-C., Claveau, Y., Kelly, C., and Lechowicz, M.J. 1999. Functional ecology of advance regeneration in relation to light in boreal forests. Canadian Journal of Forest Research 29(6): 812–823.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A., and Kent, J. 2000. Biodiversity hotspots for conservation priorities. Nature 403(6772): 853–858. doi:10.1038/35002501.

Norstedt, G., Bader, P., and Ericson, L. 2001. Polypores as indicators of conservation value in Corsican pine forests. Biological Conservation 99(3): 347–354. doi:10.1016/S0006-3207(00)00220-2.

Oldeman, R.A.A. 2012. Forests: Elements of Silvology. Springer Science & Business Media.

Otjen, L., and Blanchette, R.A. 1984. *Xylobolus frustulatus* decay of oak: patterns of selective delignification and subsequent cellulose removal. Applied and Environmental Microbiology 47(4): 670–676.

Ottosson, E., Kubartova, A., Edman, M., Jonsson, M., Lindhe, A., Stenlid, J., and Dahlberg, A. 2015. Diverse ecological roles within fungal communities in decomposing logs of *Picea abies*. FEMS Microbiology Ecology 91(3). doi:10.1093/femsec/fiv012.

Paillet, Y., Bergès, L., Hjältén, J., óDor, P., Avon, C., Bernhardt-Römermann, M., Bijlsma, R.-J., De Bruyn, L., Fuhr, M., Grandin, U., Kanka, R., Lundin, L., Luque, S., Magura, T., Matesanz, S., Mészáros, I., Sebastià, M.-T., Schmidt, W., Standovár, T., Tóthmérész, B., Uotila, A., Valladares, F., Vellak, K., and Virtanen, R. 2010. Biodiversity differences between managed and unmanaged forests: meta-analysis of species richness in Europe. Conservation Biology 24(1): 101–112. doi:10.1111/j.1523-1739.2009.01399.x.

Panaïotis, C., Carcaillet, C., and M'Hamedi, M. 1997. Determination of the natural mortality age of holm oak (*Quercus ilex* L.) stand in Corsica (Mediterranean Island). Acta Oecologica 18(5): 519–530. doi:10.1016/S1146-609X(97)80038-0.

Panaïotis, C., Loisel, R., and Paradis, G. 1995. Dating natural gaps in the holm oak forest (*Quercus ilex* L) in Fango MAB Reserve (Corsica) by reading rings of maquis components. Annals of Forest Science 52: 477–487.

Panaïotis, C., Loisel, R., and Roux, M. 1998. Analyse de la réponse de la végétation aux trouées naturelles dans une futaie âgée de *Quercus ilex* L. en Corse (île Méditerranéenne). Canadian Journal of Forest research 28(8): 1125–1134. doi:10.1139/x98-070.

Papp, V. 2011. Adatok a *Xylobolus* Nemzetség Magyarországi El\hofordulásáról. Mikológiai Közlemények, Clusiana 50(2).

Parmasto, E. 2001. Fungi as indicators of primeval and old-growth forests deserving protection. *In* Fungal conservation, issues and solutions, Cambridge University Press. pp. 81–88.

Penttilä, R., Siitonen, J., and Kuusinen, M. 2004. Polypore diversity in managed and old-growth boreal *Picea abies* forests in southern Finland. Biological Conservation 117(3): 271–283. doi:10.1016/j.biocon.2003.12.007.

Quézel, P., and Médail, F. 2003. Ecologie et biogéographie des forêts du bassin méditerranéen. Elsevier, Paris, France.

Rackham, O., and Grove, A.T. 2001. The nature of Mediterranean Europe: an ecological history. Yale Univ. Press, New Haven, CT.

Rajala, T., Peltoniemi, M., Pennanen, T., and Mäkipää, R. 2012. Fungal community dynamics in relation to substrate quality of decaying Norway spruce (*Picea abies* [L.] Karst.) logs in boreal forests. FEMS Microbiology Ecology 81(2): 494–505. doi:10.1111/j.1574-6941.2012.01376.x.

Rayner, A.D., and Boddy, L. 1988. Fungal decomposition of wood. Its biology and ecology. John Wiley & Sons Ltd.

Richard, F., Moreau, P.-A., Selosse, M.-A., and Gardes, M. 2004. Diversity and fruiting patterns of ectomycorrhizal and saprobic fungi in an old-growth Mediterranean forest dominated by *Quercus ilex* L. Can. J. Bot. 82(12): 1711–1729. doi:10.1139/b04-128.

Richard, F., Selosse, M.-A., and Gardes, M. 2009. Facilitated establishment of *Quercus ilex* in shrub-dominated communities within a Mediterranean ecosystem: do mycorrhizal partners matter? FEMS Microbiology Ecology 68(1): 14–24. doi:10.1111/j.1574-6941.2009.00646.x.

Sacchelli, S., De Meo, I., and Paletto, A. 2013. Bioenergy production and forest multifunctionality: A trade-off analysis using multiscale GIS model in a case study in Italy. Applied Energy 104: 10–20. doi:10.1016/j.apenergy.2012.11.038.

Smith, J.E., Molina, R., Huso, M.M., Luoma, D.L., McKay, D., Castellano, M.A., Lebel, T., and Valachovic, Y. 2002. Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, U.S.A. Canadian Journal of Botany 80(2): 186–204. doi:10.1139/b02-003.

Stokland, J.N., and Larsson, K.-H. 2011. Legacies from natural forest dynamics: Different effects of forest management on wood-inhabiting fungi in pine and spruce forests. Forest Ecology and Management 261(11): 1707–1721. doi:10.1016/j.foreco.2011.01.003.

Wilson, E.O. 1999. The diversity of life. WW Norton & Company.

Worrall, J.J., Lee, T.D., and Harrington, T.C. 2005. Forest dynamics and agents that initiate and expand canopy gaps in *Picea–Abies* forests of Crawford Notch, New Hampshire, USA. Journal of Ecology 93(1): 178–190. doi:10.1111/j.1365-2745.2004.00937.x.

Table 1: GenBank accession numbers of ITS sequences generated for the present work.

Gap	Species	Accession number
12	*X. subpileatus*	KX578072
19	-*X. subpileatus*	KX578074
20	-*X. subpileatus*	KX578075
38	-*X. subpileatus*	KX578080
45	-*X. subpileatus*	KX578077
52	-*X. subpileatus*	KX578076
57	-*X. subpileatus*	KX578083
59	-*X. subpileatus*	KX578086
60	-*X. subpileatus*	KX578078
62	-*X. subpileatus*	KX578073
63	-*X. subpileatus*	KX578079
64	-*X. subpileatus*	KX578085
68	-*X. subpileatus*	KX578082
75	-*X. subpileatus*	KX578084
67	*Stereum hirsutum*	KX578081

Table 2: Summary of the generalized linear model of the presence of basidiome of *Xylobolus subpileatus* and *Stereum hirsutum* according to the age gaps (n=59; 22 logs with *X. subpileatus*, 7 with *S hirsutum*).

	Stereum hirsutum		***Xylobolus subpileatus***	
	Estimate ± SE	*p*	Estimate ± SE	*p*
(Intercept)	-1.03 ± 0.654	0.112	-1.85 ± 0.550	**<0.001**
Age	-0.09 ± 0.053	0.0936	0.07 ± 0.024	**<0.001**

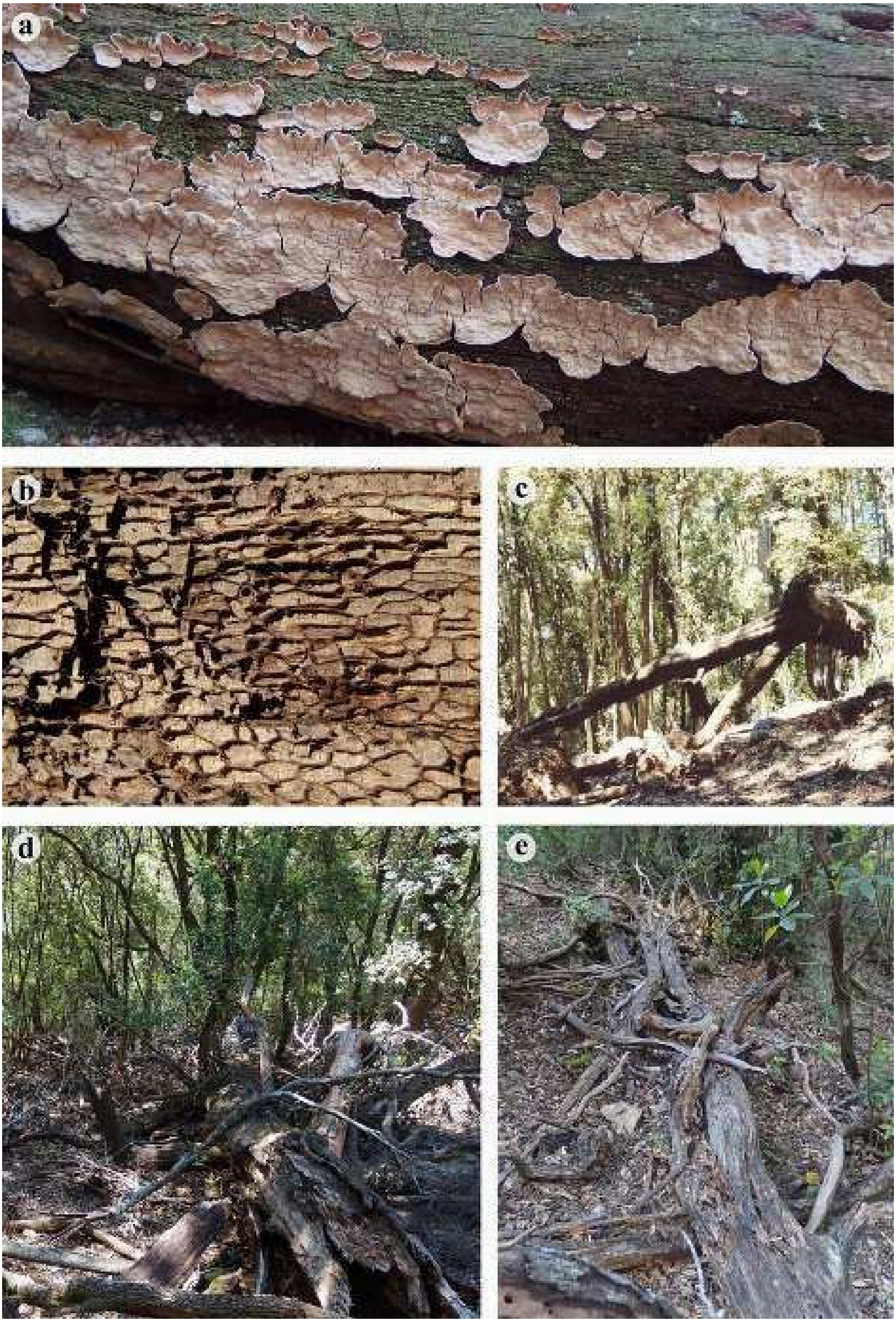

a
b
c
d
e

Fig. 1: (**a**) Reproductive structure (basidiomes) of *Xylobolus subpileatus* on *Quercus Ilex* logs. **(b)** Typical honeycomb decomposition pattern of decayed wood. View of decaying logs from the forest edge in a 1- **(c)**, 5- **(d)** and 20-years old **(e)** canopy gap.

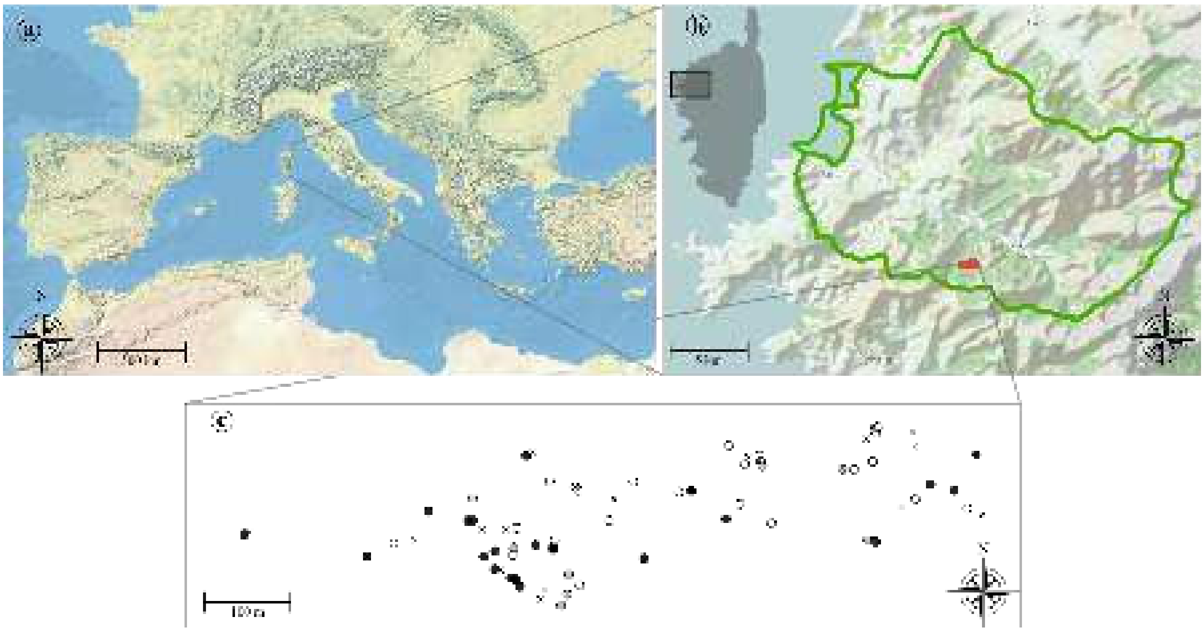


Fig. 2: Location of the study site in the northwestern Mediterranean **(a),** and more precisely in Corsica island **(b),** and detailed pattern of the sampling area in red **(c)**. The green area delimits the UNESCO's Man and Biosphere reserve of Fango. Black, grey and open circles respectively indicate the presence of *Xylobolus subpileatus*, (•) the presence of *Stereum hirsutum* (•) and the absence of both species (◦) on *Quercus ilex*logs. Crosses (+) symbolize gaps created by other woody species. The grey rectangle represents the area where surface of *X. subpileatus* fruitbodies and *Q. ilex* logs were measured.

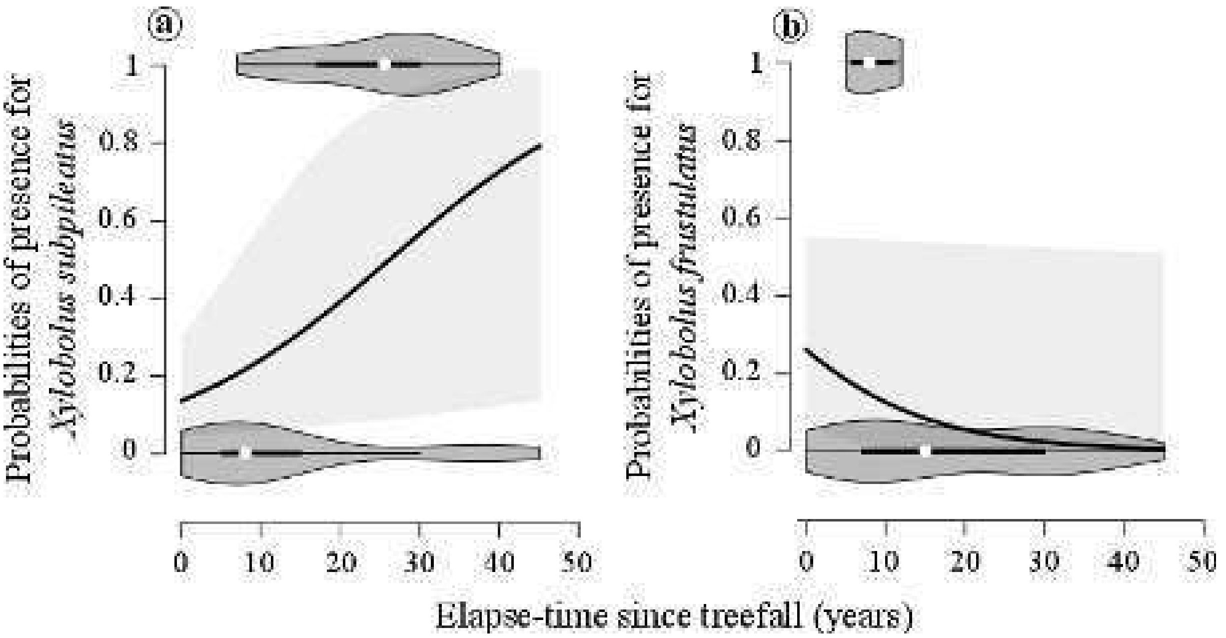


Fig. 3: Probability of fruiting of **(a)** *Xylobolus subpileatus* and **(b)** *Stereum hirsutum* on tree logs in function of the year numbers since tree fall. The distribution of presence/absence is represented by violin plot: the surrounding envelop is a kernel density estimation of the distribution, the white point represents the median and the rectangle the interquartile range. Black lines depict the linear model and the associates grey shades delimit 95% confidence intervals.

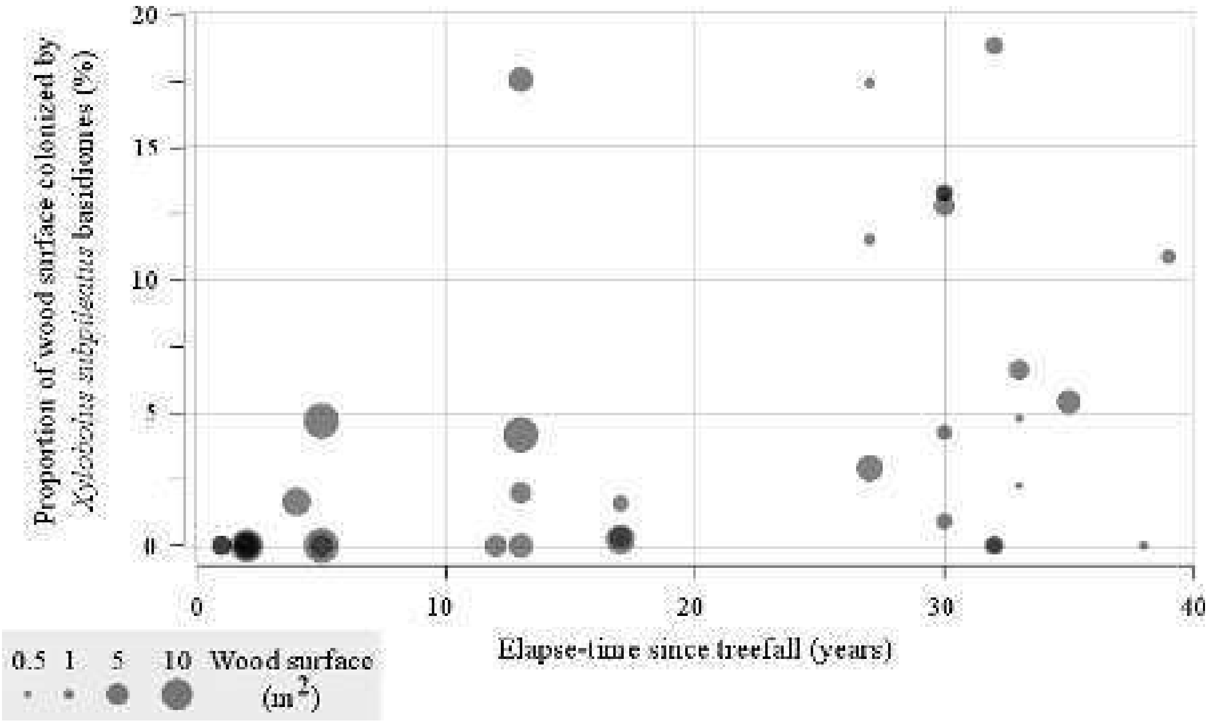


Fig. 4: Proportion of wood surface colonized by *Xylobolus subpileatus* fruitbodies in function of the time elapse since treefall. The size of each circle is proportional (square root transformed) to wood surface on which the *Xylobolus subpileatus* fruitbodies were found.

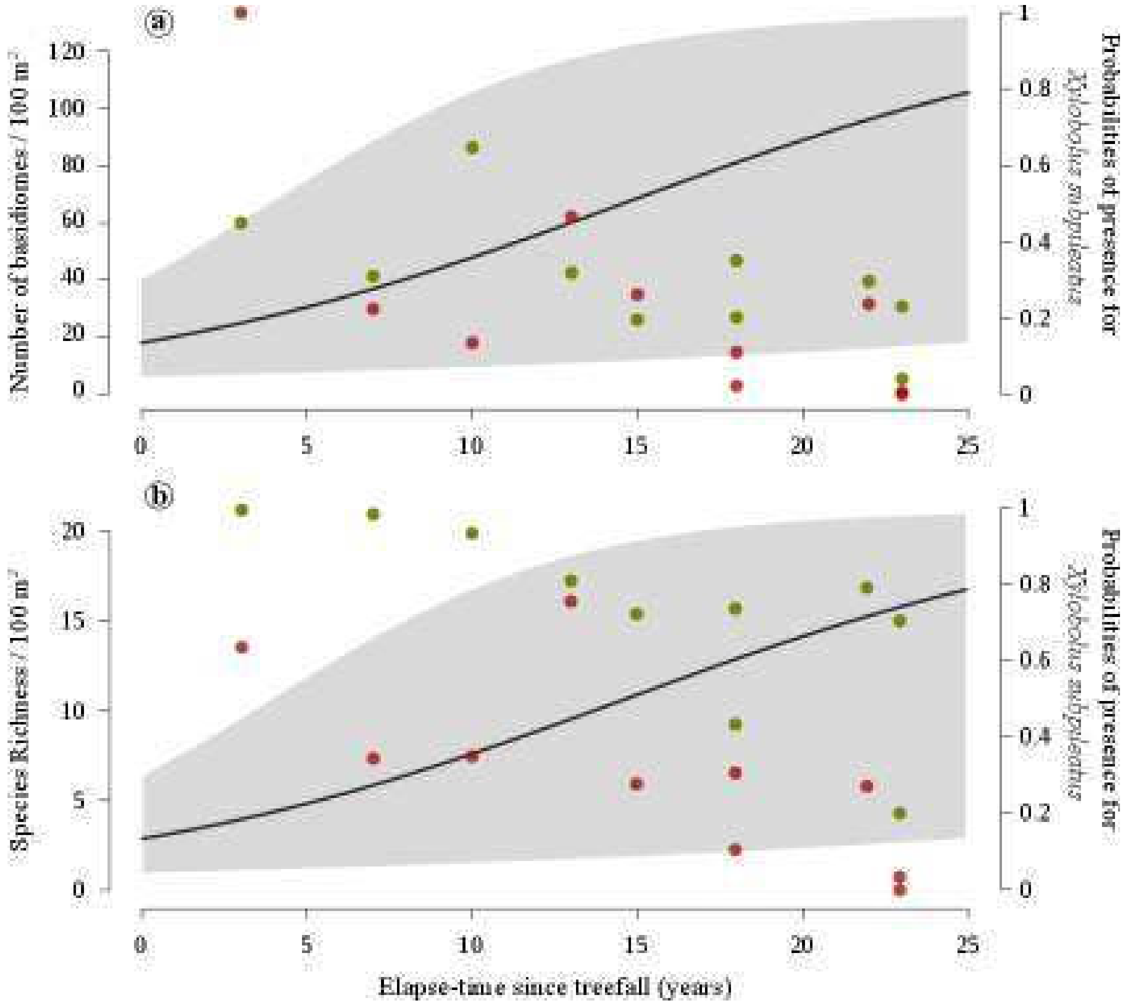


Fig. 5: Fruitbody numbers **(a)** and Species richness 100m²per **(b)** of saproxylic (in red) and ectomycorrhizal fungus (in green) according to the agecanopy gap s. Black lines represent the linear model of the probabilities of presence of *Xylobolus subpileatus* in function of the age of the gap and the associates grey shades delimit 95% confidence intervals.

Supplementary Materials

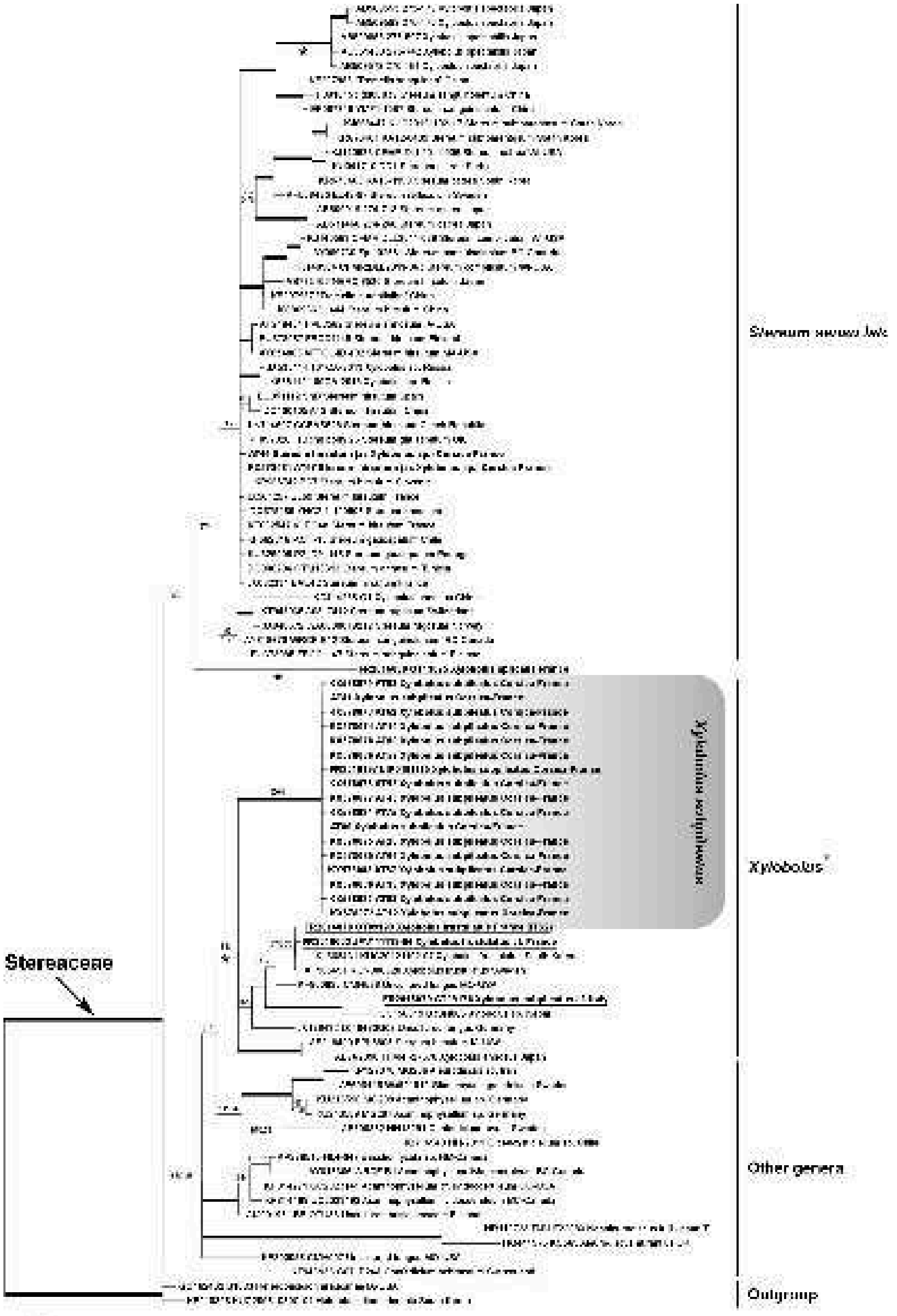


Fig. S1: ITS phylogeny of Stereaceae, depicting the polyphyly of genus *Xylobolus* (main lineages marked with *) and the monophyly of the Corsican *X. subpileatus* clade (in red) within the main *Xylobolus* lineage ("Xylobolus"). Collections studied and sequenced for this work are highlighted in bold, reference sequences are further underlined. Thick branches are supported by BPP values ≥ 95% and SH-aLRT > 0.8, other ones display BI (BPP, as %) and ML (SH-aLRT) support values, respectively.

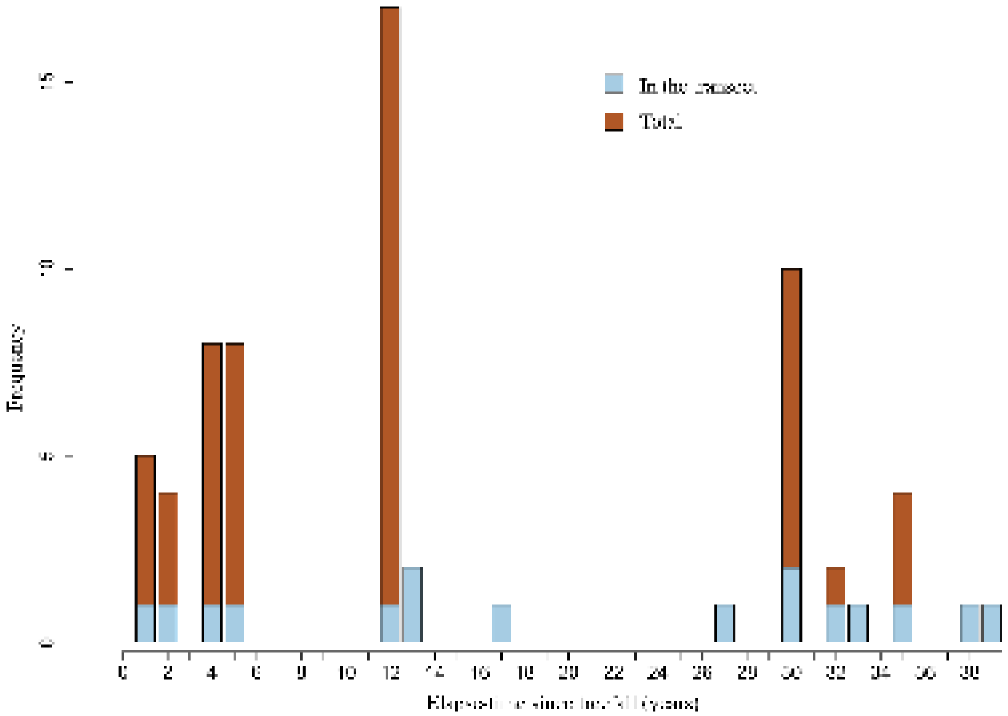


Fig S2: Age distribution of gaps in years. Blue bars symbolize the 16 gaps where the surface of *Xylobolus subpileatus* fruitbodies and *Quercus ilex* logs were measured.

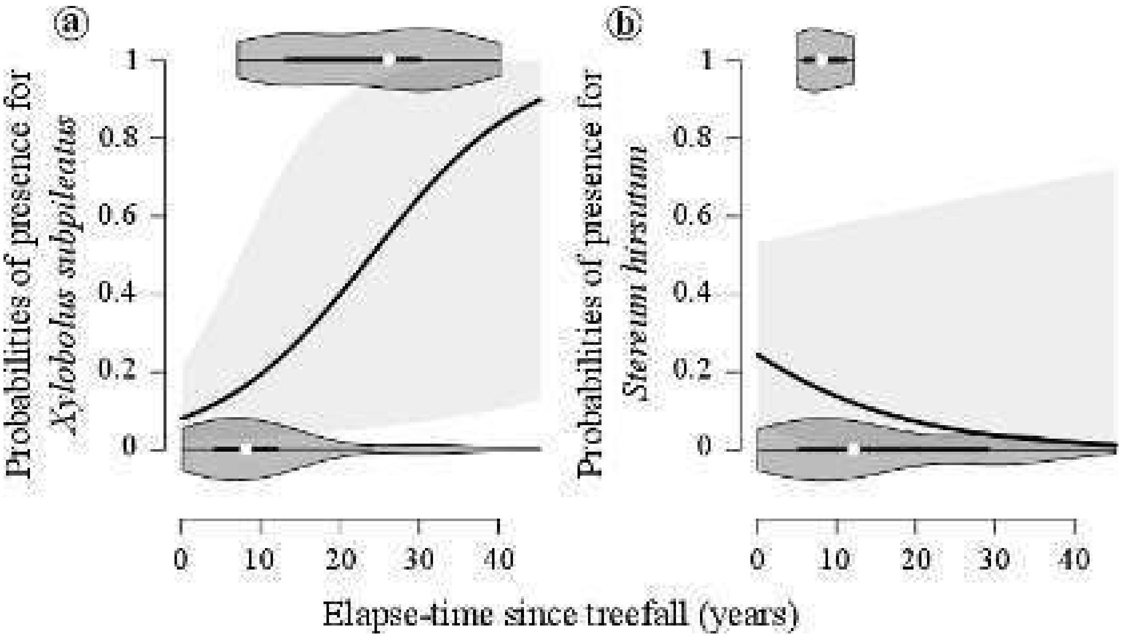


Fig S3: See legend figure 3. Here, eleven gaps approximately dated were removed from the analysis.

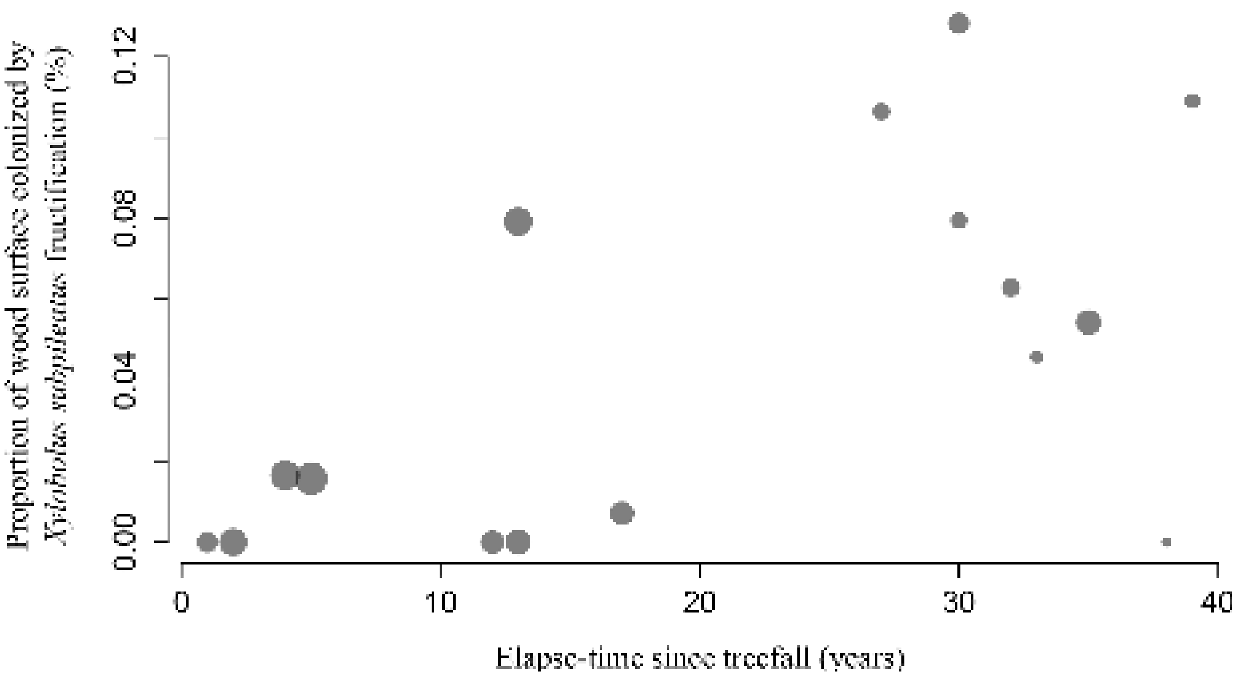


Fig S4: See legend figure 4. Grey points are the mean proportion of wood surface colonized by *Xylobolus subpileatus* fruitbodies by gap.

Table S1: Summary of the generalized linear model of the presence of *X. subpileatus* and *S. hirsutum* in function of the age of the gap (n=48). Here, eleven gaps approximately dated were removed from the analysis in contrast to table 1.

	Stereum hirsutum		***Xylobolus subpileatus***	
	Estimate ± SE	*p*	Estimate ± SE	*p*
(Intercept)	-1.14 ± 0.663	0.087	-2.44 ± 0.661	**<0.001**
Age	-0.07 ± 0.056	0.199	0.10 ± 0.032	**0.0016**

Table S2: Dataset

Gap number	**Log number**	**Log length (cm)**	**Log maximum diameter (cm)**	**Log minimum diameter (cm)**	**Surface of fruitbodies of *Xylobolus subpileatus* (cm²)**	**Age of the gap**
1	1	342	39	30	0	32
1	3	267	29	23	0	32
1	2	400	29	23	6160	32
2	1	210	83	63	0	5
2	3	830	47	47	0	5
2	2	830	48	48	6059.48	5
3	1	383	23	13	2359.44	39
4	2	341	15	13	1730.55	27
4	3	250	15	13	1917.3	27
4	1	759	30	30	2128.95	27
5	3	600	40	8	900	13
5	1	470	80	80	5360.42	13
5	2	810	40	9	10956	13
6	1	202	19	8	0	38
7	1	830	32	6	0	12
8	1	780	22	10	0	1
8	2	820	22	10	0	1
9	1	697	21	18	5472	30
10	3	830	18	3	252	30
10	4	840	15	3	1015	30
10	1	300	25	21	2880	30
10	2	980	18	3	4300.8	30
11	1	992	30	8	3213	35
12	1	1005	21	17	0	13
13	3	125	20	10	134	33
13	2	155	18	11	340	33
13	1	582	24	21	2726.55	33
14	1	470	68	76	0	2
14	2	1600	15	1	0	2
14	3	1600	27	1	0	2
14	4	1600	28	1	0	2
15	1	1400	30	7	1344	4
16	1	270	45	60	137.8	17
16	2	750	37	37	212.5	17
16	3	250	37	27	399.99	1

3 "Soil saprobic fungal community assembly: effect of fire and forest species composition" (Projet de manuscrit J)

Coexistence de hêtres et de pins laricio dans la forêt de Ghisoni.

IN A NUTSHELL

This is a work in progress. See section works progress and draft J in appendix ⚙.

Rationale: Soil saprobic fungi (SF) are essential in nutrient cycling. Diversity and community composition of SF determine ecosystem functioning and community of organisms with which they interact. We took advantage of our sampling scheme and soil ITS sequencing corresponding to manuscripts D and G in Corsican pine forest to test three hypotheses presented in table VI.1.

Type of interaction: Saprobic fungi including wood decomposers

Ecological filters of fungal communities: Wildfire in interaction with depth; Dominant tree of the forest; Plant coexistence in mixed forest

Main results:

◕ *Taxonomy of dominant trees affect SF composition but not diversity*

Tree composition doesn't impact soil SF diversity (Fig. VI.1a). However, the taxonomy of dominant tree drives marked shift in SF communities (Fig. VI.2a). Fungal communities in mixed forests are made of a mix of communities associated with monospecific stands, with similar diversity (Fig. VI.2a).

Moreover, dominant trees significantly shape the abundance of 25 OTUs belonging in five different families (Fig. VI.3) and structure the co-occurence network of fungal OTUs (Fig. VI.4).

◕ *Fire affects SF composition but not diversity*

As for tree taxonomy, fire severity is not correlated with a change in SF diversity (Fig. VI.1b) but markedly drives SF community composition (Fig. VI.2b).

◕ *SF communities respond differently to fire, depending on soil depth*

All diversity metrics are lower in the mineral layer than in the organic layer (Fig. VI.1b). The effect of fire on SF communities is buffer in the mineral layer compared to the organic one (Fig. VI.2b). At the phylum level, fire induces changes in abundances but does not change species richness (Fig. VI.5). Surface fire decreases the number of detected sequences belonging in Zygomycota, in favor of Ascomycota in the organic layer and in favor of both Ascomycota and Basidiomycota in the mineral horizon (Fig. VI.5a). Destructive fire induces a shift in organic soil layer only from communities dominated by Zygomycota sequences to communities dominated by Ascomycota sequences (Fig. VI.5a).

Main conclusions: Fire history of Corsican pine forest still structures soil saprobic fungal communities 15 years after the fire. Moreover, the effect is more important in the organic layer than in the mineral layer.

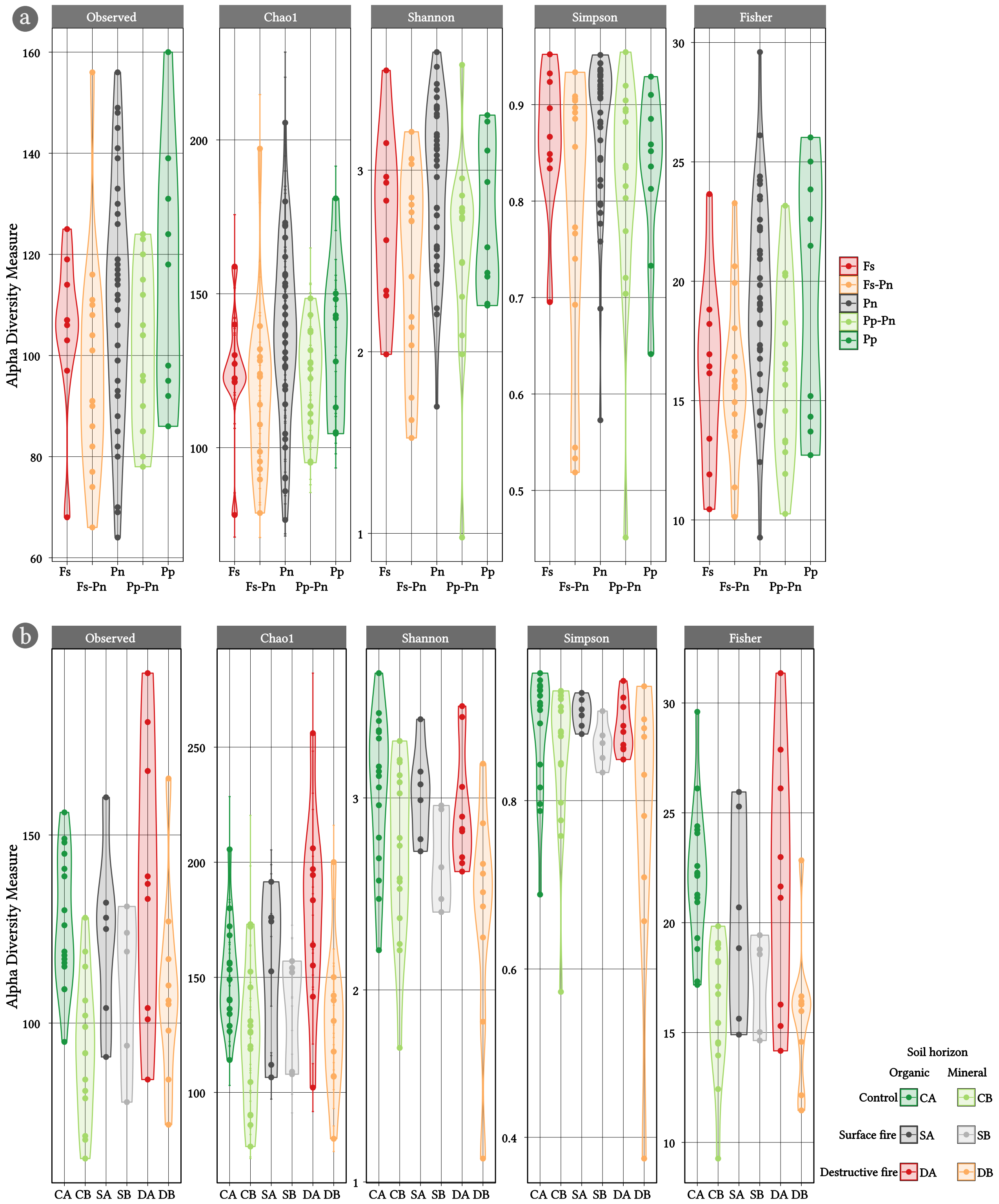


Figure VI.1 – Saprobic fungal diversity in function of (a) vegetation and (b) fire severity: Vioplot represent density of points. (**a**) Abbreviations used: Fs: *Fagus sylvatica*; Pn: *Pinus nigra* subsp. *laricio*; Pp: *Pinus pinaster*. (**b**) Diversity in function of fire severity in *Pinus nigra* subsp. *laricio* stand: control (green), surface-fire (gray) and destructive fire (red). Strong colors indicate organic layers and clearer colors indicate mineral layers.

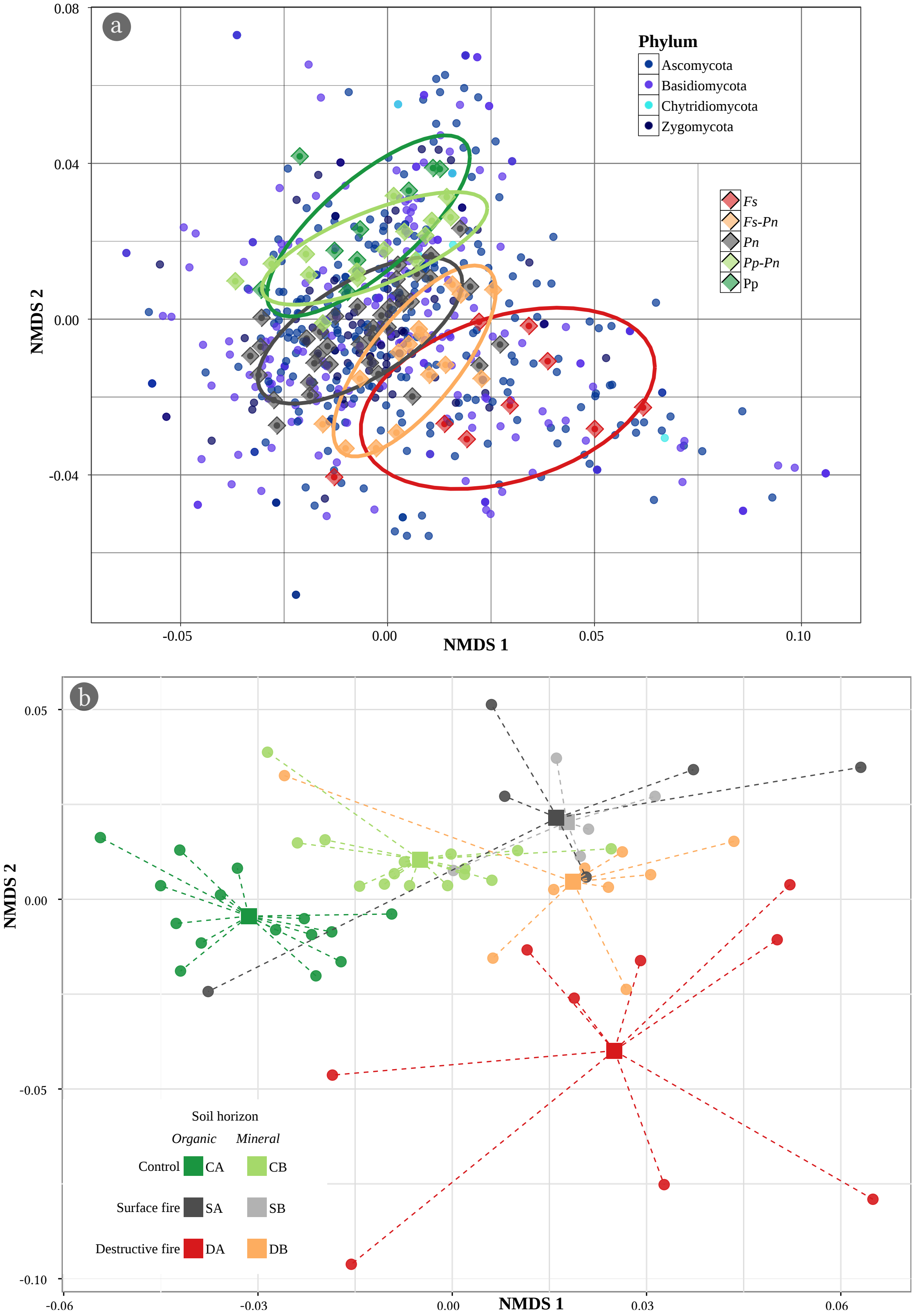


Figure VI.2 – Non metric multidimensional scaling (Gower distances) showing the difference in species composition (a) among forest dominate by different tree and (b) among forest with different fire history: Same legend as figure VI.1. The NMDS stress is equal to 22.1% for panel **a** and 18.6% for panel **b**.

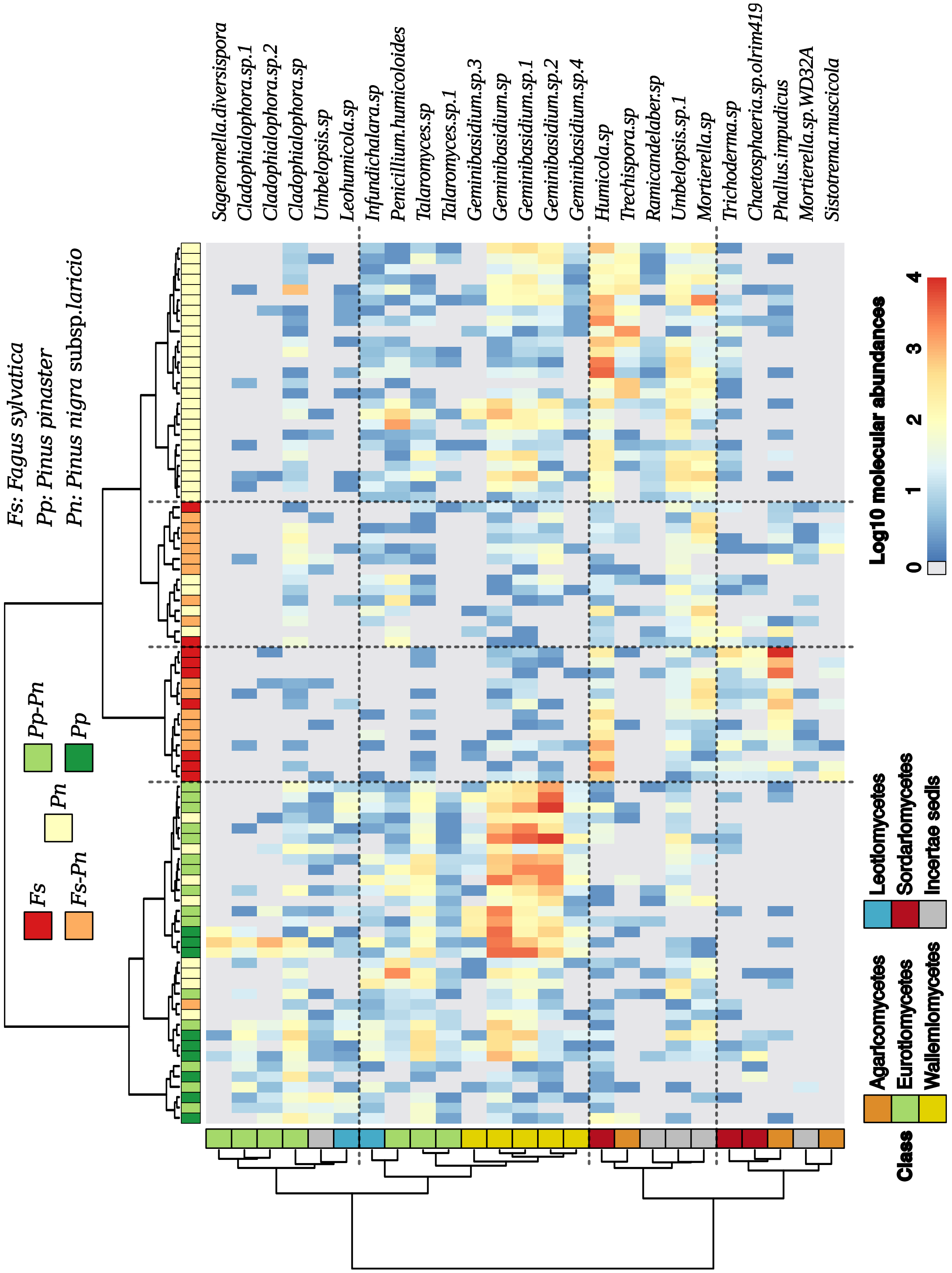


Figure VI.3 – Distribution of fungal taxa significantly affected by the taxonomy of dominant tree: Colors indicate dominant tree(s) in columns, fungal class in rows and read number (log10-transformed) in cells. Rows and columns are ordered using hierarchical clustering (ward method). Dashed lines split the data in four groups given the hierarchical clustering trees.

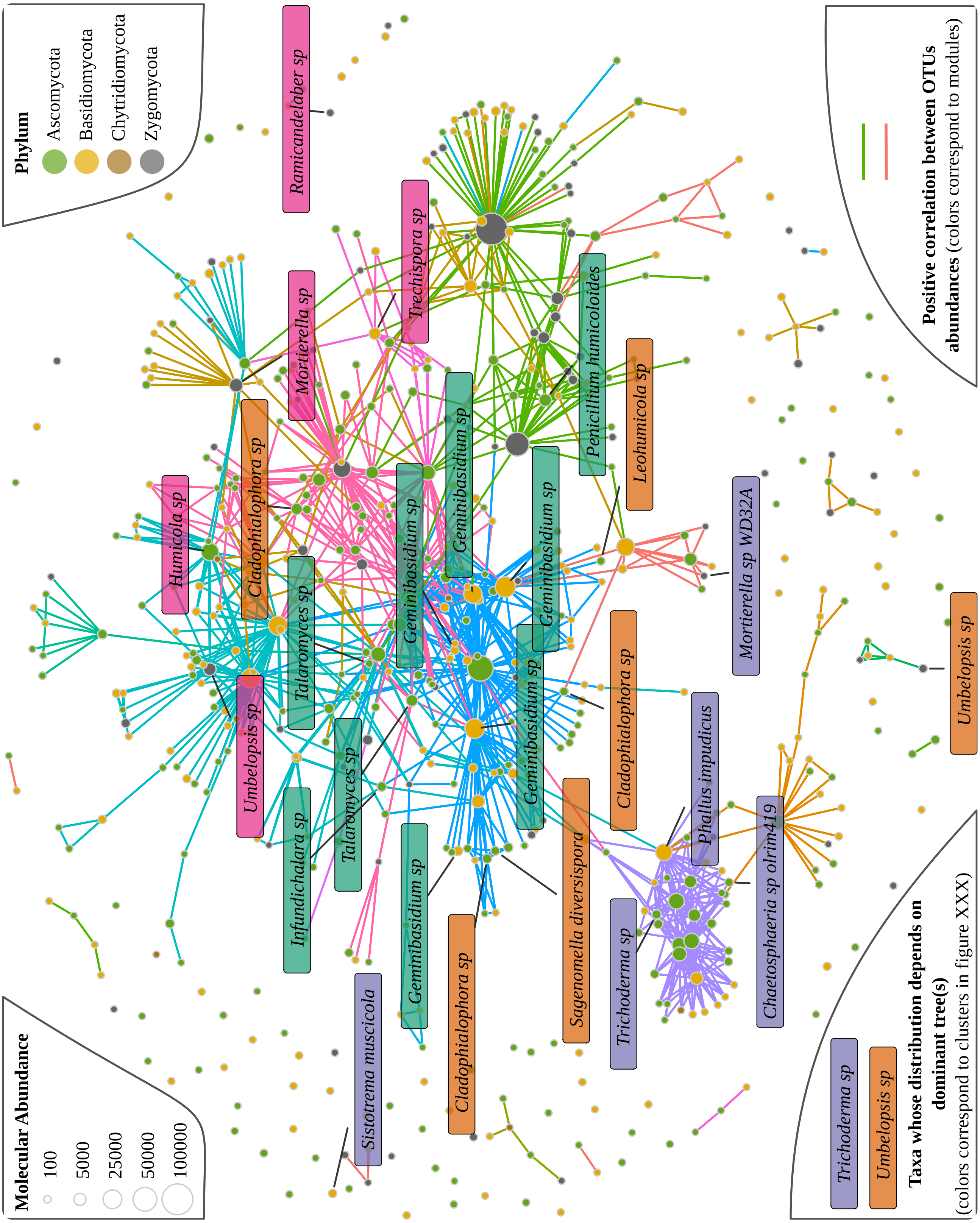


Figure VI.4 – Correlation network among OTUs: Network linking the 581 saprotrophic OTUs. Each link represents a positive and significant correlation between two OTUs (p-value < 0.05 and Standardized effect size values > 6). Link colors code for modules (function *cluster_louvain* in package *igraph*). Each OTU is represented by a circle whose size is proportional to the number of read and color represent the Phylum. Taxa names are given for OTUs significantly affected by dominant tree(s) taxonomy (function *mt* in package *phyloseq*). Filled color of boxes correspond to the cluster from figure VI.3.

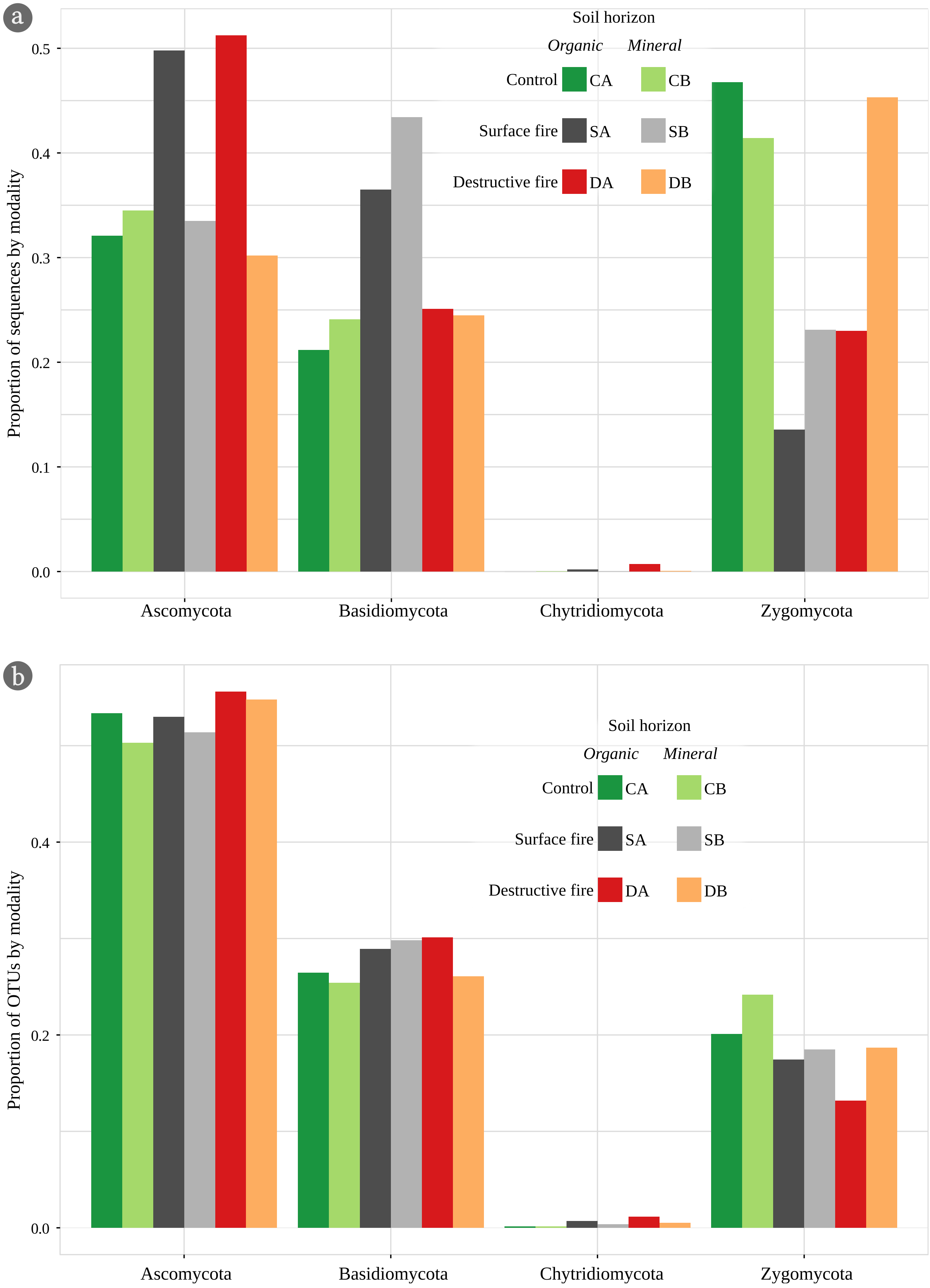


Figure VI.5 – Distribution of the number of sequences (a) and OTUs (b) by phylum for each fire × layer modality.

Table VI.1 – Hypothesis tested regarding saprobic fungal diversity in soil: ✔, ⊗, and (✔) indicate respectively that we confirm, infirm or partially confirm our hypothesis.

(**I**) Soil SF diversity is higher …	**Ia**) … in mixed forest than in pure one	⊗
	Ib) … in the organic layer than in the mineral layer in laricio forest	✔
	Ic) … in unburnt P. nigra forest than in forest that undergone a surface fire than in forest entirely destructed by fire	⊗
(**II**) Soil SF communities composition is shaped by dominant tree(s) identity	**IIa**) SF communities of mixed forests is made of a mix of the communities present in corresponding pure stand	✔
	IIb) Forests dominated by the two pine species harbor more similar SF communities than pine forests with beech one	(✔)
(**III**) Fire shape soil SF communities...	**IIIa**) … and this effect is stronger after a destructive fire than a surface fire	(✔)
	IIIb) … and this effect is stronger in the soil organic layer than in the mineral layer	✔

Table VI.2 – Number of sites, samples, OTUs and sequences for each analysis.

	Sites	**Soil samples**	**Samples analysed**	**OTUs**	**Sequences**
Vegetation	3	87	85	581	810 471
Fire	6	72	60	558	638 133

Chapitre VII

General discussion

« *- Bien sûr. De toute façon, y a plusieurs réponses possibles hein.*

- Ah bah oui, mais comme on peut répondre ni par oui, ni par non, ni par sans opinion, la difficulté est majorée. »

Alexandre Astier, *Kaamelott Livre VI (épisode 8)*, 2006

This work documents the patterns of community assembly of different fungal guilds (Fig. VII.1) in Corsican forests. Here, we called community assembly rules the patterns describing the relation between fungal diversity (either α-, β- or γ- diversity) and environmental variables – including disturbance regimes, abiotic environment parameters and biotic interactions.

In the first section of this discussion, we compare community assembly rules found in diverse fungal guilds. We first summarize the results for each guild (1.1) and then discuss the role of dispersion (1.2) and selection processes (1.3; *sensu* Vellend 2010) on fungal community assembly in the studied forests. We then propose perspectives to better understand fungal community ecology in the light of drift and speciation processes (1.4). Finally, we challenge our methodology based on guilds using example of fungal species which show guild shifting during their biological cycle (1.5).

In the second section, we focus on ectomycorrhizal (ECM) interactions and the the high intimacy and dependency that characterizes both plants and fungi in ECM interactions. We first define the concept of niche mirror and we discuss its the implication for experimental studies (2.1). We expose the consequences of this intermingling of partners' niche for ECM plant ecology at various spatio-temporal scales (2.2). Finally, we rely on the concept of niche mirror to propose tools for stakeholder to conserve conjointly ECM plants and fungal species (2.3).

In the third section, we develop what we think are the main issues and the most promising directions for fungal community ecology. We argue for the necessary shift from community assembly rules to processes (3.1). Next we emphasize the challenges and questions generated by the applying of new techniques and methods to fungal communities, using the network framework as an example (3.2). In the last section, we suggest some research directions for fungal ecology by getting inspiration from plant ecology (3.3).

Finally, we propose perspectives based on the identification of the scale of homogeneity in fungal community assemblages. Identifying scales at which variation among fungal communities is low allows to (i) identify scales at which patterns are mostly driven by deterministic processes (i.e. non stochastic), and (ii) implement relevant sampling schemes for future studies by sampling the right number of samples by variable modality.

* * *

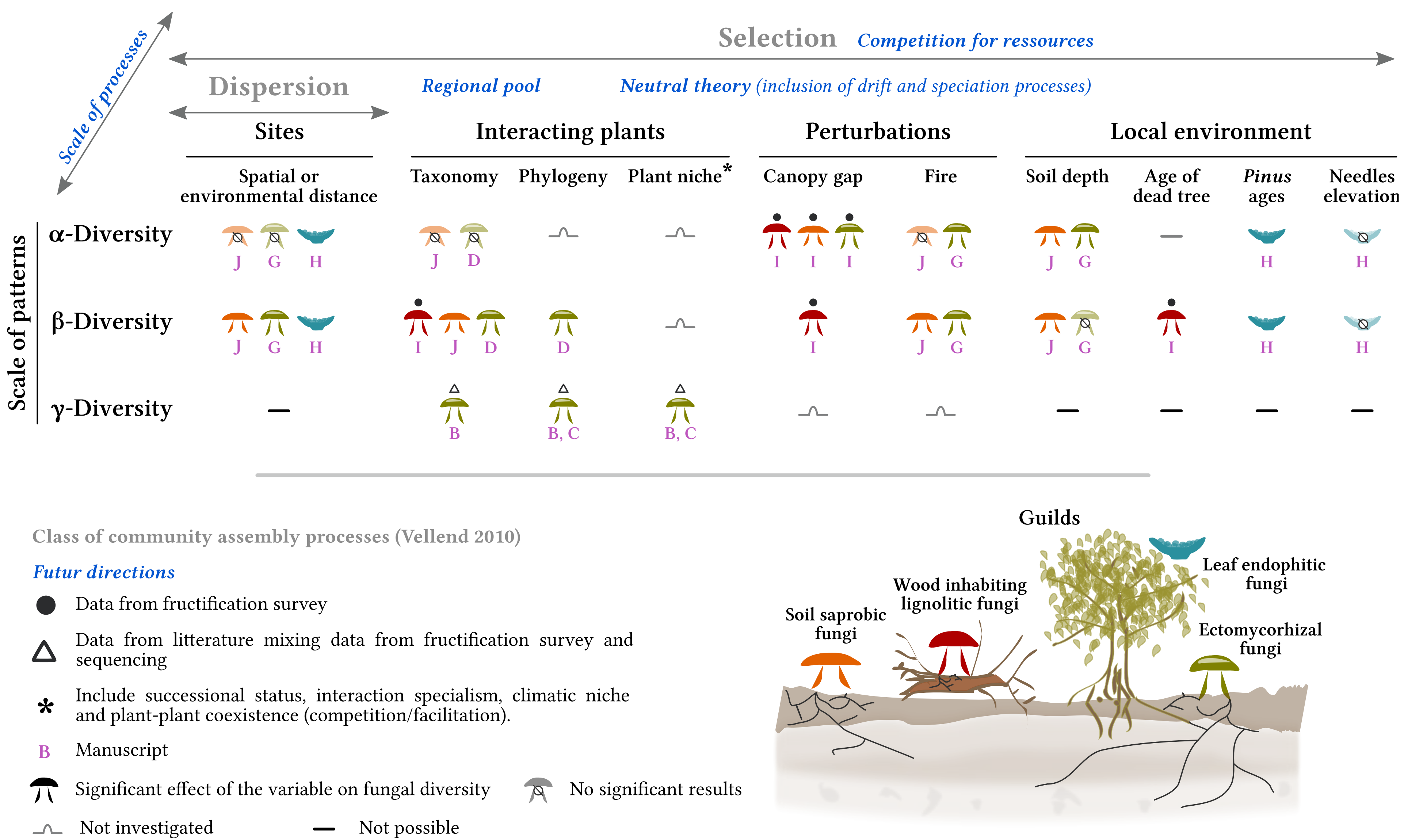


Figure VII.1 – Summary of the thesis results: Perspectives discussed in this section are noted in blue. Symbol colors correspond to guilds. Purple letters indicate the related manuscripts.

1 Are community assembly rules identical across fungal guilds?

1.1 Summary of the thesis results

Insights into community assembly rules of ECM fungi

Study of community ecology of ECM fungi starts in the 1990's for the below-ground[1] component of the symbioses (Gardes and Bruns 1996; Dahlberg 2001). Since then, ECM fungal community ecology has developed regularly to provide a more comprehensive overview of the below-ground patterns of ECM diversity and the factors governing the spatio-temporal distribution of species (reviewed in Dahlberg 2001; Peay *et al.* 2008; Kennedy 2010; Koide *et al.* 2011). In the present work, we focus on the effect of ECM plant ecology (e.g. position during ecological succession and level of coexistence with other plants) and disturbance (fire and canopy gap) on ECM fungal communities.

First, we explore the effect of host specificity at several scales (Corsica Island, separated valley and forest stands) and the effect of plant ecology on the community of ECM fungi at these scales (chapter III). We find that both host specificity and plant successional status drive ECM ecological networking and therefore, constrain the potential ECM fungal community at a given location (manuscript B). Moreover, we demonstrate the effect of plant coexistence on both potential (manuscript C) and soil (draft D) ECM fungal communities. The last step will be to explore the realized ECM fungal communities from root tips sequencing (draft D).

Second, we explore the effect of fire on ECM fungal communities in chapter IV. A review of the literature, integral to the present thesis (manuscript F), highlights the effect of wildfire on community composition but not on diversity (draft G). As a consistent pattern, reviewed studies reveal that 15 years after a surface wildfire , ECM fungal communities are still different in composition when compared to unburnt stands, and the effect is even stronger after a destructive fire (Fig. IV.3). In marked contrast, ECM fungal communities of Corsican pine in soil were not driven by the interaction between fire severity and soil depth (draft G). On one hand, fire induces an increase of the molecular abundance of four ECM fungal species (assigned to *Geopora sp.* and *Rhizopogon* aff. *rubescens*). On the other hand, 21 OTUs presented a decrease in molecular abundance after a destructive fire (Fig. IV.4). Again, further analysis of ECM root tips is still in process and will complete our knowledge of the response of ECM communities to fire. Finally, in chapter V, we underline the dynamics of the above-ground ECM fungal diversity (sporocarps survey) after canopy gap in an emblematic old growth forest in Corsica (draft I). In this descriptive and fruitbody-restricted study, we reveal that in response to the disturbance, the taxonomic diversity is maximal after tree fall and decreases until the canopy recovery.

---

[1] Before the advent of molecular tools, ECM fungal identification was done mostly using sporocarp surveys. The first publications on the topic (e.g. Gardes and Bruns 1996; Jonsson *et al.* 1999) rapidly pointed to the marked discrepancy between above-ground and below-ground views. See discussion on functional traits)

Insights into community assembly rules of foliar endophytic fungi (manuscript H)

Our study of the foliar endophytic fungi (FEF) communities associated to the Corsican pine reveals that spatial location (here geographically isolated valleys across the island) was the main explanatory variable of FEF community composition and diversity. This spatial effect of may be due to dispersal limitation or to unmeasured spatially structured variables (see section on environmental filtering). Consistent with the existing literature (Table I.1), age of trees had a significant but small effect on FEF assemblages. In fact, next-generation sequencing (NGS) studies bring so much information that even a very small change in community can be detected (high statistical power). In previous works on FEF, all measured variables were found to significantly drive FEF communities (Table I.1). Yet, FEF communities of Corsican pines seem poorly shaped by microclimatic variation linked to leaf position in the tree (shade vs full light exposure).

Insights into community assembly rules of soil saprobic fungi (draft J)

Both ECM vegetation composition and fire severity affect soil saprobic fungal (SF) community composition but not diversity (draft J). In soils of Corsican pine forests, organic layer displays higher SF diversity than mineral layer. SF communities of mixed forests (two coexisting dominant tree species) are made of a mix of the communities associated with each of the two kind of monospecific forests (*i.e.* dominated by one of two tree species forming the mixed stand). Finally, fire shapes SF communities by both species replacement and shift in OTU relative abundances, and this shift is more important in the organic than in the mineral layer.

Insights into community assembly rules of wood-inhabiting fungi (manuscript I)

We document here the temporal dynamics of *Xylobolus subpileatus* and *Stereum hirsutum* fruiting across the complete decaying process of *Quercus ilex* logs, using space for time substitution chronosequences. *X. subpileatus* exclusively fruits on *Quercus ilex* logs, and never on *Arbutus*, *Erica*, *Phyllirea* and *Fraxinus* woody debris. The probability of fruiting of *X. subpileatus* positively correlates with the age of canopy gaps and reaches its maximum at the very end of the decaying process. On the contrary, *Stereum hirsutum* fruits only in young canopy gaps.

This temporal pattern of *X. subpileatus* sporocarp occurrence indicates either (i) its affinity for deep shade environments, (ii) its adaptation to high concentration of lignin (Blanchette 1984) or (iii) its highly competitive ecological strategy regarding ecological succession of fungal guilds on logs (more details are available in the discussion of manuscript I).

1.2 Role of dispersion processes

For a long time, microbiologists followed a paradigm summarized by the famous sentence from Baas Becking (1934) "*Everything is everywhere,* but*, the environment selects*" [2] (Baas Becking

---

[2] The dutch original sentence ("*alles is overal :* maar *het milieu selecteert*") use italic to underline the word *marr* ("*but*"; de Wit and Bouvier 2006)

1934, cited in de Wit and Bouvier 2006). Consistent with Baas Becking (1934), Green *et al.* (2004) and Queloz *et al.* (2011) established the cosmopolitanism of soil Ascomycota and root fungi, respectively. However, numerous recent publications found spatially structured communities of microorganisms and attributed these spatial patterns to dispersal limitations (reviewed in Martiny *et al.* 2006, see also introduction *in french*). In any case, numerous fungal species have been found to be cosmopolitan.

Given its worldwide distribution, its tight ecological requirement and the patchiness of its habitat, high dispersal abilities of *Xylobolus subpileatus* seem to be overriding its survival rate (manuscript I). However, population genetics studies are still lacking for this particular species, to ascertain the role of sexual reproduction and fruiting in the biological cycle of *X. subpileatus.*

The role of dispersal in community ecology is difficult to assess due to potential spatial correlation of environmental variables that can result in spatial patterns identical to what would be expected in the case of dispersal limitation. This work is not the exception to the rule. In all sampling scheme, we tried to choose sites as homogeneous as possible. Yet, we find spatial structure in community of SF, ECM and FEF fungi (Fig. VII.1; Table VII.1). As we sampled a limited number of sites, and did not investigate spatial distance gradient, we are not able to disentangle the role of dispersal ("Everything is everywhere [. . .]") and spatially correlated environmental variables ("[. . .] but, the environment selects").

The case of foliar endophytic fungi (FEF) is an illuminating example. FEF communities in needles of 15 years-old Corsican pines were more similar to the FEF communities in needles collected in the surrounding old individuals (more than 200 years) in the same site than FEF communities in pines of the same age in other sites. In other words, spatial structure (*sensu lato*) was thus more important in FEF community structure than other variables we expected to be important, namely age of trees (biotic parameter) and leaf elevation within the tree (microclimatic parameter). By identifying the scale of variation in FEF communities of Corsican pine needles, manuscript H paves the way for further studies dealing explicitly with the role of dispersion and sites environmental variables on community ecology of foliar microorganisms.

By causing variation in fungal fruiting, disturbance such as fire and treefall may impact dispersal processes. Moreover, perturbation may also drives drift and selection processes. Moving to a broader spatial scale, disturbance regime plays a central role in metacommunity theory (Calcagno *et al.* 2006). The study of fungal metacommunities is out of reach in the present work but is certainly a crucial step to better understand fungal communities assemblages (see Halme *et al.* 2013 for an example with wood-inhabiting fungi).

1.3 Role of selection processes (*sensu* Vellend 2010)

Interacting plants matter

For all guilds handled in this work, plants with which fungi interact shape the community of their partners (Fig. VII.1).

Table VII.1 – Comparison of mean β-diversity and proportion of variance explained by sites, fire and soil depth for three fungal guilds: Value in brackets are not significant using PerManova test. Note that ECM and SF communities were assessed exactly in the same soil core, whereas the sampling of FEF was fully independent.

	ECM	**SF**	**FEF**
Sites (df = 2)	6%	8.9%	13.6%
Fire severity (df = 2)	7.7%	8.7%	—
Depth (df = 1)	(1.5%)	5.8%	—
Mean β-Bray	0.985	0.967	0.978
Mean β-Sørensen	0.918	0.872	0.910

Age of interacting plants matters

Corsican pine age drives the composition of FEF communities. The existing literature (table 1 in manuscript H) also documents the effect of host taxonomy and phylogeny on FEF community composition (e.g. Kembel and Mueller 2014). Several questions arise from this pattern. When getting older, trees may experience a change in physiological trade-off (e.g. more investment in reproduction but less in growth; change in resistance to herbivory while growing). On one hand, these physiological changes and their biochemical consequences could govern changes in their receptivity towards FEF fungal partners along tree life. On the other hand, FEF communities of pines experience their own dynamics (dependent and nested ageing processes during both concomitant needle and tree ageing) and may experience competition/colonization dynamics. Does tree ageing correlate with physiological changes and does those changes imply a different active choice of FEF species by the host? Or do FEF communities change during tree life through the establishment of late-successional species (colonization) and the extinctions of existing ones (competition and drift) ?

Wood-inhabiting saproxylic fungi also depend on tree ontology. In the Mediterranean old-growth forest dominated by holm oak (*Quercus ilex*), oak individuals naturally fall at age of 170 ± 46 years (Panaıotis *et al.* 1997, 1998). Then, the presence of *Xylobolus subpileatus* basidiomes reaches a maximum after 40 subsequent years. Thus, *Xylobolus subpileatus* requires a total period of 210 years devoid of human intervention on the forest stand to produce its reproductive structure and finally disperse sexual spores. This information is of interest for both conservation actors and forest managers, in a context of high pressure on marge woody debris by human societies (see manuscript I).

Taxonomy and phylogeny of interacting plants matters

ECM fungal partners differ among plant species at several levels. The survey of the literature in manuscript B reinforces the idea that ECM fungal species show preference regarding the taxonomy of their hosts (e.g. Molina and Horton 2015). At local scale, forest dominated by different ECM hosts exhibit different ECM fungal communities (manuscript D).

Above species levels, phylogenetic relationships also drive regional[3] (manuscript C) and local ECM interactions (manuscript D). Indeed, plant species share more partners at the regional scale with co-generic and, in a lesser extent, with co-familial species. This conservatism in the ECM symbiosis

---

[3] Regional ECM interactions refer to interspecific interactions at the scale of the whole island of Corsica.

is also present at the local scale since forest stands of *Pinus pinaster* and *Pinus nigra* harbor ECM fungal communities that are more similar than those of pine forests compared with those associated to*Fagus sylvatica* (Fig. III.3). Soil saprobic fungi (SF) communities are also different regarding the tree species composition of the forest (draft J). In the case of both SF and ECM fungal species, the observed turnover is due to both changes in abundance and species replacement, without any change in fungal diversity (Fig. VII.1). Moreover, the proportion of variance explained[4] by the taxonomy of dominant tree is higher in the case of SF fungal communities (11.8%) than in ECM communities (8.9%).

The higher part of variance explained by tree species in SF communities than in ECM communities is surprising when considering the widely accepted interaction-specialization of ECM species (see table I.5). However, the present result on ECM communities are based on soil sampling that may include inactive fungal species. Here we point to a putative confounding outcome of deep sequencing methods, which make possible the description of fungal diversity out of both active mycelia and inactive propagules (resistance forms such as spores and sclerotia). Those participate to the potential of interaction at the sampling site, before host specificity filtering process. In other words, ECM soil communities described in soils may be partially filtered by vegetation composition, and the result on the ongoing analysis of ECM root tips in the same soils may provide useful information about the effect of vegetation composition on the actual assemblages from an ECM pool.

Based on both literature and the manuscript I, the wood-inhabiting species *Xylobolus subpileatus* evince a high specificity on *Quercus ilex* logs. Whether this specificity results from an adaptation of *X. subpileatus* to particular chemical or biotic composition of decayed *Quercus ilex* logs remains to be solved.

The importance of disturbance in selection processes

SF and ECM fungal diversities present contrasted response to fire

ECM fungal diversity[5], but not SF diversity, is affected by fire severity. Indeed, ECM diversity increases after surface fire with no change in species richness (i.e. the number of species). One potential explanation for this positive effect of low severity fires rely on some arguments of the intermediate disturbance hypothesis[6]: When surface fires occur, competition among ECM individuals may decrease due to direct or indirect mortality of some individuals. In these conditions, post-fire growth from surviving roots may provide opportunities for early colonizer fungi to co-occur (at the sampling scale) with late-stage and/or competitive ECM species already established in these soils. A remarkable result of our work is the possibility of a long positive effect of fire on local ECM diversity, as 15 years would not be sufficient to erode ECM diversity through competitive exclusion. Adequate fire chronosequence would be usefully sampled to fully address this issue.

---

[4] Using a PerManova test with 9 999 permutations

[5] Here, diversity refers to metric that take into account abundances (e.g. Shannon index, Hill number with $q \neq 0$).

[6] Otherwise debated in Fox 2013a,b; Sheil and Burslem 2013.

SF and ECM fungal communities present contrasted response to fire

Both ECM and saprobic fungal communities present in soil are still hallmarked by fire 15 years after the disturbance. However, the two guilds show contrasted responses. The community shift seems to be higher for SF communities than for ECM fungal communities (Table VII.1). Moreover, the expected buffering effect of soil depth on fire-induced SF community change could not be detected for ECM fungi . These discrepancies between fungal guilds can be explained by contrasted ecologies. First, ECM species, protected against fire in root tips structure, may display a higher resistance to fire in organic layer than numerous SF fungal species. Second, SF species may display a lower resilience than ECM species because they feed on organic matter partially destroyed by fire, whereas ECM fungal species may rely on plant host for carbon supply. Then, ECM species may recolonize faster organic layer from mineral layer.

Dynamic of fungal communities after canopy gap openings

Above-ground (sporocarps) diversity of both ECM and SF fungi increases in canopy gaps the year immediately following tree fall and then progressively decreases (manuscript I). Thus, canopy opening seems to be an important signal in the biological cycle of numerous ECM and SF fungal species. In marked contrast with this pattern, *Xylobolus subpileatus*, a rare and worldwide fungal saproxylic species, requires much more time (mean: 23.5 ± 10.5 years) to sexually reproduce in natural canopy gaps. Sporocarp production is a crucial step in the biological cycle of numerous fungi and is often needed for fungi to disperse. In many populations, fungal fitness depends on sporocarp production (Vincenot *et al.* 2012; Taschen *et al.* 2016) and therefore, fungal fruitbody survey is not useless and should be considered as a suitable tool to explore dispersal processes and functional traits linked to reproduction.

The importance of abiotic environment (environmental filtering)

Unmeasured variables . . .

Selection processes driven by abiotic environment play a decisive role in fungal ecology (Dahlberg 2001; Lilleskov *et al.* 2002; Courty *et al.* 2016). In the majority of ecological studies, variables of interest are coarse integrative variables (e.g. island size, altitude, latitude...) grouping a bundle of covariate variables, the so-called "responsible for confounding effects". We already discussed above the difficulty to split the evidenced site effects into dispersal and selection processes through spatially structured variables.

Along the same line, studying the effect of fire, soil depth, alive tree age or dead log lifespan, we actually measured the merged effect of multiple co-varying variables. Disentangling all these sources of covariation is one way to get closer to processes. However, one must weigh up gain and costs to measure a high number of variables. Drafts G and J inform us on the effect of fire on fungal communities without untangling the mechanisms at work. Yet, this information is relevant for both researchers and stakeholder.

Abiotic variables linked to disturbance

Fire can affect fungi either (i) by direct mortality or weakening (direct biotic effect) or (ii) by positive/negative effect on interacting organisms (indirect biotic effect) and (iii) by changing abiotic environment (abiotic indirect effect). These fire-induced habitat shifts modify chemical and physical characteristics of the soil organic layer (Certini 2005), leading to the selection of adapted fungi over maladaptive ones. In our case study in Corsica, we did not find variation in nitrogen and carbon soil content 15 years after fire (master report in appendix), but some other physicochemical variables may impact the observed shift in fungal assemblages due to fire. This work has still to be done in our ecosystems, at various scales of space and time, before drawing any definitive conclusion.

Abiotic variables may also drive fruiting dynamics of the wood-inhabiting saproxylic fungi *Xylobolus subpileatus* on *Quercus ilex* logs. Indeed, during the decaying processes of tree logs in old-growth forest, the probability of *X. subpileatus* fruiting increases along with modifications of wood composition, in particular during the increase of the lignin/cellulose ratio. Because *X. subpileatus* shows particularly high abilities to hydrolyse polyphenols, we may expect other fungi to be less competitive than *X. subpileatus* when lignin concentration increases. Thus, the temporal dynamics of *X. subpileatus* fruiting may be driven by abiotic filtering (chemical niche dimension) occurring in natural canopy gaps.

Intra- and inter-guild interactions

Several patterns in community composition may arise from interactions – both negative and positive – among fungi. Importance of intra- (e.g. competition among ECM fungi and succession among saproxylic fungi; Kennedy 2010; Rajala *et al.* 2012) and inter-guild interactions (Ottosson *et al.* 2014; Fernandez and Kennedy 2015b) are widely accepted in fungal ecology, and may explain some of the patterns revealed in the present work.

Intra-guild interactions

One hypothesis to explain the late fruiting of *Xylobolus subpileatus* and the scarcity of other species' sporocarps at this time, lies on interactions among wood-decaying fungi (I). *Xylobolus subpileatus* is able to decay wood with a very high $\frac{lignin}{cellulose}$ ratio. As very few micro-organisms are able to degrade lignin, this ratio keeps increasing over the decaying process. We may hypothesize that *Xylobolus subpileatus* is a poor competitor when cellulose availability is sufficient, but when lignin concentration increases, *Xylobolus subpileatus* outcompetes other wood-decaying species, but also an increasingly abundant pool of late-stage and wood-inhabiting ECM fungi (Ottosson *et al.* 2015), to reproduce sexually in complex inter-guild assemblages.

The abundance of some ECM species increases after fire (*Suillus sp*, *Rhizopogon sp* and *Geopora sp*). In *Pinus ponderosa* forests in California, Glassman *et al.* (2015) failed to find an effect of fire on the composition of ECM spore bank. Thus, the high abundance of these vegetative post-fire species[7] is unlikely to be explained by resistance or dispersal abilities, but rather by the relaxation

---

[7] See discussion on post-fire concept in fungal ecology.

of intra-ECM fungal competitive interactions (similarly, plant pioneer species are favored by the decreasing competition after disturbance). This hypothesis is reinforced by the fact that *Rhizopogon spp* possess long-living spores and are often absent from mature forests (Gardes and Bruns 1996; Baar *et al.* 1999; Bruns and Kennedy 2009).

Questioning the magnitude of the biotic internal filter, including competition and facilitation among ECM fungi, may benefit from the functional ecology framework (see discussion on functional traits). Indeed, the competition among ECM fungi may be driven by several mechanisms including chemical deterrence and resource depletion (Kennedy 2010). In the case of high competition by resource depletion in ECM fungal communities, differences in fungal enzymatic activities may decrease the competition for resources through niche differentiation. However, in manuscript N, we did not find a significant influence of niche differentiation in ECM community assembly at the very local scale (Courty *et al.* 2016).

Inter-guild interactions

Competition for soil nitrogen between ECM fungi and free-living microbial decomposers, including soil saprobic fungi (SF), is an important driver of carbon balance in forest ecosystems (Averill and Hawkes 2016). ECM and SF play a central role in the breakdown of soil organic matter. A phenomenon known as the "Gadgil effect" describes the effect of competition between these two fungal guilds on decomposition rates (Fernandez and Kennedy 2015b). Several studies support the Gadgil effect, even if the outcome of the competition[8] is variable (reviewed in Fernandez and Kennedy 2015b). Given that fire drives both ECM and SF communities, it remains an open question to understand the direction and extent of fire impact on inter-guild competition. This is not only a fascinating question, but also a functional key to understand the effect of fire on carbon cycling in a systemic view. We here wish that future studies dealing with fire and carbon cycling will integrate the combined analysis of ECM and SF communities. From a methodological point of view, NGS clearly offers this opportunity.

In addition to soil, intra-guild interactions also take place in decaying wood. Several publications document the presence of ECM fungi at the end of the decaying process in wood (Harvey *et al.* 1979; Rajala *et al.* 2012; Ottosson *et al.* 2015). Even if we did not sample ECM fungi in *Quercus ilex* the wood of decaying logs, we suspect that *Xylobolus subpileatus* may co-occur with ECM species at the end of the decaying process. In addition, its capacity to massively fruit at this time may be partially explained by remarkable competitive abilities towards ECM fungi.

1.4 *Quid* of speciation and drift processes in fungal community assemblage? Incorporate neutral theory in fungal ecology.

---

[8] The Gadgil effect posits an increase of carbon storage due to ECM-SF competition, but the reverse was also found.

« *The strategy behind neutral theory is to see how far one can get with the simplification of assuming ecological equivalence before introducing more complexity.* »

Hubbell 2006

Whereas many ecologists first opposed niche and neutral theory in their publications, a recent relative consensus emerged stating that the two theories address complementary aspects of community dynamics (Adler *et al.* 2007). The majority of ecologists also agree with the continuum hypothesis (Gravel *et al.* 2006), stating that all communities are shaped by both neutral and niche-based processes. Moreover, neutral theory is based on ecological equivalence rather than on the absence of niche. This ecological equivalence can emerge at local and regional scales from niche-based processes, through equalizing and stabilizing mechanisms (Chesson 2000; Munoz and Huneman 2016).

Even so, opposition between niche and neutral theory continues to be heavily debated in fungal ecology (e.g. Dumbrell *et al.* 2010b). The majority of mycological studies refute neutral theory on the basis of niche-based processes such as environmental filtering (e.g. Maherali and Klironomos 2007; Lekberg *et al.* 2007). In addition, Adler *et al.* (2007) state that: "Ecologists now recognize that controversy over the relative importance of niches and neutrality cannot be resolved by analyzing species abundance patterns". But mycologists keep going exactly that way (e.g. Dumbrell *et al.* 2010b; Feinstein and Blackwood 2012). Moreover, we are not aware of studies modelling explicitly neutral theory and dealing specifically with the fungal Kingdom and its characteristics.

A major contribution of the Hubbell's book *The unified neutral theory of biodiversity and biogeography* (Hubbell 2001) is to underline the underestimation of the importance of drift and dispersal in community assembly. This underestimation is still present in the study of fungal ecology (including all the work presented here). A deeper rooting of mycologists into neutral theory is necessary to overcome the misuse of the neutral concept. As neutral theory is historically based on abundance, how to measure fungal abundance will be one major question in the future of fungal ecology (*cf.* discussion on abundances in mycology).

1.5 The limit of the guild concept: Fungal species pertaining to different guild across biological cycle

Some fungus passes through diverse guilds during their biological cycles

All along this work, we define community using the concept of guild. However, some fungi drastically change their niche (e.g. change in carbon source and/or habitat) during ontogeny. Aquatic hyphomycetes illustrate perfectly this phenomenon (Selosse *et al.* 2008; Chauvet *et al.* 2016). Hyphomycetes fungi live in a wide variety of habitat from aquatic deposits of litter to forest litter and tree roots and leaves. Chauvet *et al.* (2016) proposed that "Aquatic hyphomycetes may have a double life in terrestrial and aquatic environments, i.e. as endophytes in plant aerial parts and roots and saprotrophs on plant detritus in streams and rivers, respectively". This is not the only case of multi-guild fungi. The debate around the saprothrophic abilities of some ECM species (Lindahl and

Tunlid 2015; Shah *et al.* 2015) and the capacity of FEF to decompose leaves (Yuan and Chen 2014) further illustrates the putative porosity of the concept of guilds.

Does post-fire recolonization of ECM and saprobic fungi fall out of the sky?

With this in mind, we hypothesize that some post-fire fungi such as *Morchella* can wait for fire in the tree canopy as FEF. In our hypothesis, fungus reproduces asexually in trees leaves. After a fire, scorched leaves, with living mycelium or resistance structures, fall down on the ground. Post-fire fungal species then develop under favorable conditions (low competition, high quantity of carbon on ground) and fruit after a sexual reproduction. However, even using the strength of deep sequencing, we coulddid not find a single sequence of post-fire Ascomycota fungi (manuscript H) such as *Morchella* and *Geopyxis* in Corsican pine needles. Thus, post-fire Ascomycota are presumably devoid of vegetative life as FEF within leaf tissues. Nonetheless, our hypothesis is not completely rejected considering that post-fire Ascomycota fungi may live in other part of the tree less damaged by fire such as bark and roots.

2 The "niche mirror" concept : ECM plants and fungal communities are reciprocally dependent

THE high intimacy of ECM symbiosis leads to a huge dependency of ECM plants for ECM fungi and conversely: we call this dependency niche mirror. Thus, ECM plant and fungal ecologies are intermingled and need to be studied together.

2.1 Intimacy blurs mutualism status

Given this high degree of intimacy and dependency between ECM partners it is difficult to assess the advantage offered by the ECM symbiosis to both plant and fungal partners. Indeed, ECM interactions are almost obligatory for partners, probably since millions of years. Thus, ECM plant and fungal evolution are tightly linked and both partners may accumulate phenotypes that will be counter-selected in the absence of the partner, reinforcing their inter-dependency. Experiments consisting in impeding the ECM interaction may bring out these counter-selected traits rather than inform us about the outcome of the ECM symbiosis.

2.2 Implication for ECM plant ecology

Measuring the importance of interaction niche in plant coexistence

The first implication of this high intimacy is the complexity of the ECM symbiosis and the predictable failure of reductionism to study the ecology of ECM partners. Indeed, experimental approaches consisting in precluding ECM interaction will tell us more about the strong interdependence of partners than about the natural benefits of the symbiosis. We must embrace complexity through field studies to make hidden patterns emerge.

A large part of the ECM plant niche is defined by the interaction niche (manuscripts C and H). Thus, the incorporation of this "ECM interaction niche" in the discipline of plant ecology, and in particular concerning plant coexistence, should not be delayed. This was one of the objective of the manuscript C (fundamental interaction niche) and the draft D (realized interaction niche). Forthcoming studies need to assess plant – and if possible fungal – performance traits (e.g. leaf nitrogen content, fungal biomass) to go beyond descriptive and correlative study of the ECM interaction niche.

Incorporating scale of interaction in the study of ECM interactions

A central tenet of ecology is the spatial scaling of ecological pattern [9] (e.g. Wiens 1989; Levin 1992; Hubbell 2001) including ecological patterns caused by biotic interactions (Araújo and Rozenfeld 2014). Along the chapter III, we document the scaling of ECM interactions from regional to individual tree scales. We still miss the ECM root tip results to document the complete set of scales we were interesting in.

We propose the term of ecological mycorrhizal network (EMN; manuscript B) to define the mutualistic interaction network linking together plant and fungal entities (e.g. individuals, populations, species) able to establish a mycorrhizal connection in at least one ecological context and during one ontological stage. The ecological ECM network studied here at the species level (Figure 1 in manuscript 3) is qualitative (binary links) and only informs on the potentiality of two species to interact (Taudiere *et al.* 2015). This definition thus includes the below-ground network where fungal mycelia physically connect roots of different plant individuals (common mycorrhizal network; Simard *et al.* 2012) but also interaction at larger taxonomic, temporal and spatial scales. We hope that the work initiated in manuscripts C and D will offer new perspectives about scaling of ECM ecological networks and their role in partners' ecology.

2.3 Implications for conservation

Conserving plants through protected habitats without protecting also their symbiotic partners is a dead end. Fungal conservation is urgent and arduous. The joint conservation of both partners through the analysis of the ECM interaction network is certainly one solution to move forward in forest biodiversity conservation. We develop this idea in the manuscript E.

3 What are the main issues and the most promising directions in fungal community ecology?

---

[9]. The spatial scale where the researcher consider a pattern.

3.1 The necessary shift from patterns description to mechanisms understanding

Intrinsic difficulty in Ecology is the pattern-process relationship (Vellend 2010), especially for non cultivable microorganisms such as the majority of fungi. One pattern can stem from multiple very different processes. For example [10], dispersal limitations can disfavor species with low dispersal abilities (e.g. hypogeous [11] fungi); but low abundance or diversity of hypogeous fungi can be explained by other mechanisms like high abundance of mycophagous mammals specialized on hypogeous fungi (e.g. mycophagous marsupial Vernes *et al.* 2001; Trappe *et al.* 2006). Moreover, interactions between processes and trade-off among genotypic and phenotypic traits can further complicate the inference of processes from patterns (Laughlin and Messier 2015).

Getting closer to processes is the announced aim of functional ecology (Lavorel and Garnier 2002; McGill *et al.* 2006). To achieve this goal, fungal ecologists can thus rest on functional traits (e.g. enzymatic activities, Courty *et al.* 2016); but also on newly available tools such network-based methods – including network null models (e.g. Chagnon *et al.* 2012; Taudiere *et al.* 2015) – and next-generation sequencing (NGS; e.g. Buée *et al.* 2009; Martinová *et al.* 2016). By providing more robust (e.g. by multiplying the number of sequences per sample) and new patterns, these approaches may reveal ecological processes. Indeed, studying multiple patterns jointly may allow disentangling processes that gave similar results for some patterns but not for others. Another promising way to infer processes is to measure the relative importance of community assembly rules (e.g. dispersal *vs* competition for N) rather than only a list of significant patterns [12].

3.2 The network mirage

First, graph theory was, is and will be a tremendous source of problem solving and inspiration in manifold disciplines. But, if misused, network analysis may worsen the "statistical machismo" in ecology denounced by B. Bolker in a post on the blog dynamic ecology [13] (11-09-2012). In other words, using network objects is sometimes a way to sacrifice clarity to fashion, communication and publishable [14]. In the special case of ECM network, numerous seminal and interesting papers on ECM interactions do not use network theory to study specialization (e.g. Horton and Bruns 1998; Bruns

---

[10] Vellend (2010) (p. 199) provides another illustrative example: the species-area relationships.

[11] Hypogeous fungi fruit underground. The most famous hypogeous fungal species are certainly truffle species.

[12] We specifically underline this guidance in manuscript F.

[13] B. Bolker defines those "must-use" approaches to (i) be vastly more complex to apply, (ii) be understood by a much narrower circle of readers (and reviewers), (iii) often require additional data that is impossible or expensive to obtain, than a well-known simple alternative. He also adds that the majority of "macho" approaches (iv) reduce the power in a statistical sense, downgrading the p-value and (v) have not, in the grand sweep over many papers, fundamentally changed our understanding of ecology. In some papers, network analyses are vastly more complex, understandable and require a higher amount of data compared to classical statistical tools. However, network analysis does not always reduce statistical power and has definitely changed our way we are studying Ecology.

[14] B. Bolker also stated that "ecologists have a long list of 'must use' approaches to statistics that are more complicated than simpler methods but don't necessarily change the outcome [. . .] this is a machismo attitude to statistics – 'my paper is better because I used tougher statistics'. It has a Red-Queen dynamics – eventually what starts as a signal of being superior turns into something reviewers expect in every paper."

et al. 2002). And some recent papers on ECM network misuse network-level metrics by computing them out of very small networks (e.g. Bahram *et al.* 2014; Torrecillas *et al.* 2014; see Table I.4 for other examples of small ECM networks) or by interpreting these metrics outside mycorrhizal framework (e.g. Toju *et al.* 2015, see Chagnon 2016 for a discussion about this problem).

Chagnon (2016) recently proposed a thorough opinion paper on the incorporation of network theory in mycorrhizal ecology. He warns ecologists against misuse of network-based tools for three major reasons. First, he underlines the difficulty to infer mechanisms from network patterns because multiple processes can yield a given network-level pattern (See also our discussion on this subject). Second, modeling studies of the biology of mycorrhizal symbiosis are not enough advanced to predict future dynamics of mycorrhizal communities using network. Third, analogies between mycorrhizal networks and other types of networks may be tricky if we take into account only similarities, neglecting discrepancies among different kinds of networks.

3.3 Bridging the gap with and get inspired (reasonably) from plant ecology

Abundances

Numerous plant ecology theories are based on abundances (e.g. neutral theory [Hubbell 2001] previously discussed, species-abundance distributions [Gaston and Blackburn 2000]). Information on abundance is also necessary to compute most α- (e.g. Shannon and Simpson index, Hill numbers) and β-diversity indices (e.g. Bray-Curtis and Morisita metrics). Fungal ecologists were interested in abundance from the beginning, mostly using sporocarp counts. However, the development of molecular techniques for species identification (White *et al.* 1990; Gardes and Bruns 1993) and environmental sampling (e.g. NGS, Buée *et al.* 2009; qPCR, Taschen *et al.* 2015) gave birth to other measures of abundance.

There is mainly two types of abundance measures: absolute and relative ones. Absolute measures can be easily transformed into relative ones, but are more time-consuming and even sometimes impossible. Researchers interested in ecosystem processes such as biogeochemical cycling often need absolute measures (e.g. absolute number of individuals, or biomass) whereas community ecologists are satisfied in most cases with relative measures (e.g. relative DNA abundancy in samples, relative number of root tips for ECM fungi).

Individual counts

The oldest and most intuitive measure of abundance is to count "individuals". In particular, counting or weighting sporocarps has been the only way to measure fungal abundance for a long time. However, many fungal species form invisible or sparse fructifications. In addition, there is high inconsistency between abundance measured on sexual tissues (mostly above-ground fruitbodies) and on vegetative tissues (belowground; Gardes and Bruns 1996; Dahlberg *et al.* 1997; Rajala *et al.* 2012). Finally, a single fungal physical individual may form a huge number of sporocarps. Sporocarp count by itself is consequently insufficient to measure fungal abundance. So, if we want to count individuals, we must define what is a fungal individual (physical *vs* genetic entity; Pepper and Herron 2008) and how to define the limits of individuals in practice.

Individuals often refer to a physically delimited entity in biology (but see Pepper and Herron 2008 for other definitions). Yet, in the case of filamentous fungi, how to define the individual when physical limits change over time and space through physical disconnection and anastomosis [15] (fusion of encountering vegetative hyphae)?

One way is to define an important structure to count. In the case of ECM fungi, the number of root tips is often used to measure fungal abundance. However, such a structure is often difficult to identify at species level and is not present in most fungal guilds. Another way to define individuals is to switch from the physical definition of individuals (the ramet) to a genetic definition (the genet). Currently, the genetic concept of "individual" is far more relevant in practice, thanks to the numerous available molecular tools.

Number of sequences

Next-generation sequencing (NGS) yields millions of reads of environmental samples. This large amount of information allows the comparison of (i) the number of sequences of a given taxon in function of variables of interest, and (ii) the relative number of sequences among taxa in function of the variables. Using mock communities, Amend *et al.* (2010) showed that sequence abundance is a good proxy of DNA concentration within but not between species. In our work using Illumina sequencing, we consequently always run double test (quantitative and binary) when using abundance of multiple taxa. In all cases studied here, community analysis gave the same results using either binary or abundance data.

Molecular rarity (low number of sequences for a given taxon, despite high total number of sequences) is considered with caution in most ecological studies using NGS (e.g. Bálint *et al.* 2014). By sequencing a mix of equimolar amounts of DNA from 27 species, Nguyen *et al.* (2015a) showed that two species were never found in the 11 489 sequences. This can be partially explained by the variation in the copy number of ITS within and among fungal species and may limit the relevance of molecular-based abundances (Amend *et al.* 2010).

Beyond true "rarity", PCR and sequencing biases can also lead to a very low number of sequences representing a given taxon (Lindahl *et al.* 2013; Brown *et al.* 2015). Whether we need to "scrap the bottom of the barrel" [16] depends on the biological questions. If we are interested in the presence of a known rare species or group of species, we need to take a special look at those molecularly rare taxa. Yet, the rare sequences are unlikely to greatly affect ecological metrics, in particular regarding β-diversity. Thus, community ecologists often discard these molecularly rare taxa from their community analyses.

---

[15] Chagnon (2014) proposed a review on the implications of hyphal anastomosis in arbuscular mycorrhizal fungi for population ecology and evolution.

[16] Brown *et al.* 2015

To summarize, further work on molecular abundance is still needed to accurately measure abundance using NGS, although the number of reads certainly yield information about some biological and ecological traits.

Biomass

One problem with individual count is the absence of individual weighted in function of its size/biomass. A lot of abundance metrics are weighted by biomass (e.g. using the diameter at breast height [DBH] in plant ecology). One way to do that in fungal ecology is qPCR (q for quantitative). This technics is based on the comparison of DNA concentrations for one species with previous calibration. So far, the major limitation of qPCR is the possibility to study species only one by one.

Frequency in samples

Finally, there is a last way to measure abundance. If it is difficult to compare the number of sequences in one sample, one can score the presence/absence of a given sequence within a bigger sampling. By increasing the number of samples, one can approach a measure of abundance. However, this method is time-consuming and could increase the abundance of spatially diffuse organisms to the detriment of spatially clustered species. Thus, choosing the scale of sampling is particularly important (see section perspectives).

Functional Traits

What is a functional trait?

McGill *et al.* (2006) proposed to rebuild plant community ecology from functional traits. In a seminal paper for functional ecology, Lavorel and Garnier (2002) distinguished between effect traits (effects on ecosystem) and response traits (responses to the environment). Community ecologists mostly use the response traits concept and define functional traits as "morpho-physio-phenological traits which impact fitness indirectly via their effects on growth, reproduction and survival, the three components of individual performance" (Violle *et al.* 2007). In short, any measurable variable linked to fitness is a functional trait.

Linking ecosystem process to community composition

Functional ecology is also interested in the effect of traits on ecosystems (effect traits, Lavorel and Garnier 2002). Studying functional traits of ectomycorrhizal fungi that drive both community structure and ecosystem functioning was done in several contexts (Koide *et al.* 2014; Clemmensen *et al.* 2015; Fernandez and Kennedy 2015a; Treseder and Lennon 2015. In the case of fire, the ability to make sclerotia or melanized cell walls may be a response trait and an effect trait. On one hand, sclerotia and melanin protect hyphae from death during fire. On the other hand, fungal necromass decomposition is strongly negatively affected by increasing melanin concentration (Fernandez and Kennedy 2015a) and C accumulation in soil organic content is positively correlated to abundance of melanized fungi (Clemmensen *et al.* 2015) and sclerotia (Koide *et al.* 2014).

Grasping fungal niche and interspecific competition

Several papers already proposed to study fungi through their functional traits. Crowther *et al.* (2014) proposed several fungal response traits to study niche processes in the fungal Kingdom (e.g. spore size, enzymes production, cell wall thickness). Chagnon *et al.* (2015) proposed a trait-based framework to study specifically endomycorrhizal fungi (AM for arbuscular mycorrhizal). One of his recommendations is to apply the C-S-R framework to AM fungi. Understanding the fungal response trait implicated in fire resistance and recolonization after fire is a promising road to understand which mechanisms are responsible forof the patterns reported in this thesis.

Kennedy (2010) underlined the importance of interspecific competition in the distribution of ECM fungal species. Understanding the determinants of competition output should benefit from studying functional traits thought to be implicated in community competition. Two groups of traits may be of particular importance for competition among ECM fungi. First, fungal species differ in terms of exploration strategies (Agerer 2001; see Fig. I.11 for illustrations). We can reasonably hypothesize that competition is higher between two fungi staying close to the roots (contact exploration) than that between one of this fungi and a long-distance exploration fungi. Second, enzymatic activities can also drive competitive output. Yet, Courty *et al.* (2016) (manuscript N) found environmental filter but not niche differentiation in enzymatic traits.

What about intra-specific and intra-individual variation?

Recent studies in plant ecology underline the extent (Albert *et al.* 2010; Siefert *et al.* 2015) and importance of intraspecific variation (Clark 2010; Albert *et al.* 2011; Bolnick *et al.* 2011; Violle *et al.* 2012) for both theoretical [17] and empirical [18]reasons. Intraspecific variation in ECM fungal traits is also well known (Wallander 2000; Selosse *et al.* 2001; Johnson *et al.* 2012; Courty *et al.* 2016) and may be important in numerous ecosystems and ecological processes.

Intraspecific variation includes both genetic causes and plasticity. As a single genotype may split into several ramets, fungal ecology is a perfect model to test for plasticity only, including lab experiments using the few cultivable fungal species. Moreover, even a single ramet can differ in functional traits at different places of the mycelium (Cairney 1999; Johnson *et al.* 2012). For example, we can hypothesize that different regions of a same ECM fungal mycelium have distinct enzymatic activities depending on whether this region is close to plant roots or not. This intraspecific variation needs to be taken into account when studying competitive exclusion and niche partition in fungal communities.

Fungi are not plant: misuse of analogy concerning post-fire fungi

Post-fire concept arose first in plant ecology to describe plant species which abundance increases after a fire (Pausas *et al.* 2016). Post-fire plants are splited into post-fire resprouters (plants able to

---

[17] The notion of competitive exclusion in niche theory is based on niche overlap among species and is therefore based intrinsically on intraspecific variation (MacArthur and Levins 1967; Violle *et al.* 2012).

[18] Interactions take place at the individual level, not at the species level. Thus, when intraspecific variance is high, ecologists need to take into account this variability.

resprout), post-fire seeders (fire-resistant seeds and often fire- induced germination) and post-fire colonizers (recolonize through seeds from adjoining areas).

Post-fire fungi are classically defined as species which massively fruit within the first year after fire. Documented cases mostly concern saprobic macromycetes such as morels (*Morchella spp.*), *Gyromitra spp.*, *Sphaerosporella spp.*, and *Tricharina spp.* (Miller *et al.* 1998; Richard *et al.* 2015; Larson *et al.* 2016) whose ephemeral fruitbodies cover forest soil immediately after fires.

In marked contrast, very few ECM fungi are considered as post-fire species (Dahlberg 2001, manuscript F). Yet, some ECM species are clearly more abundant after fire, whether by resistance (e.g. in the form of sclerotia; Torres and Honrubia 1997) or by recolonization from soil bank spores (Glassman *et al.* 2015) or from new arrival of spores. In Corsican pine forests (draft G), we identified OTUs belonging to the genus *Suillus*, *Rhizopogon* and *Geopora* that display higher abundance in forests having suffered a fire 15 years ago (Fig. IV.4).

Thus, some ECM species are vegetatively favored by fire at both short (less than 1 year; Clemmensen *et al.* 2015) and middle term (15 years; draft G). Given the high discrepancies between sexual and vegetative measures of abundance, the post-fire concept should be revisited and adapted to fungi. The partition into post-fire resprouters (e.g. from sclerota), seeders (fire-resistant spores) and colonizers is certainly relevant for fungal ecology. However, we propose to add the notion of post-fire fruiters to encompass all possible fungal positive responses to fire. The ECM symbiosis offers an ideal model for conceptualizing the notion of post-fire fungi using both reproductive (basidiome abundance) and vegetative (soil mycelia and ECM root tip dynamics) responses of ECM fungal species.

4 Perspectives: focus on the scale of homogeneity

In ecology, finding the scale of homogeneity is of prime importance for at least two reasons. First, the scale at wich community composition is homogeneous between samples marks a threshold below which community assembly rules are deterministic. Second, this scale knowing, it allows relaxing sampling intensity in order to maximize statistical power and thus biological signal. Identifying these scales of homogeneity is even more crucial in microbiology where most studies found differences between samples at all scales (e.g. see table I.2 for the case of foliar endophytic fungi).

4.1 Where homogeneity is, stochasticity is limited

As previously discussed (sections on neutral theory and pattern *vs* processes relationship), multiple processes – whether deterministic or stochastic – can lead to identical patterns. One way to assess the importance of neutral stochasticity in ecological communities is to study the temporal dynamics of communities (Vellend *et al.* 2014). In the case of the fungal communities studied here, we only get a snapshot of different communities at one point along their trajectories.

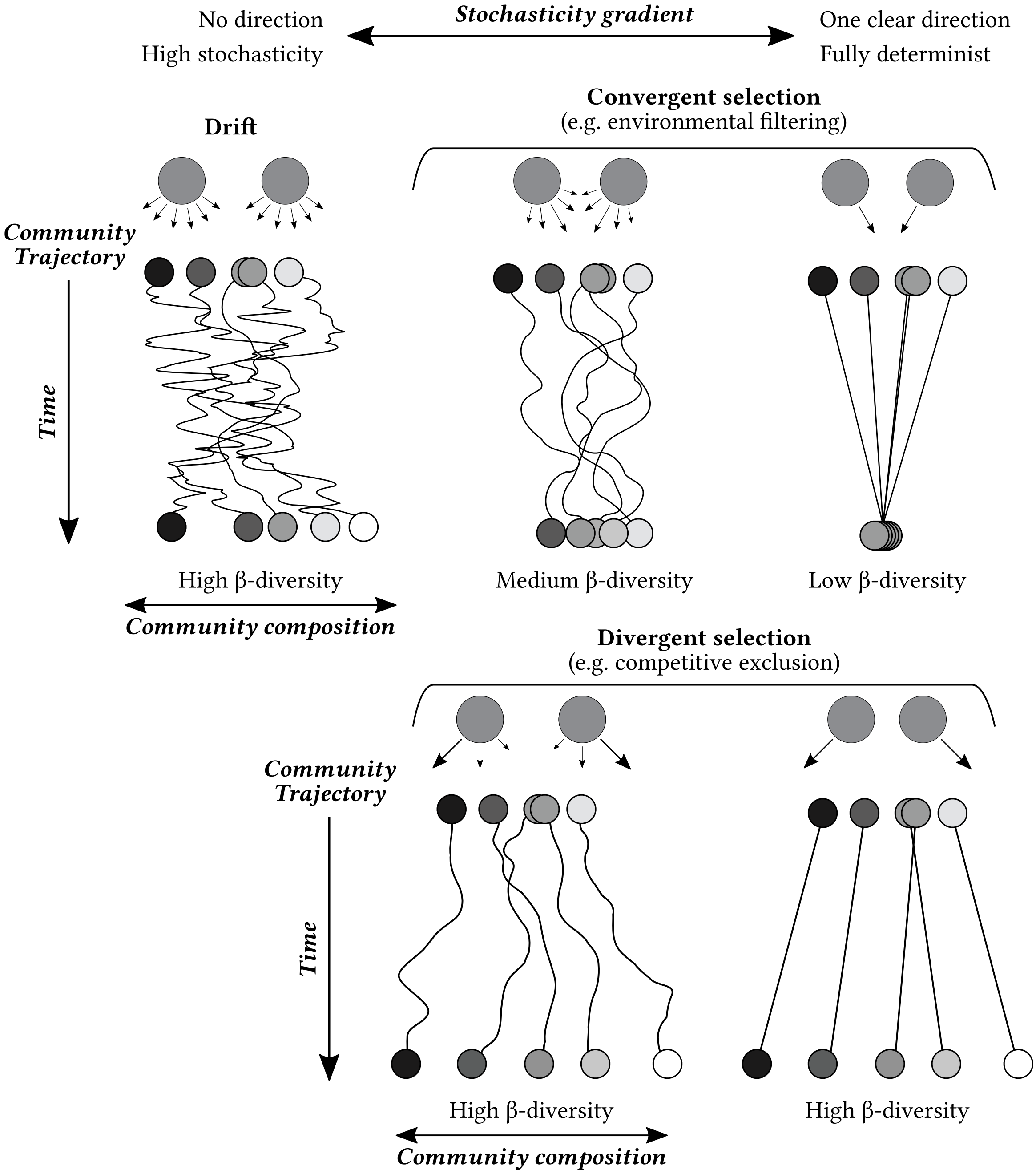


Figure VII.2 – Variation among communities (β-diversity) depends on community trajectory: Illustration of community trajectory in case of drift, convergent selection (*sensu* Vellend 2010), divergent selection and interaction among selection deterministic processes and stochastic processes (e.g. drift). Circles represents communities. The closer Close colours of the circles colours are, the closer indicate close the community compositions are. A low β-diversity attests a low importance of stochasticity in community assembly processes. Figure inspired from Drake *et al.* 2001 (page 243 in Weiher and Keddy 2001 and Vellend *et al.* 2014). Figure inspired from Drake *et al.* 2001 (page 243 in Weiher and Keddy 2001 and Vellend *et al.* 2014).

High variation can arise from both deterministic niche-based (e.g. competitive exclusion; Fig. VII.2) and stochastic processes (e.g. drift; Fig. VII.2). Processes mixing niche-based and stochasticity (e.g. community assembly history; Fukami *et al.* 2010) are also a source of variation. On the contrary, homogeneity in species rich communities can only arise from deterministic processes (e.g. habitat filtering and frequency-dependent stabilizing processes; Fig. VII.2). Thus, identifying the scale of homogeneity is a central goal in community ecology, especially as variation is the rule and homogeneity the exception in the majority of empiric studies (*cf.* Fig. VII.3).

4.2 Improving statistical power using previous identification of scale(s) of homogeneity

To measure the effect of variables on biodiversity, community ecologists sample communities of organisms along environmental gradients (both abiotic and biotic) or in contrasted environments (Anderson *et al.* 2011). Their sampling schemes need to make a compromise between the number of samples (increasing statistical power) and the number of variables to consider and measure (increasing biological information). For instance, in fungal community ecology, NGS allows obtaining much information from few samples but at the expanse of samples number.

One solution to improve statistical power, and thus decrease the number of required samples for a given number of variables is to optimize the ratio $\frac{\sigma_{inter-group}}{\sigma_{intra-group}}$ with $\sigma_{inter-group}$ the variance among samples in the same group, i.e. with identical or very close variables values and with $\sigma_{intra-group}$ the variance among sample groups. When inter-group variation or sampling effort is low, it is primordial to sample at the scale with the lowest variance within group.

4.3 Scale of homogeneity for foliar endophytic fungi (FEF)

The choice to sample a bunch of pine needles resulted from the reported high variability of FEF among leaves (Porras-Alfaro and Bayman 2011; Cordier *et al.* 2012). As we were interested in larger scales than a leaf, we reduced the *a priori* high variability by grouping several needles per sample. We found high homogeneity of FEF communities within a given pine tree, whatever the elevation in the tree. Thus, to take into account intra-tree variability, future studies on FEF of Corsican pine can include only two samples per tree individual. Given the low variability among trees of different ages, two individuals per cohort would have been sufficient to describe satisfactorily FEF communities in our study. This low number of samples per individual (2) and forest stand (3) would have allowed sampling many more sites and therefore to provide a stronger test of the effect of spatial/environmental distances among sites on FEF communities [19].

The homogeneity of FEF communities within sites and the heterogeneity among sites may be explained by both dispersal limitation and environmental filtering (see specific discussions on the role of dispersion and environmental filtering in fungal community assembly). However, these processes decrease diversity by either limiting the number of species reaching the community or by selecting for only the subset of species adapted to the local environment. Given the high diversity in FEF communities, it is likely that homogeneity is caused by density-dependent stabilizing mechanisms of coexistence (Chesson 2000). By increasing negative intraspecific interactions relative to negative interspecific interactions, stabilizing mechanisms allow the coexistence of numerous species together.

---

[19] We develop the idea that FEF are ideal candidates to test (again) the Baas-Becking hypothesis in manuscript H.

The next step towards deciphering mechanisms driving FEF local diversity is to identify those density-dependent processes that could stabilize FEF communities at the scale of a single site.

* * *

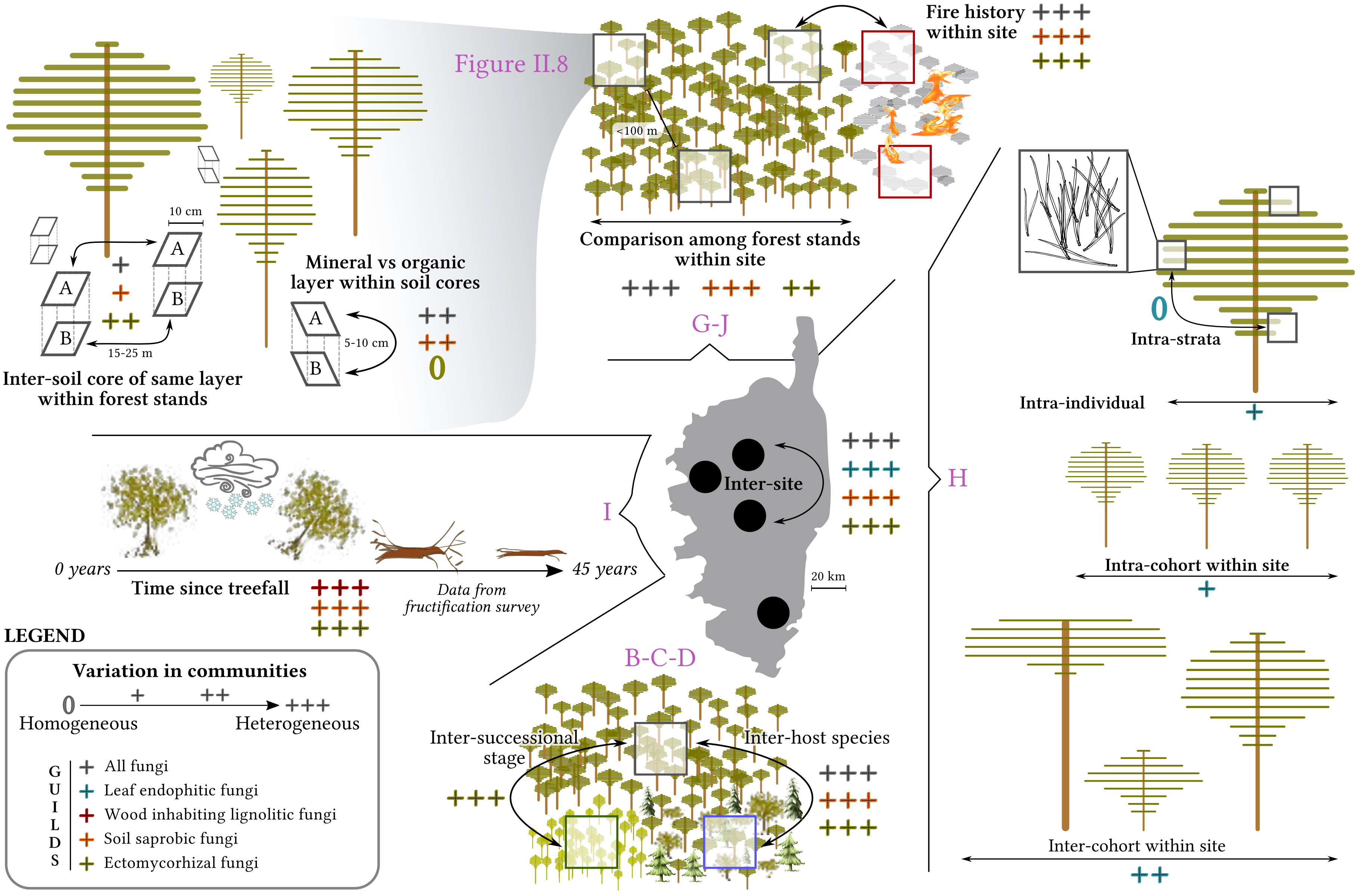


Figure VII.3 – Qualitative importance of variation in fungal community across scales: The qualitative importance of variation in community composition is set on a scale from **0** (homogenous) to **+++** (heterogeneous). Colours correspond to guilds. Purple letters indicate the corresponding manuscripts. Location of sites in Corsica are only indicative. All manuscripts except I are based on multiple sites. Clip arts sources: clipartpanda and Ilona34.

Bibliographie

Abarenkov, K. et coll. (2010). « The UNITE database for molecular identification of fungi –recent updates and future perspectives ». *New Phytologist* 186.2, p. 281–285 (cité p. 57, 58).

Abdelfattah, A., M. G. Li Destri Nicosia, S. O. Cacciola, S. Droby et L. Schena (2015). « Metabarcoding analysis of fungal diversity in the phyllosphere and carposphere of olive (*Olea europaea*) ». *PLoS ONE* 10.7, e0131069 (cité p. 46).

Adler, P. B., J. HilleRisLambers et J. M. Levine (2007). « A niche for neutrality ». *Ecology Letters* 10.2, p. 95–104 (cité p. 272).

Agerer, R., éd. (1987). *Colour Atlas of Ectomycorrhizae.* 1th. Schwäbisch Gmünd : Einhorn-Verl. Dietenberger (cité p. 27).

— (2001). « Exploration types of ectomycorrhizae ». *Mycorrhiza* 11.2, p. 107–114 (cité p. 31, 33, 106, 279, 315).

Albert, C. H., F. Grassein, F. M. Schurr, G. Vieilledent et C. Violle (2011). « When and how should intraspecific variability be considered in trait-based plant ecology ? ». *Perspectives in Plant Ecology, Evolution and Systematics* 13.3, p. 217–225 (cité p. 279).

Albert, C. H., W. Thuiller, N. G. Yoccoz, A. Soudant, F. Boucher, P. Saccone et S. Lavorel (2010). « Intraspecific functional variability : extent, structure and sources of variation ». *Journal of Ecology* 98.3, p. 604–613 (cité p. 279).

Allen, M. F. (2007). « Mycorrhizal fungi : highways for water and nutrients in arid soils ». *Vadose Zone Journal* 6.2, p. 291–297 (cité p. 31).

Allison, S. D., D. S. LeBauer, M. R. Ofrecio, R. Reyes, A.-M. Ta et T. M. Tran (2009). « Low levels of nitrogen addition stimulate decomposition by boreal forest fungi ». *Soil Biology and Biochemistry* 41.2, p. 293–302 (cité p. 51).

Amend, A. S., K. A. Seifert et T. D. Bruns (2010). « Quantifying microbial communities with 454 pyrosequencing : does read abundance count ? ». *Molecular Ecology* 19.24, p. 5555–5565 (cité p. 277, 314).

Anderson, M. J. (2001). « A new method for non-parametric multivariate analysis of variance ». *Austral ecology* 26.1, p. 32–46 (cité p. 142, 191).

Anderson, M. J. et coll. (2011). « Navigating the multiple meanings of β diversity : a roadmap for the practicing ecologist ». *Ecology Letters* 14.1, p. 19–28 (cité p. 282).

Araújo, M. B. et A. Rozenfeld (2014). « The geographic scaling of biotic interactions ». *Ecography* 37.5, p. 406–415 (cité p. 274).

Arfi, Y., M. Buée, C. Marchand, A. Levasseur et E. Record (2012). « Multiple markers pyrosequencing reveals highly diverse and host-specific fungal communities on the mangrove trees *Avicennia marina* and *Rhizophora stylosa* ». *FEMS Microbiology Ecology* 79.2, p. 433–444 (cité p. 46).

Arnold, A. E. et F. Lutzoni (2007). « Diversity and host range of foliar fungal endophytes : Are tropical leaves biodiversity hotspots ? ». *Ecology* 88.3, p. 541–549 (cité p. 44).

Averill, C. et C. V. Hawkes (2016). « Ectomycorrhizal fungi slow soil carbon cycling ». *Ecology Letters* 19.8, p. 937–947 (cité p. 271).

Averill, C., B. L. Turner et A. C. Finzi (2014). « Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage ». *Nature* 505.7484, p. 543–545 (cité p. 30, 51).

Ayres, E., H. Steltzer, S. Berg et D. H. Wall (2009). « Soil biota accelerate decomposition in high-elevation forests by specializing in the breakdown of litter produced by the plant species above them ». *Journal of Ecology* 97.5, p. 901–912 (cité p. 51).

Baar, J., T. R. Horton, A. M. Kretzer et T. D. Bruns (1999). « Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire ». *New Phytologist* 143.2, p. 409–418 (cité p. 271).

Baas Becking, L. G. M. (1934). *Geobiologie of inleiding tot de milieukunde.* Den Haag : W.P. Van Stockum & Zoon (cité p. 39, 43–45, 265, 266).

Babikova, Z., L. Gilbert, T. J. A. Bruce, M. Birkett, J. C. Caulfield, C. Woodcock, J. A. Pickett et D. Johnson (2013). « Underground signals carried through common mycelial networks warn neighbouring plants of aphid attack ». *Ecology Letters* 16.7, p. 835–843 (cité p. 38, 106).

Bahram, M., H. Harend et L. Tedersoo (2014). « Network perspectives of ectomycorrhizal associations ». *Fungal Ecology* 7, p. 70–77 (cité p. 61, 63, 276).

Bálint, M., L. Bartha, R. B. O'Hara, M. S. Olson, J. Otte, M. Pfenninger, A. L. Robertson, P. Tiffin et I. Schmitt (2015). « Relocation, high-latitude warming and host genetic identity shape the foliar fungal microbiome of poplars ». *Molecular Ecology* 24.1, p. 235–248 (cité p. 44–46).

Bálint, M., P.-A. Schmidt, R. Sharma, M. Thines et I. Schmitt (2014). « An Illumina metabarcoding pipeline for fungi ». *Ecology and Evolution* 4.13, p. 2642–2653 (cité p. 277).

Bálint, M., P. Tiffin, B. Hallström, R. B. O'Hara, M. S. Olson, J. D. Fankhauser, M. Piepenbring et I. Schmitt (2013). « Host genotype shapes the foliar fungal microbiome of balsam poplar (*Populus balsamifera*) ». *PLOS ONE* 8.1, e53987 (cité p. 46).

Bálint, M. et coll. (2016). « Millions of reads, thousands of taxa : microbial community structure and associations analyzed via marker genes ». *FEMS Microbiology Reviews*, fuw017 (cité p. 57).

Barabási, A.-L. (2016). *Network Science.* UK : Cambridge University Press. 498 p. (cité p. 60).

— (2012). « The network takeover ». *Nature Physics* 8.1, p. 14 (cité p. 60).

Bascompte, J. et P. Jordano (2007). « Plant-animal mutualistic networks : The architecture of biodiversity ». *Annual Review of Ecology, Evolution, and Systematics* 38.1, p. 567–593 (cité p. 60).

Bascompte, J., P. Jordano, C. J. Melián et J. M. Olesen (2003). « The nested assembly of plant–animal mutualistic networks ». *Proceedings of the National Academy of Sciences* 100.16, p. 9383–9387 (cité p. 60).

Beiler, K. J., S. W. Simard et D. M. Durall (2015). « Topology of tree–mycorrhizal fungus interaction networks in xeric and mesic Douglas-fir forests ». *Journal of Ecology* 103.3, p. 616–628 (cité p. 62).

Bendel, M., F. Kienast et D. Rigling (2006). « Genetic population structure of three *Armillaria* species at the landscape scale : a case study from Swiss *Pinus mugo* forests ». *Mycological Research* 110.6, p. 705–712 (cité p. 10).

Bender, S. F., R. B. d. S. Valadares et A. Taudiere (2014). « Mycorrhizas : dynamic and complex networks of power and influence ». *New Phytologist* 204.1, p. 15–18 (cité p. 32).

Bengtsson-Palme, J. et coll. (2013). « Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other Eukaryotes for analysis of environmental sequencing data ». *Methods in Ecology and Evolution* 4.10, p. 914–919 (cité p. 98).

Berger, S. (2016). *Gideon Guide to Medically Important Yeasts.* Edition 2016. [S.l.] : Gideon Informatics Inc (cité p. 224).

Bernard-Verdier, M., M.-L. Navas, M. Vellend, C. Violle, A. Fayolle et E. Garnier (2012). « Community assembly along a soil depth gradient : contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland ». *Journal of Ecology* 100.6, p. 1422–1433 (cité p. 21).

Bersier, L.-F. (2007). A history of the study of ecological networks. *Biological networks.* World Scientific. T. 3. Complex Systems and Interdisciplinary Science. F. Képès, p. 365–421 (cité p. 19).

Bever, J. D., I. A. Dickie, E. Facelli, J. M. Facelli, J. Klironomos, M. Moora, M. C. Rillig, W. D. Stock, M. Tibbett et M. Zobel (2010). « Rooting theories of plant community ecology in microbial interactions ». *Trends in Ecology & Evolution* 25.8, p. 468–478 (cité p. 26, 31, 38).

Bidartondo, M. I. et T. D. Bruns (2001). « Extreme specificity in epiparasitic Monotropoideae (Ericaceae) : widespread phylogenetic and geographical structure ». *Molecular Ecology* 10.9, p. 2285–2295 (cité p. 33).

Bingham, M. A. et S. W. Simard (2012). « Mycorrhizal networks affect ectomycorrhizal fungal community similarity between conspecific trees and seedlings ». *Mycorrhiza* 22.4, p. 317–326 (cité p. 36).

Blackwell, M. (2011). « The Fungi : 1, 2, 3 … 5.1 million species ? ». *American Journal of Botany* 98.3, p. 426–438 (cité p. 10).

Blanchette, R. A. (1984). « Screening wood decayed by white rot fungi for preferential lignin degradation ». *Applied and Environmental Microbiology* 48.3, p. 647–653 (cité p. 265).

Blondel, J. (2003). « Guilds or functional groups : does it matter ? ». *Oikos* 100.2, p. 223–231 (cité p. 19, 316).

Boccaletti, S., V. Latora, Y. Moreno, M. Chavez et D. Hwang (2006). « Complex networks : Structure and dynamics ». *Physics Reports* 424 (4-5), p. 175–308 (cité p. 60).

Boddy, L. (1993). « Saprotrophic cord-forming fungi : warfare strategies and other ecological aspects ». *Mycological Research* 97.6, p. 641–655 (cité p. 31, 49, 315).

Bolnick, D. I., P. Amarasekare, M. S. Araújo, R. Bürger, J. M. Levine, M. Novak, V. H. Rudolf, S. J. Schreiber, M. C. Urban et D. A. Vasseur (2011). « Why intraspecific trait variation matters in community ecology ». *Trends in Ecology & Evolution* 26.4, p. 183–192 (cité p. 279).

Bond, W. J. et J. E. Keeley (2005). « Fire as a global 'herbivore' : the ecology and evolution of flammable ecosystems ». *Trends in Ecology & Evolution* 20.7, p. 387–394 (cité p. 65).

Bonello, P., T. D. Bruns et M. Gardes (1998). « Genetic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens* ». *New Phytologist* 138.3, p. 533–542 (cité p. 11).

Botella, L. et J. J. Diez (2011). « Phylogenic diversity of fungal endophytes in Spanish stands of *Pinus halepensis* ». *Fungal Diversity* 47.1, p. 9–18 (cité p. 46).

Bourcet, J. (1996). « Aperçu sur deux siècles d'histoire forestière en Corse ». *Revue Forestière Française* 48.6, p. 563–580 (cité p. 69).

Briscoe, C. B. ; (1959). « Early results of mycorrhizal inoculation of pine in Puerto Rico ». *Carribean Forester* 1959, p. 73–77 (cité p. 35).

Brown, S. P., A. M. Veach, A. R. Rigdon-Huss, K. Grond, S. K. Lickteig, K. Lothamer, A. K. Oliver et A. Jumpponen (2015). « Scraping the bottom of the barrel : are rare high throughput sequences artifacts ? ». *Fungal Ecology* 13, p. 221–225 (cité p. 277).

Bruno, J. F., J. J. Stachowicz et M. D. Bertness (2003). « Inclusion of facilitation into ecological theory ». *Trends in Ecology & Evolution* 18.3, p. 119–125 (cité p. 17).

Bruns, T. D., T. M. Szaro, M. Gardes, K. W. Cullings, J. J. Pan, D. L. Taylor, T. R. Horton, A. Kretzer, M. Garbelotto et Y. Li (1998). « A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis ». *Molecular Ecology* 7.3, p. 257–272 (cité p. 34, 35).

Bruns, T. D., M. I. Bidartondo et D. L. Taylor (2002). « Host specificity in ectomycorrhizal communities : what do the exceptions tell us ? ». *Integrative and Comparative Biology* 42.2, p. 352–359 (cité p. 275).

Bruns, T. D. et P. G. Kennedy (2009). « Individuals, populations, communities and function : the growing field of ectomycorrhizal ecology ». *New Phytologist* 182.1, p. 12–14 (cité p. 271).

Bruns, T. D. et R. P. Shefferson (2004). « Evolutionary studies of ectomycorrhizal fungi : recent advances and future directions ». *Canadian Journal of Botany* 82.8, p. 1122–1132 (cité p. 35).

Buée, M., M. Reich, C. Murat, E. Morin, R. H. Nilsson, S. Uroz et F. Martin (2009). « 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity ». *New Phytologist* 184.2, p. 449–456 (cité p. 275, 276).

Bugg, T. D. H., M. Ahmad, E. M. Hardiman et R. Rahmanpour (2011). « Pathways for degradation of lignin in bacteria and fungi ». *Natural Product Reports* 28.12, p. 1883 (cité p. 49, 50).

Bulgarelli, D., K. Schlaeppi, S. Spaepen, E. V. L. van Themaat et P. Schulze-Lefert (2013). « Structure and functions of the bacterial microbiota of plants ». *Annual Review of Plant Biology* 64.1, p. 807–838 (cité p. 44).

Bullington, L. S. et B. G. Larkin (2015). « Using direct amplification and next-generation sequencing technology to explore foliar endophyte communities in experimentally inoculated western white pines ». *Fungal Ecology* 17, p. 170–178 (cité p. 46).

Buscardo, E., S. Rodriguez-Echeverria, H. Freitas, P. De Angelis, J. S. Pereira et L. A. H. Muller (2015). « Contrasting soil fungal communities in Mediterranean pine forests subjected to different wildfire frequencies ». *Fungal Diversity* 70.1, p. 85–99 (cité p. 36).

Cairney, J. W. G. (1999). « Intraspecific physiological variation : implications for understanding functional diversity in ectomycorrhizal fungi ». *Mycorrhiza* 9.3, p. 125–135 (cité p. 279).

Cairney, J. W. G. (2011). « Ectomycorrhizal fungi : the symbiotic route to the root for phosphorus in forest soils ». *Plant and Soil* 344 (1-2), p. 51–71 (cité p. 31).

Cairney, J. W. (2012). « Extramatrical mycelia of ectomycorrhizal fungi as moderators of carbon dynamics in forest soil ». *Soil Biology and Biochemistry* 47, p. 198–208 (cité p. 31).

Calcagno, V., N. Mouquet, P. Jarne et P. David (2006). « Coexistence in a metacommunity : the competition–colonization trade-off is not dead ». *Ecology Letters* 9.8, p. 897–907 (cité p. 266).

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson et S. P. Holmes (2016). « DADA2 : High-resolution sample inference from Illumina amplicon data ». *Nature Methods* 13.7, p. 581–583 (cité p. 99).

Campbell, N., J. Reece, L. Urry, M. Cain, S. Wasserman, P. Minorsky et R. Jackson (2012). *Biologie*. 9e édition. Saint-Laurent ; Montréal : Pearson. 1570 p. (cité p. 8).

Capitanio, R. et C. Carcaillet (2008). « Post-fire Mediterranean vegetation dynamics and diversity : A discussion of succession models ». *Forest Ecology and Management* 255 (3-4), p. 431–439 (cité p. 66).

Caporaso, J. G. et coll. (2010). « QIIME allows analysis of high-throughput community sequencing data ». *Nature Methods* 7.5, p. 335–336 (cité p. 99).

Caruso, T., M. C. Rillig et D. Garlaschelli (2012). « On the application of network theory to arbuscular mycorrhizal fungi–plant interactions : the importance of basic assumptions ». *New Phytologist* 194.4, p. 891–894 (cité p. 63).

Certini, G. (2005). « Effects of fire on properties of forest soils : a review ». *Oecologia* 143.1, p. 1–10 (cité p. 65, 270).

Chagnon, P.-L. (2014). « Ecological and evolutionary implications of hyphal anastomosis in arbuscular mycorrhizal fungi ». *FEMS Microbiology Ecology* 88.3, p. 437–444 (cité p. 277).

— (2016). « Seeing networks for what they are in mycorrhizal ecology ». *Fungal Ecology, In press* (cité p. 63, 276).

Chagnon, P.-L., R. L. Bradley et J. N. Klironomos (2015). « Trait-based partner selection drives mycorrhizal network assembly ». *Oikos* 124.12, p. 1609–1616 (cité p. 61, 279).

— (2012). « Using ecological network theory to evaluate the causes and consequences of arbuscular mycorrhizal community structure ». *New Phytologist* 194.2, p. 307–312 (cité p. 61, 275).

Chalmandrier, L., T. Münkemüller, L. Gallien, F. de Bello, F. Mazel, S. Lavergne et W. Thuiller (2013). « A family of null models to distinguish between environmental filtering and biotic interactions in functional diversity patterns ». *Journal of Vegetation Science* 24.5, p. 853–864 (cité p. 21).

Chambers, S. M., C. J. Hitchcock et J. W. Cairney (2005). « Ectomycorrhizal mycobionts of *Pisonia grandis* on coral cays in the Capricorn-Bunker group, Great Barrier Reef, Australia ». *Mycological Research* 109.10, p. 1105–1111 (cité p. 33).

Chauvet, E., J. Cornut, K. R. Sridhar, M.-A. Selosse et F. Bärlocher (2016). « Beyond the water column : aquatic hyphomycetes outside their preferred habitat ». *Fungal Ecology* 19, p. 112–127 (cité p. 272).

Cheng, T. C. (1991). Is parasitism symbiosis ? A definition of terms and the evolution of concepts. *Parasite-Host Associations : Coexistence or Conflict ?* Oxford University Press. Toft C.A, Aeschlimann A, Bolis L., p. 15–36 (cité p. 14).

Chesson, P. (2000). « Mechanisms of maintenance of species diversity ». *Annual Review of Ecology and Systematics* 31, p. 343–366 (cité p. 272, 282).

Christopoulou, A., P. Z. Fulé, P. Andriopoulos, D. Sarris et M. Arianoutsou (2013). « Dendrochronology-based fire history of *Pinus nigra* forests in Mount Taygetos, Southern Greece ». *Forest Ecology and Management* 293, p. 132–139 (cité p. 66, 80).

Claridge, A. (1992). « Is the relathionship among mycophagous marsupials, mycorrhizal fungi and plants dependent on fire ? ». *Australian Journal of Ecology* 17.2, p. 223–225 (cité p. 40).

Clark, J. S. (2010). « Individuals and the Variation Needed for High Species Diversity in Forest Trees ». *Science* 327.5969, p. 1129–1132 (cité p. 279).

Clemente, J., L. Ursell, L. Parfrey et R. Knight (2012). « The impact of the gut microbiota on human health : An integrative view ». *Cell* 148.6, p. 1258–1270 (cité p. 43).

Clements, F. E. (1916). *Plant Succession : An Analysis of the Development of Vegetation*. Carnegie Institution of Washington. 242 p. (cité p. 22).

Clemmensen, K. E., R. D. Finlay, A. Dahlberg, J. Stenlid, D. A. Wardle et B. D. Lindahl (2015). « Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests ». *New Phytologist* 205.4, p. 1525–1536 (cité p. 87, 278, 280).

Cockle, K. L., K. Martin et G. Robledo (2012). « Linking fungi, trees, and hole-using birds in a Neotropical tree-cavity network : Pathways of cavity production and implications for conservation ». *Forest Ecology and Management* 264, p. 210–219 (cité p. 16).

Coince, A., T. Cordier, J. Lengellé, E. Defossez, C. Vacher, C. Robin, M. Buée et B. Marçais (2014). « Leaf and root-associated fungal assemblages do not follow similar elevational diversity patterns ». *PLoS ONE* 9.6, e100668 (cité p. 44–46).

Coley, P. D. et J. A. Barone (1996). « Herbivory and plant defenses in tropical forests ». *Annual Review of Ecology and Systematics*, p. 305–335 (cité p. 17).

Collectif (2006a). *Contribution à la conduite des peuplements de pin laricio et habitats associés. Tome 1 : patrimoine et richesses.* Réd. par D. Soulé et S. Guy. ONF, PNRC, CTC, OEC. 249 p. (cité p. 69).

— (2006b). *Contribution à la conduite des peuplements de pin laricio et habitats associés. Tome 2 : Enjeux et gestion.* Réd. par D. Soulé et S. Guy. ONF, PNRC, CTC, OEC. 249 p. (cité p. 71).

Colwell, R. K. et T. F. Rangel (2009). « Hutchinson's duality : The once and future niche ». *Proceedings of the National Academy of Sciences* 106 (Supplement 2), p. 19651–19658 (cité p. 20).

Cordier, T., C. Robin, X. Capdevielle, M.-L. Desprez-Loustau et C. Vacher (2012). « Spatial variability of phyllosphere fungal assemblages : Genetic distance predominates over geographic distance in a European beech stand (*Fagus sylvatica*) ». *Fungal Ecology* 5.5, p. 509–520 (cité p. 44, 46, 282).

Courty, P.-E., M. Buée, A. G. Diedhiou, P. Frey-Klett, F. Le Tacon, F. Rineau, M.-P. Turpault, S. Uroz et J. Garbaye (2010). « The role of ectomycorrhizal communities in forest ecosystem processes : New perspectives and emerging concepts ». *Soil Biology and Biochemistry* 42.5, p. 679–698 (cité p. 30).

Courty, P.-E. et coll. (2016). « Into the functional ecology of ectomycorrhizal communities : Environmental filtering of enzymatic activities ». *Journal of Ecology* (cité p. 33, 40, 45, 106, 269, 271, 275, 279).

Craine, J. M. et R. Dybzinski (2013). « Mechanisms of plant competition for nutrients, water and light ». *Functional Ecology* 27.4, p. 833–840 (cité p. 17).

Crowther, T. W., D. S. Maynard, T. R. Crowther, J. Peccia, J. R. Smith et M. A. Bradford (2014). « Untangling the fungal niche : the trait-based approach ». *Terrestrial Microbiology* 5, p. 579 (cité p. 279).

Dahlberg, A. (2001). « Community ecology of ectomycorrhizal fungi : an advancing interdisciplinary field ». *New Phytologist* 150.3, p. 555–562 (cité p. 264, 269, 280).

Dahlberg, A., D. R. Genney et J. Heilmann-Clausen (2010). « Developing a comprehensive strategy for fungal conservation in Europe : Current status and future needs ». *Fungal Ecology* 3.2, p. 50–64 (cité p. 16).

Dahlberg, A., L. Jonsson et J.-E. Nylund (1997). « Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden ». *Canadian Journal of Botany* 75.8, p. 1323–1335 (cité p. 27, 33, 276).

Dahlberg, A. et G. M. Mueller (2011). « Applying IUCN red-listing criteria for assessing and reporting on the conservation status of fungal species ». *Fungal Ecology* 4.2, p. 147–162 (cité p. 16).

Dahlberg, A. et J. Stenlid (1994). « Size, distribution and biomass of genets in populations of *Suillus bovinus* (L. : Fr.) Roussel revealed by somatic incompatibility ». *New Phytologist* 128.2, p. 225–234 (cité p. 33).

Daniel, G. et T. Nilsson (1998). Developments in the study of soft rot and bacterial decay. *Forest products biotechnology.* Taylor & Francis Ltd. London - Bristol : A. Bruce & J.W. Palfreyman, p. 37–62 (cité p. 53).

Daniel, T. C. et coll. (2012). « Contributions of cultural services to the ecosystem services agenda ». *Proceedings of the National Academy of Sciences* 109.23, p. 8812–8819 (cité p. 69).

Dawkins, R. (1989). *The Extended Phenotype : The Long Reach of the Gene.* Oxford ; New York : Oxford Univ Pr. 318 p. (cité p. 30, 43).

De Queiroz, K. (2007). « Species concepts and species delimitation ». *Systematic Biology* 56.6, p. 879–886 (cité p. 59).

De Wit, R. et T. Bouvier (2006). « 'Everything is everywhere, but, the environment selects' ; What did Baas Becking and Beijerinck really say ? ». *Environmental Microbiology* 8.4, p. 755–758 (cité p. 43, 265, 266).

Devictor, V., J. Clavel, R. Julliard, S. Lavergne, D. Mouillot, W. Thuiller, P. Venail, S. Villéger et N. Mouquet (2010). « Defining and measuring ecological specialization ». *Journal of Applied Ecology* 47.1, p. 15–25 (cité p. 20).

Dickie, I. A., B. Xu et R. T. Koide (2002). « Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis ». *New Phytologist* 156.3, p. 527–535 (cité p. 27).

Dighton, J. (2003). *Fungi in Ecosystem Processes.* Mycology series v. 17. New York : M. Dekker. 432 p. (cité p. 16).

Dinan, T. G., R. M. Stilling, C. Stanton et J. F. Cryan (2015). « Collective unconscious : How gut microbes shape human behavior ». *Journal of Psychiatric Research* 63, p. 1–9 (cité p. 43).

Dooley, S. R. et K. K. Treseder (2012). « The effect of fire on microbial biomass : a meta-analysis of field studies ». *Biogeochemistry* 109 (1-3), p. 49–61 (cité p. 52).

Dormann, C. F., J. Frund, N. Bluthgen et B. Gruber (2009). « Indices, graphs and null models : Analyzing bipartite ecological networks ». *The Open Ecology Journal* 2.1, p. 7–24 (cité p. 60).

Douhan, G. W., L. Vincenot, H. Gryta et M.-A. Selosse (2011). « Population genetics of ectomycorrhizal fungi : from current knowledge to emerging directions ». *Fungal Biology* 115.7, p. 569–597 (cité p. 11).

Drake, J. A., C. R. Zimmerman, T. Purucker et C. Rojo (2001). On the nature of the assembly trajectory. *Ecological assembly rules : perspectives, advances, retreats.* Cambridge University Press, p. 233–50 (cité p. 281).

Dumbrell, A. J., M. Nelson, T. Helgason, C. Dytham et A. H. Fitter (2010a). « Idiosyncrasy and overdominance in the structure of natural communities of arbuscular mycorrhizal fungi : is there a role for stochastic processes ? ». *Journal of Ecology* 98.2, p. 419–428 (cité p. 44).

— (2010b). « Relative roles of niche and neutral processes in structuring a soil microbial community ». *The ISME Journal* 4.3, p. 337–345 (cité p. 272).

Dunne, J. A., R. J. Williams et N. D. Martinez (2002). « Network structure and biodiversity loss in food webs : robustness increases with connectance ». *Ecology Letters* 5.4, p. 558–567 (cité p. 60).

Dupouey, J.-L., E. Dambrine, J.-D. Laffite et C. Moares (2002). « Irreversible impact of past land use on forest soils and biodiversity ». *Ecology* 83.11, p. 2978–2984 (cité p. 52).

Edgar, R. C. (2010). « Search and clustering orders of magnitude faster than BLAST ». *Bioinformatics* 26.19, p. 2460–2461 (cité p. 99).

— (2013). « UPARSE : highly accurate OTU sequences from microbial amplicon reads ». *Nature Methods* 10.10, p. 996–998 (cité p. 99).

Egli, S. et I. Brunner (2002). « Les mycorhizes ». *Notice pour le praticien* 35.8 (cité p. 29).

Ekblad, A. et coll. (2013). « The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils : role in carbon cycling ». *Plant and Soil* 366 (1-2), p. 1–27 (cité p. 33).

Elton, C. (1927). *Animal Ecology.* Sidgwick and Jackson. London (cité p. 20).

Encinas-Viso, F., D. Alonso, J. N. Klironomos, R. S. Etienne et E. R. Chang (2016). « Plant–mycorrhizal fungus co-occurrence network lacks substantial structure ». *Oikos* 125.4, p. 457–467 (cité p. 61).

Erwin, D. (2008). « Macroevolution of ecosystem engineering, niche construction and diversity ». *Trends in Ecology & Evolution* 23.6, p. 304–310 (cité p. 21).

Farré-Armengol, G., I. Filella, J. Llusia et J. Peñuelas (2016). « Bidirectional interaction between phyllospheric microbiotas and plant volatile emissions ». *Trends in Plant Science* (*In Press*) (cité p. 44).

Feinstein, L. M. et C. B. Blackwood (2012). « Taxa–area relationship and neutral dynamics influence the diversity of fungal communities on senesced tree leaves ». *Environmental Microbiology* 14.6, p. 1488–1499 (cité p. 272).

Fellbaum, C. R., J. A. Mensah, A. J. Cloos, G. E. Strahan, P. E. Pfeffer, E. T. Kiers et H. Bücking (2014). « Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants ». *New Phytologist* 203, p. 646–656 (cité p. 32).

Fenchel, T. et B. J. Finlay (2004). « The ubiquity of small species : Patterns of local and global diversity ». *BioScience* 54.8, p. 777–784 (cité p. 43).

Fernandez, C. W. et P. G. Kennedy (2015a). « Moving beyond the black-box : fungal traits, community structure, and carbon sequestration in forest soils ». *New Phytologist* 205.4, p. 1378–1380 (cité p. 16, 51, 278).

— (2015b). « Revisiting the 'Gadgil effect' : do interguild fungal interactions control carbon cycling in forest soils ? ». *New Phytologist* 209.4, p. 1382–1394 (cité p. 270, 271).

Field, K. J., S. Pressel, J. G. Duckett, W. R. Rimington et M. I. Bidartondo (2015). « Symbiotic options for the conquest of land ». *Trends in Ecology & Evolution* 30.8, p. 477–486 (cité p. 30).

Fisher, M. C., D. A. Henk, C. J. Briggs, J. S. Brownstein, L. C. Madoff, S. L. McCraw et S. J. Gurr (2012). « Emerging fungal threats to animal, plant and ecosystem health ». *Nature* 484.7393, p. 186–194 (cité p. 15, 16).

Fodor, E. (2013). « Linking biodiversity to mutualistic networks–woody species and ectomycorrhizal fungi ». *Annals of Forest Research* 56.1, p. 53–78 (cité p. 61).

Fontaine, C., P. R. Guimarães, S. Kéfi, N. Loeuille, J. Memmott, W. H. van der Putten, F. J. F. van Veen et E. Thébault (2011). « The ecological and evolutionary implications of merging different types of networks ». *Ecology Letters* 14.11, p. 1170–1181 (cité p. 14).

Fortuna, M. A., D. B. Stouffer, J. M. Olesen, P. Jordano, D. Mouillot, B. R. Krasnov, R. Poulin et J. Bascompte (2010). « Nestedness versus modularity in ecological networks : two sides of the same coin ? ». *Journal of Animal Ecology* (cité p. 318).

Fox, J. W. (2013a). « The intermediate disturbance hypothesis is broadly defined, substantive issues are key : a reply to Sheil and Burslem ». *Trends in Ecology & Evolution* 28.10, p. 572–573 (cité p. 268).

— (2013b). « The intermediate disturbance hypothesis should be abandoned ». *Trends in Ecology & Evolution* 28.2, p. 86–92 (cité p. 268).

Frank, B. (1887). « Ueber neue Mycorhiza-Formen ». *Berichte der Deutschen Botanischen Gesellschaft* 5.8, p. 395–409 (cité p. 25).

Frank, J. L., S. Anglin, E. M. Carrington, D. S. Taylor, B. Viratos et D. Southworth (2009). « Rodent dispersal of fungal spores promotes seedling establishment away from mycorrhizal networks on *Quercus garryana* ». *Botany* 87.9, p. 821–829 (cité p. 40).

Fukami, T., I. A. Dickie, J. Paula Wilkie, B. C. Paulus, D. Park, A. Roberts, P. K. Buchanan et R. B. Allen (2010). « Assembly history dictates ecosystem functioning : evidence from wood decomposer communities ». *Ecology Letters* 13.6, p. 675–684 (cité p. 17, 53, 281).

Fulé, P. Z., M. Ribas, E. Gutiérrez, R. Vallejo et M. W. Kaye (2008). « Forest structure and fire history in an old *Pinus nigra* forest, eastern Spain ». *Forest Ecology and Management* 255 (3-4), p. 1234–1242 (cité p. 66, 71, 80).

Gamisans, J. (1999). *La Végétation de La Corse.* Aix-en-Provence, France : Edisud. 391 p. (cité p. 68).

Garbaye, J. et J. A. Fortin (2013). *La symbiose mycorhizienne : Une association entre les plantes et les champignons.* Quae éditions. 256 p. (cité p. 25).

Gardes, M. et T. D. Bruns (1996). « Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest : above- and below-ground views ». *Canadian Journal of Botany* 74.10, p. 1572–1583 (cité p. 27, 264, 271, 276).

— (1993). « ITS primers with enhanced specificity for Basidiomycetes - application to the identification of mycorrhizae and rusts ». *Molecular Ecology* 2.2, p. 113–118 (cité p. 25, 26, 33, 57, 276, 315).

Gardes, M., G. M. Mueller, J. A. Fortin et B. R. Kropp (1991). « Mitochondrial DNA polymorphisms in *Laccaria bicolor*, *L. laccata*, *L. proxima* and *L. amethystina* ». *Mycological Research* 95.2, p. 206–216 (cité p. 27).

Gaston, K. J. et T. M. Blackburn (2000). *Pattern and Process in Macroecology.* Oxford, OX ; Malden, MA, USA : Blackwell Science. 377 p. (cité p. 276).

Gazis, R. et P. Chaverri (2015). « Wild trees in the Amazon basin harbor a great diversity of beneficial endosymbiotic fungi : is this evidence of protective mutualism ? ». *Fungal Ecology* 17, p. 18–29 (cité p. 46).

Geml, J., B. Gravendeel, K. J. van der Gaag, M. Neilen, Y. Lammers, N. Raes, T. A. Semenova, P. de Knijff et M. E. Noordeloos (2014). « The contribution of DNA metabarcoding to fungal conservation : diversity assessment, habitat partitioning and mapping red-listed fungi in protected coastal *Salix repens* communities in the netherlands ». *PLOS ONE* 9.6, e99852 (cité p. 16).

Giraud, T., G. Refrégier, M. Le Gac, D. M. de Vienne et M. E. Hood (2008). « Speciation in fungi ». *Fungal Genetics and Biology* 45.6, p. 791–802 (cité p. 59).

Girvan, M. et M. E. Newman (2002). « Community structure in social and biological networks ». *Proceedings of the National Academy of Sciences* 99.12, p. 7821–7826 (cité p. 318).

Glassman, S. I., C. R. Levine, A. M. DiRocco, J. J. Battles et T. D. Bruns (2015). « Ectomycorrhizal fungal spore bank recovery after a severe forest fire : some like it hot ». *The ISME Journal* (cité p. 270, 280).

Gleason, H. A. (1926). « The individualistic concept of the plant association ». *Bulletin of the Torrey Botanical Club* 53.1, p. 7–26 (cité p. 22).

Gobat, J.-M., M. Aragno, W. Mathey, Collectif et R. Bally (2010). *Le sol vivant : Bases de pédologie, Biologie des sols.* 3e édition revue et augmentée. Lausanne : PPUR. 817 p. (cité p. 50, 315).

Gómez-Aparicio, L., R. Zamora, J. M. Gómez, J. A. Hódar, J. Castro et E. Baraza (2004). « Applying plant facilitation to forest restoration : a meta-analysis of the use of shrubs as nurse plants ». *Ecological Applications* 14.4, p. 1128–1138 (cité p. 17).

Goodell, B., Y. Qian et J. Jellison (2008). Fungal Decay of Wood : Soft Rot—Brown Rot—White Rot. *Development of Commercial Wood Preservatives.* Sous la dir. de T. P. Schultz, H. Militz, M. H. Freeman, B. Goodell et D. D. Nicholas. T. 982. Washington, DC : American Chemical Society, p. 9–31 (cité p. 50).

Gravel, D., C. D. Canham, M. Beaudet et C. Messier (2006). « Reconciling niche and neutrality : the continuum hypothesis ». *Ecology Letters* 9.4, p. 399–409 (cité p. 272).

Green, J. L., A. J. Holmes, M. Westoby, I. Oliver, D. Briscoe, M. Dangerfield, M. Gillings et A. J. Beattie (2004). « Spatial scaling of microbial eukaryote diversity ». *Nature* 432.7018, p. 747–750 (cité p. 44, 266).

Griffin, E. A. et W. P. Carson (2015). « The ecology and natural history of foliar bacteria with a focus on tropical forests and agroecosystems ». *The Botanical Review* 81.2, p. 105–149 (cité p. 44).

Grime, J. P. (1977). « Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory ». *The American Naturalist* 111.982, p. 1169 (cité p. 45).

Grinnell, J. (1917). « The niche-relationships of the california thrasher ». *The Auk* 34.4, p. 427–433 (cité p. 20).

Guimerà, R. et L. A. N. Amaral (2005). « Cartography of complex networks : modules and universal roles ». *Journal of Statistical Mechanics* (P02001) nihpa35573, p. 1–13 (cité p. 62).

Guimerà, R., M. Sales-Pardo et L. A. N. Amaral (2004). « Modularity from fluctuations in random graphs and complex networks ». *Physical Review E* 70.2 (cité p. 62).

Hale, A. N., L. Lapointe et S. Kalisz (2016). « Invader disruption of belowground plant mutualisms reduces carbon acquisition and alters allocation patterns in a native forest herb ». *New Phytologist* 209.2, p. 542–549 (cité p. 35).

Halme, P., P. Ódor, M. Christensen, A. Piltaver, M. Veerkamp, R. Walleyn, I. Siller et J. Heilmann-Clausen (2013). « The effects of habitat degradation on metacommunity structure of wood-inhabiting fungi in European beech forests ». *Biological Conservation* 168, p. 24–30 (cité p. 266).

Hammel, K. E. (1997). Fungal degradation of lignin. *Driven by nature : plant litter quality and decomposition.* CABI, p. 33–45 (cité p. 49).

Handelsman, J., M. R. Rondon, S. F. Brady, J. Clardy et R. M. Goodman (1998). « Molecular biological access to the chemistry of unknown soil microbes : a new frontier for natural products ». *Chemistry & Biology* 5.10, R245–R249 (cité p. 314).

Hansen, M. C. et coll. (2013). « High-resolution global maps of 21st-century forest cover change ». *Science* 342.6160, p. 850–853 (cité p. 83).

Hanski, I. (1998). « Metapopulation dynamics ». *Nature* 396.6706, p. 41–49 (cité p. 316).

Hardoim, P. R., L. S. van Overbeek, G. Berg, A. M. Pirttilä, S. Compant, A. Campisano, M. Döring et A. Sessitsch (2015). « The hidden world within plants : ecological and evolutionary considerations for defining functioning of microbial endophytes ». *Microbiology and Molecular Biology Reviews* 79.3, p. 293–320 (cité p. 43, 44).

Hart, M. M., K. Aleklett, P.-L. Chagnon, C. Egan, S. Ghignone, T. Helgason, Y. Lekberg, M. Öpik, B. J. Pickles et L. Waller (2015). « Navigating the labyrinth : a guide to sequence-based, community ecology of arbuscular mycorrhizal fungi ». *New Phytologist* 207.1, p. 235–247 (cité p. 58).

Harvey, A. E., M. J. Larsen et M. F. Jurgensen (1979). « Comparative distribution of ectomycorrhizae in soils of three western Montana forest habitat types ». *Forest Science* 25.2, p. 350–358 (cité p. 271).

Hawksworth, D. L. (2012). « Global species numbers of fungi : are tropical studies and molecular approaches contributing to a more robust estimate ? ». *Biodiversity and Conservation* 21.9, p. 2425–2433 (cité p. 10).

— (1991). « The fungal dimension of biodiversity : magnitude, significance, and conservation ». *Mycological Research* 95.6, p. 641–655 (cité p. 10).

Hawksworth, D. L. (2001). « The magnitude of fungal diversity : the 1.5 million species estimate revisited ». *Mycological Research* 105.12, p. 1422–1432 (cité p. 10).

Healy, R. A. et coll. (2013). « High diversity and widespread occurrence of mitotic spore mats in ectomycorrhizal Pezizales ». *Molecular Ecology* 22.6, p. 1717–1732 (cité p. 31).

Hebert, P. D. N., A. Cywinska, S. L. Ball et J. R. deWaard (2003). « Biological identifications through DNA barcodes. ». *Proceedings of the Royal Society B : Biological Sciences* 270.1512, p. 313–321 (cité p. 58).

Heilmann-Clausen, J. (2005). *Diversity of Saproxylic Fungi on Decaying Beech Wood in Protected Forests in the County of Halland*. Länsstyrelsen Halland. Report from Länsstyrelsen Halland and HabitatVision. Örjan Fritz. 68 p. (cité p. 53).

Heilmann-Clausen, J., E. S. Barron, L. Boddy, A. Dahlberg, G. W. Griffith, J. Nordén, O. Ovaskainen, C. Perini, B. Senn-Irlet et P. Halme (2015). « A fungal perspective on conservation biology ». *Conservation Biology* 29.1, p. 61–68 (cité p. 16).

Hibbett, D. S., L.-B. Gilbert et M. J. Donoghue (2000). « Evolutionary instability of ectomycorrhizal symbioses in Basidiomycetes ». *Nature* 407.6803, p. 506–508 (cité p. 26, 34, 35).

Hibbett, D. S. et coll. (2007). « A higher-level phylogenetic classification of the Fungi ». *Mycological Research* 111.5, p. 509–547 (cité p. 9).

Higgins, K. L., A. E. Arnold, P. D. Coley et T. A. Kursar (2014). « Communities of fungal endophytes in tropical forest grasses : highly diverse host- and habitat generalists characterized by strong spatial structure ». *Fungal Ecology* 8, p. 1–11 (cité p. 44).

Hobbie, E. A., L. T. A. van Diepen, E. A. Lilleskov, A. P. Ouimette, A. C. Finzi et K. S. Hofmockel (2014). « Fungal functioning in a pine forest : evidence from a 15N-labeled global change experiment ». *New Phytologist* 201.4, p. 1431–1439 (cité p. 40).

Hoeksema, J. D. (2010). « Ongoing coevolution in mycorrhizal interactions : Tansley review ». *New Phytologist* 187.2, p. 286–300 (cité p. 15).

Hoeksema, J. D. et coll. (2010). « A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi ». *Ecology Letters* 13.3, p. 394–407 (cité p. 31).

Holden, S. R., A. Gutierrez et K. K. Treseder (2013). « Changes in soil fungal communities, extracellular enzyme activities, and litter decomposition across a fire chronosequence in Alaskan Boreal forests ». *Ecosystems* 16.1, p. 34–46 (cité p. 52).

Holden, S. R., B. M. Rogers, K. K. Treseder et J. T. Randerson (2016). « Fire severity influences the response of soil microbes to a boreal forest fire ». *Environmental Research Letters* 11.3, p. 035004 (cité p. 52).

Holden, S. R. et K. K. Treseder (2013). « A meta-analysis of soil microbial biomass responses to forest disturbances ». *Frontiers in Microbiology* 4, p. 163 (cité p. 52).

Hollingsworth, P. M. et coll. (2009). « A DNA barcode for land plants ». *Proceedings of the National Academy of Sciences* 106.31, p. 12794–12797 (cité p. 58).

Holyoak, M., M. A. Leibold et R. D. Holt (2005). From metacommunities to meta-ecosystems. *Metacommunities : Spatial Dynamics and Ecological Communities*. University of Chicago Press (cité p. 22, 23).

Horton, T. R. et T. D. Bruns (1998). « Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*) ». *New Phytologist* 139.2, p. 331–339 (cité p. 275).

— (2001). « The molecular revolution in ectomycorrhizal ecology : peeking into the black-box ». *Molecular Ecology* 10.8, p. 1855–1871 (cité p. 25, 26, 33, 34).

Horton, T. R., T. D. Bruns et V. T. Parker (1999). « Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment ». *Canadian Journal of Botany* 77.1, p. 93–102 (cité p. 36).

Howard, R. et N. A. Gow (2013). Biomechamics of invasive hyphal growth. *Biology of the Fungal Cell.* Springer Science & Business Media, p. 3–18 (cité p. 11).

Huang, Y.-L., M. M. N. Devan, J. M. U'Ren, S. H. Furr et A. E. Arnold (2016). « Pervasive effects of wildfire on foliar endophyte communities in montane forest trees ». *Microbial Ecology* 71.2, p. 452–468 (cité p. 45, 46).

Hubbell, S. P. (2006). « Neutral theory and the evolution of ecological equivalence ». *Ecology* 87.6, p. 1387–1398 (cité p. 272).

— (2001). *The Unified Neutral Theory of Biodiversity and Biogeography.* Monographs in population biology 32. Princeton : Princeton University Press. 375 p. (cité p. 22, 23, 272, 274, 276).

Hunt, G. A. et J. M. Trappe (1987). « Seasonal hypogeous sporocarp production in a western Oregon Douglas-fir stand ». *Canadian Journal of Botany* 65.3, p. 438–445 (cité p. 33).

Hutchinson, G. E. (1957). Concluding Remarks. *Cold Spring Harbor Symposia on Quantitative Biology.* T. 22, p. 415–427 (cité p. 20, 23, 316).

Hutchison, L. J. (1989). « Absence of conidia as a morphological character in ectomycorrhizal fungi ». *Mycologia* 81.4, p. 587 (cité p. 31).

Jacquemyn, H., R. Brys, M. Waud, P. Busschaert et B. Lievens (2015). « Mycorrhizal networks and coexistence in species-rich orchid communities ». *New Phytologist* 206.3, p. 1127–1134 (cité p. 61).

Jacquemyn, H., O. Honnay, B. P. A. Cammue, R. Brys et B. Lievens (2010). « Low specificity and nested subset structure characterize mycorrhizal associations in five closely related species of the genus *Orchis* ». *Molecular Ecology* 19.18, p. 4086–4095 (cité p. 61).

Jacquemyn, H., V. Merckx, R. Brys, D. Tyteca, B. P. A. Cammue, O. Honnay et B. Lievens (2011). « Analysis of network architecture reveals phylogenetic constraints on mycorrhizal specificity in the genus *Orchis* (Orchidaceae) ». *New Phytologist* 192.2, p. 518–528 (cité p. 61).

James, T. Y. et coll. (2006). « Reconstructing the early evolution of Fungi using a six-gene phylogeny ». *Nature* 443.7113, p. 818–822 (cité p. 34, 35).

Jeffery, S., C. Gardi, A. Jones, L. Montanarella, L. Marmo, L. Miko, K. Ritz, G. Peres, J. Römbke et W. H. Van Der Putten (2013). *Atlas Européen de La Biodiversité Des Sols.* Luxembourg : Bureau des publications de l'Union européenne : Centre commun de recherche (joint Research Centre) (cité p. 49, 50).

Johnson, D., F. Martin, J. W. G. Cairney et I. C. Anderson (2012). « The importance of individuals : intraspecific diversity of mycorrhizal plants and fungi in ecosystems : *Tansley review* ». *New Phytologist* 194.3, p. 614–628 (cité p. 11, 279).

Johnson, N. C., C. H. Graham et F. A. Smith (1997). « Functioning of mycorrhizal associations along the mutualism–parasitism continuum ». *New Phytologist* 135.4, p. 575–585 (cité p. 32).

Jones, E. I. et coll. (2015). « Cheaters must prosper : reconciling theoretical and empirical perspectives on cheating in mutualism ». *Ecology Letters* 18.11, p. 1270–1284 (cité p. 32).

Jonsson, L., A. Dahlberg, M. C. Nilsson, O. Zackrisson et O. Karen (1999). « Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire ». *Molecular Ecology* 8.2, p. 205–215 (cité p. 264).

Jordano, P., J. Bascompte et J. M. Olesen (2003). « Invariant properties in coevolutionary networks of plant–animal interactions ». *Ecology Letters* 6.1, p. 69–81 (cité p. 60).

Jumpponen, A. et K. L. Jones (2009). « Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere ». *New Phytologist* 184.2, p. 438–448 (cité p. 46).

Karst, J., L. Marczak, M. D. Jones et R. Turkington (2008). « The mutualism-parasitism continuum in ectomycorrhizas : a quantitative assessment using meta-analysis ». *Ecology* 89.4, p. 1032–1042 (cité p. 15).

Kaul, S., S. Gupta, M. Ahmed et M. K. Dhar (2012). « Endophytic fungi from medicinal plants : a treasure hunt for bioactive metabolites ». *Phytochemistry Reviews* 11.4, p. 487–505 (cité p. 10).

Keeley, J. E., éd. (2012). *Fire in Mediterranean Ecosystems : Ecology, Evolution and Management.* Cambridge, UK ; New York : Cambridge University Press. 515 p. (cité p. 316, 317).

Kembel, S. W. et R. C. Mueller (2014). « Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities ». *Botany* 92.4, p. 303–311 (cité p. 46, 267).

Kembel, S. W., T. K. O'Connor, H. K. Arnold, S. P. Hubbell, S. J. Wright et J. L. Green (2014). « Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest ». *Proceedings of the National Academy of Sciences* 111.38, p. 13715–13720 (cité p. 45).

Kemler, M., J. Garnas, M. J. Wingfield, M. Gryzenhout, K.-A. Pillay et B. Slippers (2013). « Ion Torrent PGM as tool for fungal community analysis : a case study of endophytes in *Eucalyptus grandis* reveals high taxonomic diversity ». *PLOS ONE* 8.12, e81718 (cité p. 46).

Kendrick, B. (2001). *The Fifth Kingdom.* 3 edition. Newburyport, MA : Focus. 386 p. (cité p. 15).

Kennedy, P. (2010). « Ectomycorrhizal fungi and interspecific competition : species interactions, community structure, coexistence mechanisms, and future research directions ». *New Phytologist* 187.4, p. 895–910 (cité p. 264, 270, 271, 279).

Kennedy, P. G., K. G. Peay et T. D. Bruns (2009). « Root tip competition among ectomycorrhizal fungi : are priority effects a rule or an exception ? ». *Ecology* 90.8, p. 2098–2107 (cité p. 17).

Kiers, E. T., M. Duhamel, Y. Beesetty, J. A. Mensah, O. Franken, E. Verbruggen, C. R. Fellbaum, G. A. Kowalchuk, M. M. Hart et A. Bago (2011). « Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis ». *Science* 333.6044, p. 880–882 (cité p. 32).

Kirk, P. M., P. F. Cannon, D. W. Minter et J. A. Stalpers (2008). *Dictionary of the Fungi.* CABI - CBS. CSIRO Publishing. 640 p. (cité p. 10).

Klironomos, J., M. Zobel, M. Tibbett, W. D. Stock, M. C. Rillig, J. L. Parrent, M. Moora, A. M. Koch, J. M. Facelli et E. Facelli (2011). « Forces that structure plant communities : quantifying the importance of the mycorrhizal symbiosis ». *New Phytologist* 189.2, p. 366–370 (cité p. 31).

Kohler, A. et coll. (2015). « Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists ». *Nature Genetics* 47.4, p. 410–415 (cité p. 34).

Koide, R. T., C. Fernandez et G. Malcolm (2014). « Determining place and process : functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function ». *New Phytologist* 201, p. 433–439 (cité p. 36, 278).

Koide, R. T., C. Fernandez et K. Petprakob (2011). « General principles in the community ecology of ectomycorrhizal fungi ». *Annals of Forest Science* 68.1, p. 45–55 (cité p. 140, 264).

Koide, R. T., J. N. Sharda, J. R. Herr et G. M. Malcolm (2008). « Ectomycorrhizal fungi and the biotrophy–saprotrophy continuum ». *New Phytologist* 178.2, p. 230–233 (cité p. 34).

Koide, R. T., B. Xu, J. Sharda, Y. Lekberg et N. Ostiguy (2004). « Evidence of species interactions within an ectomycorrhizal fungal community ». *New Phytologist* 165.1, p. 305–316 (cité p. 25).

Kõljalg, U. et coll. (2013). « Towards a unified paradigm for sequence-based identification of fungi ». *Molecular Ecology* 22.21, p. 5271–5277 (cité p. 57, 99, 315).

Kraft, N. J. B., P. B. Adler, O. Godoy, E. C. James, S. Fuller et J. M. Levine (2015). « Community assembly, coexistence and the environmental filtering metaphor ». *Functional Ecology* 29.5, p. 592–599 (cité p. 316).

Krebs, P., G. B. Pezzatti, S. Mazzoleni, L. M. Talbot et M. Conedera (2010). « Fire regime : history and definition of a key concept in disturbance ecology ». *Theory in Biosciences* 129.1, p. 53–69 (cité p. 23, 67).

Kretzer, A. M., M. I. Bidartondo, L. C. Grubisha, J. W. Spatafora, T. M. Szaro et T. D. Bruns (2000). « Regional specialization of *Sarcodes sanguinea* (Ericaceae) on a single fungal symbiont from the *Rhizopogon ellenae* (Rhizopogonaceae) species complex ». *American Journal of Botany* 87.12, p. 1778 (cité p. 33).

Kubartová, A., E. Ottosson, A. Dahlberg et J. Stenlid (2012). « Patterns of fungal communities among and within decaying logs, revealed by 454 sequencing ». *Molecular Ecology* 21.18, p. 4514–4532 (cité p. 53).

Lacourt, I., M. Girlanda, S. Perotto, M. Del Pero, D. Zuccon et A. M. Luppi (2001). « Nuclear ribosomal sequence analysis of *Oidiodendron* : towards a redefinition of ecologically relevant species ». *New Phytologist* 149.3, p. 565–576 (cité p. 224).

Landeweert, R., P. Leeflang, T. W. Kuyper, E. Hoffland, A. Rosling, K. Wernars et E. Smit (2003). « Molecular identification of ectomycorrhizal mycelium in soil horizons ». *Applied and Environmental Microbiology* 69.1, p. 327–333 (cité p. 40).

Langley, J. A., S. K. Chapman et B. A. Hungate (2006). « Ectomycorrhizal colonization slows root decomposition : the post-mortem fungal legacy ». *Ecology Letters* 9.8, p. 955–959 (cité p. 31).

Langley, J. A. et B. A. Hungate (2003). « Mycorrhizal controls on belowground litter quality ». *Ecology* 84.9, p. 2302–2312 (cité p. 31).

Laroche, F. (2014). Etude de Patrons de Diversité, Des Gènes Aux Espèces, Dans Les Métacommunautés : Apports D'une Théorie Quasi-Neutre. Rapport de Thèse. AgroParisTech. 228 p. (cité p. 20, 23, 316).

Larson, A. J., C. A. Cansler, S. G. Cowdery, S. Hiebert, T. J. Furniss, M. E. Swanson et J. A. Lutz (2016). « Post-fire morel (*Morchella*) mushroom abundance, spatial structure, and harvest sustainability ». *Forest Ecology and Management* 377, p. 16–25 (cité p. 280).

Latapy, M., C. Magnien et N. D. Vecchio (2008). « Basic notions for the analysis of large two-mode networks ». *Social Networks* 30.1, p. 31–48 (cité p. 317).

Laughlin, D. C. et J. Messier (2015). « Fitness of multidimensional phenotypes in dynamic adaptive landscapes ». *Trends in Ecology & Evolution* (cité p. 275).

Lavoir, A. V., E. Ormeño, V. Pasqualini, L. Ferrat, S. Greff, C. Lecareux, B. Vila, J. P. Mévy et C. Fernandez (2013). « Does prescribed burning affect leaf secondary metabolites in pine stands ? ». *Journal of Chemical Ecology* 39.3, p. 398–412 (cité p. 71).

Lavorel, S. et E. Garnier (2002). « Predicting changes in community composition and ecosystem functioning from plant traits : revisiting the Holy Grail ». *Functional Ecology* 16.5, p. 545–556 (cité p. 275, 278).

Leake, J., D. Johnson, D. Donnelly, G. Muckle, L. Boddy et D. Read (2004). « Networks of power and influence : the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning ». *Canadian Journal of Botany* 82.8, p. 1016–1045 (cité p. 11, 26, 31, 33).

Lebeis, S. L. (2015). « Greater than the sum of their parts : characterizing plant microbiomes at the community-level ». *Current Opinion in Plant Biology* 24, p. 82–86 (cité p. 43).

Lecointre, G., H. L. Guyader et D. Visset (2006). *Classification phylogénétique du vivant.* 3e édition revue et augmentée. Paris : Belin. 559 p. (cité p. 8).

Lekberg, Y., R. T. Koide, J. R. Rohr, L. Aldrich-Wolfe et J. B. Morton (2007). « Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities ». *Journal of Ecology* 95.1, p. 95–105 (cité p. 272).

LePage, B., R. Currah, R. Stockey et G. Rothwell (1997). « Fossil ectomycorrhizae from the Middle Eocene. ». *American Journal of Botany* 84.3, p. 410–410 (cité p. 34).

Letunic, I. et P. Bork (2016). « Interactive tree of life (iTOL) v3 : an online tool for the display and annotation of phylogenetic and other trees ». *Nucleic Acids Research* (cité p. 9).

Levin, S. A. (1992). « The Problem of Pattern and Scale in Ecology : The Robert H. MacArthur Award Lecture ». *Ecology* 73.6, p. 1943 (cité p. 274).

Leys, B., C. Carcaillet, L. Dezileau, A. A. Ali et R. H. Bradshaw (2013). « A comparison of charcoal measurements for reconstruction of Mediterranean paleo-fire frequency in the mountains of Corsica ». *Quaternary Research* 79.3, p. 337–349 (cité p. 66).

Leys, B., W. Finsinger et C. Carcaillet (2014). « Historical range of fire frequency is not the Achilles' heel of the Corsican black pine ecosystem ». *Journal of Ecology* 102.2, p. 381–395 (cité p. 66, 71).

Lilleskov, E., E. Hobbie et T. Horton (2011). « Conservation of ectomycorrhizal fungi : exploring the linkages between functional and taxonomic responses to anthropogenic N deposition ». *Fungal Ecology* 4.2, p. 174–183 (cité p. 16).

Lilleskov, E. A. et T. D. Bruns (2005). « Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs ». *Mycologia* 97.4, p. 762–769 (cité p. 40).

Lilleskov, E. A., T. D. Bruns, T. R. Horton, D. L. Taylor et P. Grogan (2004). « Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities ». *FEMS Microbiology Ecology* 49.2, p. 319–332 (cité p. 44, 87).

Lilleskov, E. A., T. J. Fahey, T. R. Horton et G. M. Lovett (2002). « Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska ». *Ecology* 83.1, p. 104–115 (cité p. 269).

Lindahl, B. D. et A. Tunlid (2015). « Ectomycorrhizal fungi –potential organic matter decomposers, yet not saprotrophs ». *New Phytologist* 205.4, p. 1443–1447 (cité p. 34, 50, 272, 315, 316).

Lindahl, B. D. et coll. (2013). « Fungal community analysis by high-throughput sequencing of amplified markers - a user's guide ». *New Phytologist* 199.1, p. 288–299 (cité p. 26, 57, 58, 277).

Lindner, D. L. et M. T. Banik (2011). « Intragenomic variation in the ITS rDNA region obscures phylogenetic relationships and inflates estimates of operational taxonomic units in genus *Laetiporus* ». *Mycologia* 103.4, p. 731–740 (cité p. 59).

Loizides, M., J.-M. Bellanger, P. Clowez, F. Richard et P.-A. Moreau (2016). « Combined phylogenetic and morphological studies of true morels (Pezizales, Ascomycota) in Cyprus reveal significant diversity, including *Morchella arbutiphila* and *M. disparilis* spp. nov. ». *Mycological Progress* 15.4, p. 1–28 (cité p. 10).

Lonsdale, D., M. Pautasso et O. Holdenrieder (2008). « Wood-decaying fungi in the forest : conservation needs and management options ». *European Journal of Forest Research* 127.1, p. 1–22 (cité p. 16).

Looijen, R. C. et J. van Andel (1999). « Ecological communities : conceptual problems and definitions ». *Perspectives in Plant Ecology, Evolution and Systematics* 2.2, p. 210–222 (cité p. 19).

Lorch, J. M. et coll. (2011). « Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome ». *Nature* 480.7377, p. 376–378 (cité p. 224).

Lortie, C. J., R. W. Brooker, P. Choler, Z. Kikvidze, R. Michalet, F. I. Pugnaire et R. M. Callaway (2004). « Rethinking plant community theory ». *Oikos* 107.2, p. 433–438 (cité p. 21).

MacArthur, R. H. et E. O. Wilson (1967). *The Theory of Island Biogeography.* Princeton : Princeton University Press. 203 p. (cité p. 39).

MacArthur, R. et R. Levins (1967). « The limiting similarity, convergence, and divergence of coexisting species ». *The American Naturalist* 101.921, p. 377–385 (cité p. 279).

Mahé, F., T. Rognes, C. Quince, C. de Vargas et M. Dunthorn (2014). « Swarm : robust and fast clustering method for amplicon-based studies ». *PeerJ* 2, e593 (cité p. 99).

— (2015). « Swarm v2 : highly-scalable and high-resolution amplicon clustering ». *PeerJ* 3 (cité p. 99).

Maherali, H. et J. N. Klironomos (2007). « Influence of phylogeny on fungal community assembly and ecosystem functioning ». *Science* 316.5832, p. 1746–1748 (cité p. 28, 272).

Martin, F., S. Duplessis, F. Ditengou, H. Lagrange, C. Voiblet et F. Lapeyrie (2001). « Developmental cross talking in the ectomycorrhizal symbiosis : signals and communication genes ». *New Phytologist* 151.1, p. 145–154 (cité p. 77).

Martin, F. et coll. (2008). « The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis ». *Nature* 452.7183, p. 88–92 (cité p. 26, 77).

Martinová, V., M. van Geel, B. Lievens et O. Honnay (2016). « Strong differences in *Quercus robur*-associated ectomycorrhizal fungal communities along a forest-city soil sealing gradient ». *Fungal Ecology* 20, p. 88–96 (cité p. 275).

Martiny, J. B. H. et coll. (2006). « Microbial biogeography : putting microorganisms on the map ». *Nature Reviews Microbiology* 4.2, p. 102–112 (cité p. 44, 266).

Martos, F., F. Munoz, T. Pailler, I. Kottke, C. Gonneau et M.-A. Selosse (2012). « The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids ». *Molecular Ecology* 21.20, p. 5098–5109 (cité p. 61).

Mataix-Solera, J., C. Guerrero, F. Garcia-Orenes, G. M. Bárcenas et M. P. Torres (2009). Forest fire effects on soil microbiology. *Fire effects on soils and restoration strategies.* Science Publishers, Inc. Enfield, New Hampshire USA : Cerdà, A., Robichaud, P., p. 133–175 (cité p. 52).

Maupassant, G. de (1882). *Un Bandit Corse.* Texte Publié Dans La Revue Gil Blas Sous La Signature de Maufrigneuse (cité p. 69).

McGill, B., B. Enquist, E. Weiher et M. Westoby (2006). « Rebuilding community ecology from functional traits ». *Trends in Ecology & Evolution* 21.4, p. 178–185 (cité p. 36, 275, 278).

McGuire, K. L. (2007). « Common ectomycorrhizal networks may maintain monodominance in a tropical rain forest ». *Ecology* 88.3, p. 567–574 (cité p. 36).

Médail, F. et P. Quézel (1999). « Biodiversity hotspots in the Mediterranean Basin : setting global conservation priorities ». *Conservation biology* 13.6, p. 1510–1513 (cité p. 36).

Mikola, P. (1970). Mycorrhizal inoculation in afforestation. *International Review of Forestry Research.* Elsevier. Romberger JA, Mikola P, p. 123–196 (cité p. 35).

Millberg, H., J. Boberg et J. Stenlid (2015). « Changes in fungal community of Scots pine (*Pinus sylvestris*) needles along a latitudinal gradient in Sweden ». *Fungal Ecology* 17, p. 126–139 (cité p. 44–46).

Miller, S. L., T. M. McClean, N. L. Stanton et S. E. Williams (1998). « Mycorrhization, physiognomy, and first-year survivability of conifer seedlings following natural fire in Grand Teton National Park ». *Canadian Journal of Forest Research* 28.1, p. 115–122 (cité p. 280).

Milo, R. (2002). « Network motifs : simple building blocks of complex networks ». *Science* 298.5594, p. 824–827 (cité p. 60).

Minnis, A. M. et D. L. Lindner (2013). « Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America ». *Fungal Biology* 117.9, p. 638–649 (cité p. 224).

Moeller, H. V., K. G. Peay et T. Fukami (2013). « Ectomycorrhizal fungal traits reflect environmental conditions along a coastal California edaphic gradient ». *FEMS Microbiology Ecology* 87.3, p. 797–806 (cité p. 27, 40).

Molina, R. et T. R. Horton (2015). Mycorrhiza specificity : Its role in the development and function of common mycelial networks. *Mycorrhizal Networks.* Dordrecht : Springer Netherlands, p. 1–39 (cité p. 38, 106, 267).

Molina, R. et J. M. Trappe (1982). « Lack of mycorrhizal specificity by the ericaceous hosts *Arbutus menziesii* and *Arctostaphylos uva-ursi* ». *New Phytologist* 90.3, p. 495–509 (cité p. 38).

Montesinos-Navarro, A., J. G. Segarra-Moragues, A. Valiente-Banuet et M. Verdú (2012). « The network structure of plant-arbuscular mycorrhizal fungi ». *New Phytologist* 194.2, p. 536–547 (cité p. 61).

Moore, D., M. Nauta, E. Shelley et M. Rotheroe (2001). *Fungal Conservation : Issues and Solutions.* British Mycological Society symposium series v. 22. Cambridge, UK ; New York, NY, USA : Cambridge University Press. 262 p. (cité p. 16).

Morales-Castilla, I., M. G. Matias, D. Gravel et M. B. Araújo (2015). « Inferring biotic interactions from proxies ». *Trends in Ecology & Evolution* 30.6, p. 347–356 (cité p. 17).

Moreau, P.-A., J. Rochet, F. Richard, F. Chassagne, S. Manzi et M. Gardes (2011). « Taxonomy of *Alnus*-associated hypogeous species of *Alpova* and *Melanogaster* (Basidiomycota, Paxillaceae) in Europe ». *Cryptogamie, Mycologie* 32.1, p. 33–62 (cité p. 33, 34).

Moritz, M. A. et coll. (2014). « Learning to coexist with wildfire ». *Nature* 515.7525, p. 58–66 (cité p. 52, 66, 71).

Mouquet, N. et coll. (2012). « Ecophylogenetics : advances and perspectives ». *Biological Reviews* 87.4, p. 769–785 (cité p. 36).

Mummey, D. L. et M. C. Rillig (2008). « Spatial characterization of arbuscular mycorrhizal fungal molecular diversity at the submetre scale in a temperate grassland ». *FEMS Microbiology Ecology* 64.2, p. 260–270 (cité p. 44).

Munoz, F. et p. Huneman (2016). « From the neutral theory to a comprehensive and multiscale theory of ecological equivalence ». *The Quarterly Review of Biology* 91.3, p. 321–342 (cité p. 272).

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. Da Fonseca et J. Kent (2000). « Biodiversity hotspots for conservation priorities ». *Nature* 403.6772, p. 853–858 (cité p. 36).

Nara, K. (2006). « Ectomycorrhizal networks and seedling establishment during early primary succession ». *New Phytologist* 169.1, p. 169–178 (cité p. 36, 106).

Neary, D. G., C. C. Klopatek, L. F. DeBano et P. F. Ffolliott (1999). « Fire effects on belowground sustainability : a review and synthesis ». *Forest ecology and management* 122.1, p. 51–71 (cité p. 52, 65).

Newman, M. (2010). *Networks : An Introduction.* 1 edition. Oxford ; New York : Oxford University Press. 720 p. (cité p. 60).

Nguyen, N. H., D. Smith, K. Peay et P. Kennedy (2015a). « Parsing ecological signal from noise in next generation amplicon sequencing ». *New Phytologist* 205.4, p. 1389–1393 (cité p. 277, 314).

Nguyen, N. H., Z. Song, S. T. Bates, S. Branco, L. Tedersoo, J. Menke, J. S. Schilling et P. G. Kennedy (2015b). « FUNGuild : An open annotation tool for parsing fungal community datasets by ecological guild ». *Fungal Ecology* (cité p. 100, 316).

Nicoletti, R. et M. De Stefano (2012). « *Penicillium Restrictum* as an antagonist of plant pathogenic fungi ». *Dynamic Biochemistry, Process Biotechnology and Molecular Biology* 6, p. 61–9 (cité p. 224).

Nilsson, L. O., E. Bååth, U. Falkengren-Grerup et H. Wallander (2007). « Growth of ectomycorrhizal mycelia and composition of soil microbial communities in oak forest soils along a nitrogen deposition gradient ». *Oecologia* 153.2, p. 375–384 (cité p. 33).

Nilsson, R. H., E. Kristiansson, M. Ryberg, N. Hallenberg et K.-H. Larsson (2008). « Intraspecific ITS variability in the Kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification ». *Evolutionary Bioinformatics* 4, p. 193–201 (cité p. 58, 59).

Norstedt, G., P. Bader et L. Ericson (2001). « Polypores as indicators of conservation value in Corsican pine forests ». *Biological Conservation* 99.3, p. 347–354 (cité p. 16, 17, 67).

North, M. P., S. L. Stephens, B. M. Collins, J. K. Agee, G. Aplet, J. F. Franklin et P. Z. Fule (2015). « Reform forest fire management ». *Science* 349.6254, p. 1280–1281 (cité p. 66).

Nuñez, M. A., J. Hayward, T. R. Horton, G. C. Amico, R. D. Dimarco, M. N. Barrios-Garcia et D. Simberloff (2013). « Exotic mammals disperse exotic fungi that promote invasion by exotic trees ». *PLoS ONE* 8.6, e66832 (cité p. 31, 40).

Nuñez, M. A., T. R. Horton et D. Simberloff (2009). « Lack of belowground mutualisms hinders Pinaceae invasions ». *Ecology* 90.9, p. 2352–2359 (cité p. 35, 39).

Olesen, J. M., J. Bascompte, Y. L. Dupont et P. Jordano (2007). « The modularity of pollination networks ». *Proceedings of the National Academy of Sciences* 104.50, p. 19891–19896 (cité p. 60).

Oliver, A. K., M. A. Callaham Jr. et A. Jumpponen (2015). « Soil fungal communities respond compositionally to recurring frequent prescribed burning in a managed southeastern US forest ecosystem ». *Forest Ecology and Management* 345, p. 1–9 (cité p. 52).

Oono, R., F. Lutzoni, A. E. Arnold, L. Kaye, J. M. U'Ren, G. May et I. Carbone (2014). « Genetic variation in horizontally transmitted fungal endophytes of pine needles reveals population structure in cryptic species ». *American Journal of Botany* 101.8, p. 1362–1374 (cité p. 44).

Oono, R., E. Lefèvre, A. Simha et F. Lutzoni (2015). « A comparison of the community diversity of foliar fungal endophytes between seedling and adult loblolly pines (*Pinus taeda*) ». *Fungal Biology* 119.10, p. 917–928 (cité p. 45, 46).

Öpik, M., A. Vanatoa, E. Vanatoa, M. Moora, J. Davison, J. M. Kalwij, Ü. Reier et M. Zobel (2010). « The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota) ». *New Phytologist* 188.1, p. 223–241 (cité p. 58).

Ordóñez, J. L., R. Molowny-Horas et J. Retana (2006). « A model of the recruitment of *Pinus nigra* from unburned edges after large wildfires ». *Ecological Modelling* 197 (3–4), p. 405–417 (cité p. 71).

Ottosson, E., A. Kubartova, M. Edman, M. Jonsson, A. Lindhe, J. Stenlid et A. Dahlberg (2015). « Diverse ecological roles within fungal communities in decomposing logs of *Picea abies* ». *FEMS Microbiology Ecology* 91.3 (cité p. 50, 54, 270, 271).

Ottosson, E., J. Nordén, A. Dahlberg, M. Edman, M. Jönsson, K.-H. Larsson, J. Olsson, R. Penttilä, J. Stenlid et O. Ovaskainen (2014). « Species associations during the succession of wood-inhabiting fungal communities ». *Fungal Ecology* 11, p. 17–28 (cité p. 270).

Paillet, Y. et coll. (2010). « Biodiversity differences between managed and unmanaged forests : meta-analysis of species richness in Europe ». *Conservation Biology* 24.1, p. 101–112 (cité p. 52).

Paine, R. T. (1980). « Food webs : linkage, interaction strength and community infrastructure ». *The Journal of Animal Ecology*, p. 667–685 (cité p. 60).

Panaiotis, C., C. Carcaillet et M. M'Hamedi (1997). « Determination of the natural mortality age of holm oak (*Quercus ilex* L.) stand in Corsica (Mediterranean Island) ». *Acta Oecologica* 18.5, p. 519–530 (cité p. 224, 267).

Panaiotis, C., R. Loisel et M. Roux (1998). « Analyse de la réponse de la végétation aux trouées naturelles dans une futaie âgée de *Quercus ilex* L. en Corse (île Méditerranéenne) ». *Canadian Journal of Forest Research* 28.8, p. 1125–1134 (cité p. 267).

Parmasto, E. (2001). Fungi as indicators of primeval and old-growth forests deserving protection. *Fungal conservation, issues and solutions*. Cambridge University Press, p. 81–88 (cité p. 16).

Parrent, J. L. et R. Vilgalys (2007). « Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO2 and nitrogen fertilization ». *New Phytologist* 176.1, p. 164–174 (cité p. 33).

Partida-Martinez, L. P. P. et M. Heil (2011). « The microbe-free plant : fact or artifact ? ». *Frontiers in Plant Science* 2 (cité p. 43).

Pausas, J. G. (2015). « Evolutionary fire ecology : lessons learned from pines ». *Trends in Plant Science* 20.5, p. 318–324 (cité p. 65, 71).

Pausas, J. G. et S. Fernández-Muñoz (2012). « Fire regime changes in the Western Mediterranean basin : from fuel-limited to drought-driven fire regime ». *Climatic Change* 110 (1-2), p. 215–226 (cité p. 66).

Pausas, J. G. et J. E. Keeley (2009). « A burning story : the role of fire in the history of life ». *BioScience* 59.7, p. 593–601 (cité p. 65).

Pausas, J. G., J. Llovet, A. Rodrigo et R. Vallejo (2008). « Are wildfires a disaster in the Mediterranean Basin ? –A review ». *International Journal of Wildland Fire* 17.6, p. 713 (cité p. 71).

Pausas, J. G., R. B. Pratt, J. E. Keeley, A. L. Jacobsen, A. R. Ramirez, A. Vilagrosa, S. Paula, I. N. Kaneakua-Pia et S. D. Davis (2016). « Towards understanding resprouting at the global scale ». *New Phytologist* 209.3, p. 945–954 (cité p. 65, 279).

Peay, K. G., M. I. Bidartondo et A. Elizabeth Arnold (2010). « Not every fungus is everywhere : scaling to the biogeography of fungal–plant interactions across roots, shoots and ecosystems ». *New Phytologist* 185.4, p. 878–882 (cité p. 39).

Peay, K. G. et T. D. Bruns (2014). « Spore dispersal of basidiomycete fungi at the landscape scale is driven by stochastic and deterministic processes and generates variability in plant-fungal interactions ». *New Phytologist* 204.1, p. 180–191 (cité p. 39).

Peay, K. G., T. D. Bruns, P. G. Kennedy, S. E. Bergemann et M. Garbelotto (2007). « A strong species-area relationship for eukaryotic soil microbes : island size matters for ectomycorrhizal fungi ». *Ecology Letters* 10.6, p. 470–480 (cité p. 39, 44).

Peay, K. G., P. G. Kennedy et T. D. Bruns (2008). « Fungal community ecology : a hybrid beast with a molecular master ». *BioScience* 58.9, p. 799–810 (cité p. 11, 15, 57, 264).

Peay, K. G., P. G. Kennedy et J. M. Talbot (2016). « Dimensions of biodiversity in the Earth mycobiome ». *Nature Reviews Microbiology* 14.7, p. 434–447 (cité p. 314, 316).

Peay, K. G., M. G. Schubert, N. H. Nguyen et T. D. Bruns (2012). « Measuring ectomycorrhizal fungal dispersal : macroecological patterns driven by microscopic propagules ». *Molecular Ecology* 21.16, p. 4122–4136 (cité p. 33, 39, 40, 44).

Pepper, J. W. et M. D. Herron (2008). « Does biology need an organism concept ? ». *Biological Reviews* 83.4, p. 621–627 (cité p. 277).

Pickles, B. J., M. A. Gorzelak, D. S. Green, K. N. Egger et H. B. Massicotte (2015). « Host and habitat filtering in seedling root-associated fungal communities : taxonomic and functional diversity are altered in ‘novel’ soils ». *Mycorrhiza* 25.7, p. 517–531 (cité p. 40).

Pimm, S. L., J. H. Lawton et J. E. Cohen (1991). « Food web patterns and their consequences ». *Nature* 350.6320, p. 669–674 (cité p. 60).

Pocheville, A. (2009). Chap 26 : La niche écologique : Histoire et controverses récentes. *Les mondes darwiniens*. Réd. par T. Heams, P. Huneman, G. Lecointre et M. Silberstein. Éditions Matériologiques. T. 1. Sciences & philosophie. Paris, p. 734 (cité p. 20).

Poisot, T., E. Canard, N. Mouquet et M. E. Hochberg (2012). « A comparative study of ecological specialization estimators ». *Methods in Ecology and Evolution* 3.3, p. 537–544 (cité p. 61).

Porras-Alfaro, A. et P. Bayman (2011). « Hidden fungi, emergent properties : endophytes and microbiomes ». *Annual Review of Phytopathology* 49.1, p. 291–315 (cité p. 44, 282).

Pringle, A., J. D. Bever, M. Gardes, J. L. Parrent, M. C. Rillig et J. N. Klironomos (2009). « Mycorrhizal symbioses and plant invasions ». *Annual Review of Ecology, Evolution, and Systematics* 40.1, p. 699–715 (cité p. 31, 34).

Puillandre, N., A. Lambert, S. Brouillet et G. Achaz (2012). « ABGD, Automatic Barcode Gap Discovery for primary species delimitation ». *Molecular Ecology* 21.8, p. 1864–1877 (cité p. 58).

Queloz, V., T. N. Sieber, O. Holdenrieder, B. A. McDonald et C. R. Grünig (2011). « No biogeographical pattern for a root-associated fungal species complex ». *Global Ecology and Biogeography* 20.1, p. 160–169 (cité p. 44, 266).

Raffaelli, D. (2007). Food Webs, Body Size and the Curse of the Latin Binomial. *From Energetics to Ecosystems : The Dynamics and Structure of Ecological Systems*. Sous la dir. de N. Rooney, K. S. McCann et D. L. G. Noakes. Springer Netherlands, p. 53–64 (cité p. 36, 311).

Rajala, T., M. Peltoniemi, T. Pennanen et R. Mäkipää (2012). « Fungal community dynamics in relation to substrate quality of decaying Norway spruce (*Picea abies* [L.] Karst.) logs in boreal forests ». *FEMS Microbiology Ecology* 81.2, p. 494–505 (cité p. 53, 54, 270, 271, 276).

Rameau, J.-C., D. Mansion, G. Dumé et C. Gauberville (2008). *Flore forestière française Tome 3*. Paris : Institut pour le développement forestier. 2432 p. (cité p. 66).

Rastogi, G., G. L. Coaker et J. H. J. Leveau (2013). « New insights into the structure and function of phyllosphere microbiota through high-throughput molecular approaches ». *FEMS Microbiology Letters* 348.1, p. 1–10 (cité p. 44).

Rayner, A. D. et L. Boddy (1988). *Fungal Decomposition of Wood. Its Biology and Ecology*. John Wiley & Sons Ltd. 587 p. (cité p. 53).

Rayner, M. C. (1926). « Mycorrhiza ». *New Phytologist* 25.1, p. 1–50 (cité p. 25).

— (1927). « Mycorrhiza ». *New Phytologist* 26.2, p. 85–114 (cité p. 25).

Read, D. J. et J. Perez-Moreno (2003). « Mycorrhizas and nutrient cycling in ecosystems –a journey towards relevance ? ». *New Phytologist* 157.3, p. 475–492 (cité p. 33).

Reinhart, K. O. et R. M. Callaway (2006). « Soil biota and invasive plants ». *New Phytologist* 170.3, p. 445–457 (cité p. 35).

Remy, W., T. N. Taylor, H. Hass et H. Kerp (1994). « Four hundred-million-year-old vesicular arbuscular mycorrhizae ». *Proceedings of the National Academy of Sciences* 91.25, p. 11841–11843 (cité p. 30).

Richard, F., J.-M. Bellanger, P. Clowez, K. Hansen, K. O'Donnell, A. Urban, M. Sauve, R. Courtecuisse et P.-A. Moreau (2015). « True morels (*Morchella*, Pezizales) of Europe and North America : evolutionary relationships inferred from multilocus data and a unified taxonomy ». *Mycologia* 107.2, p. 359–382 (cité p. 280).

Richard, F., S. Millot, M. Gardes et M.-A. Selosse (2005). « Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex* ». *New Phytologist* 166.3, p. 1011–1023 (cité p. 36).

Richard, F., M.-A. Selosse et M. Gardes (2009). « Facilitated establishment of *Quercus ilex* in shrub-dominated communities within a Mediterranean ecosystem : do mycorrhizal partners matter ? ». *FEMS Microbiology Ecology* 68.1, p. 14–24 (cité p. 36, 106, 224).

Richards, T. A., M. D. Jones, G. Leonard et D. Bass (2012). « Marine fungi : Their ecology and molecular diversity ». *Annual Review of Marine Science* 4.1, p. 495–522 (cité p. 10).

Rillig, M. C. et D. L. Mummey (2006). « Mycorrhizas and soil structure ». *New Phytologist* 171.1, p. 41–53 (cité p. 31).

Robinson, D. et A. Fitter (1999). « The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network ». *Journal of Experimental Botany* 50.330, p. 9–13 (cité p. 38).

Rochet, J., P.-A. Moreau, S. Manzi et M. Gardes (2011). « Comparative phylogenies and host specialization in the alder ectomycorrhizal fungi *Alnicola*, *Alpova* and *Lactarius* (Basidiomycota) in Europe ». *BMC evolutionary biology* 11.1, p. 40 (cité p. 32).

Rodriguez, R. J., J. F. White Jr, A. E. Arnold et R. S. Redman (2009). « Fungal endophytes : diversity and functional roles ». *New Phytologist* 182.2, p. 314–330 (cité p. 44, 315).

Romani, A. M., H. Fischer, C. Mille-Lindblom et L. J. Tranvik (2006). « Interactions of bacteria and fungi on decomposing litter : differential extracellular enzyme activities ». *Ecology* 87.10, p. 2559–2569 (cité p. 49).

Rousseau, J. V. D., D. M. Sylvia et A. J. Fox (1994). « Contribution of ectomycorrhiza to the potential nutrient-absorbing surface of pine ». *New Phytologist* 128.4, p. 639–644 (cité p. 33).

Roy, M., M.-P. Dubois, M. Proffit, L. Vincenot, E. Desmarais et M.-A. Selosse (2008). « Evidence from population genetics that the ectomycorrhizal basidiomycete *Laccaria amethystina* is an actual multihost symbiont ». *Molecular Ecology* 17.12, p. 2825–2838 (cité p. 17, 33, 34).

Roy-Bolduc, A., E. Laliberté et M. Hijri (2016). « High richness of ectomycorrhizal fungi and low host specificity in a coastal sand dune ecosystem revealed by network analysis ». *Ecology and Evolution* 6.1, p. 349–362 (cité p. 61, 63).

Rubini, A., B. Belfiori, V. Passeri, L. Baciarelli Falini, S. Arcioni, C. Riccioni et F. Paolocci (2011). « The AD-type ectomycorrhizas, one of the most common morphotypes present in truffle fields, result from fungi belonging to the *Trichophaea woolhopeia* species complex ». *Mycorrhiza* 21.1, p. 17–25 (cité p. 27).

Ryberg, M., E. Kristiansson, E. Sjökvist et R. H. Nilsson (2009). « An outlook on the fungal internal transcribed spacer sequences in GenBank and the introduction of a web-based tool for the exploration of fungal diversity ». *New Phytologist* 181.2, p. 471–477 (cité p. 57).

San-Miguel-Ayanz, J., J. M. Moreno et A. Camia (2013). « Analysis of large fires in European Mediterranean landscapes : Lessons learned and perspectives ». *Forest Ecology and Management* 294, p. 11–22 (cité p. 66).

Scarascia-Mugnozza, G., H. Oswald, P. Piussi et K. Radoglou (2000). « Forests of the Mediterranean region : gaps in knowledge and research needs ». *Forest Ecology and Management* 132.1, p. 97–109 (cité p. 66).

Schigel, D. S. (2012). « Fungivory and host associations of Coleoptera : a bibliography and review of research approaches ». *Mycology* 3.4, p. 258–272 (cité p. 16, 17).

Schloss, P. D. et coll. (2009). « Introducing mothur : open-source, platform-independent, community-supported software for describing and comparing microbial communities ». *Applied and Environmental Microbiology* 75.23, p. 7537–7541 (cité p. 99).

Schoch, C. L., K. A. Seifert, S. Huhndorf, V. Robert, J. L. Spouge, C. A. Levesque, W. Chen, E. Bolchacova, K. Voigt et P. W. Crous (2012). « Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi ». *Proceedings of the National Academy of Sciences* 109.16, p. 6241–6246 (cité p. 26, 58, 315).

Scholz, M. B., C.-C. Lo et P. S. Chain (2012). « Next generation sequencing and bioinformatic bottlenecks : the current state of metagenomic data analysis ». *Current Opinion in Biotechnology* 23.1, p. 9–15 (cité p. 314).

Schweitzer, J. A., J. K. Bailey, D. G. Fischer, C. J. LeRoy, E. V. Lonsdorf, T. G. Whitham et S. C. Hart (2008). « Plant–soil–microorganism interactions : heritable relationship between plant genotype and associated soil microorganisms ». *Ecology* 89.3, p. 773–781 (cité p. 51).

Selosse, M.-A. et F. Le Tacon (1998). « The land flora : a phototroph-fungus partnership ? ». *Trends in Ecology & Evolution* 13.1, p. 15–20 (cité p. 29).

Selosse, M.-A. (2000). *La symbiose*. Paris : Vuibert. 154 p. (cité p. 17, 30).

Selosse, M.-A., F. Martin et F. Le Tacon (2001). « Intraspecific variation in fruiting phenology in an ectomycorrhizal *Laccaria* population under Douglas fir ». *Mycological Research* 105.5, p. 524–531 (cité p. 279).

Selosse, M.-A., F. Richard, X. He et S. W. Simard (2006). « Mycorrhizal networks : des liaisons dangereuses ? ». *Trends in Ecology & Evolution* 21.11, p. 621–628 (cité p. 15, 36).

Selosse, M.-A. et M. Roy (2009). « Green plants that feed on fungi : facts and questions about mixotrophy ». *Trends in Plant Science* 14.2, p. 64–70 (cité p. 38).

Selosse, M.-A., L. Vincenot et M. Öpik (2016). « Data processing can mask biology : towards better reporting of fungal barcoding data ? ». *New Phytologist* 210.4, p. 1159–1164 (cité p. 58).

Selosse, M.-A., M. Vohnik et E. Chauvet (2008). « Out of the rivers : are some aquatic hyphomycetes plant endophytes ? ». *New Phytologist* 178.1, p. 3–7 (cité p. 272).

Shah, F. et coll. (2015). « Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors ». *New Phytologist* 209.4, p. 1705–1719 (cité p. 34, 273).

Shahin, O., N. M.-S. Paul, S. Rambal, R. Joffre et F. Richard (2013). « Ectomycorrhizal fungal diversity in *Quercus ilex* Mediterranean woodlands : variation among sites and over soil depth profiles in hyphal exploration types, species richness and community composition ». *Symbiosis* 61.1, p. 1–12 (cité p. 36, 40, 87).

Sheil, D. et D. F. Burslem (2013). « Defining and defending Connell's intermediate disturbance hypothesis : a response to Fox ». *Trends in Ecology & Evolution* 28.10, p. 571–572 (cité p. 268).

Shokralla, S., J. L. Spall, J. F. Gibson et M. Hajibabaei (2012). « Next-generation sequencing technologies for environmental DNA research ». *Molecular Ecology* 21.8, p. 1794–1805 (cité p. 58).

Siddique, A. B. et M. Unterseher (2016). « A cost-effective and efficient strategy for Illumina sequencing of fungal communities : A case study of beech endophytes identified elevation as main explanatory factor for diversity and community composition ». *Fungal Ecology* 20, p. 175–185 (cité p. 46).

Sieber, T. N. (2007). « Endophytic fungi in forest trees : are they mutualists ? ». *Fungal Biology Reviews* 21 (2-3), p. 75–89 (cité p. 44).

Siefert, A. et coll. (2015). « A global meta-analysis of the relative extent of intraspecific trait variation in plant communities ». *Ecology Letters* 18.12, p. 1406–1419 (cité p. 279).

Silvertown, J. (2004). « Plant coexistence and the niche ». *Trends in Ecology & Evolution* 19.11, p. 605–611 (cité p. 21, 52, 316).

Simard, S. W., K. J. Beiler, M. A. Bingham, J. R. Deslippe, L. J. Philip et F. P. Teste (2012). « Mycorrhizal networks : Mechanisms, ecology and modelling ». *Fungal Biology Reviews* 26.1, p. 39–60 (cité p. 38, 106, 274).

Simard, S. W. et D. M. Durall (2004). « Mycorrhizal networks : a review of their extent, function, and importance ». *Canadian Journal of Botany* 82.8, p. 1140–1165 (cité p. 38).

Simard, S. W., D. A. Perry, M. D. Jones, D. D. Myrold, D. M. Durall et R. Molina (1997). « Net transfer of carbon between ectomycorrhizal tree species in the field ». *Nature* 388.6642, p. 579–582 (cité p. 25, 26, 31, 38, 39, 62).

Smith, M. E., T. W. Henkel et J. A. Rollins (2015). « How many fungi make sclerotia ? ». *Fungal Ecology* 13, p. 211–220 (cité p. 31, 33).

Smith, M. L., J. N. Bruhn et J. B. Anderson (1992). « The fungus *Armillaria bulbosa* is among the largest and oldest living organisms ». *Nature* 356.6368, p. 428–431 (cité p. 10).

Smith, S. E. et D. J. Read (2008). *Mycorrhizal Symbiosis.* 3rd. Academic Press. 800 p. (cité p. 17, 26, 29–34, 40).

Soberón, J. (2007). « Grinnellian and Eltonian niches and geographic distributions of species ». *Ecology Letters* 10.12, p. 1115–1123 (cité p. 20).

Soulères, O. (2000). « Les incendies de Haute-Corse ». *Revue Forestière Française* 5, p. 401–406 (cité p. 71).

Staniczenko, P. P. A., J. C. Kopp et S. Allesina (2013). « The ghost of nestedness in ecological networks ». *Nature Communications* 4, p. 1391 (cité p. 318).

Steidinger, B. S. et J. D. Bever (2014). « The coexistence of hosts with different abilities to discriminate against cheater partners : an evolutionary game-theory approach ». *The American Naturalist* 183.6, p. 762–770 (cité p. 32).

Sueur, C. (2015). *Analyse Des Réseaux Sociaux Appliquée À L'éthologie et L'écologie.* 1re éd. Modélisations, simulations, systèmes complexes. Paris : Éditions Matériologiques. 514 p. (cité p. 60).

Suz, L. M. et coll. (2015). « Monitoring ectomycorrhizal fungi at large scales for science, forest management, fungal conservation and environmental policy ». *Annals of Forest Science* 72.7, p. 877–885 (cité p. 16).

Talbot, J. M., S. D. Allison et K. K. Treseder (2008). « Decomposers in disguise : mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change ». *Functional Ecology* 22.6, p. 955–963 (cité p. 31).

Tardieu, C. (2011). « La bonne orthographe du mot taxinomie ». *PALEO. Revue d'archéologie préhistorique* 22, p. 331–334 (cité p. 10).

Taschen, E., F. Rousset, M. Sauve, L. Benoit, M.-P. Dubois, R. F et M. .-.-A. Selosse (2016). « How the truffle got its mate : insights from genetic structure in spontaneous and planted Mediterranean populations of Tuber melanosporum ». *Molecular Ecology*, n/a–n/a (cité p. 269).

Taschen, E., M. Sauve, A. Taudiere, J. Parlade, M.-A. Selosse et F. Richard (2015). « Whose truffle is this ? Distribution patterns of ectomycorrhizal fungal diversity in *Tuber melanosporum* brûlés developed in multi-host Mediterranean plant communities ». *Environmental Microbiology* 17.8, p. 2747–2761 (cité p. 276).

TAUDIERE, A., F. MUNOZ, A. LESNE, A.-C. MONNET, J.-M. BELLANGER, M.-A. SELOSSE, P.-A. MOREAU et F. RICHARD (2015). « Beyond ectomycorrhizal bipartite networks : projected networks demonstrate contrasted patterns between early- and late-successional plants in Corsica ». *Frontiers in Plant Science* 6 (cité p. 33, 38, 39, 61, 274, 275).

TAUDIERE, A. et C. VIOLLE (2016). « Cati : an R package using functional traits to detect and quantify multi-level community assembly processes ». *Ecography* 39.7, p. 699–708 (cité p. 21).

TAYLOR, A. F. (2002). Fungal diversity in ectomycorrhizal communities : sampling effort and species detection. *Diversity and Integration in Mycorrhizas.* Springer, p. 19–28 (cité p. 33).

TAYLOR, D. L., T. N. HOLLINGSWORTH, J. W. MCFARLAND, N. J. LENNON, C. NUSBAUM et R. W. RUESS (2014). « A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning ». *Ecological Monographs* 84.1, p. 3–20 (cité p. 40, 52).

TAYLOR, T. N., H. KERP et H. HASS (2005). « Life history biology of early land plants : deciphering the gametophyte phase ». *Proceedings of the National Academy of Sciences of the United States of America* 102.16, p. 5892–5897 (cité p. 30).

TEDERSOO, L., T. JAIRUS, B. M. HORTON, K. ABARENKOV, T. SUVI, I. SAAR et U. KÕLJALG (2008). « Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers ». *New Phytologist* 180.2, p. 479–490 (cité p. 57).

TESTE, F. P., S. W. SIMARD et D. M. DURALL (2009). « Role of mycorrhizal networks and tree proximity in ectomycorrhizal colonization of planted seedlings ». *Fungal Ecology* 2.1, p. 21–30 (cité p. 36).

THIBAULT, J.-C., A. CIBOIS, R. PRODON et E. PASQUET (2016). « Quaternary history of an endemic passerine bird on Corsica Island : Glacial refugium and impact of recent forest regression ». *Quaternary Research* 85.2, p. 271–278 (cité p. 67).

THIET, R. K. et R. E. J. BOERNER (2007). « Spatial patterns of ectomycorrhizal fungal inoculum in arbuscular mycorrhizal barrens communities : implications for controlling invasion by *Pinus virginiana* ». *Mycorrhiza* 17.6, p. 507–517 (cité p. 35).

THOMAS, F., T. LEFEVRE et M. RAYMOND (2010). *Biologie évolutive.* Bruxelles : De Boeck (cité p. 14, 32).

TOJU, H., P. R. GUIMARAES, J. M. OLESEN et J. N. THOMPSON (2015). « Below-ground plant-fungus network topology is not congruent with above-ground plant-animal network topology ». *Science Advances* 1.9, e1500291–e1500291 (cité p. 63, 276).

TORRECILLAS, E., M. del MAR ALGUACIL, A. ROLDÁN, G. DIAZ, A. MONTESINOS-NAVARRO et M. P. TORRES (2014). « Modularity reveals the tendency of arbuscular mycorrhizal fungi to interact differently with generalist and specialist plant species in gypsum soils ». *Applied and Environmental Microbiology* 80.17, p. 5457–5466 (cité p. 276).

TORRES, P. et M. HONRUBIA (1997). « Changes and effects of a natural fire on ectomycorrhizal inoculum potential of soil in a *Pinus halepensis* forest ». *Forest Ecology and Management* 96.3, p. 189–196 (cité p. 280).

TRAPPE, J. M., A. O. NICHOLLS, A. W. CLARIDGE et S. J. CORK (2006). « Prescribed burning in a *Eucalyptus* woodland suppresses fruiting of hypogeous fungi, an important food source for mammals ». *Mycological Research* 110, p. 1333–1339 (cité p. 275).

TRESEDER, K. K. et J. T. LENNON (2015). « Fungal traits that drive ecosystem dynamics on land ». *Microbiology and Molecular Biology Reviews* 79.2, p. 243–262 (cité p. 278).

UNTERSEHER, M., D. PERŠOH et M. SCHNITTLER (2013). « Leaf-inhabiting endophytic fungi of European Beech (*Fagus sylvatica* L.) co-occur in leaf litter but are rare on decaying wood of the same host ». *Fungal Diversity* 60.1, p. 43–54 (cité p. 46).

U'REN, J. M., F. LUTZONI, J. MIADLIKOWSKA, A. D. LAETSCH et A. E. ARNOLD (2012). « Host and geographic structure of endophytic and endolichenic fungi at a continental scale ». *American Journal of Botany* 99.5, p. 898–914 (cité p. 44).

U'REN, J. M., J. M. RIDDLE, J. T. MONACELL, I. CARBONE, J. MIADLIKOWSKA et A. E. ARNOLD (2014). « Tissue storage and primer selection influence pyrosequencing-based inferences of diversity and community composition of endolichenic and endophytic fungi ». *Molecular Ecology Resources* 14.5, p. 1032–1048 (cité p. 46).

USHIO, M., R. WAGAI, T. C. BALSER et K. KITAYAMA (2008). « Variations in the soil microbial community composition of a tropical montane forest ecosystem : Does tree species matter ? ». *Soil Biology and Biochemistry* 40.10, p. 2699–2702 (cité p. 51).

Valentini, A., F. Pompanon et P. Taberlet (2009). « DNA barcoding for ecologists ». *Trends in Ecology & Evolution* 24.2, p. 110–117 (cité p. 314).

Van der Heijden, M. G. A. et T. R. Horton (2009). « Socialism in soil ? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems ». *Journal of Ecology* 97.6, p. 1139–1150 (cité p. 31, 36, 39).

Van der Heijden, M. G. A., F. M. Martin, M.-A. Selosse et I. R. Sanders (2015). « Mycorrhizal ecology and evolution : the past, the present, and the future ». *New Phytologist* 205.4, p. 1406–1423 (cité p. 30–33, 38).

Vandenkoornhuyse, P., A. Quaiser, M. Duhamel, A. Le Van et A. Dufresne (2015). « The importance of the microbiome of the plant holobiont ». *New Phytologist* 206.4, p. 1196–1206 (cité p. 43).

Varese, G. C. et coll. (2011). « *Ex Situ* conservation and exploitation of fungi in Italy ». *Plant Biosystems* 145.4, p. 997–1005 (cité p. 16).

Varnaitė, R., A. Paškevičius et V. Raudonienė (2008). « Cellulose degradation in rye straw by micromycetes and their complexes ». *Ekologija* 54.1, p. 29–31 (cité p. 224).

Vazquez, D. P., N. Bluthgen, L. Cagnolo et N. P. Chacoff (2009). « Uniting pattern and process in plant-animal mutualistic networks : a review ». *Annals of Botany* 103.9, p. 1445–1457 (cité p. 60).

Vellend, M. (2010). « Conceptual synthesis in community ecology ». *The Quarterly Review of Biology* 85.2, p. 183–206 (cité p. 19, 22–24, 43, 45, 262, 275, 281).

Vellend, M. et coll. (2014). « Assessing the relative importance of neutral stochasticity in ecological communities ». *Oikos* 123.12, p. 1420–1430 (cité p. 280, 281).

Vernes, K., M. Castellano et C. N. Johnson (2001). « Effects of season and fire on the diversity of hypogeous fungi consumed by a tropical mycophagous marsupial ». *Journal of Animal Ecology* 70.6, p. 945–954 (cité p. 275).

Vincenot, L., K. Nara, C. Sthultz, J. Labbé, M.-P. Dubois, L. Tedersoo, F. Martin et M.-A. Selosse (2012). « Extensive gene flow over Europe and possible speciation over Eurasia in the ectomycorrhizal basidiomycete "*Laccaria amethystina*" complex ». *Molecular Ecology* 21.2, p. 281–299 (cité p. 269).

Vincent, J. B., G. D. Weiblen et G. May (2016). « Host associations and beta diversity of fungal endophyte communities in New Guinea rainforest trees ». *Molecular Ecology* 25.3, p. 825–841 (cité p. 46).

Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung et J. Messier (2012). « The return of the variance : intraspecific variability in community ecology ». *Trends in Ecology & Evolution* 27.4, p. 244–252 (cité p. 21, 22, 279).

Violle, C., M.-L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel et E. Garnier (2007). « Let the concept of trait be functional ! ». *Oikos* 116.5, p. 882–892 (cité p. 36, 278).

Volis, S. et M. Blecher (2010). « Quasi in situ : a bridge between ex situ and in situ conservation of plants ». *Biodiversity and Conservation* 19.9, p. 2441–2454 (cité p. 15).

Vorholt, J. A. (2012). « Microbial life in the phyllosphere ». *Nature Reviews Microbiology* 10.12, p. 828–840 (cité p. 44).

Wallander, H. (2000). « Uptake of P from apatite by *Pinus sylvestris* seedlings colonised by different ectomycorrhizal fungi ». *Plant and Soil* 218 (1-2), p. 249–256 (cité p. 279).

Wallander, H., H. Göransson et U. Rosengren (2004). « Production, standing biomass and natural abundance of 15N and 13C in ectomycorrhizal mycelia collected at different soil depths in two forest types ». *Oecologia* 139.1, p. 89–97 (cité p. 33).

Wallander, H. et coll. (2013). « Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils –A review ». *Soil Biology and Biochemistry* 57, p. 1034–1047 (cité p. 11).

Wan, S., D. Hui et Y. Luo (2001). « Fire effects on nitrogen pools and dynamics in terrestrial ecosystems : a meta-analysis ». *Ecological Applications* 11.5, p. 1349–1365 (cité p. 65).

Wang, B. et Y.-L. Qiu (2006). « Phylogenetic distribution and evolution of mycorrhizas in land plants ». *Mycorrhiza* 16.5, p. 299–363 (cité p. 29, 30).

Wang, Q., G. M. Garrity, J. M. Tiedje et J. R. Cole (2007). « Naıve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy ». *Applied and Environmental Microbiology* 73.16, p. 5261–5267 (cité p. 99).

Weiher, E. et P. Keddy (2001). *Ecological Assembly Rules : Perspectives, Advances, Retreats.* Cambridge University Press. 440 p. (cité p. 20, 281).

White, T. J., T. Bruns, S. Lee et J. W. Taylor (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Innis, M.A., Gelfand, DH, Sninsky, JJ et White, TJ. *PCR protocols : A guide to methods and applications.* T. 18. New York : Academic Press, Inc., p. 315–322 (cité p. 57, 276, 315).

Whitfield, J. (2007). « Fungal roles in soil ecology : underground networking ». *Nature* 449.7159, p. 136–138 (cité p. 35).

Whittaker, R. H. (1969). « New concepts of kingdoms of organisms ». *Science* 163.3863, p. 150–160 (cité p. 9).

Wiens, J. A. (1989). « Spatial scaling in ecology ». *Functional ecology* 3.4, p. 385–397 (cité p. 274).

Wolfe, B. E., D. L. Mummey, M. C. Rillig et J. N. Klironomos (2007). « Small-scale spatial heterogeneity of arbuscular mycorrhizal fungal abundance and community composition in a wetland plant community ». *Mycorrhiza* 17.3, p. 175–183 (cité p. 44).

Wolfe, B. E. et A. Pringle (2012). « Geographically structured host specificity is caused by the range expansions and host shifts of a symbiotic fungus ». *The ISME journal* 6.4, p. 745–755 (cité p. 34).

Wolfe, B. E., F. Richard, H. B. Cross et A. Pringle (2010). « Distribution and abundance of the introduced ectomycorrhizal fungus *Amanita phalloides* in North America ». *New Phytologist* 185.3, p. 803–816 (cité p. 34).

Wolfe, B. E., V. L. Rodgers, K. A. Stinson et A. Pringle (2008). « The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range ». *Journal of Ecology* 96.4, p. 777–783 (cité p. 35).

Wolfe, B. E., R. E. Tulloss et A. Pringle (2012). « The irreversible loss of a decomposition pathway marks the single origin of an ectomycorrhizal symbiosis ». *PLoS ONE* 7.7, e39597 (cité p. 35).

Wu, Q. (2000). « Phylogenetic and biogeographic relationships of eastern Asian and eastern north American disjunct *Suillus* species (fungi) as inferred from nuclear ribosomal RNA ITS sequences ». *Molecular Phylogenetics and Evolution* 17.1, p. 37–47 (cité p. 34).

Wu, T. (2011). « Can ectomycorrhizal fungi circumvent the nitrogen mineralization for plant nutrition in temperate forest ecosystems ? ». *Soil Biology and Biochemistry* 43.6, p. 1109–1117 (cité p. 31).

Yang, H., Q. Zhang, R. T. Koide, J. D. Hoeksema, J. Tang, X. Bian, S. Hu et X. Chen (2016a). « Taxonomic resolution is a determinant of biodiversity effects in arbuscular mycorrhizal fungal communities ». *Journal of Ecology* (cité p. 46).

Yang, T., P. Weisenhorn, J. A. Gilbert, Y. Ni, R. Sun, Y. Shi et H. Chu (2016b). « Carbon constrains fungal endophyte assemblages along the timberline ». *Environmental Microbiology* 18.8, p. 2455–2469 (cité p. 45).

Yuan, Z. et L. Chen (2014). « The role of endophytic fungal individuals and communities in the decomposition of *Pinus massoniana* needle litter ». *PLOS ONE* 9.8, e105911 (cité p. 273).

Zhang, Z.-Z., Y.-G. Lou, D.-J. Deng, M. M. Rahman et Q.-S. Wu (2015). « Effects of common mycorrhizal network on plant carbohydrates and soil properties in trifoliate orange–white clover association ». *PLoS ONE* 10.11, e0142371 (cité p. 46).

Zhou, D. et K. D. Hide (2001). « Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi ». *Mycological Research* 105.12, p. 1449–1457 (cité p. 51).

Zimmerman, N. B. et P. M. Vitousek (2012). « Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape ». *Proceedings of the National Academy of Sciences* 109.32, p. 13022–13027 (cité p. 45, 46).

* * *

Liste des logiciels et ressources en ligne utilisés

Traitement de texte : Latex, Kile, TeXworks

Dessins, clip-arts et photos : Inkscape, Gephi, Interactive Tree Of Life, Digikam, Ilona34

Bioinformatique : Virtualbox avec la distribution Biolinux (basée sur Ubuntu)

Analyses statistiques et développement logiciel : R, Rstudio, Github

Bibliographie : Zotero, Jabref

Dictionnaires en ligne (sens, étymologie et synonymie) : Le grand dictionnaire terminologique, Centre de Recherche Inter-langues sur la Signification en COntexte (CRISCO), Centre National de Ressources Textuelles et Lexicales (CNRTL), Le wiktionnaire

Taxinomie des champignons : MycoBank

Liste des abréviations

c. : Du latin *circa* ; à peu près, environ

c.-à-d. : c'est à dire ; ***i.e.*** en anglais

🅭 : Licence *creative commons*

CEF - FEF : Champignons Endophytiques Foliaires – *Foliar Endophitic Fungi*

cf. : Du latin *Confer* ; se reporter à, comparer à. Utiliser uniquement dans les parties en anglais. Nous lui avons préféré l'utilisation du mot « voir » en français.

com. pers. : communication personnelle

def. : Définition

ECM : Ectomycorhize ; par extension fait office d'adjectif : ectomycorhizien(ne(s)).

et coll. - ***et al.*** : et collaborateurs – *and collaborators*

Fig. : Figure

ha : Hectare

ITS : De l'anglais *Internal Transcribed Spacer* ; voir glossaire et encadré 5

NGS : De l'anglais *Next-Generation Sequencing*. Méthodes de séquençage à haut débit, voir glossaire.

OTU : De l'anglais *Operational Taxonomic Unit*. Se reporter au glossaire et à la section bioinformatique.

par ex. : par exemple ; **e.g.** en anglais

RFLP : De l'anglais *Restriction fragment length polymorphism*. Méthode moléculaire d'identification grâce au polymorphisme des longueurs de fragments de restriction suite à une digestion enzymatique.

SF : *Saprobic Fungi*

W : Wikipédia

* * *

Table des figures

Liste des tableaux

Liste des encadrés

* * *

Chapitre VIII

Annexes

> « *Pour la plupart des gens, la Corse est la terre natale d'un empereur qui a laissé dans l'Histoire des pages aussi indélébiles que celles inspirées par notre vieux complice Jules César.*
>
> *C'est aussi le berceau d'un chanteur de charme à la longue et prestigieuse carrière, dont les refrains où il est question de Marinella et d'une belle Catarineta, tchi tchi, ont fait le tour du monde.*
>
> *C'est aussi le pays de la vendetta, de la sieste, des jeux politiques compliqués, des fromages vigoureux, des cochons sauvages, des châtaignes, des succulents merles moqueurs et des vieillards sans âge qui regardent passer la vie.*
>
> *Mais la Corse, c'est plus que tout cela. Elle fait partie de ces endroits privilégiés du globe qui ont un caractère, une forte personnalité, que ni le temps ni les hommes n'arrivent à entamer.*
>
> *C'est un des plus beaux pays du monde, qui justifie pleinement son appellation d'île de Beauté.*
>
> *Mais pourquoi ce préambule, nous demanderez-vous. Parce que les Corses, que l'on décrit comme individualistes — alliant l'exubérance à la maîtrise de soi — nonchalants, hospitaliers, loyaux, fidèles en amitié, attachés à leurs pays natal, éloquents et courageux, sont, eux aussi, plus que tout cela.*
>
> *Ils sont susceptibles.* »
>
> René Goscinny & Albert Uderzo, *Asterix en Corse – Préambule à la version française*, 1973

Glossaire thématique

- Biologie moléculaire et bioinformatique
- Champignon
- Écologie
- Feu
- Forêt et végétation
- Mycorhize
- Réseau

Biologie moléculaire et bioinformatique

Abondance moléculaire : Nombre de séquences d'une OTU dans un échantillon. Le passage de l'abondance moléculaire à l'abondance réelle (nombre d'individu ou biomasse) est compliqué en raison des biais de séquençages. De nombreux travaux de séquençage de communautés connues ont montré l'inconsistance entre les deux abondances (Nguyen et coll. 2015a). Une des solutions est la PCR quantitative, mais cette technique s'applique uniquement pour des communautés très pauvres. Cependant, l'information portée par le nombre de séquences peut nous informer sur les abondances relatives entre les échantillons d'une OTUs (Amend et coll. 2010). Il est donc conseillé de faire les analyses basées sur les incidences et sur les abondances et de comparer les résultats.

Métagénomique : Terme utilisé pour la première fois dans une publication de Handelsman et coll. (1998) qui définit ainsi ce terme : « the genomes of the total microbiota found in nature ». Il s'agit de l'étude de l'ensemble des séquences issues d'un échantillon environnemental. La métagénomique se divise en deux grands groupes d'analyses (Scholz et coll. 2012) : (i) le séquençage de parties entières du génome d'un ou quelques organismes et (ii) le séquençage d'une courte zone d'ADN spécifique, chez une grande diversité d'organismes. Le séquençage de ces séquences code-barres est également appelé *barcoding* (Valentini et coll. 2009).

NGS, HTS : De l'anglais *Next-Generation Sequencing*. Désigne l'ensemble des méthodes de séquençage (en particulier les méthodes Roche 454, Illumina, Ion Torrent et SOLiD) qui ont permis dans les 20 dernières années de produire un nombre de séquences très largement supérieur au séquençage de Sanger avec un coût par séquence très inférieur (Peay et coll. 2016). Ces méthodes sont très utilisées pour étudier les communautés de micro-organismes, y compris des champignons (voir Fig. I.9). L'abréviation HTS (pour *High-Throughput Sequencing*) est parfois utilisée en lieu et place de NGS (Fig. I.17).

OTUs : Abréviations de l'anglais *Operational taxonomic Unit*. Une OTU correspond à une espèce d'un point de vue moléculaire. Par convention, les OTUs pour la plupart des lignées fongiques sont définies comme des groupes de séquences ayant moins de 3% de différence. Il s'agit d'une définition opérationnelle qui prend tout son sens en écologie des communautés mais qui a des limites importantes pour les taxinomistes.

Champignon

Chitine : Du Grec ancien *khitôn* (« tunique »). Polysaccharide azoté (polymère de N-acetylglucosamine) présent particulièrement dans les parois des champignons et dans l'exosquelette des arthropodes (Gobat et coll. 2010). La présence de chitine est un des rares caractères dérivés partagés chez les Eumycètes.

Décomposition : Transformation biologique de la matière organique morte à la fois par désagrégation de la matière organique en molécules plus petites et par modification de la composition chimique de la matière organique (Lindahl et Tunlid 2015 ; plus de détails autour du concept de décomposition tableau I.2).

Endophyte : Du Grec ancien *éndon* : (« dans ») et *phyton* (« plante »). Organisme dont au moins une partie du cycle de vie se déroule à l'intérieur des tissus végétaux. Les endophytes appartiennent aux groupes des Bactéries, des Eucaryotes (principalement des champignons) et des Archées. On distingue les endophytes selon les organes qu'ils habitent (par ex. feuilles, fruits, racines) et selon leur groupe fonctionnel (Rodriguez et coll. 2009). En français courant, la notion d'endophyte est souvent associée par atavisme au parasitisme. Les endophytes ont été observés pour la première fois dans un contexte scientifique où la présence d'organisme à l'intérieur d'un corps ne pouvait être que délétère. Ainsi, le Centre National de Ressources Textuelles et Lexicales (CNRTL) définit ainsi le terme endophyte : « Parasite végétal qui vit à l'intérieur du corps de son hôte ». L'adjectif associé est « endophytique».

Eumycètes : Du Grec ancien *eu* (« vrai ») et mykês (« champignon »). Clade (au sens de groupe monophylétique) rassemblant l'ensemble des champignons au sens phylogénétique du terme. Ainsi, sont exclus du clade des Eumycètes les Oomycètes et les Myxomycètes.

Génet et **ramet :** Un génet est un groupe d'individus physiques (les ramets) originaire d'un ancêtre individuel commun et vivant dans un endroit donné. D'un point de vue opérationnel, un génet correspond à un clone (identité génétique) local (identité géographique).

Hyphe : Du Grec ancien *huphê* (« tissu »). Filament à structure cellulaire, dépourvu de chlorophylle, à partir duquel est constitué, par enchevêtrement, le mycélium des champignons supérieurs et des lichens (source CNRTL). L'ensemble des hyphes forme la partie végétative du champignon : le **mycélium**. Voir figure I.11 pour une illustration de la structure en filament des champignons.

ITS : *Internal Transcribed Spacer* Région de l'ADN ribosomique utilisée comme marqueur universel chez les champignons (Gardes et Bruns 1993 ; Kõljalg et coll. 2013 ; Schoch et coll. 2012 ; White et coll. 1990). Voir encadré 5.

Mycélium : Voir à Hyphe.

Pourriture blanche (fibreuse) et **pourriture brune (cubique) :** Faciès de décomposition (Lindahl et Tunlid 2015) caractérisés par la dégradation de la lignine et de la cellulose (pourriture fibreuse) ou par la dégradation de la cellulose uniquement (pourriture cubique). Voir tableau I.2 et figure I.13.

Rhizomorphe : Du Grec ancien *rhíza* : (« racine ») et *morphé* (« forme »). Structure composée de mycélium agglutiné que l'on trouve chez certains Eumycètes. Les rhizomorphes supportent des transferts de molécules (C, N, P, hormones, etc) à longues distances, autorisant ainsi une exploration efficace des zones éloignées de la mycorhize (Agerer 2001 ; Boddy 1993) et participant activement à la relocalisation des ressources (eau et minéraux) dans le sol à l'échelle de plusieurs mètres (photo I.13a).

Saprotrophe : *sapros* (« putride ») et *trophikós* (« nourrissant »). Organisme hétérotrophe dont la majorité du carbone provient de la décomposition de matière organique morte (Lindahl et Tunlid 2015 ; plus de détails tableau I.2).

Saproxylique : Du Grec ancien *sapros* (« putride ») et *xylon* (« bois »). Organisme hétérotrophe dont la majorité du carbone provient de la décomposition de bois mort (plus de détails tableau I.2).

Écologie

Communauté : Ensemble d'organismes n'ayant pas de relation de prédation directe ou indirecte entre eux, partageant un ou plusieurs facteurs limitants (ressource(s), prédateur(s),. . .) et occupant un même espace de petite dimension au regard de leur mobilité et de leur dispersion (Laroche 2014). Voir la section de l'introduction sur le sujet.

Filtre environnemental : Ensemble des processus −liés à l'environnement − qui restreignent la distribution des espèces. Le plus souvent on utilise la notion de filtre environnemental pour souligner le caractère abiotique de la variable responsable du processus de filtre (Kraft et coll. 2015).

Guilde : Groupe d'organismes qui utilise la même ressource, peu importe leur relation taxinomique (Blondel 2003). On s'attend à ce que des organismes d'une même guilde expérimentent une compétition interspécifique plus forte que des organismes de guildes différents (Silvertown 2004). Chez les champignons on utilise souvent le terme guilde pour différencier les champignons mycorhiziens, saprotrophes, (zoo-, myco-, phyto-) parasites ou encore lichénisant (Nguyen et coll. 2015b ; Peay et coll. 2016).

Limitation de la similarité : La limitation de la similarité décrit un patron : la tendance qu'ont les espèces qui coexistent à occuper des niches différentes. Ce concept est étroitement relié au processus de déplacement de caractères. Le déplacement de caractères correspond à l'accentuation évolutive des différences de traits entre les espèces sympatriques causée par une compétition pour les ressources (Silvertown 2004).

Métacommunauté : Ensemble de communautés reliées entre elles par des flux d'individus (Hanski 1998).

Niche : Hypervolume à *n* dimension, où chaque dimension est une condition environnementale (abiotique ou biotique) qui limite la viabilité des individus de l'espèce (Hutchinson 1957). Voir la section de l'introduction sur le sujet.

Feu

Feu de couronne : Un feu de couronne est un feu qui monte dans le houppier et brûle les arbres jusqu'à la canopée. Un feu de couronne est très souvent plus sévère qu'un feu de surface.

Feu de surface : Un feu de surface est un feu qui brûle la litière, les plantes de sous bois et les branches basses des arbres sans monter dans la canopée. Les feux de surface sont très souvent moins sévère qu'un feu de couronne.

Fréquence : La fréquence d'un feu est le nombre d'occurrence du feu dans une aire circonscrite et durant une période donnée (Keeley 2012). La fréquence de feu est couramment exprimée à travers le temps de rotation du feu (temps requis pour brûler l'équivalent d'une surface spécifiée) ou à travers le temps de retour de feu (temps entre les feux dans la zone spécifiée ; Keeley 2012).

Intensité : Quantité d'énergie dégagée par un feu (Keeley 2012).

Régime de feu : Ensemble des paramètres qui caractérise un ou des feux. Ces paramètres incluent notamment la fréquence, la durée, l'intensité, ou encore la surface du feu. Voir encadré 6.

Sévérité : Impact du feu sur la perte de biomasse végétale aérienne et souterraine (Keeley 2012). Plus largement, décrit la magnitude de l'effet d'un feu sur l'écosystème. La sévérité dépend de nombreux paramètres dont la composition végétale, le climat la topographie et le type de feu (feu de surface ou feu de couronne). De plus, la sévérité est d'autant plus grande que l'intensité du feu est grande, et que la vitesse de propagation du feu est faible.

Forêt et végétation

Chablis : « Arbre ou groupe d'arbres déracinés ou rompus dans le bas du tronc sous l'effet d'événements climatiques ou de l'âge » (Le grand dictionnaire terminologique). On exclue du terme chablis tous les phénomènes impliquant l'intervention de l'homme.

Forêt : « Vaste étendue de terrain peuplée d'arbres ; ensemble des arbres qui couvrent cette étendue » (CNRTL). « Un arbre est une plante vasculaire ligneuse qui doit atteindre minimalement cinq mètres à maturité et dont le tronc unique ne se ramifie qu'à une certaine hauteur au dessus du sol » (Le grand dictionnaire terminologique).

Parcelle forestière : Une zone continue de forêt dans laquelle la composition spécifique et démographique est relativement homogène.

Mycorhize

Apex : Voir à Ectomycorhize

Ectomycorhize : Du Grec ancien *ektos* (« au dehors »), *mykês* (« champignon ») et *rhíza* (« racine »). Zone d'échange entre la racine d'une plante et le mycélium d'un champignon lors du mutualisme ectomycorhizien. L'**apex** est un synonyme d'ectomycorhize qui est utile pour préciser que l'on parle bien de l'organe (par ex. Fig. I.8) et non de l'interaction. L'abréviation de « ectomycorhize », **ECM**, fait office d'adjectif par extension [ectomycorhizien(ne(s))].

Mycélium mycorhizien extraracinaire : Structure végétative des champignons mycorhiziens composée d'hyphes externes aux racines. Cette structure permet aux champignons mycorhiziens d'explorer le sol à la recherche d'eau et de nutriments. C'est grâce à ce mycélium mycorhizien extraracinaire que la plante mycorhizienne augmente sensiblement sa surface d'échange avec le sol.

Spécificité ectomycorhizienne : Terme général décrivant l'étendue des symbiontes avec lesquels une plante ou un champignon forment des ectomycorhizes. Des organismes généralistes s'associent avec un grand nombre de partenaires tandis que les organismes spécialistes ont peu de partenaires. La spécificité est le plus souvent établie à l'échelle des espèces.

Réseau

Biparti (réseau) : Réseau constitué de deux classes de partenaires qui n'interagissent qu'avec des partenaires de l'autre classe. Les nœuds sont alors de deux types, les liens ne devenant possibles qu'entre deux nœuds de classes différentes (Latapy et coll. 2008). Les interactions sont donc toutes inter-classes mais jamais intra-classes. Dans le cas des réseaux bipartis ECM, les plantes forment une classe et interagissent uniquement avec des membres de l'autre classe : les champignons.

Coefficient de participation : Le coefficient de participation est indice qui rend compte de la position des nœuds dans les modules. Il indique l'importance du nœud dans les liens entre modules en mesurant l'équitabilité des liens du nœud entre les différents modules. Autrement dit, le coefficient de participation est maximum lorsque les liens du nœud sont distribués équitablement entre les modules.

Connectance : Proportion de lien possible qui sont effectivement présent dans le réseau. Correspond dans la majorité des réseaux au nombre de liens divisé par le carré du nombre de nœuds. Dans le cas des réseaux bipartis ECM, la connectance se calcule selon l'équation $\frac{L}{p \times c}$ avec L le nombre de liens total dans le réseau, p le nombre de plantes et c le nombre de champignon.

Degré relatif intra-module : Le degré relatif intra-module est indice qui rend compte de la position des nœuds dans les modules. Le degré relatif intra-module est un indice qui renseigne sur l'importance du nœud dans son module en mesurant la part de lien du module qui dépend de ce nœud.

Imbrication : En français courant, l'imbrication est une disposition particulière des choses qui se recouvrent partiellement les unes les autres comme les écailles d'un poisson ou les tuiles d'un toit (CNRTL). En science des réseaux l'imbrication (*nestedness* en anglais) correspond également à une disposition particulière des partenaires en « poupée russe ». Plus un réseau est imbriqué, plus les partenaires des espèces spécialistes sont également partenaires des espèces généralistes. Dans le cas des plantes ECM, un réseau imbriqué parfait serait constitué de cortèges de champignons des plantes complètement emboîtés. L'imbrication est souvent corrélée à la modularité dans les réseaux écologiques empiriques et le sens de la corrélation dépend de la connectance du réseau (Fortuna et coll. 2010). L'imbrication peut être calculée sur des données qualitatives ou quantitatives (par ex. Staniczenko et coll. 2013).

Modulaire (réseau) : Un réseau modulaire est un réseau dans lequel les nœuds sont reliés entre eux dans des groupes bien unis, les modules, entre lesquelles peu de connexion existent (Girvan et Newman 2002). La modularité mesure l'inclination du réseau à présenter une tel structure.

Projeté (réseau) : Le réseau projeté d'un réseau biparti correspond à un réseau formé d'une seule classe de nœuds. L'utilisation de réseaux projetés permet d'avoir des informations sur les relations indirectes entre membres d'une même classe via les partenaires de l'autre classe. Il existe deux réseaux projetés différents pour un réseau biparti. Par exemple, dans le cas du réseau plantes-champignons ECM, le réseau projeté des plantes relient les plantes qui ont des partenaires fongiques en commun entre elles.

Manuscrits en projet - *Drafts in progress*

Projet de manuscrit D : "Effect of host taxonomy and coexistence on ectomycorrhizal fungal communities"

Coexistence du pin laricio et du hêtre.

ECM symbiosis as plant extended niche: fungal partners sharing among plants at regional and local scale

Adrien Taudière [1, 2*], Christopher Carcaillet [3], Annick Lesne [4, 5], François Munoz [6, 7] & Franck Richard [2]

1/ École Pratique des Hautes Études (EPHE) & Paris Research University, Paris, France

2/ UMR 5175, CEFE – CNRS – Université de Montpellier, Montpellier, France

3/ Laboratoire d'Écologie des Hydrosystèmes Naturels et Anthropisés (UMR5023 CNRS), Université Lyon 1, 69622 Villeurbanne, France

4/ CNRS, LPTMC UMR 7600, Université Pierre et Marie Curie-Paris 6, Sorbonne Universités, Paris, France

5/ CNRS, IGMM UMR 5535, Université de Montpellier, Montpellier, France

6/ UM2, UMR AMAP, Montpellier, France

7/ French Institute of Pondicherry, Pondicherry, India

* Corresponding authors: adrien.taudiere@cefe.cnrs.fr

Introduction

Most Temperate, Boreal and Mediterranean tree are dependent of the ectomycorrhizal (ECM) mutualism. The ECM symbiosis is essential for both plant and fungal associates. ECM fungi feeds on host carbon and provide water, nutrient and root protection to the host (Smith and Read 2008).

Beyond the benefit for individuals, ECM interactions are implicated in direct and indirect plant-plant interactions. ECM fungi are though to be important in facilitation processes during both primary (Nara 2006) and secondary succession (Horton *et al.* 1999; Richard *et al.* 2009; Bingham and Simard 2012). Other plant-plant interactions involving ECM symbiosis include mutualism disruption by invasive species (*Alliaria petiolata*, Wolfe *et al.* 2008), carbon transfer through CMN (Common Mycorrhizal Network; Simard *et al.* 1997) and information exchange among plants (Song *et al.* 2015).

ECM fungal association are governed by host preference (Molina and Horton 2015) that sometimes turn into strict specificity (e.g., *Alpova spp* on *Alnus*; Moreau *et al.* 2011). Moreover, ECM fungal species possess different capabilities in terms of enzymatic activities (Courty *et al.* 2016), exploration (Agerer 2001) and nutrient uptake (Wallander 2000). To summarize, plant species differ in terms of fungal associates which themselves differ in terms of ecology. Thus, the plant species extended phenotype (Dawkins 1989) is shaped by ECM fungal partners, and difference in associations patterns among plants may play a central role in plant community assembly (e.g., through facilitation) and plant coexistence (e.g. through limiting similarity relaxing competition).

For each plant species (Fig. 1), we compare (i) potential fungal communities at the regional scale (*akin* regional pool in functional ecology, Lessard *et al.* 2012), (ii) locally available fungal species in soil (part of the plant fundamental niche) and (iii) realized ECM fungal communities in the form of apex in pure stand and mixed forest (part of the plant realized niche).

Materials and Methods

Sites description

Soil sampling and sequencing

We present the sampling scheme in Taudiere *et al.* (*in prep.*; manuscript J in the thesis). In short, we sample soil in *Fagus sylvatica* (3 sites), *Pinus pinaster* (3 sites) and *Pinus nigra* subsp. *laricio* (4 sites) pure stands (Tab. 1). We also sample soil in mixed forest of F. *sylvatica* – *P. nigra* (3 sites) and *P. pinaster* – *P. nigra* (3 sites). For pure stand samples, we sample five soil cores in three different plot inside each sites and we mixed soil cores (Tab. 1). In each mixed forest, we sample five cores in which we can determine the presence of roots of the two co-occurring species (Tab. 1). Note that in one site of pure *P. nigra* we didn't mix the soil cores to test the effect of mixing soil core in each plot. We then blend and filtered soil samples for further extraction.

Root tips sampling

Using the same soil core than for soil DNA, we extracted roots and exhaustively (*i.e.* without morphotyping) picked mycorrhizas under binocular microscope. For each pure stand sample, we sampled 200 root tips using at least three different root fragments. For mixed forest, we separate roots of the different species before to picked the maximum number of root tips until reaching 200 for each species. Ecological analysis will take into account the bias due to the difference in number of root tips in few samples.

Sequencing and bioinformatics processing

Sequencing and bioinformatic processing is detailed in Taudiere et al (*in prep.*; manuscript J in the thesis). Briefly, we extract soil/root tip DNA using the same protocle of the FastDNA™ SPIN Kit for Soil (*MP Biomedicals*) and we send samples to the MiSeq plateform after a protocol of ethanol precipitation (detailed online). We amplify ITS-1 and ITS-2 regions using the primer ITS-2 and ITS-4.

Here, we summarize the bioinformatic pipeline detailed in Taudiere et al (*in prep.*; manuscript J in the thesis). Sequences were quality filtered (*sickle*), quality checked (*FastQC*; Andrews 2010) and separated into ITS-1 and ITS-2 (*fqgrep*; Indraniel & Gosselin 2016). Then for both ITS-1 and ITS-2 in parallel, we demultiplexed, dereplicated, removed chimeras (*Usearch*; Edgar *et al.* 2011) using the Unite database (dynamic v.7; Kõljalg *et al.* 2013) and discarded non ITS sequences (*ITSx*; Bengtsson-Palme *et al.* 2013). Sequences were then clustered into OTUs thanks to the Uparse software (Edgar 2013). We also applied other clustering methods that gave similar results in terms of ecological response (Sup. Mat. of Taudiere et al i*n prep.*; manuscript J in the thesis). Finally, we assessed taxonomic and ecological assignation using respectively *RPD classifier* (Wang *et al.* 2007; compute under *Qiime* software [Caporaso *et al.* 2010] using the Unite database [Kõljalg *et al.* 2013] as reference) and *FUNGuild* (Nguyen *et al.* 2015).

We kept only samples with at least 10 000 sequences and OTUs represented by more than 4 sequences and classify as ectomycorrhizal by *FUNGuild*. This lead to a total of XXX OTUs accounting for a total of XXX sequences among the XXX samples (Table 1). All analysis were computed with *R 3.2.4* (R Core Team, 2016), mostly thanks to the *Phyloseq* package v. 1.12.2 (McMurdie, 2013).

Regional association

We use the published ECM interaction network at the scale of a whole Island (Corsica, France; Taudiere *et al.* 2015). We extract fungal taxa associate, at the regional scale, with at least one of the three trees species studied in this paper (namely *Fagus sylvatica*, *Pinus pinaster* and *Pinus nigra* subsp. *laricio*).

Statistical analysis

Fungal diversity and richness were assessed using the observed number of OTUs, Chao1, Fisher. Simpson and Shannon index. Difference in fungal diversity were tested using linear model and Tuckey post-hoc tests. To control for difference in number of sequences by samples, the number of sequences was set as the first explanatory variable of the linear model (Bálint *et al.* 2015). We explore beta-diversity using NMDS (non-metric multidimensional scaling on Bray distance) and PerManova (Anderson 2001; function adonis of the vegan package; 9 999 permutations).

Results

Soil ECM α-diversity

All ECM fungal diversity indicators are identical regarding host species present in the forest (Fig. 2a; adjusted p-values > 0.05 using Tuckey test). In particular, mixed forests present the same ECM diversity than pure one.

Soil ECM β-diversity

Soil ECM communities are shaped by sites and host identity (Fig. 3; Table 2). Sites and sites interactions with host also drive significant shift in ECM soil communities (Table 2). Moreover, soil ECM fungal assemblage of mixed *Fagus-Pinus* forests are intermediary between fungal communities of pure *Fagus* and pure *Pinus* forests.

ECM root tips α-diversity

ECM root tips β-diversity

Discussion

References

Agerer R. 2001. Exploration types of ectomycorrhizae. *Mycorrhiza* 11: 107–114.

Anderson MJ. **2001**. A new method for non-parametric multivariate analysis of variance. *Austral ecology* **26**: 32–46.

Bálint M, Bartha L, O'Hara RB, Olson MS, Otte J, Pfenninger M, Robertson AL, Tiffin P, Schmitt I. **2015**. Relocation, high-latitude warming and host genetic identity shape the foliar fungal microbiome of poplars. *Molecular Ecology* **24**: 235–248.

Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, Godhe A, De Wit P, Sánchez-García M, Ebersberger I, de Sousa F, *et al.* **2013**. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution* **4**: 914–919.

Bingham MA, Simard SW. **2012**. Mycorrhizal networks affect ectomycorrhizal fungal community similarity between conspecific trees and seedlings. *Mycorrhiza* **22**: 317–326.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, *et al.* **2010**. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**: 335–336.

Courty P-E, Munoz F, Selosse M-A, Duchemin M, Criquet S, Ziarelli F, Buée M, Plassard C, Taudière A, Garbaye J, *et al.* **2016**. Into the functional ecology of ectomycorrhizal communities: environmental filtering of enzymatic activities. *Journal of Ecology*.

Dawkins R. **1989**. *The Extended Phenotype: The Long Reach of the Gene*. Oxford ; New York: Oxford Univ Pr.

Edgar RC. **2013**. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**: 996–998.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. **2011**. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.

Horton TR, Bruns TD, Parker VT. **1999**. Ectomycorrhizal fungi associated with Arctostaphylos contribute to *Pseudotsuga menziesii* establishment. *Canadian Journal of Botany* **77**: 93–102.

Indraniel D, Gosselin T. **2016**. *fqgrep: v0.4.4*.

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, *et al.* **2013**. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* **22**: 5271–5277.

Lessard J-P, Belmaker J, Myers JA, Chase JM, Rahbek C. **2012**. Inferring local ecological processes amid species pool influences. *Trends in Ecology & Evolution* **27**: 600–607.

Lortie CJ, Brooker RW, Choler P, Kikvidze Z, Michalet R, Pugnaire FI, Callaway RM. **2004**. Rethinking plant community theory. *Oikos* **107**: 433–438.

McMurdie PJ, Holmes S. **2013**. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data (M Watson, Ed.). *PLoS ONE* **8**: e61217.

Molina R, Horton TR. **2015**. Mycorrhiza specificity: Its role in the development and function of common mycelial networks. Mycorrhizal Networks. Dordrecht: Springer Netherlands, 1–39.

Moreau P-A, Rochet J, Richard F, Chassagne F, Manzi S, Gardes M. **2011**. Taxonomy of Alnus-associated hypogeous species of Alpova and Melanogaster (Basidiomycota, Paxillaceae) in Europe. *Cryptogamie, Mycologie* **32**: 33–62.

Nara K. **2006**. Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytologist* **169**: 169–178.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. **2015**. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*.

Richard F, Selosse M-A, Gardes M. **2009**. Facilitated establishment of *Quercus ilex* in shrub-dominated communities within a Mediterranean ecosystem: do mycorrhizal partners matter? *FEMS Microbiology Ecology* **68**: 14–24.

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R. **1997**. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **388**: 579–582.

Smith SE, Read DJ. **2008**. *Mycorrhizal Symbiosis*. Academic Press.

Song YY, Simard SW, Carroll A, Mohn WW, Zeng RS. **2015**. Defoliation of interior Douglas-fir elicits carbon transfer and stress signalling to ponderosa pine neighbors through ectomycorrhizal networks. *Scientific Reports* **5**.

Taudiere A, Munoz F, Lesne A, Monnet A-C, Bellanger J-M, Selosse M-A, Moreau P-A, Richard F. **2015**. Beyond ectomycorrhizal bipartite networks: projected networks demonstrate contrasted patterns between early- and late-successional plants in Corsica. *Frontiers in Plant Science* **6**.

The R Core Team. **2016**. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Wallander H akan. **2000**. Uptake of P from apatite by Pinus sylvestris seedlings colonised by different ectomycorrhizal fungi. *Plant and Soil* **218**: 249–256.

Wang Q, Garrity GM, Tiedje JM, Cole JR. **2007**. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Applied and Environmental Microbiology* **73**: 5261–5267.

Wolfe BE, Rodgers VL, Stinson KA, Pringle A. **2008**. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. *Journal of Ecology* **96**: 777–783.

Table 1: Number of sites, plots, soil cores, samples and sequences for each modalities.

ECM tree species	Sites	Plots	Cores	Samples	Mycorrhizas	Soil seq.	Mycorrhizal seq.
F. sylvatica	3	9	45	9	1 800		
P. nigra subsp. *laricio*	4	12	60	43	8 600		
P. pinaster	3	9	45	9	1 800		
F. sylvatica – P. nigra	3	3	15	15	~4 500		
P. pinaster – P. nigra	3	3	15	15	~4 500		

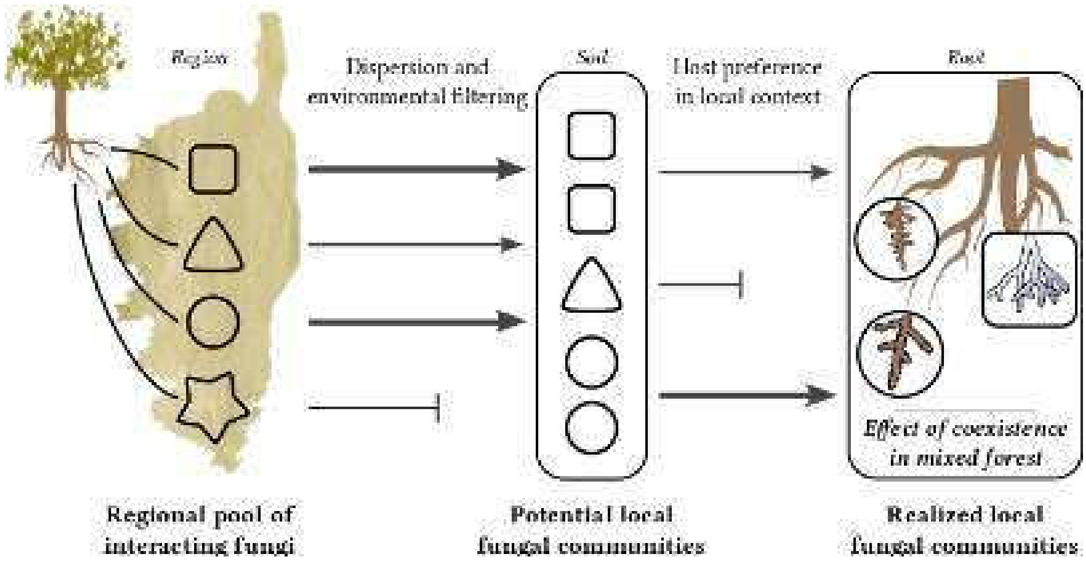
Table 2: PerManova result of ECM communities (a) in soil and (b) in roots tips.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Host	4	3.430	0.85748	2.0514	0.08888	**0.0001**
Sites	5	3.280	0.65598	1.5693	0.08499	**0.0001**
Host:Sites	6	3.039	0.50654	1.2118	0.07875	**0.0034**
Residuals	69	28.842	0.41800		0.74738	
Total	84	38.591			1.0000	

Table 3: Indicator ECM fungal species in soil regarding host composition of the forest. This table result from the *multipatt* function in the *indicspecies* R package (de Cáceres and Legendre, 2009) using 1000 permutations. Abbreviations used: *Fs*: *Fagus sylvatica*; *Pn*: *Pinus nigra* subsp. *laricio*; *Pp*: *Pinus pinaster.*

	Family	Genus	Fs	Fs – Pn	Pn	Pn – Pm	Pm	IndVal.g	p-value
Ascomycota									
OTU_7838	Pyronemataceae	*Humaria sp.*	1	0	0	0	0	0.554	0.0039
OTU_1567	Pyronemataceae	*Humaria sp.*	1	0	0	0	0	0.471	0.0159
OTU_593	Pyronemataceae	*Geopora sp.*	0	0	0	1	1	0.576	0.0449
OTU_856	Gloniaceae	*Cenococcum sp.*	0	0	0	0	1	0.920	0.0009
OTU_2348	Gloniaceae	*Cenococcum sp.*	0	0	0	0	1	0.471	0.0169
OTU_2261	Pyronemataceae	*Geopora sp.*	0	0	0	0	1	0.577	0.0009
OTU_996	Incertae sedis	*Meliniomyces sp.*	0	0	0	0	1	0.424	0.0349
OTU_1585	Pezizaceae	*Terfezia pseudoleptoderma*	0	0	0	0	1	0.471	0.0099
Basidiomycota									
OTU_591	Rhizopogonaceae	*Rhizopogon* aff. *rubescens*	1	1	0	1	1	0.710	0.0189
OTU_561	Suillaceae	*Suillus sp.*	1	1	0	1	1	0.745	0.0329
OTU_3291	Hysterangiaceae	*Hysterangium pompholyx*	1	1	0	0	0	0.563	0.0129
OTU_168	Hydnaceae	*Sistotrema sp.*	1	1	0	0	0	0.689	0.0099
OTU_4846	Thelephoraceae	*Tomentella lapida*	1	1	0	0	0	0.5	0.0069
OTU_849	Thelephoraceae	*Tomentella sp.*	1	1	0	0	0	0.520	0.0479
OTU_200	Hygrophoraceae	*Hygrophorus eburneus*	1	0	0	0	0	0.481	0.0129
OTU_2444	Inocybaceae	*Inocybe sp.*	1	0	0	0	0	0.471	0.0159
OTU_804	Russulaceae	*Lactarius blennius*	1	0	0	0	0	0.575	0.0039
OTU_2468	Thelephoraceae	*Pseudotomentella sp.*	1	0	0	0	0	0.471	0.0169
OTU_5423	Gomphaceae	*Ramaria stricta*	1	0	0	0	0	0.471	0.0189
OTU_780	Russulaceae	*Russula globispora*	1	0	0	0	0	0.471	0.0209
OTU_1195	Hydnaceae	*Sistotrema muscicola*	1	0	0	0	0	0.706	0.0009
OTU_2778	Thelephoraceae	*Tomentella bryophila*	1	0	0	0	0	0.555	0.0009
OTU_523	Thelephoraceae	*Tomentella galzinii*	1	0	0	0	0	0.470	0.0299
OTU_2201	Thelephoraceae	*Tomentella punicea*	1	0	0	0	0	0.576	0.0029
OTU_3435	Atheliaceae	*Tylospora sp.*	0	1	1	1	1	0.942	0.0189
OTU_7573	Russulaceae	*Lactifluus vellereus*	0	0	1	1	0	0.846	0.0209
OTU_616	Atheliaceae	*Amphinema sp.*	0	0	1	0	1	0.775	0.0039
OTU_5907	Atheliaceae	*Amphinema sp.*	0	0	1	0	1	0.707	0.0169
OTU_173	Cortinariaceae	*Cortinarius stillatitius*	0	0	1	0	0	0.636	0.0189
OTU_93	Inocybaceae	*Inocybe rimosa*	0	0	1	0	0	0.592	0.0279
OTU_6311	Inocybaceae	*Inocybe rimosa*	0	0	1	0	0	0.568	0.0399
OTU_32	Russulaceae	*Russula sp.*	0	0	1	0	0	0.568	0.0279
OTU_2399	Thelephoraceae	*Tomentella sp.*	0	0	1	0	0	0.537	0.0099
OTU_779	Gomphidiaceae	*Chroogomphus rutilus*	0	0	0	1	1	0.521	0.0199
OTU_1892	Cortinariaceae	*Cortinarius sp.*	0	0	0	1	1	0.537	0.0169
OTU_594	Inocybaceae	*Inocybe sp.*	0	0	0	1	1	0.499	0.0459
OTU_1735	Russulaceae	*Lactarius deliciosus*	0	0	0	1	1	0.637	0.0019
OTU_243	Russulaceae	*Russula sardonia*	0	0	0	1	1	0.643	0.0229
OTU_493	Thelephoraceae	*Pseudotomentella sp.*	0	0	0	1	0	0.585	0.0319
OTU_7893	Hydnaceae	*Sistotrema sp.*	0	0	0	1	0	0.447	0.0199
OTU_697	Clavulinaceae	*Clavulina sp.*	0	0	0	0	1	0.573	0.0279
OTU_3283	Bankeraceae	*Hydnellum ferrugineum*	0	0	0	0	1	0.471	0.0119
OTU_498	Inocybaceae	*Inocybe assimilata*	0	0	0	0	1	0.470	0.0389
OTU_2878	Inocybaceae	*Inocybe petiginosa*	0	0	0	0	1	0.459	0.0239
OTU_822	Rhizopogonaceae	*Rhizopogon sp.*	0	0	0	0	1	0.861	0.0009
OTU_534	Russulaceae	*Russula sp.*	0	0	0	0	1	0.466	0.0159
OTU_2106	Thelephoraceae	*Tomentella cinerascens*	0	0	0	0	1	0.465	0.0119
OTU_562	Thelephoraceae	*Tomentella sp.*	0	0	0	0	1	0.573	0.0129
OTU_1234	Thelephoraceae	*Tomentella sp.*	0	0	0	0	1	0.575	0.0039
OTU_2288	Tricholomataceae	*Tricholoma terreum*	0	0	0	0	1	0.471	0.0099

Fig. 1: Schematic view of fungal communities interacting with plant at different scales. Shapes indicate fungal species. Arrow width are proportional to the relative abundance of each species pass through ecological filter. Note that in this study the network as regional pool depict qualitative interactions whereas soil and root tip composition is quantitative (or semi-quantitative; see discussion) thanks to the molecular abundances.



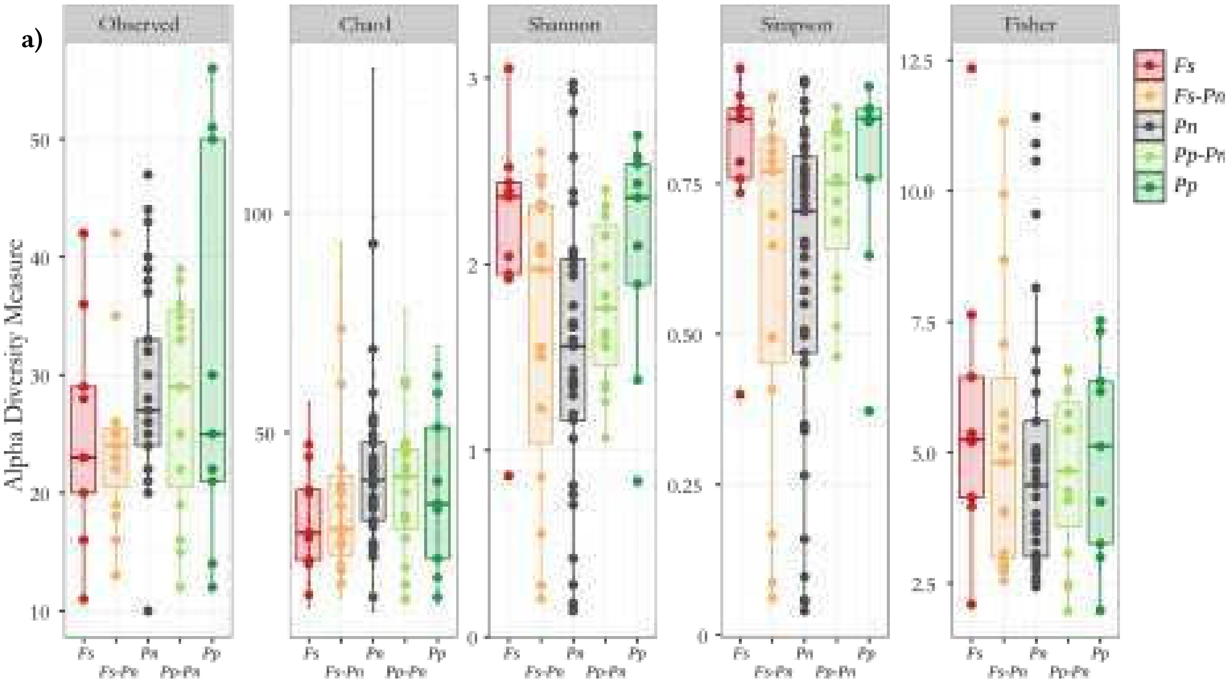


Fig. 2: Diversity of ECM taxa (a) in soil and (b) in roots tips in function of host. Abbreviations used: *Fs*: *Fagus sylvatica*; *Pn*: *Pinus nigra* subsp. *laricio*; *Pp*: *Pinus pinaster*.

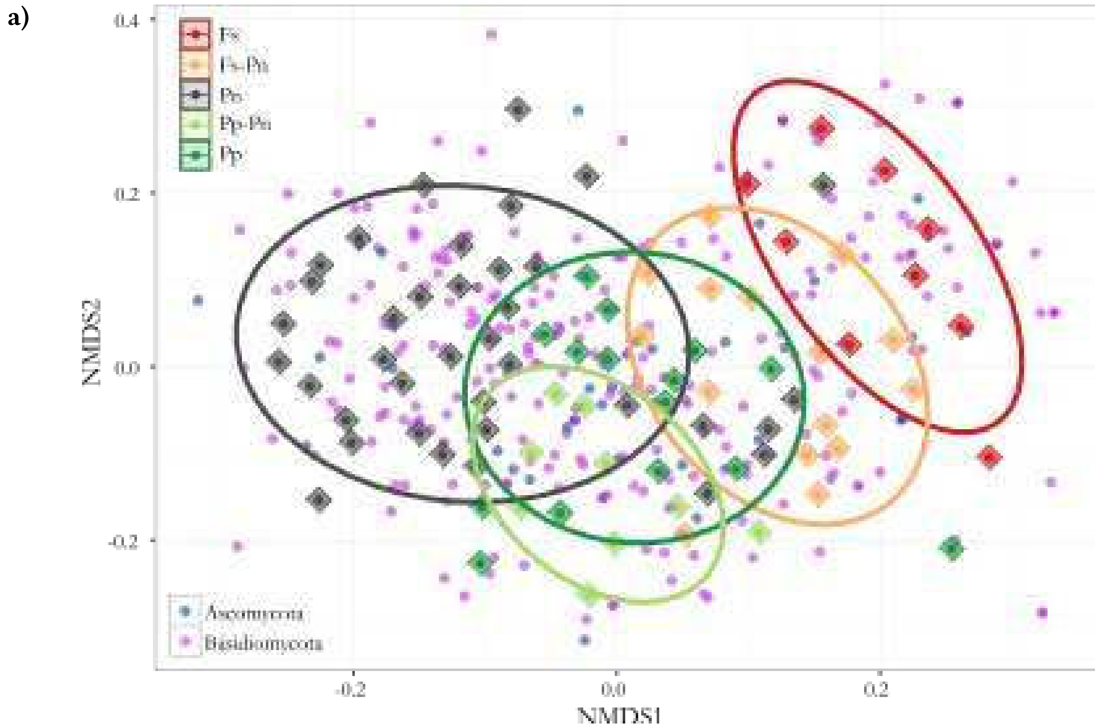


Fig. 3: Non metric multidimensional scaling (Bray distances) showing the difference in species composition (a) in soil and (b) in roots tips in function of host. Abbreviations used: *Fs*: *Fagus sylvatica*; *Pn*: *Pinus nigra* subsp. *laricio*; *Pp*: *Pinus pinaster*. Stress is equal to 29.5% for panel (a) and XX% for panel (b).

Supplementary Materials

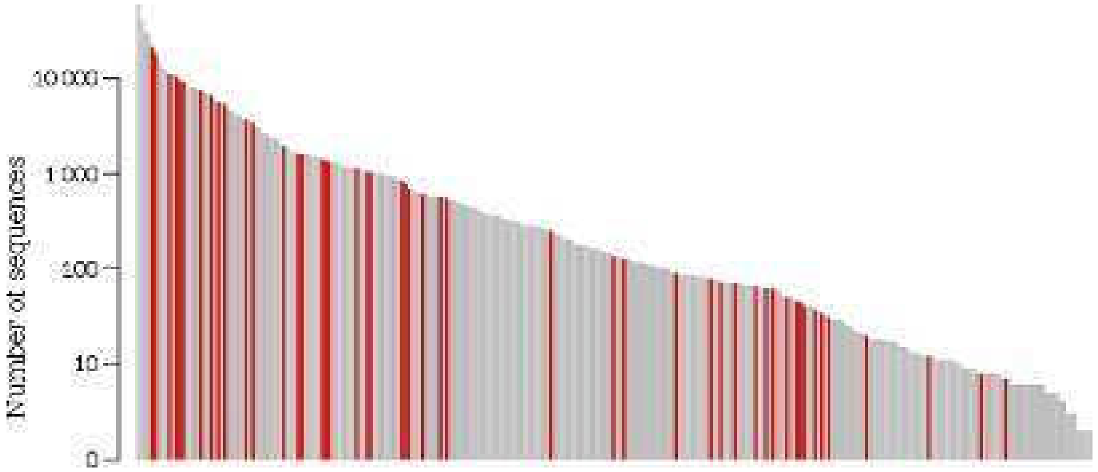


Fig. S1: Molecular abundance of OTUs. Indicator OTUs (table 3) are indicated in red.

Projet de manuscrit G : "Effect of fire on ECM fungal communities in soil and roots of *Pinus nigra* subsp. *laricio* (Corsica, France)"

Col de Rospa Sorba 15 ans après les feux de 2000.

Effect of fire on ECM fungal communities in soil and roots of *Pinus nigra* subsp. *laricio* (Corsica, France)

***Adrien Taudière** [1, 2*]**, Christopher Carcaillet** [2] **& Franck Richard** [3]*

1/ École Pratique des Hautes Études (EPHE) & Paris Research University, Paris, France

2/ Laboratoire d'Écologie des Hydrosystèmes Naturels et Anthropisés (UMR5023 CNRS), Université Lyon 1, 69622 Villeurbanne, France

3/ UMR 5175, CEFE – CNRS – Université de Montpellier, Montpellier, France

* Corresponding authors: adrien.taudiere@cefe.cnrs.fr

Introduction

On one hand, fire is crucial in driving forest dynamic including forest disappearance. One the other hand, most temperate, Boreal and Mediterranean trees rely on ectomycorrhizal (ECM) symbiosis for water and nutrients uptake, and consequently, for healthy growth. Given the current change in fire regime worldwide (Moritz et al. 2012) and the economic/ecologic importance of ECM forests, studying fire effect on ECM symbiosis is of prime importance. Fire affects multiple facets of ECM symbiosis including species richness, diversity, colonization rate, fruit bodies production and community composition of ECM fungi (Dahlberg 2002; Taudiere et al. *submitted* [manuscript F in the thesis]).

However, ECM fungal response to the interaction between fire severity, time since fire and soil depth was never assessed. Moreover, previous studies studied either active ECM fungi (e.g., using fruit bodies identification or root tips sequencing) or the combination of latent and active communities of ECM fungi (e.g., soil sequencing or bioassay using soil from the burn ecosystems [Glassman et al. 2015]).

Here we characterize active ECM fungal communities using root tips sampling and the combination of latent and active communities by soil sampling. All samples were sequenced using Illumina technics on MiSeq platform. Thus, the sampling of both soil and root tips allow disentangling the latent and active part of ECM fungal communities in soil.

Materials and Methods

Pinus nigra subsp. *laricio* is an endemic pine living in mountain forest (900 – 1 800 m; Rameau et al. 2008) of Corsica (France). Laricio forests are protected by the European Community (CEE 92/43, 1992, natura 2000 code: 9530). All materials and methods are described in manuscripts B and J of the thesis.

Results

Remaining effect of wildfire on ECM fungi in soil after 15 years

Surface fire increase soil ECM fungal diversity

ECM fungal diversity in *Pinus nigra* subsp. *laricio* forest are higher 15 years after a surface fire than in the absence of fire or 15 years after a destructive fire. This is true for the three Hill numbers (H^0, H^1 and H^2) and only the difference in the H^2 between surface fire and destructive fire is not significant (Fig. 1). Moreover, ECM fungal diversity but not specific richness is higher in the deeper layer (Fig. 1).

Wildfire severity shape soil ECM fungal communities irrespective of soil depth

Sites and fire severity significantly structure ECM fungal communities (PerManova; Table 1 and Fig. 2 and 3). On the contrary, depth and interaction between depth and fire don't affect ECM fungal communities (PerManova; Table 1, Fig. 4).

Wildfire affect soil OTUs molecular abundance

The majority of OTUs whose abundance change regarding fire severity are disfavored by fire (Fig. 3 and 4; e.g., *Amanita*, *Cenococcum*, *Lactifluus*, *Sebacina*). In particular, OTUs belonging to Russulales and Agaricales family are more abundant in the absence of fire. On the other hand, two OTUs in *Rhizopogon* genus one in *Geospora* as well as one in *Suillus* are favored by fire (Fig. 3 and 4). Interestingly, *Geospora sp* (Ascomycetes) is favored by destructive fire but not by surface fire.

Effect of wildfire on ECM root tips

Time of resilience for ECM fungi facing wildfire

Discussion

References

Dahlberg A. 2002. Effects of fire on ectomycorrhizal fungi in fennoscandian boreal forests. *Silva Fennica* 36: 69–80.

Glassman SI, Levine CR, DiRocco AM, Battles JJ, Bruns TD. **2015**. Ectomycorrhizal fungal spore bank recovery after a severe forest fire: some like it hot. *The ISME Journal.*

Moritz MA, Batllori E, Bradstock RA, Gill AM, Handmer J, Hessburg PF, Leonard J, McCaffrey S, Odion DC, Schoennagel T, *et al.* 2014. Learning to coexist with wildfire. *Nature* **515**: 58–66.

Rameau J-C, Mansion D, Dumé G, Gauberville C. **2008**. *Flore forestière française Tome 3.* Paris: Institut pour le développement forestier.

Table 1: PerManova result of ECM communities (a) in soil and (b) in roots tips in relation to fire severity.

a)	**Df**	**SumsOfSqs**	**MeanSqs**	**F.Model**	**R2**	**Pr(>F)**
Sites	2	1.6097	0.80485	1.88789	**0.05994**	**0.0001**
Fire severity	2	2.0577	1.02886	2.41334	**0.07662**	**0.0001**
Depth	1	0.4019	0.40187	0.94266	0.01496	0.5736
Fire severity × Depth	2	0.6183	0.30913	0.72511	0.02302	0.9929
Residuals	52	22.1687	0.42632		0.82546	
Total	59	26.8563			1.00000	

Table 2: PerManova result of ECM communities (a) in soil and (b) in roots tips in relation to time since fire.

Table 3: Indicator fungal ECM species in soil regarding fire severity. This table result from the *multipatt* function in the *indicspecies* R package (de Cáceres and Legendre, 2009) using 1 000 permutations. **C**: control forest without fire; **S**: surface fire; **D**: destructive fire. Notes that p-value are not corrected for multiple testing.

	Family	Genus	C	S	D	stat	p-value
Ascomycota							
OTU_4297	Gloniaceae	Cenococcum	0	0	1	0.408	0.0469
OTU_172	Pyronemataceae	Wilcoxina	0	1	1	0.924	0.0019
OTU_593	Pyronemataceae	Geopora	0	0	1	0.745	0.0009
Basidiomycota							
OTU_1294	Atheliaceae	Amphinema	0	1	1	0.637	0.0229
OTU_7536	Atheliaceae	Tylospora	0	0	1	0.520	0.0119
OTU_629	Cortinariaceae	Cortinarius	0	1	0	0.470	0.0279
OTU_1445	Cortinariaceae	Cortinarius	0	1	0	0.426	0.0289
OTU_779	Gomphidiaceae	Chroogomphus	0	0	1	0.577	0.0019
OTU_1050	Hydnaceae	Hydnum	0	1	0	0.425	0.0439
OTU_422	Inocybaceae	Inocybe	0	1	0	0.664	0.0259
OTU_3802	Inocybaceae	Inocybe	0	1	0	0.603	0.0029
OTU_205	Inocybaceae	Inocybe	0	1	0	0.586	0.0169
OTU_1651	Inocybaceae	Inocybe	0	1	0	0.425	0.0199
OTU_8081	Inocybaceae	Inocybe	0	1	0	0.422	0.0249
OTU_167	Lyophyllaceae	Lyophyllum	0	1	0	0.426	0.0499
OTU_591	Rhizopogonaceae	Rhizopogon	0	1	1	0.868	0.0009
OTU_822	Rhizopogonaceae	Rhizopogon	0	1	1	0.806	0.0009
OTU_7573	Russulaceae	Lactifluus	1	0	0	0.835	0.0049
OTU_312	Russulaceae	Russula	1	1	0	0.635	0.0459
OTU_32	Russulaceae	Russula	1	0	0	0.621	0.0219
OTU_7701	Russulaceae	Russula	0	1	0	0.528	0.0469
OTU_1735	Russulaceae	Lactarius	0	1	1	0.523	0.0359
OTU_2223	Russulaceae	Russula	0	1	0	0.415	0.0209
OTU_561	Suillaceae	Suillus	0	1	1	0.828	0.0019
OTU_539	Suillaceae	Suillus	0	0	1	0.471	0.0169
OTU_239	Thelephoraceae	Tomentella	0	1	0	0.724	0.0069

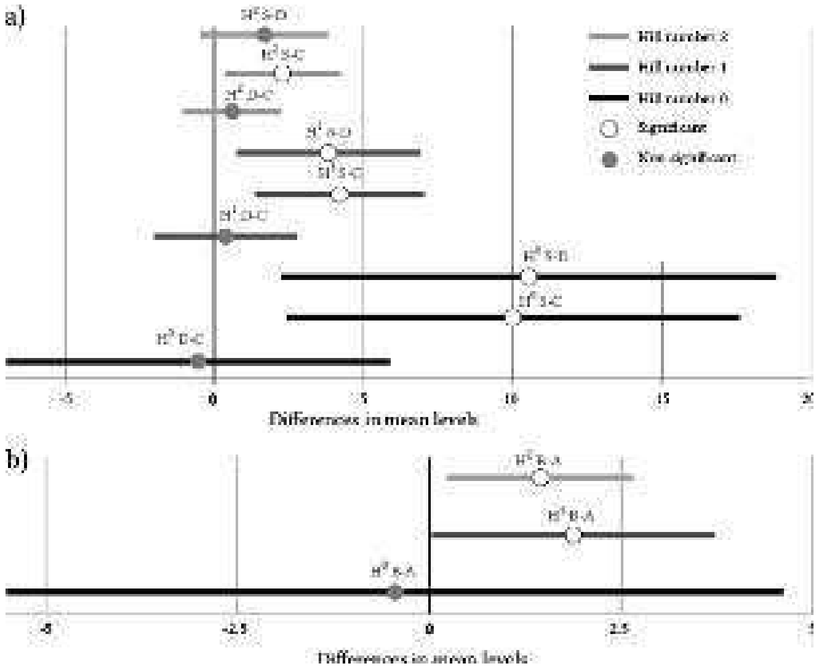


Fig. 1: Effect of (a) fire severity (C: control; S: surface fire; D: destructive crown fire) and (b) depth (A: organic layer and B: mineral layer) on ECM fungal diversity in soil. X-axes indicate the difference in mean levels and confidence interval using Tukey honest significant difference on linear models involving Hill numbers (H^0, H^1 et H^2) in function of sample size (square root of the number of sequence) and either fire severity or depth.

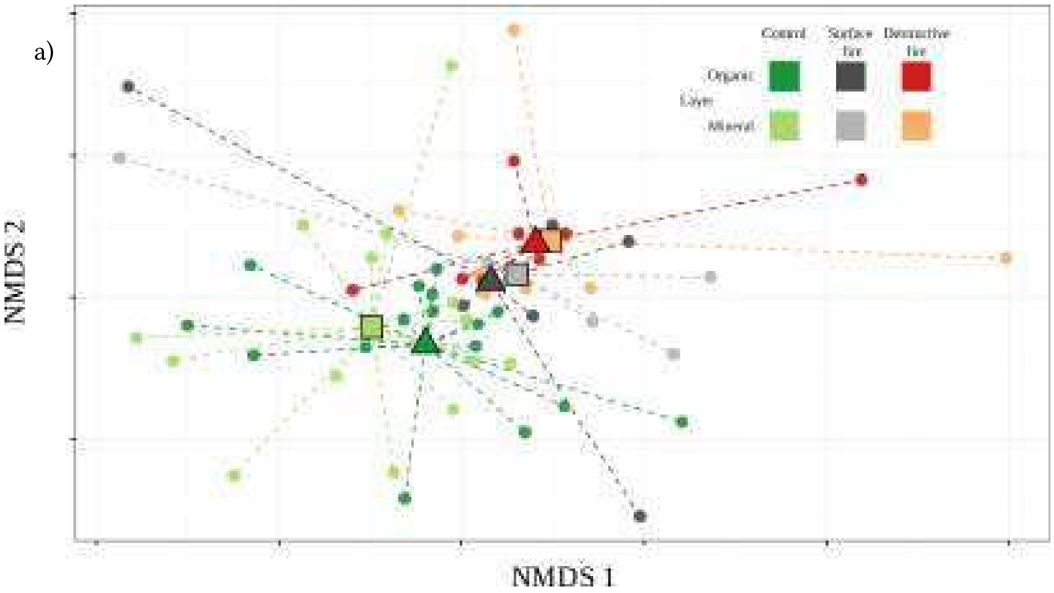


Fig. 2: Non metric multidimensional scaling (using Gower distances) showing the difference in species composition (a) in soil and (b) in roots tips in function of host. Stress is equal to 25.4% for panel (a).

Fig. 3: Change in molecular abundance of ECM OTUs present in soil in function of fire severity. Each point is an OTU whose abundance significantly shift regarding fire severity (*Deseq2* R package). Left panel compare abundance after surface fire and without fire. Middle panel compare abundance after destructive fire and surface fire. Right panel compare abundance after destructive fire and without fire. Colors indicate fungal Order. Note the scale of y-axis are different for each panel.

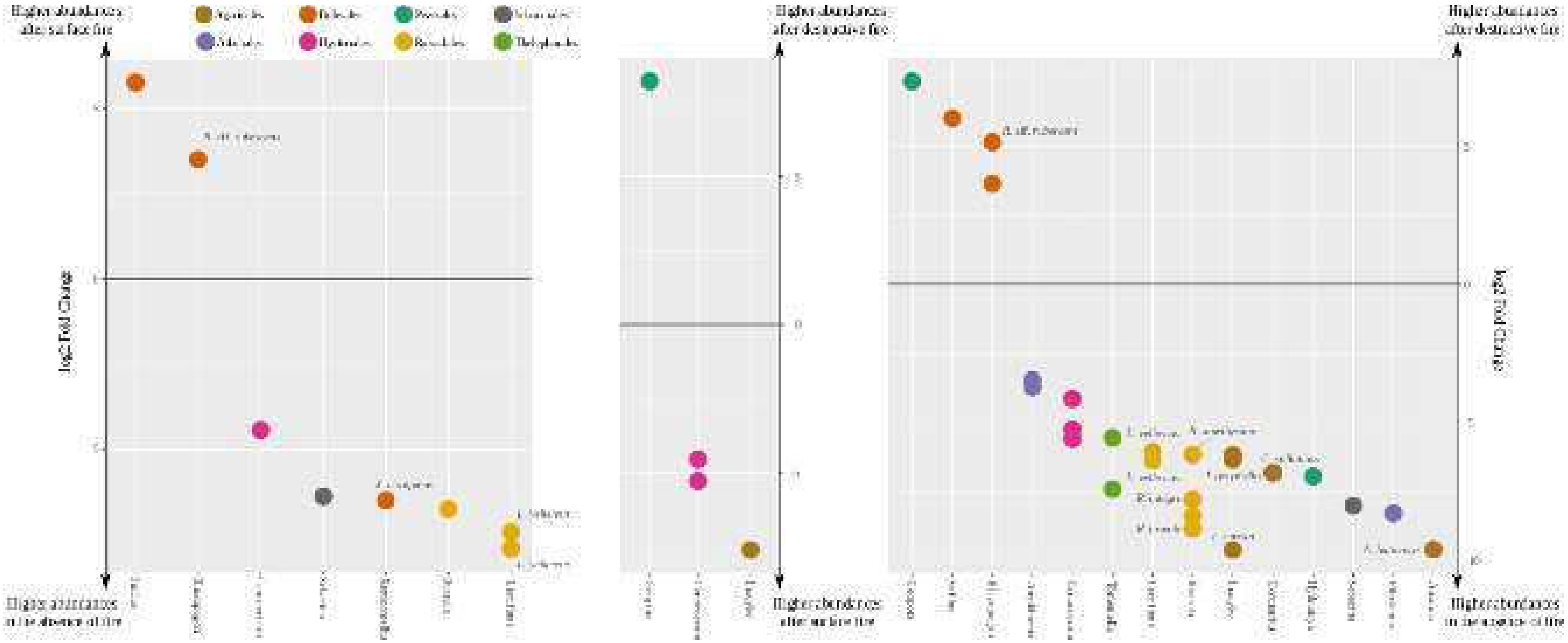
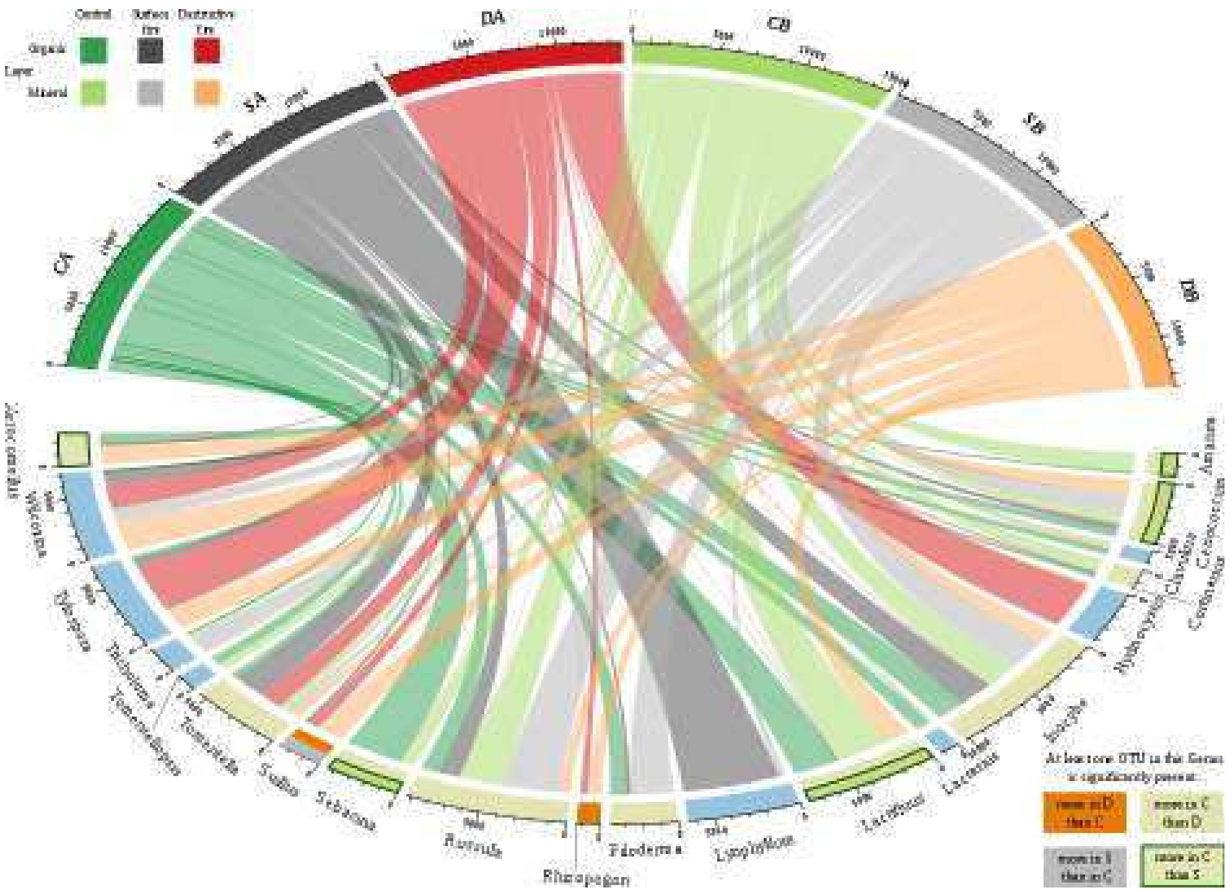


Fig. 4: Distribution of the number of sequences for fungal Genus in function of fire severity and depth. All modalities were first rarefy at the same sequences numbers. Only fungal genus representing more than 1% of the total number of sequences are represented. See SM for other representation. Modalities and ribbons colors are the same as figure 1. Fungal genus with at least one OTU that differ in molecular abundance (using *DESeq2* package) among different fire severity are colored following the schema in the left down corner. Genus in blue do not contain any OTU showing significant preference regarding fire severity.



Projet de manuscrit J : "Soil saprobic fungal community assembly: effect of fire and forest species composition"

Fructification de morille (Morchella sp) après feu dans une forêt d'arbousier.

Soil saprobic fungal community assembly: effect of fire and forest species composition

Adrien Taudière [1, 4*], **Christopher Carcaillet** [2], **Sebastian Leidel** [3] **& Franck Richard** [4]

1/ École Pratique des Hautes Études (EPHE) & Paris Research University, Paris, France

2/ Laboratoire d'Écologie des Hydrosystèmes Naturels et Anthropisés (UMR5023 CNRS), Université Lyon 1, 69 622 Villeurbanne, France

3/ Max Planck Research Group for RNA Biology, Max Planck Institute for Molecular Biomedicine, Von-Esmarch-Strasse 54, 48 149 Muenster, Germany

4/ UMR 5175, CEFE – CNRS – Université de Montpellier, Montpellier, France

* Corresponding authors: adrien.taudiere@cefe.cnrs.fr

Abstract:

Saprobic fungi (SF) are basic component of nutrient cycling. Diversity and community composition of SF determine ecosystem functioning and community of organisms with which they interact. Moreover, SF showed to be useful to understand community assembly process. Factors shaping SF communities are therefore crucial for both applied and fundamental research. Using Illumina sequencing of soil DNA, we characterize SF communities (i) in forests dominated by different tree species, including pure and mixed forests (72 samples), (ii) in *Pinus nigra* subsp. *laricio* stands who undergone either the absence of fire since decades, a surface fire or a destructive crown fire (87 samples).

1 448 604 sequences were clustered into 632 SF Operational Taxonomic Unit (OTUs). Both taxonomy of dominant tree and fire severity affect SF community composition but not diversity. Contrariwise, organic layer of *Pinus nigra* forest harbor more SF diversity than mineral layer. SF communities of mixed forests (two coexisting dominant tree species) is made of a mix of the communities present in pure forest of the two tree species. Finally, fire shape SF communities by both species replacement and shift in OTUs relative abundance, and this shift is more important in the organic layer than in the mineral layer.

Keywords: disturbance, soil microorganisms, NGS, mixed forest, Corsica, saprobic fungi

Introduction

Saprobic organisms, in particular microorganisms one, are basic component of nutrient cycling and ecosystem functioning due to their ability to decompose dead organic matters (McGuire and Treseder 2010; Bardgett and van der Putten 2014; Treseder and Lennon 2015). Regarding decomposition ability, saprobic fungi (SF) differ from Bacteria in terms of extracellular enzyme activities (Romani et al. 2006) and capacity to aggregate soil (Lehmann and Rillig 2015). In particular, only some fungal species, mainly in the Basidiomycota phylum, are able to massively decompose lignine (Hammel 1997; Bugg et al. 2011; Floudas et al. 2012).

Diversity (Hooper et al. 2012, Bardgett and van der Putten 2014) and community composition (McGuire and Treseder 2010) of microorganisms are though to be important for ecosystem functioning. Moreover, works on SF recently shed light into community ecology of microorganisms. For instance, Fukami et al. (2010) used wood-decaying fungi to study assembly history effect on ecosystem functioning, Taylor et al. (2014) studies niche partitioning of soil fungal communities and Tu et al. (2015) highlight community reassembly of fungi in response to long-term elevated CO^2. Consequently, understanding factors shaping SF communities are crucial for both applied and fundamental research. We focus here on the SF present in forest soil excluding the litter that harbor different SF communities (Baldrian et al. 2012).

Both tree genetic (Schweitzer et al. 2008) and species identity (Ushio et al. 2008) drives composition of soil microorganisms. In forest, dominant tree(s) could affect SF through direct fungal preference (host recurrence of Zhou and Hide 2001) for the dead tissues of one or several plant species, but also through the chemical, physical and biological properties of the forest soil where dominant tree(s) live. Both tree density and species identity drives soil chemical and physical properties (Guckland et al. 2009; Langenbruch et al. 2012); and soil properties can in turn drives biota composition (Lauber et al. 2008).

Fire is a major driver of ecosystem composition and functioning worldwide (Bond and Keeley 2005) and particularly in the Mediterranean biome (Keeley 2012). The high socioeconomic importance (Moritz et al. 2014) and the ecological impact of fire on biotic (Neary et al. 1999) and abiotic (Certini 2005) soil properties is indisputable. For example, in a meta-analysis, Holden and Treseder (2013) found a reduction of 55.2% in soil fungal biomass due to fire (n = 13 publications). Fire affect soil microorganisms through multiple pathway including direct death of microorganisms, short and long terms modification of soil aggregation and chemical composition, and death of interacting species (e.g. plants and predators; Neary 1999; Mataix-Solera et al. 2009; Dooley and Treseder 2012).

In Corsica (France), a Mediterranean Island of 8 681 km², fire play a major role in nature including human activities. In particular, in 2000, fire damaged 848 ha of *Pinus nigra* ssp *laricio* (Thibault et al. 2004). This endemic and emblematic tree is pivotal for numerous species including the endemic bird *Sitta whiteheadi* (Thibault et al. 2004). *P nigra* ssp *laricio* forests (900 m − 1 800 m, Rameau et al. 2008) are considered as priority habitats for Europe. *Pinus pinaster* stand were also affected by fire but this species is far better adapted to fire and regeneration of *P pinaster* stand are fast. The last considered species, *Fagus sylvatica* grows in place unfavorable for fire and reinforce the fire-resistance of its environment by decreasing the probability of fire ignition through its particular litter.

In this study we test several hypotheses of the impact of fire in *Pinus nigra* ssp *laricio* forest and forest composition on soil SF: diversity and composition (Table 1). We hypothesize that SF diversity is higher in mixed forest than in pure one (Hyp. **Ia**), in the organic layer than in the mineral layer and (Hyp. **Ia**) and in unburnt forest than in forest that undergone a surface fire than in forest entirely destructed by fire (Hyp. **Ic**). Concerning SF community composition of mixed forests (beech − laricio pine and laricio pine − maritime pine), we supposed that its is made of a mix of SF communities present in pure stand (Hyp. **IIa**). We also hypothesize that forests dominated by the two pine species harbor more similar communities than pine forests with beech one (Hyp. **IIb**). Finally, we hypothesize that fire shape SF communities of *Pinus nigra* ssp *laricio* forest by both species replacement and shift in taxa relative abundance (Hyp. **IIIa**) and that this shift is stronger in the organic layer than in the mineral layer (Hyp. **IIIb**).

Materials and Methods

Sites description and soil sampling

Fire severity

We sampled soil fungal community in three sites burnt in 2000 (*Ghisoni*, *Rospa Sorba* and *Restonica*). In each sites we choose 3 plots for each of the 3 modalities: controls (unburnt; not available in *Restonica* forest), surface fire and destructive fire. In each plot, we sampled 5 soils cores separating organic layer (A) from mineral layer (B). As our sampling encompass very different soil depth and type, a purely metric methods is not applicable to recognize the two layers. Moreover, our objective is not to map fine spatial grain but to sample two contrasted depth. Thus, we visually defined the two layers on the fields and we always excavated more than 3 cm between layers. Finally, we mixed the 5 soils cores in samples for 2 sites and we keep each soils cores intact for the plots at *Ghisoni*. The total number of soil samples is 72 (Ghisoni: [3 plots * 2 modalities + 3 controls plots * 5 cores] * 2 depths; *Rospa Sorba:* 3 plots * 3 modalities * 2 depths; *Restonica*: 3 plots * 2 modalities * 2 depths).

Dominant tree(s)

We sampled soil fungal community in (i) three sites for pure beech forest (*Fagus sylvatica*) and mixed forest *F. sylvatica* and *Pinus nigra* subsp. *laricio* (*Ghisoni*, *Manganello* and *Vizavonna*); (ii) three sites for pure *Pinus pinaster* stand and mixed forest *P. pinaster* and *P. nigra* subsp. *laricio* (*Ghisoni*, *Manganello* and *Verghello*); (iii) 4 sites for pure *P. nigra* subsp. *laricio* stand (*Ghisoni*, *Manganello, Rospa Sorba* and, *Vizavonna*).

For each pure stand modalities, we collected 5 soil cores in three different locations (plot) in each site. We then mixed the five soil cores in one soil sample by plots For soil of mixed forest, we collect five soil cores by sites (see Taudiere et al. *unpublished* [manuscript G in the thesis] for the rationale of this sampling scheme). To summarize we collected 3 plots in the 3 sites for the 2 modalities of pure stand, 3 plots in one sites for *P. nigra* subsp. *laricio* pure stand and 5 cores in the 3 sites for mixed forest (18 + 3 + 30 = 51 samples). We add 36 samples of unburnt pure *P. nigra* subsp. *laricio* used as controls in the "fire" analysis leading to a total of 87 samples.

Sequencing and bioinformatics processing

Soil samples were blent and filtered using coffee filter. We extract DNA from 1.5 ml of soil using the FastDNA™ SPIN Kit for Soil (*MP Biomedicals*). In addition, we made an ethanol precipitation (detailed ***online***) before to send samples to MiSeq plateform.

The detailed workflow from raw sequences to OTUs tables and taxonomy is available in supplementary materials (Fig. S1 and script S2). To summarize, sequences were quality filtered using *sickle* (single pe command, quality threshold = 20). We checked also the quality profiles before and after quality filtering using *FastQC* (Andrews 2010; Fig. S3 and S4). Then we separated ITS-1 and ITS-2 using custom bash script and *fqgrep* (Indraniel et al. 2016). Then we applied the same workflow in parallel for both markers. Sequences were demultiplexed (same methods as Balint et al. 2014) and dereplicated. Chimeras removing were assessed using *Uchime* (Edgar et al. 2011) and the Unite database (dynamic v.7 release of 01-08-2015; Kõljalg et al. 2013), while non ITS sequences were removed using *ITSx* (Bengtsson-Palme et al. 2013). Sequences were clustered into OTUs using *Uparse* (Edgar 2013). Other clustering methods, namely Swarm (Mahé et al. 2015) and Uclust (Edgar 2010) gave very closed ecological results (SM 7).

Then, *RPD classifier* (Wang et al. 2007) was used to assign taxonomy for each representative sequences (function *assign_taxonomy.py* of *Qiime* software [Caporaso et al. 2010]; bootstrap threshold -c 80% following recommendation of Claesson et al. 2009; Unite database). We attribute guild (e.g. lichenized) and trophic mode (e.g. saprotrophe). *FUNGuild* (Nguyen et al. 2015) is a software and a database that allow attributing functional guild to OTUs using taxonomic information.

For subsequent ecological analysis we kept samples containing more than 10 000 sequences and OTUs represented by at least 5 sequences (Brown et al. 2015) and determined as—at least partially—saprotrophic by *FUNGuild.* This lead to a total of 810 471 (638 133) sequences distributed in 581 (558) OTUs and 85 (60) samples for dominant "tree analysis" ("fire analysis"; Table 2). All analysis were computed with *R 3.2.4* (R Core Team, 2016) and the *Phyloseq* package 1.12.2 (McMurdie et al. 2013). A dynamic report script (SM 7) allow running again all the analysis using different parameters of filtering (as in Taudiere et al. *in prep.;* Manuscript H in the thesis).

Statistical analysis

We measure fungal richness using observed number of OTUs but also using Chao and Fisher alpha metrics. Biodiversity was assessed by Simpson and Shannon index. We use linear model and Tuckey post-hoc tests to test for sample variables (fire, depth, dominant tree(s) and sites) effect of diversity metrics. Even if we do not detect difference in sampling depth depending of samples variables (SM 7), we account for difference in sampling depth by using the number of read as the first explanatory variable in the linear model following Bálint et al. (2015). We explore taxonomic diversity of OTUs and read number in function of modalities using the software Krona (Ondov et al. 2011; Fig. S5 and S6).

We test the effect of sample variables on beta-diversity using PerManova (Anderson 2001; 9 999 permutations, function *adonis* of the *vegan* package [Oksanen et al. 2016]). We used non-metric multidimensional scaling (NMDS) using Gower distance to visualize b-diversity in function of sample variables (see SM 7 for alternative multivariate analysis).

To determine OTUs whose abundances depend of sample variables, we use non parametric (rank-based) multiple testing (function *mt* in package *phyloseq*) using F-statistic. Test of significant correlation among OTUs were assessed using a method developed by Morueta-Holme et al. (2015) with 999 null models and alpha = 0.01 (*make_netassoc_network* in package *netassoc* [Blonder and Morueta-Holme 2015]). Modules definition in the networks were computed using a method (function *cluster_louvain* in package *igraph* [Csardi and Nepusz 2015]) of Blondel et al. (2008). We recognize that others methods may be more precise, but as modules are only used in graphical representation, we think this method is far sufficient. Finally, we used hierarchical clustering of samples and OTUs (function *hclust*).

Results

Taxonomy of dominant trees affect fungal composition but not diversity

The composition of dominant tree is not correlates with fungal saprobic diversity (adjusted p-value > 0.05 in Tuckey post-hoc tests following linear models on the five metrics of Fig. 1; SM 7). The only exception is the higher diversity in *Pinus nigra* pure stand compared to mixed *Fagus sylvatica-Pinus nigra* stand (Shannon adjusted p-values = 0.017; Simpson adjusted p-values = 0.049).

On the other hand, dominant tree(s) taxonomy drives OTUs composition (Fig. 2a) and explain 11.75% of b-diversity patterns (PerManova; Table 3). Dominant tree(s) significantly shape the abundances of 25 OTUs (R function *mt*; 13 using the parametric test; SM 7) belonging to five different Families (Fig. 3). Consequently, dominant tree shape co-occurence network of fungal OTUs (Fig. 4).

Mixed forest harbor the same diversity than pure one. Moreover, mixed forest fungal composition is made of a mix of taxa present in pure forest of tree present in the mixed forest (NMDS; Fig. 2a).

Fire affects fungal composition but not diversity

Saprothophic fungal diversity is not affected by fire 15 years after the fire (adjusted p-value > 0.05 in Tuckey post-hoc tests following linear models on the five metrics of Fig. 1;

SM 7). In contrast, all diversity metrics are lower in the mineral layer than in the organic layer (adjusted p-value < 0.05 using Tuckey post-hoc tests).

Fire affects saprophytic fungal composition and this effect is buffer by depth (Fig 2b and Fig. 5). At the phylum level, this shift is due to change in abundances rather than change in species richness (Fig. 5a and b). In particular, the genus *Mortierella* (Zygomycota) represent 44% of sequences in the organic layer of control and only 11% in the organic layer respectively after a surface (21% after a destructive fire; Fig. S6). Contrastingly, sequences belonging to fungi of the genus *Penicillium* increase in frequency from 11% in the control organic layer to 33% and 35% in the organic layer after surface and destructive fire respectively (Fig. S6).

Surface fire induce a decrease of the number of sequences belonging to Zygomycota in favor of Ascomycota in Organic layer and in favor of both Ascomycota and Basidiomycota (Fig. 5a). Destructive fire induce a shift in organic soil layer only from communities dominated by Zygomycota sequences to communities dominated by Ascomycota sequences (Fig. 5a).

For both layers, community composition of saprobic fungi after a surface fire is close to the composition of the mineral layer after a destructive fire or without fire (Fig. 2b).

Discussion

Soil SF communities of mixed forest are made of a mixture of communities from pure stand without an increase in diversity

Fire drives a marked shift in soil SF communities by both species replacement and change in relative abundance

References

Anderson MJ. **2001**. A new method for non-parametric multivariate analysis of variance. *Austral ecology* **26**: 32–46.

Andrews S, others. **2010**. FastQC: A quality control tool for high throughput sequence data. *Reference Source.*

Baldrian P, Kolařík M, Štursová M, Kopecký J, Valášková V, Větrovský T, Žifčáková L, Šnajdr J, Rídl J, Vlček Č, *et al.* **2012**. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *The ISME Journal* **6**: 248–258.

Bálint M, Bartha L, O'Hara RB, Olson MS, Otte J, Pfenninger M, Robertson AL, Tiffin P, Schmitt I. **2015**. Relocation, high-latitude warming and host genetic identity shape the foliar fungal microbiome of poplars. *Molecular Ecology* **24**: 235–248.

Bálint M, Schmidt P-A, Sharma R, Thines M, Schmitt I. **2014**. An Illumina metabarcoding pipeline for fungi. *Ecology and Evolution* **4**: 2642–2653.

Bardgett RD, van der Putten WH. **2014**. Belowground biodiversity and ecosystem functioning. *Nature* **515**: 505–511.

Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, Godhe A, De Wit P, Sánchez-García M, Ebersberger I, de Sousa F, *et al.* **2013**. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution* **4**: 914–919.

Bond WJ, Keeley JE. **2005**. Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology & Evolution* **20**: 387–394.

Brown SP, Veach AM, Rigdon-Huss AR, Grond K, Lickteig SK, Lothamer K, Oliver AK, Jumpponen A. **2015**. Scraping the bottom of the barrel: are rare high throughput sequences artifacts? *Fungal Ecology* **13**: 221–225.

Bugg TDH, Ahmad M, Hardiman EM, Rahmanpour R. **2011**. Pathways for degradation of lignin in bacteria and fungi. *Natural Product Reports* **28**: 1883.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, *et al.* **2010**. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**: 335–336.

Certini G. **2005**. Effects of fire on properties of forest soils: a review. *Oecologia* **143**: 1–10.

Claesson MJ, O'Sullivan O, Wang Q, Nikkilä J, Marchesi JR, Smidt H, Vos WM de, Ross RP, O'Toole PW. **2009**. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PLOS ONE* **4**: e6669.

Dooley SR, Treseder KK. **2012**. The effect of fire on microbial biomass: a meta-analysis of field studies. *Biogeochemistry* **109**: 49–61.

Edgar RC. **2010**. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460–2461.

Edgar RC. **2013**. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**: 996–998.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. **2011**. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–2200.

Floudas D, Binder M, Riley R, Barry K, Blanchette RA, Henrissat B, Martinez AT, Otillar R, Spatafora JW, Yadav JS, *et al.* **2012**. The paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science* **336**: 1715–1719.

Fukami T, Dickie IA, Paula Wilkie J, Paulus BC, Park D, Roberts A, Buchanan PK, Allen RB. **2010**. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. *Ecology Letters* **13**: 675–684.

Guckland A, Jacob M, Flessa H, Thomas FM, Leuschner C. **2009**. Acidity, nutrient stocks, and organic-matter content in soils of a temperate deciduous forest with different abundance of European beech (*Fagus sylvatica* L.). *Journal of Plant Nutrition and Soil Science* **172**: 500–511.

Hammel KE. **1997**. Fungal degradation of lignin. *Driven by nature: plant litter quality and decomposition*: 33–45.

Holden SR, Treseder KK. **2013**. A meta-analysis of soil microbial biomass responses to forest disturbances. *Frontiers in Microbiology* **4**.

Hooper DU, Adair EC, Cardinale BJ, Byrnes JEK, Hungate BA, Matulich KL, Gonzalez A, Duffy JE, Gamfeldt L, O'Connor MI. **2012**. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*.

Indraniel D, Gosselin T. **2016**. *fqgrep: v0.4.4*.

Keeley JE (Ed.). **2012**. *Fire in Mediterranean ecosystems: ecology, evolution and management*. Cambridge, UK ; New York: Cambridge University Press.

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, *et al.* **2013**. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* **22**: 5271–5277.

Langenbruch C, Helfrich M, Flessa H. **2011**. Effects of beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*) and lime (*Tilia* spec.) on soil chemical properties in a mixed deciduous forest. *Plant and Soil* **352**: 389–403.

Lauber CL, Strickland MS, Bradford MA, Fierer N. **2008**. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry* **40**: 2407–2415.

Lehmann A, Rillig MC. **2015**. Understanding mechanisms of soil biota involvement in soil aggregation: A way forward with saprobic fungi? *Soil Biology and Biochemistry* **88**: 298–302.

Mahé F, Rognes T, Quince C, de Vargas C, Dunthorn M. **2015**. *Swarm v2: highly-scalable and high-resolution amplicon clustering*. PeerJ PrePrints.

McGuire KL, Treseder KK. **2010**. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. *Soil Biology and Biochemistry* **42**: 529–535.

McMurdie PJ, Holmes S. **2013**. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data (M Watson, Ed.). *PLoS ONE* **8**: e61 217.

Moritz MA, Batllori E, Bradstock RA, Gill AM, Handmer J, Hessburg PF, Leonard J, McCaffrey S, Odion DC, Schoennagel T, *et al.* **2014**. Learning to coexist with wildfire. *Nature* **515**: 58–66.

Morueta-Holme N, Blonder B, Sandel B, McGill BJ, Peet RK, Ott JE, Violle C, Enquist BJ, Jørgensen PM, Svenning J-C. **2015**. A network approach for inferring species associations from co-occurrence data. *Ecography*: *In press*

Neary DG, Klopatek CC, DeBano LF, Ffolliott PF. **1999**. Fire effects on belowground sustainability: a review and synthesis. *Forest ecology and management* **122**: 51–71.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. **2015**. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, *et al.* **2016**. *vegan: Community Ecology Package*.

Ondov BD, Bergman NH, Phillippy AM. **2011**. Interactive metagenomic visualization in a Web browser. *BMC bioinformatics* **12**: 385.

Rameau J-C, Mansion D, Dumé G, Gauberville C. **2008**. *Flore forestière française Tome 3*. Paris: Institut pour le développement forestier.

Romaní AM, Fischer H, Mille-Lindblom C, Tranvik LJ. **2006**. Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. *Ecology* **87**: 2559–2569.

Schweitzer JA, Bailey JK, Fischer DG, LeRoy CJ, Lonsdorf EV, Whitham TG, Hart SC. **2008**. Plant–soil–microorganism interactions: heritable relationship between plant genotype and associated soil microorganisms. *Ecology* **89**: 773–781.

Taylor DL, Hollingsworth TN, McFarland JW, Lennon NJ, Nusbaum C, Ruess RW. **2014**. A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. *Ecological Monographs* **84**: 3–20.

The R Core Team. **2016**. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Thibault J-C, Prodon R, Moneglia P. **2004**. Estimation de l'impact des incendies de l'été 2000 sur l'effectif d'un oiseau endémique menacé: la sittelle corse (*Sitta whiteheadi*). *Ecol Mediterr* **30**: 195–203.

Treseder KK, Lennon JT. **2015**. Fungal traits that drive ecosystem dynamics on land. *Microbiology and Molecular Biology Reviews* **79**: 243–262.

Tu Q, Yuan M, He Z, Deng Y, Xue K, Wu L, Hobbie SE, Reich PB, Zhou J. **2015**. Fungal communities respond to long-term CO ^{2} elevation by community reassembly (JE Kostka, Ed.). *Applied and Environmental Microbiology* **81**: 2445–2454.

Ushio M, Wagai R, Balser TC, Kitayama K. **2008**. Variations in the soil microbial community composition of a tropical montane forest ecosystem: Does tree species matter? *Soil Biology and Biochemistry* **40**: 2699–2702.

Wang Q, Garrity GM, Tiedje JM, Cole JR. **2007**. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Applied and Environmental Microbiology* **73**: 5261–5267.

Zhou D, Hide KD. **2001**. Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. *Mycological Research* **105**: 1449–1457.

Table 1: Hypothesis tested in this work. ✓, ✗ and (✓) indicate respectively that we confirm, infirm or partially confirm our hypothesis.

(I) Soil SF diversity is higher ...	**Ia**) ... in mixed forest than in pure one	✗
	Ib) ... in the organic layer than in the mineral layer in laricio forest	✓
	Ic) ... in unburnt *P. nigra* forest than in forest that undergone a surface fire than in forest entirely destructed by fire	✗
(II) Soil SF communities composition is shaped by dominant tree(s) identity	**IIa**) SF communities of mixed forests is made of a mix of the communities present in corresponding pure stand	✓
	IIb) Forests dominated by the two pine species harbor more similar SF communities than pine forests with beech one	(✓)
(III) Fire shape soil SF communities...	**IIIa**) ... and this effect is stronger after a destructive fire than a surface fire	(✓)
	IIIb) ... and this effect is stronger in the soil organic layer than in the mineral layer	✓

Table 2: Number of sites, samples, OTUs and sequences for each analysis

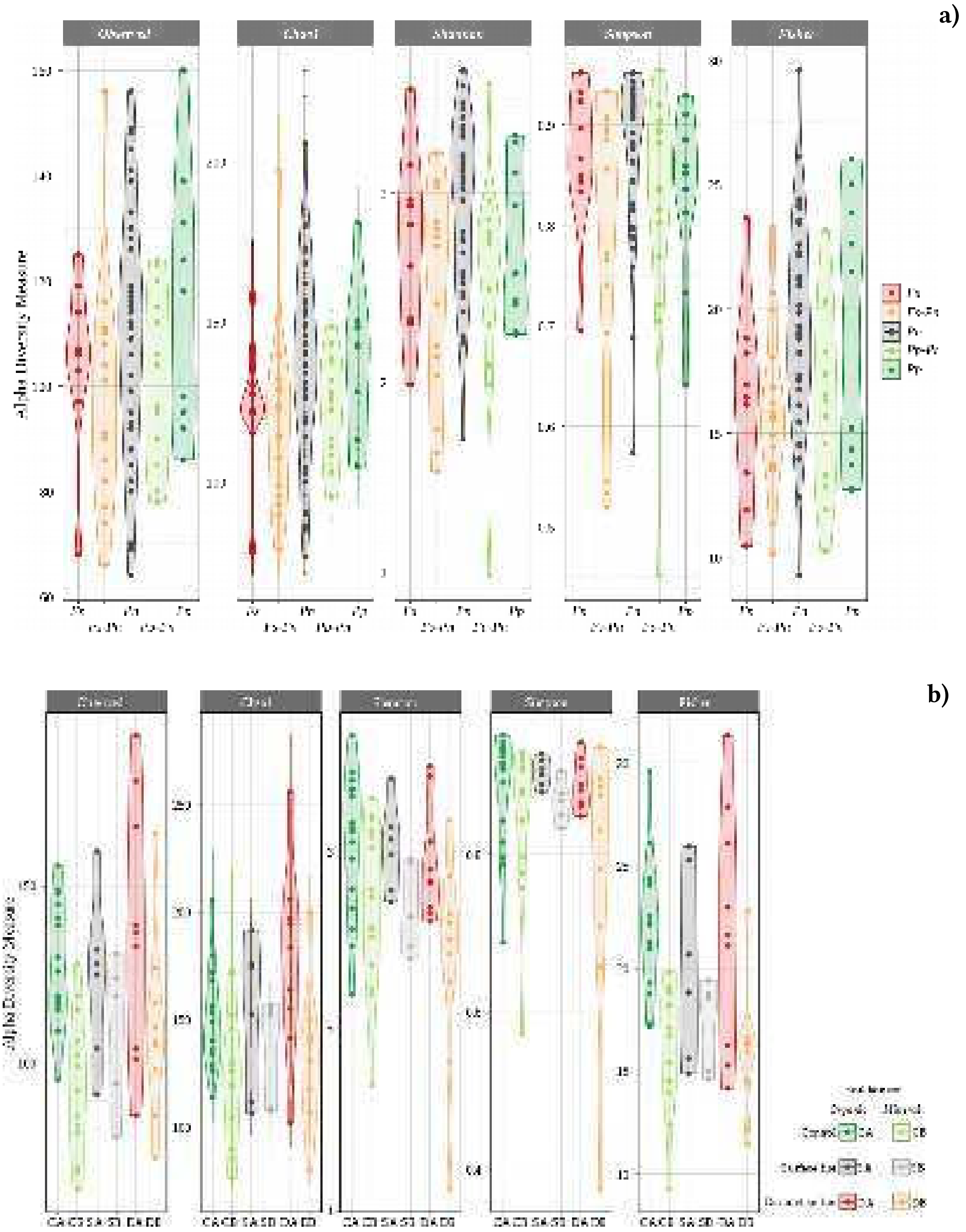
	Sites	Soil samples	Samples analysed	OTUs	Sequences
Dominant tree(s)	3	87	85	581	810 471
Fire	6	72	60	558	638 133

Table 3: Result of PerManova for (a) effect of dominant tree and (b) effect of fire severity

a)	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Dominant tree(s)	4	3.0762	0.76905	3.0821	0.11775	**0,0001**
Sites	5	3.4538	0.69076	2.7684	0.13221	**0,0001**
Dominant tree(s):Sites	6	2.3776	0.39627	1.5881	0.09101	**0,0006**
Residuals	69	17.2168	0.24952		0.65903	
Total	84	26.1244			1.00000	

b)	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Sites	2	1.5873	0.79365	3.1665	0.08875	**0.0001**
Fire	2	1.5598	0.77992	3.1117	0.08721	**0.0001**
Layer	1	1.0408	1.04083	4.1527	0.05819	**0.0002**
Fire:Layer	2	0.6640	0.33199	1.3246	0.03712	0.1136
Residuals	52	13.0333	0.25064		0.72872	
Total	59	17.8852			1.00000	

Fig. 1: Fungal diversity in function of (a) dominant tree(s) species and (b) fire severity. Vioplot represent density of points. (a) Abbreviations used: *Fs*: *Fagus sylvatica*; *Pn*: *Pinus nigra* subsp. *laricio*; *Pp*: *Pinus pinaster.* (b) Diversity in function of fire severity in *Pinus nigra* subsp. *laricio* stand: control (green), surface-fire (grey) and destructive fire (red). Strong colors indicate organic layers and clearer colors indicate mineral layers.



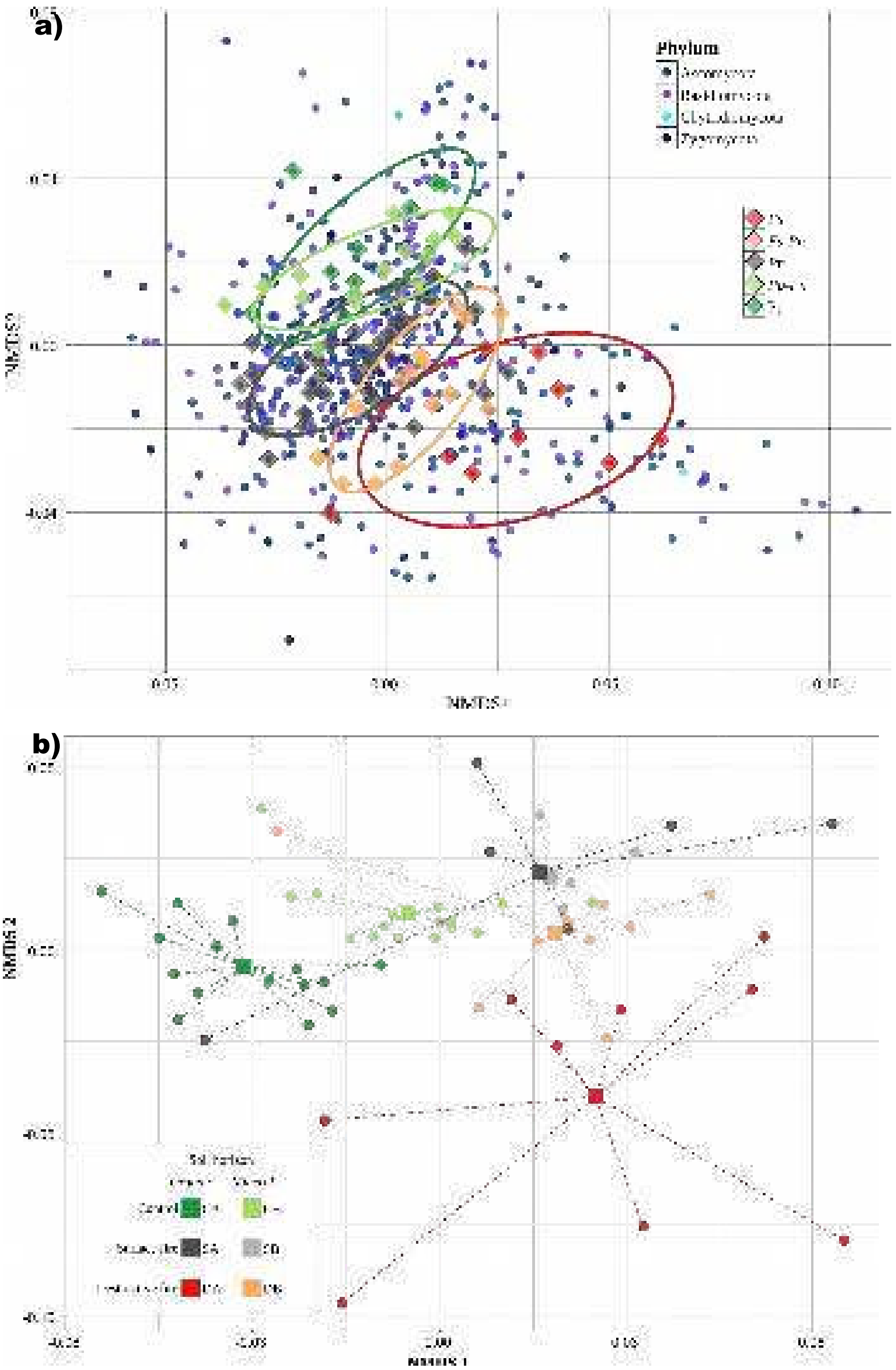


Fig. 2: Non metric multidimensional scaling (Gower distances) showing the difference in species composition (a) among forest dominate by different tree and (b) among forest with different fire history. Same legend as figure 1. The NMDS stress is equal to 22.1% for panel (a) and 18.6% for panel (b).

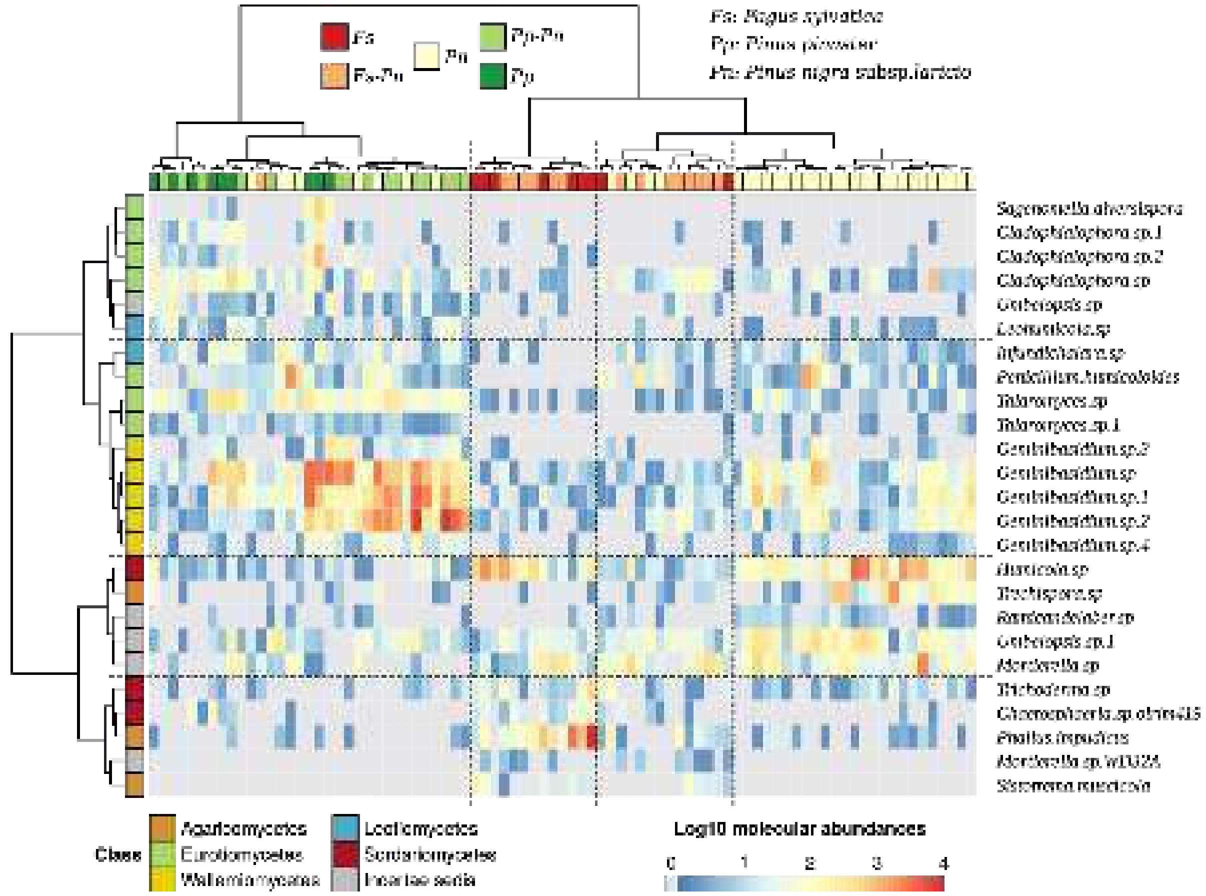


Fig. 3: Distribution of fungal taxa significantly affected by the taxonomy of dominant tree. Colors indicate dominant tree(s) in columns, fungal class in rows and read number (log10-transformed) in cells. Rows and columns are ordered using hierarchical clustering (ward method). Dashed lines split the data in four groups given hierarchical clustering trees (OTUs clusters used in Fig. 4).

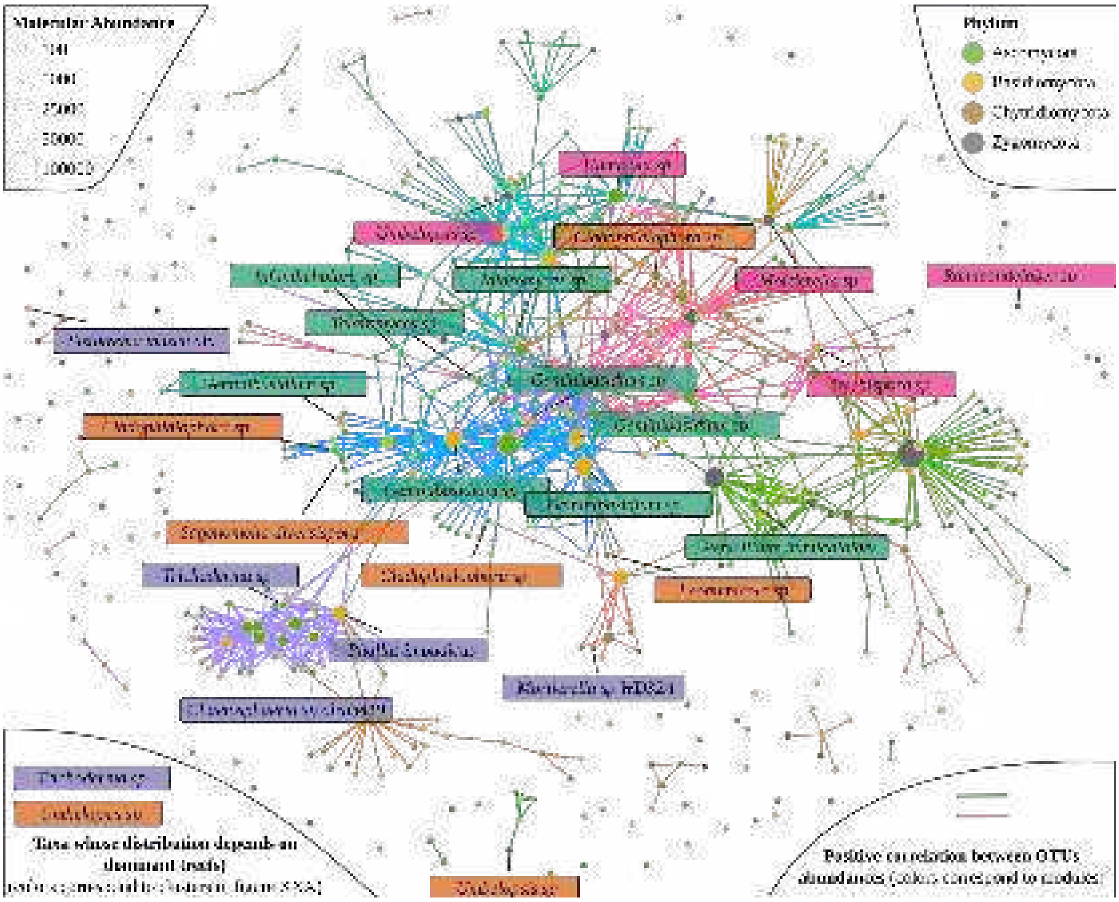


Fig. 4: Correlation network among OTUs. Network linking the 581 saprotrophic OTUs. Each link represents a positive and significant correlation between two OTUs (p-value < 0.05 and SES values > 6; see SM 7 for other cut-off values). Link colors code for modules (function *cluster_louvain* in package igraph). Each OTU is represented by a circle whose size is proportionnal to the number of read and color represent the Phylum. Taxa names are given for OTUs significantly affected by dominant tree(s) taxonomy (function *mt* in package phyloseq). Filled color of boxes correspond to the cluster in figure 3.

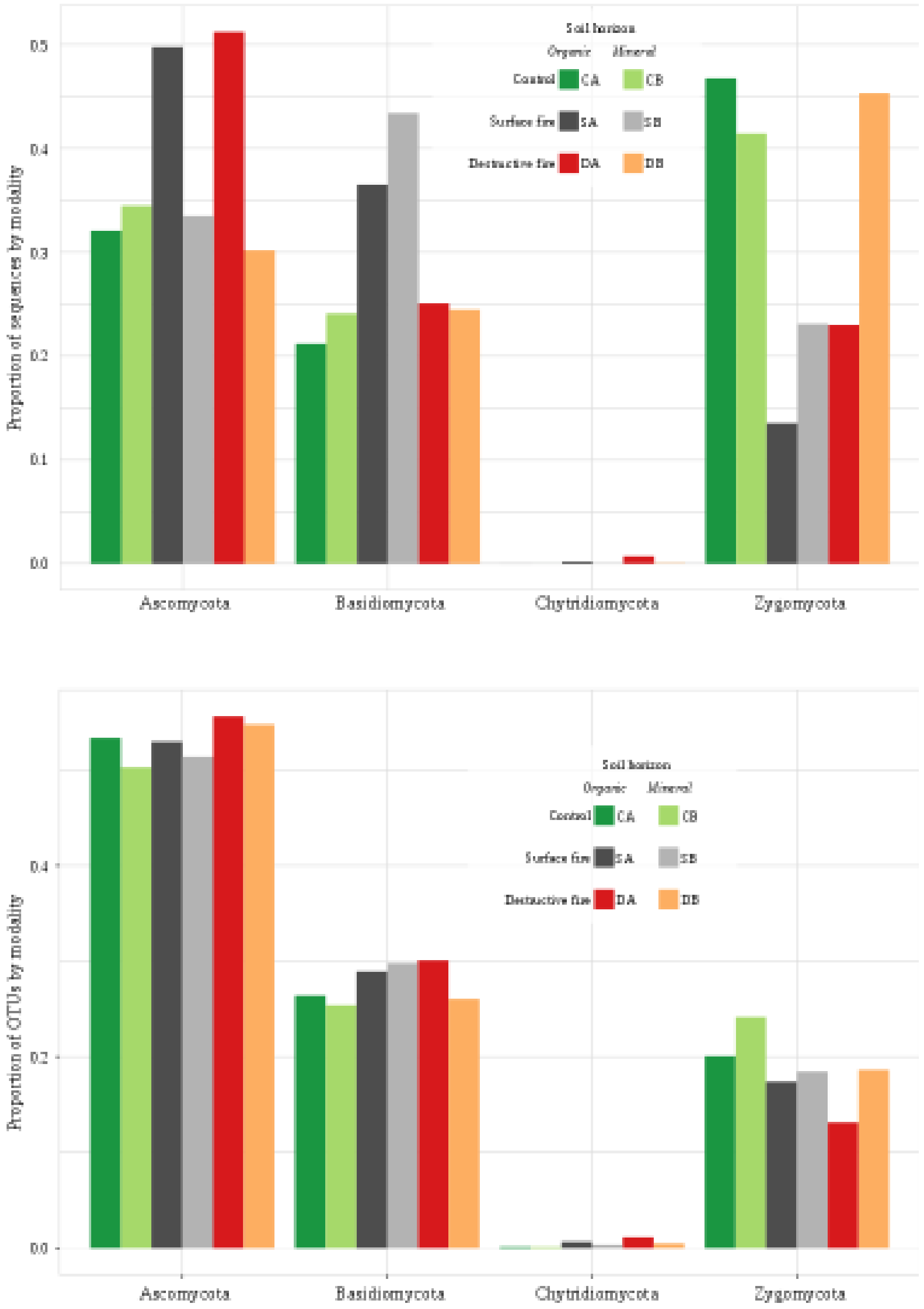


Fig. 5: Distribution of the number of sequences (a) and OTUs (b) by phylum for each fire * layer modality.

Supplementary materials:

FIG S1 (pdf): Bioinformatics pipeline

SM S2 (pdf): UNIX scripts for bioinformatics pipeline

FIG S3 (html): FastQC result of sequences quality before quality filtering. Available at: https://github.com/adrientaudiere/SF_paper/

FIG S4 (html): FastQC result of sequences quality after quality filtering. Available at: https://github.com/adrientaudiere/SF_paper/

FIG S5 (html): Comparison of SF taxonomic composition in forests dominated by different tree species. We build this figure using Krona (Ondov et al. 2011). Available at: https://github.com/adrientaudiere/SF_paper/

FIG S6 (html): Comparison of SF taxonomic composition in forests undergone different fire intensity. We build this figure using Krona (Ondov et al. 2011). Available at: https://github.com/adrientaudiere/SF_paper/

SM 7: Dynamic report script of ecological analysis in R language. Available at: https://github.com/adrientaudiere/SF_paper/

Manuscrits annexes

Manuscrit K : "cati: An R package using functional traits to detect and quantify multi-level community assembly processes"

Software notes

Ecography 38: 001–010, 2015
doi: 10.1111/ecog.01433

Subject Editor: Michael Borregaard. Editor-in-Chief: Miguel Araújo. Accepted 27 May 2015

cati: an R package using functional traits to detect and quantify multi-level community assembly processes

Adrien Taudiere and Cyrille Violle

A. Taudiere (adrien.taudiere@cefe.cnrs.fr) and C. Violle, CEFE UMR 5175, CNRS – Univ. de Montpellier – Univ. Paul-Valéry Montpellier – EPHE – 1919 route de Mende, FR-34293 Montpellier, Cedex 5, France.

Community ecologists are active in describing species by their functional traits, quantifying the functional structure of plant and animal assemblages and inferring community assembly processes with null-model analyses of trait distribution and functional diversity indices. Intraspecific variation in traits and effects of spatial scale are potentially important in these analyses.

Here, we introduce the R package cati (Community Assembly by Traits: Individuals and beyond) available on CRAN, for the analysis of community assembly with functional traits. cati builds on a recent approach to community assembly that explicitly incorporates individual differences in community assembly analyses and decomposes phenotypic variations across scales and organizational levels, based on three phenotypic variance ratios, termed the T-statistics. More generally, the cati package 1) calculates a variety of single-trait and multi-trait indices from interspecific and intraspecific trait measures; 2) it partitions functional trait variation among spatial and taxonomic levels; 3) it implements a palette of flexible null models for detecting non-random patterns of functional traits. These patterns can be used to draw inferences about hypotheses of community assembly such as environmental filtering and species interactions.

The basic input for cati is a data frame in which columns are traits, rows are species or individuals, and entries are the measured trait values. The cati package can also incorporate a square distance matrix into analyses, which could include phylogenetic or genetic distances among individuals or species. Users select from a variety of functional trait metrics and analyze these relative to a null model that specifies trait distributions in a regional source pool.

Software notes

Ecography 38: 001–010, 2015
doi: 10.1111/ecog.01433

Subject Editor: Michael Borregaard. Editor-in-Chief: Miguel Araújo. Accepted 27 May 2015

cati: an R package using functional traits to detect and quantify multi-level community assembly processes

Adrien Taudiere and Cyrille Violle

A. Taudiere (adrien.taudiere@cefe.cnrs.fr) and C. Violle, CEFE UMR 5175, CNRS – Univ. de Montpellier – Univ. Paul-Valéry Montpellier – EPHE – 1919 route de Mende, FR-34293 Montpellier, Cedex 5, France.

Community ecologists are active in describing species by their functional traits, quantifying the functional structure of plant and animal assemblages and inferring community assembly processes with null-model analyses of trait distribution and functional diversity indices. Intraspecific variation in traits and effects of spatial scale are potentially important in these analyses.

Here, we introduce the R package cati (Community Assembly by Traits: Individuals and beyond) available on CRAN, for the analysis of community assembly with functional traits. cati builds on a recent approach to community assembly that explicitly incorporates individual differences in community assembly analyses and decomposes phenotypic variations across scales and organizational levels, based on three phenotypic variance ratios, termed the T-statistics. More generally, the cati package 1) calculates a variety of single-trait and multi-trait indices from interspecific and intraspecific trait measures; 2) it partitions functional trait variation among spatial and taxonomic levels; 3) it implements a palette of flexible null models for detecting non-random patterns of functional traits. These patterns can be used to draw inferences about hypotheses of community assembly such as environmental filtering and species interactions.

The basic input for cati is a data frame in which columns are traits, rows are species or individuals, and entries are the measured trait values. The cati package can also incorporate a square distance matrix into analyses, which could include phylogenetic or genetic distances among individuals or species. Users select from a variety of functional trait metrics and analyze these relative to a null model that specifies trait distributions in a regional source pool.

Beyond species: trait distribution and individual differences in community ecology

Breaking down phenotypes into functional traits (Violle et al. 2007) has long been the basis of comparative research in ecology (Grime 1979, Weiher et al. 1999, Shipley 2007). More recently, the employment of functional traits has stimulated the study of a new facet of biodiversity: the diversity of traits within a study unit (e.g. a community), namely functional diversity (Tilman 2001, Petchey and Gaston 2002, Weiher 2010, Cadotte et al. 2011, Mason and de Bello 2013). From a community-ecology perspective, a functional characterization of species, provides a way of describing patterns and of inferring hypotheses about the processes leading to local species coexistence (Weiher and Keddy 1995, Weiher et al. 1998, 2011, McGill et al. 2006, Adler et al. 2013, Enquist et al. 2015).

Trait-based community assembly approaches generally fall into two categories (Anderson et al. 2011): 1) comparison of observed trait distributions/metrics with null distributions generated by random draws, and 2) analysis of trends in trait distributions/metrics among communities along environmental gradients. Ecologists usually propose a myriad of functional trait metrics to describe the distributions of traits within communities, namely the functional structure of communities (Mouchet et al. 2010, Schleuter et al. 2010, Aiba et al. 2013, Chalmandrier et al. 2013, Mason et al. 2013, Swenson 2014; reviewed in Supplementary material Appendix 1, Table A1). Community ecologists commonly interpret the functional structure of ecological communities as a signature of past and ongoing community assembly processes (Enquist et al. 2015). Examples include: habitat filtering (or environmental filtering, following the terminology of Kraft et al. (2014)) expected to restrict the trait distribution within a community relative to the trait distribution within the regional pool; and niche differentiation processes expected to increase phenotypic differences among individuals and/or species in a community, compared to a random situation. Null models (frequentist statistical tests that control for simple sampling effects on metrics of community patterns) help to interpret the functional structure of a community. However, a pioneering study (Jung et al. 2010 and Box 1) has shown that a lack of consideration of individual differences (e.g. using species' mean-trait values instead of accounting for intraspecific variation) in the characterization

Box 1. The Challenges of trait-based community ecology

Among the most challenging criticisms of trait-based approaches to community assembly is the illusion of assigning a specific assembly process to a specific spatial scale (Grime 2006, Cavender-Bares et al. 2009, Mayfield and Levine 2010, Violle et al. 2012). For instance, habitat filtering can be considered an 'outside-the-community' process, since an abiotic factor (e.g. a climatic factor) acts at a regional scale and thus should have a uniform effect on all the elements of a local community. However, due to micro-environmental heterogeneity and to biotic processes that can affect the local abiotic environment, this external vision of habitat filtering is caricatured, if not wrong (Adler et al. 2013, Kraft et al. 2014). Violle et al. (2012) proposed a practical way to define community assembly filters based on the identification of a generic external filter (all assembly processes taking place outside the community) and a generic internal filter (all assembly processes internal to the community). Community ecologists can further interpret these as results of biotic or abiotic processes, depending on their background knowledge of the study system. In cati, the hypotheses of randomization underlying the null models are based on this dichotomy (see also Table 3).

Recently, several authors have challenged the mean-field approach to community assembly – i.e. the use of species' trait means to describe their position along a niche axis and subsequently to test for multiple community assembly processes (Bolnick et al. 2003, 2011, Jung et al. 2010, Laughlin et al. 2012, Zaccarelli et al. 2013). Indeed, some field studies have found a considerable and unexpected amount of intraspecific variation (relative to interspecific variation) including variation in animal prey selection (Estes et al. 2003, Araújo and Gonzaga 2007), life-history traits of freshwater fishes (Blanck and Lamouroux 2007, Villéger et al. 2012), and plant functional traits (Albert et al. 2010, Messier et al. 2010, Paine et al. 2011). This implies that intraspecific variation can be central to detecting assembly processes: 1) within-species genetic variability and/or phenotypic plasticity can play a key role in explaining the actual presence of species within communities and species turnover along gradients (Jung et al. 2010, Leps et al. 2011, Schreiber et al. 2011, Siefert 2012); 2) interactions between organisms are more likely to occur among spatially-close individuals and thus should be better captured when accounting for individual phenotypic variation (Gross et al. 2009). More generally, theoretical models have considered the relative importance of intra- and interspecific phenotypic variation as a key parameter of species coexistence (MacArthur and Levins 1967). Building on these findings, Violle et al. (2012) proposed three phenotypic variance ratios, termed the T-statistics (to echo the F-statistics in population genetics, 'T' referring here to traits), to account for intraspecific variation, relative to interspecific variation, in community assembly studies. The three T-statistics are ratios of variances developed to test for internal and external filtering of a given community (Violle et al. 2012; see also Table 3). The primary goal of cati is to describe individual differences within communities (in particular through the implementation of the T-stats) and to evaluate their potential involvement in community assembly.

of the functional structure of communities and related null models, can lead to misleading interpretations of the processes driving the assembly of communities. This represents a continuing shortcoming in the functional trait-based literature, so Violle et al. (2012) proposed investigation of its consequences for community ecology through decomposition of phenotypic variation across levels of scale and organization, using three phenotypic variance ratios. These are termed the T-statistics (where T refers to traits; see also Box 1; echoing the F-statistics of population genetics).

Raison d'être and scope of cati

Here we present cati (Community Assembly by Traits: Individuals and beyond) an R package developed to meet the key challenges facing community ecology (Box 1) and based on the most recent developments in trait-based ecology.

The purpose of cati emerges from two limitations common to previous R packages and other tools and methodologies. These limitations are: 1) the absence of a specific package dedicated to trait-based analysis for community assembly analysis, and 2) the need for a tool for testing the influence of individual differences and intraspecific variation in the assembly of ecological communities.

1) A useful grouping of R packages is already available for characterizing the functional structure of communities (e.g. FD (Laliberté and Shipley 2011), entropart (Marcon and Herault 2013), hypervolume (Blonder et al. 2014)). Surprisingly, as yet, there is no R package specifically dedicated to the analysis of community assembly using functional traits (see Supplementary material Appendix 2, Table A2 for a list of R packages widely used in community and functional ecology). R users often build their own scripts, sometimes depositing these on their websites or publishing them in peer-reviewed journals. At other times, packages have been customized that were designed for other tasks, including phylogenetic or taxonomic analysis (e.g. vegan (Oksanen et al. 2013), spacodiR (Eastman et al. 2013), picante (Kembel et al. 2010) and ade4 (Dray and Dufour 2007)). Consequently, trait-based community assembly analyses remain difficult, if there is no prior knowledge of the implementation of null models or of the choice of relevant functional diversity indices sensu lato (Mouchet et al. 2010, Pavoine and Bonsall 2010, Schleuter et al. 2010, de Bello 2012, Aiba et al. 2013, Chalmandrier et al. 2013, Mason et al. 2013). Overall, the lack of a package dedicated to trait-based community ecology may already have led (and so will continue to lead) to confusing interpretations and difficulties in establishing cross-study generality.

2) There is a growing consensus on the importance of accounting for individual differences and intraspecific variation in community ecology (Box 1). However, this concern is recent and so no software exists to implement metrics

based on individual variation (including the T-statistics) and to evaluate the influence of intraspecific variation through null models. However, there are two notable exceptions to this. First, the RInSp package (Zaccarelli et al. 2013) investigates inter-individual specialization in resource use. While primarily built to characterize within- and among-population variations, the package can also be used to quantify community-wide individual differences. However, RInSp does not characterize the functional structure of ecological communities. Next, the spacodiR package (Eastman et al. 2013) was built primarily to analyze phylogenetic information. This implements the ratio of Rao's diversities at different scales, and it also associates the null models proposed by Hardy and Senterre (2007). However, spacodiR does not incorporate the decomposition of functional variance nor calculate only three metrics (cf. Supplementary material Appendix 1, Table A1). Usefully, cati and spacodiR can be used together to calculate these metrics for further comparisons (see below).

Furthermore, cati offers a tool: 1) to characterize the functional structure of ecological communities, using both classical functional-diversity descriptors and novel metrics designed to evaluate the relative importance of intra- and interspecific variation within a study unit; and 2) to implement null models that can account for individual differences. Designed to account for intraspecific variation of single traits or multi-trait spaces (functional space, hereafter), cati is flexible enough to integrate traits at higher organizational levels (e.g. populations or functional groups; Table 1). For the first time, cati provides functions to implement the T-statistics and null models that allows comparison with random expectation. Moreover, cati also provides functions to calculate other community assembly metrics available in cati or already implemented in other packages (e.g. FD, hypervolume and spacodiR; cf. Supplementary material Appendix 1, Table A1). Finally, the delineation of regional pools is a major issue in community ecology (Lessard et al. 2012) – these are essential for accurate detection of non-random assembly processes. cati proposes several alternatives to delineate the regional pool when implementing null models, using more- or less-strict delineations. Overall, cati can be considered a useful, general package for community assembly given its facility for implementing a range of metrics, regional pools and null models.

Describing and quantifying the amount of inter- and intraspecific trait variation and the functional structure of ecological communities

Overview

In a straightforward and flexible way, cati describes, quantifies and analyzes the amount of intra- and interspecific phenotypic variation in a site. With cati, several types of analyses can be envisioned through the comparison of phenotypic variation: 1) analysis of different study units (e.g. organism, population, vegetation stratum, grid cell, river, catchment, coral reef); 2) analysis of different states with time (e.g. functional structure of a community before and after a disturbance (Mouillot et al. 2013); Table 1), and 3) analysis of different organizational levels (e.g. within-individual, -guild, -functional group, -trophic) and taxonomic levels (e.g. genus, family, operational taxonomic units (OTUs), Table 1).

The critical development in cati, is the implementation of the T-statistics (new routines) to describe and analyze phenotypic variation across both scale and organizational levels. More generally, cati represents a unified platform that creates opportunities: 1) to call other community assembly metrics (existing routines) from other packages (e.g. FD, hypervolume and spacodiR: Supplementary material Appendix 1, Table A1), 2) to use a comprehensive framework of null models, and 3) to control the composition of regional pools. The 'SE' argument of the three main cati functions Tstats, ComIndex and ComIndexMulti handle measurement errors in the trait distributions within null models. It is hoped this option will encourage community ecologists to more satisfactorily manage these errors in their analyses.

Input data

Inputs (Fig. 1) required are: an individuals × traits matrix (traits), a vector assigning a site name to each individual (ind.plot), and a vector assigning a species name to each individual (sp). For example, we present here the input data for the famous Darwin's finches dataset detailed in the last section (see also Fig. 1 and 2 for other illustrations of input data).

Table 1. Examples of uses of cati with different sorts of traits and ecological distances. The three main arguments of functions – Tstats, ComIndex and ComIndexMulti – can be used to analyze different features of community assembly rules. Trait data traits can be quantitative or qualitative, including phylogenetic information. ind.plot corresponds to the vector of sites for each 'individual', allowing different definitions of a community. sp corresponds to the taxonomic affiliation of each 'individual', allowing investigation of different taxonomic scales.

Arguments	Features	Examples (possible mathematical transformations)
traits	quantitative	SLA, size, isotopic content (scaled, log)
	qualitative	dietary regime, mutualistic interactions, genetic diversity, leaf microbial diversity (pcoa of distances using Gower distance)
	evolutionary history	phylogenetic distance in the tree (pcoa of distances)
ind.plot	community described spatially	localities, strata, sea depth
	community described temporally	chronosequence, succession
	community described ecologically	environmental gradient: stress, disturbance, altitude
sp	Phylum	species, genus
	Guild	insectivore vs granivore, liana vs tree
	Individual	organ-level traits

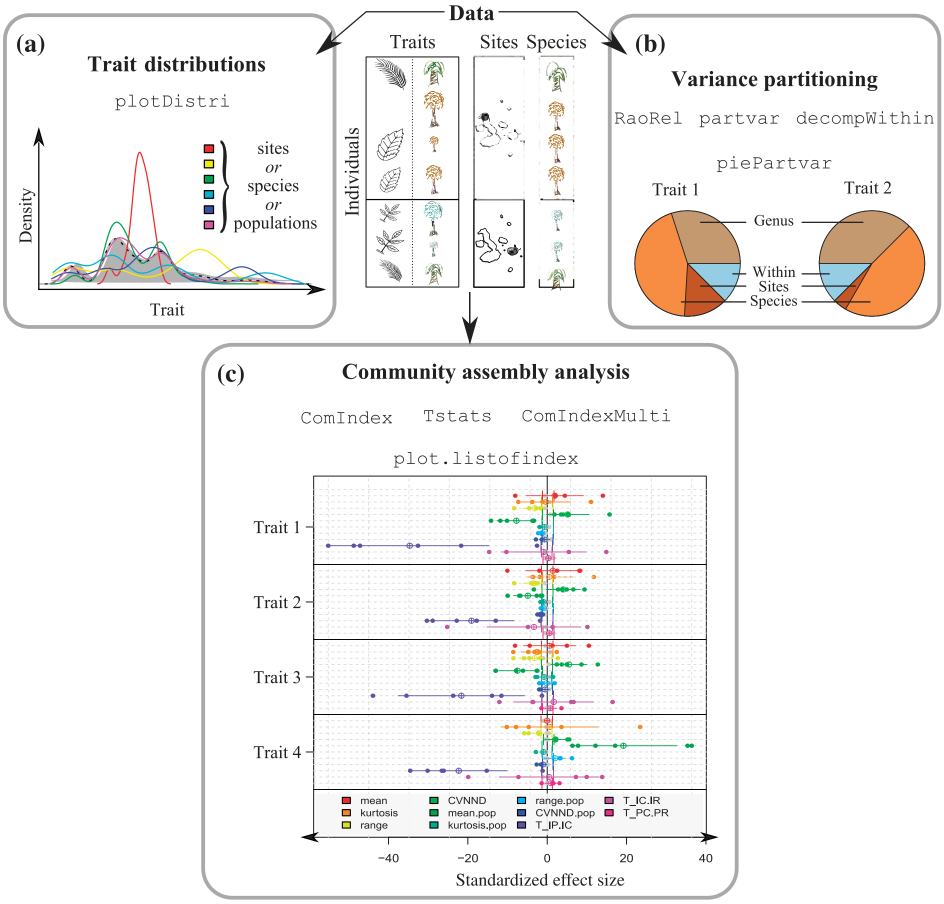
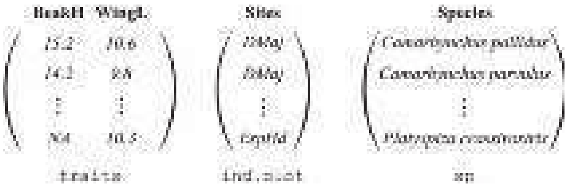


Figure 1. An example of results using cati which allows (a) plots of trait distribution using kernel density, (b) decomposition of trait variances in several ways (including decomposition across ecological scales as represented here on the pie chart) and (c) testing for the departure of observed trait distributions from randomized ones, using multiple metrics and comparing these metrics using standardized effect sizes (SES; Eq. 2). The plot illustrated here compares several metrics for each trait. See Fig. 3 for another example of this plot.



Individual censuses are often time-consuming and difficult to carry out in many projects. In this case, the vector ind.plot can be replaced by a community matrix species (or population) × sites (argument com) with or without abundances, and traits, corresponds to a species (or populations) × traits matrix.

In the case of qualitative traits, trait values must first be transformed by, e.g. using a principal coordinate analysis (function pcoa in the package ape; Paradis et al. 2004) or a Gower distances analysis (function gowdis in the package FD). cati can also integrate ecological distances (e.g. genetic or phylogenetic). Therefore, most functions of cati can accommodate continuous, integer and factor values if first transformed into a distance or a continuous vector (Table 1).

All but one (Fred) of the functions of the package are able to deal with missing values. cati incorporates basic error trapping in all complex functions and a progress bar for lengthy calculations. cati depends on the packages ade4, ape and nlme (Pinheiro et al. 2014).

Analysis of the importance of inter- and intraspecific phenotypic variation in ecological communities and implementation of the T-statistics

As a preliminary step, cati offers the opportunity to represent individual differences, and inter- and intraspecific variations within a study unit using the plot function plotDistri. plotDistri can plot the distribution of a given trait (i.e. its kernel density) within a community or at a larger scale (e.g. at the regional pool level). Several visualization tools are available, including distinct curves for all species within a community, an overall curve for a given species across communities and community-wide distribution curves (Fig. 1a).

In cati we implement the calculation of three T-statistics to quantify the relative amounts of intra- to interspecific variation, and of within-community to regional variation (see also Box 1). T-statistics partition phenotypic variances across organizational levels (individual I; population P; community C and region R; Violle et al. 2012; function Tstats; Table 2 and 3). 1) $T_{IP/IC}$ is the ratio of within-population variance

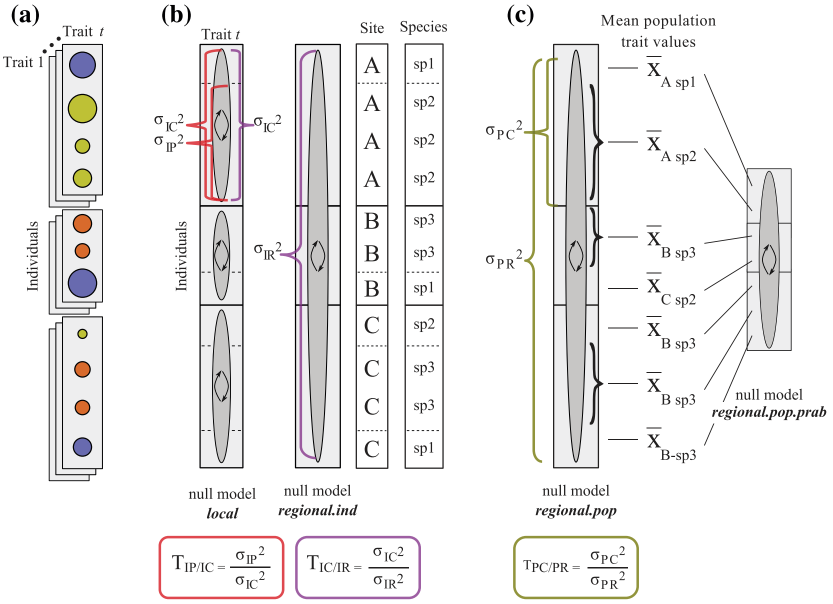


Figure 2. Schematic view of the four null models defined in Table 3. In this example, 11 individuals belonging to three species (sp1, sp2 and sp3) occur on three sites (A, B, C). (a) Distribution of individual values (circles) for the trait t. The three rectangles define the three communities, circle colors define the species, and circle size is proportional to the individual trait value. (b) Null models 'local' and 'regional.ind' use individual trait values. (c) Null models 'regional.pop' and 'regional.pop.prab' are obtained using mean values for each population (e.g. the population of species 1 in site A is labeled A sp1 in the scheme). The three ratios of variance (T-statistics) are also shown in regard to their associated null model. See Table 3 for more details on null models. See Supplementary material Appendix 2–3, Table A2 and Fig. A3 for illustration of other community assembly metrics.

(Individual within Population) to total within-community variance (Individual within Community). It measures the strength of internal filtering, i.e. the strength of niche packing among the species of the community. The higher the overlap of intraspecific trait variation (thus the higher the niche overlap among coexisting species), the higher the value of $T_{IP/IC}$ (see Hulshof et al. 2013 and Le Bagousse-Pinguet et al. 2014 for recent applications in woody and herbaceous communities). 2) $T_{IC/IR}$ is the ratio of community-wide variance (Individual within Community) to total variance in the regional pool (Individual within Region), assessed at the individual level. It measures the strength of external filtering when accounting for individual differences. The higher the overlap of community trait distributions, the higher the value of $T_{IC/IR}$. 3) $T_{PC/PR}$ is the same ratio as $T_{IC/IR}$, but with population-level means only (no intraspecific variation).

Table 2. Main functions in cati and short descriptions.

	Functions	Description	Ref.
Quantify intra-specific variation	RaoRel	The Rao function computes α, β and γ components for taxonomic, functional and/or phylogenetic diversity with: γ = mean (α) + β where γ is the diversity of the regional pool, α is the diversity of the local community and β is the turnover between local communities. Diversity is estimated using the Rao quadratic entropy indices	1, 2
	partvar	Variance partitioning across nested scales using the decomposition of variance on restricted maximum likelihood (REML) method (lme function)	3
	decompCTRE	This function decomposes the variation in community trait composition into three sources: 1) intraspecific trait variability, 2) variability due to species turnover and 3) their covariation	4
Test for community assembly	Tstats	Computes observed T-statistics (T for Traits) as three ratios of variance, namely $T_{IP/IC}$, $T_{IC/IR}$ and $T_{PC/PR}$. This function can also return the distribution of these three statistics under null models (cf. Table 3)	5
	ComIndex	Computes the moments of the trait distribution (e.g. mean and kurtosis) and other uni-traits metrics (e.g. range and CVNND) to test and quantify the non-random assembly of communities. This function allows researchers to use their own metrics and to choose a null model corresponding to each metric	
	ComIndexMulti	Computes multi-trait metrics (e.g. functional hypervolume) to test and quantify the non-random assembly of communities. This function allows researchers to use their own metrics and to choose a null model corresponding to each metric	

References: 1) de Bello et al. 2011, 2) Rao 1982, 3) Messier et al. 2010, 4) Leps et al. 2011, 5) Violle et al. 2012.

Table 3. The four types of null models implemented in cati, their related null and alternative hypotheses, randomization design and associated T-statistics. All null models can be used with the functions ComIndex and ComIndexMulti.

	Null hypothesis	Randomization procedure	Unilateral alternative hypothesis	T-statistics
local	There is no internal filtering: the distribution of trait values of all individuals within a given community does not depend on species identity	Randomization of individual trait values within the community	Internal filtering significantly affects the distribution of trait values within a given community: two individuals belonging to a population have more-similar trait values than two individuals drawn randomly from the community	$T_{IP/IC}$
regional.ind	There is no external filtering: the distribution of trait values of individuals within a given community, is a random drawing from the regional pool	Drawn without replacement of individual trait values belonging to the regional pool (keeping the actual number of individuals in each community)	Two individuals belonging to a community have more-similar trait values than two individuals drawn randomly from the regional pool	$T_{IC/IR}$
regional.pop and regional.pop.prab	There is no species-based external filtering: the distribution of mean trait values of species within a given community is a random drawing from the regional pool	1) Assigned a population-level value to each individual and 2) drawn without replacement of population-level trait values belonging to the regional pool (keeping the actual number of individuals in each community (regional.pop) or not (regional.pop.prab))	Two individuals belonging to a community have more-similar population-based trait values than two individuals drawn randomly from the regional pool with (regional.pop) or without (regional.pop.prab) taking abundance into account	$T_{PC/PR}$ (regional.pop)

It measures the strength of external filtering at the species level.

We provide three additional and commonly-used methods to decompose diversity and phenotypic variance (Fig. 1b, Table 2): 1) Rao's decomposition into alpha, beta and gamma components of any biodiversity facet (taxonomic, functional or phylogenetic; de Bello et al. 2011; function RaoRel), 2) a gradient analysis based on the decomposition of among-communities trait variation into species turnover, intraspecific trait variability and their covariation (Leps et al. 2011; function decompCTRE), and 3) variance partitioning across nested scales (Messier et al. 2010; function partvar). In summary, we set up simple generic functions and bring together several well-recognized methods in trait-based community ecology to partition phenotypic variation.

A toolbox for the calculation of indices describing the functional structure of communities

Beyond the focus on within-species variation, users of cati can choose to implement a palette of further metrics to describe and quantify trait distributions, accounting for species' abundances or not. The ComIndex function allows calculation of the moments of the distribution of any trait (e.g. mean, variance, kurtosis, skewness; Grant et al. 1985, Kraft et al. 2008, Enquist et al. 2015) and key functional diversity indices such as functional richness FRic, functional evenness FEve and functional divergence FDiv (Villéger et al. 2008; function Fred). A list of indices is required as a vector in the index option of ComIndex. For example, to calculate the variance and the functional divergence FDiv, for all traits, in all the plots studied, and to compare these with local randomizations (see below), the code is:

```
ComIndex(traits, index = c(“var(x)”,
“Fred(x, ind.plot)$FDiv”), nullmodels =
“local”, ind.plot, sp)
```

In the case of a species-based community matrix (i.e. no individual variation within species), the argument com can be used (species × sites matrix with or without abundance) instead of ind.plot (vector of sites for individuals; Table 1).

Most importantly, it is easily possible to implement any other index in ComIndex by calling other packages (e.g. FD: Laliberté and Shipley 2011; spacodiR: Eastman et al. 2013; Supplementary material Appendix 1–2, Table A1 and A2). For instance, calculating the Tst metric (the proportion of the overall trait diversity expressed among sites) of Hardy and Senterre (2007) involves the following options in ComIndex:

```
index = “spacodi.calc (table(ind.plot, 1:
length(ind.plot)), sp.traits = as.data.
frame(as.matrix(x)))$Tst”
```

with table(ind.plot, 1:length(ind.plot)), corresponding to the community matrix individuals × sites.

cati is able to calculate indices based on Euclidian space, a minimum spanning tree, a distance matrix, or a hierarchical classification tree (Supplementary material Appendix 1, Table A1). ComIndexMulti is the sister function of ComIndex, but suitable for multi-trait indices. For example, functional dispersion (FDis) and functional evenness (FEve) (existing routines of the FD package) can easily be calculated by this function. As a follow-up of multi-trait and phylogeny-based approaches to biodiversity, a new perspective is to account for the whole phenotype instead of isolated components (Laughlin 2014). Indeed, covariation among traits may occur during assembly processes, so single-trait approaches can produce contrasting results depending on the trait under examination (Bernard-Verdier et al. 2012). It is thus relevant to investigate the effects of environmental filters on the integrated phenotype that can be assessed by its functional trait space. This is because natural selection and ecological filters most likely act on ecological strategies rather than on single phenotypic traits. The functional space of a population, a community or any other grouping, can be mathematically assessed by an n-dimensional hypervolume (Blonder et al. 2014; see also Lamanna et al. 2014 for an application at local and continental scales). cati takes into account recent developments in this area by using the existing hypervolume routine (R package hypervolume, Blonder et al. 2014) in the function ComIndexMulti. Other hypervolume-like methods can be implemented in ComIndexMulti such as the calculation of the convex hull volume (Cornwell et al. 2006).

ComIndex and ComIndexMulti are generic functions used for community analysis, in this way metrics are also calculated for randomized communities. nperm = NULL returns only observed metrics. This feature speeds the calculation where the requirement is just for descriptors of the functional structure of observed communities.

Community assembly analysis: null models from local to regional scales

Four null models in cati: outlook

To compare observed patterns with random ones, cati implements two sets of null models based on different assumptions – a null model local (internal-to-the community) or one of the three nulls models regional (external-to-the community). These models can also incorporate individual differences (see Table 3 for the hypotheses and randomization procedures associated with each null model).

The null model local randomizes trait values for all individuals within a community, irrespective of taxon identity. This randomization breaks the link between taxonomic identity and trait values within the community (Fig. 2, Table 3). If individual data are not available, but only information about the abundance of each species (e.g. relative abundances in herbaceous or microbial communities), the argument com 'reinterprets' abundance data as individual-like data (internal function AbToInd). In this case, variance decomposition via the T-statistics is impossible because there is no within-species trait variation. The test can be performed with metrics such as the coefficient of variation of nearest neighboring distances (CVNND) (Jung et al. 2010; function CVNND). In the null model local, the departure from the null distribution can be interpreted as an influence of the internal filter. The internal filter tends to force two individuals belonging to a given population (in a community-wide perspective, all individuals belonging to a species in the given community) to display similar trait values compared with two individuals, randomly drawn from the same community (niche packing; Violle et al. 2012).

The null model regional.ind randomizes trait values for all individuals in all communities (or more generally in the regional pool: see below how the regional pool can be delineated in cati) while keeping the actual number of individuals of the communities constant. This randomization breaks the link between taxonomic identity and trait values at the regional scale (Fig. 2, Table 3). The results from regional.ind can be interpreted as the influence of external filtering (any ecological process outside the community that tends to narrow the trait distribution within the community; Violle et al. 2012). The external filter tends to force two individuals belonging to the same community to display more similar traits values than two individuals randomly drawn in the region, irrespective of the species (Violle et al. 2012).

The null model regional.pop mirrors regional.ind but here an average population-level trait value is assigned to each individual in a given population (Fig. 2, Table 3). regional.pop tests the implications of disregarding or averaging-out information about within-population variation, when investigating the impact of external filtering on local community structure. As discussed in Violle et al. (2012), comparing the null models regional.pop and regional.ind allows testing of the importance of accounting for intraspecific variability in community ecology. The null model regional.pop takes species abundances into account, whereas the null model regional.pop.prab does not.

Lastly, several studies use local information about species' composition but generic information about species' traits. For instance it is common to perform community assembly analyses by extracting a single mean trait value from worldwide databases. In plants, the TRY database (Kattge et al. 2011) is proving particularly useful. In this case, all individuals of a given species are assigned the same trait value. Thus, it is not possible to use T-statistics to partition inter- and intraspecific variation but other metrics can be used – for instance the range of trait values displayed by species co-occurring in a community.

Three cati functions designed to run null models

The four null models described above, can be implemented in three cati functions: Tstats, ComIndex and ComIndexMulti.

The Tstats function automatically assigns one specific null model to its related T-statistic (Table 3). Indeed, each T-statistic has been built in relation to a specific scale (see above and Fig. 2). For example, in Tstats, the value of $T_{IP/IC}$ for an observed community will be compared to *n* randomized communities (option nperm = n) having the same species composition as the observed community, based on null model local.

Users of cati can also apply the four null models with other community assembly metrics using the functions ComIndex and ComIndexMulti – the latter being for multi-trait indices (including distance-based and hypervolume-like metrics). In this case, any metric can be used with any null model (Supplementary material Appendix 3, Fig. A3). The index option selects the list of indices. The nullmodels option selects the null models to consider. Species data, including abundances data, can be included in the analyses (com and type options).

cati allows the delineation of different regional pools

Several authors have stressed the dangers of delineating the regional pool too loosely in community assembly studies

(Lessard et al. 2012). Indeed, most studies combine species (or individuals) from all the communities present in their analyses as a proxy for the regional pool. In cati too, this is the default delineation of the regional pool, though this may lead to underestimation of the regional pool values and to spurious interpretations regarding the community assembly processes at play. Therefore, cati offers several alternatives for delineating the regional pool when implementing regional null models, by allowing attribution of a specific regional pool to each community and also by extending the set of traits values to enlarge the regional pool (argument reg.pool).

Significance tests

In Tstats, ComIndex and ComIndexMulti, the significance of a metric is tested using one-tailed permutation tests. The probability that an observed value departs from the simulated values (i.e. the quantile of the null distribution in which the observed value is found) is:

$$\text{p.value} = (x + 1)/(y + 1) \qquad (1)$$

where x is the number of randomized values greater or less than the observed value and y is the total number of null values obtained with the randomization (function Pval). For example, p.value = 0.03, means that 3% of the randomized values are less (or greater) than the observed value. The magnitude of these differences is calculated based on standardized effect size (functions ses and ses.listofindex; Gotelli and McCabe 2002) as:

$$\text{SES} = (I_{obs} - I_{sim})/\sigma_{sim} \qquad (2)$$

where I_{obs} is the observed value, I_{sim} the mean of values calculated with the null model and σ_{sim} the standard deviation of these simulated values.

Graphical representations

To facilitate the use of cati's functions and the production of customized graphs, we have developed S3 methods (plot, print and summary functions) linked to classes Tstats, ComIndex, ComIndexMulti, and listofindex. The majority of plot functions represents standardized effect sizes (SES) instead of observed metrics values (e.g. Fig. 1c and Fig. 3). SES values (Eq. 2; functions ses and ses.listofindex) allow a comparison of the magnitude of departure from the null model for different metrics, communities or traits.

Test of bias, power and robustness of the T-statistics

Using simulations, we test bias, power and robustness of the T-statistics in the Supplementary material Appendix 4. We bring out a low type-I error (alpha-error is below 0.05 for all T-statistics; see Supplementary material Appendix 4 for more details). We also studied the type-II error in relation to the type of hypotheses specified in the models. The power to detect the external filter at the individual level ($T_{IC/IR}$) predominates over the power to detect it at the population level ($T_{PC/PR}$) in all simulation cases (Supplementary material Appendix 4). Therefore, users of cati must be careful when comparing $T_{IC/IR}$ and $T_{PC/PR}$. Finally, we detected no bias in the functions partvar and decomCTRE (Supplementary material Appendix 4). This appendix can be rerun with different parameters using the text document available at <https://github.com/adrientaudiere/cati/blob/Package-cati/Documentation/Appendix4/Appendix4.Rnw>.

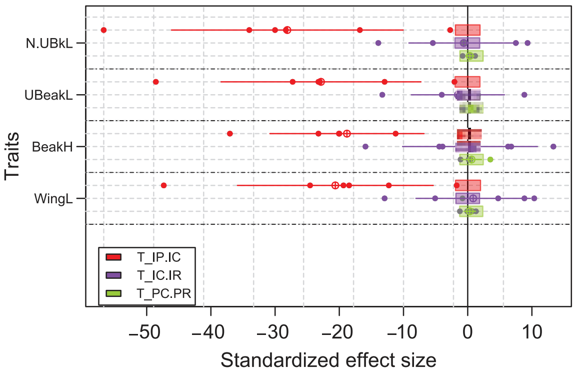


Figure 3. Standardized effect size (SES) of T-statistics for the four traits of the Darwin's finches dataset. This is the unchanged output of the command plot(res) (see main text). Four traits are represented. N.UBkL: nostril upper beak length, UBeakL: upper beak length, BeakH: beak height, and WingL: wing length. For a given trait and a given metric, each colored dot represents the SES value for one community (six communities – here islands – total) when it is different from the null model. T_IC.IR: community-wide variance relative to the total variance in the regional pool, T_IP.IC: ratio of within-population variance to total within-community variance, and T_PC.PR: inter-community variance relative to the total variance in the regional pool. The crossed circles and the segments represent, respectively, the mean and the standard deviation of the SES values for a given T-statistics and a given trait (i.e. mean and standard deviation of community values). For a given T-statistics, the mean of the SES (crossed circle) is significantly different from the null distribution if not embedded within the colored.

Application of cati functions using morphological traits of Darwin's finches

To illustrate the capabilities of cati, we provide an example using four morphological traits of Darwin's finches available online (<http://bioquest.org/birdd/morph.php>). In our case study, each island is considered a 'community'. We have deleted the island Cocos from the dataset because of the presence on it of only one species. The remaining dataset contains 13 species, 6 islands and 2513 individual measurements for four traits (N.UBkL: nostril upper beak length, UBeakL: upper beak length, BeakH: beak height and WingL: wing length). Detailed examples of analyses of traits of Darwin's finches by cati are provided in the package's reference manual and tutorial.

First, install and load cati from the Comprehensive R Archive Network (CRAN), and load the Darwin's finches dataset as an example.

```
install.packages("cati")
library(cati)
data(finch.ind)
```

Next, calculate the T-statistics on Darwin's finches data and plot the result.

```
res <- Tstats(traits.finch, ind.plot.
finch, sp.finch)
res
plot(res)
```

The comparison of the departure of the T-statistics from randomized situations (through SES values) is plotted in Fig. 3. The results are consistent among traits. $T_{IP/IC}$ is very low compared with randomized situations for the four traits. This metric reflects the degree of overlap of trait values displayed by co-occurring populations within a community. Then, at the community (island) level, each finch species is suspected to be packed along the niche axis (strong internal filtering) more often than randomly, confirming earlier observations by Darwin (1839) and successors (Grant et al. 1985). In other words, two individuals belonging to a particular population, display more similar trait values than two individuals drawn randomly from the island. This provides further evidence for the importance of biotic interactions in driving the coexistence of finches in each island. Conversely, the four traits displayed no significant pattern for the other T-statistics, either at the individual ($T_{IC/IR}$) or the population ($T_{PC/PR}$) levels. In other words, two individuals living in a particular island do not display significantly more similar trait values than two individuals randomly-drawn from the regional pool. This result suggests there is no external filtering for these traits, possibly due to few environmental differences between the islands.

Concluding remarks and future directions

cati is a package dedicated to the analysis of community assembly using functional traits. It is flexible enough: 1) to implement any uni- or multi-trait metric to describe the dispersion of traits within communities and at larger scales, 2) to partition phenotypic variation at multiple organizational levels (e.g. to account for infra-individual variations such as between-leaf phenotypic variation within a tree), and 3) to run null models with specific assumptions (e.g. by accounting for species abundances, by delineating the regional pool more or less precisely). cati can also implement various distances, including genetic and phylogenetic distances. This allows conjoint treatment of genetic, phylogenetic and phenotypic information at different organizational levels and/or spatial or temporal scales.

Online resources and data accessibility

The cati R package is available from CRAN and development versions, forum; Darwin's finches dataset and a tutorial are available at <https://github.com/adrientaudiere/cati>. The reference manual is available at <http://cran.r-project.org/web/packages/cati/cati.pdf> and the tutorial at <https://github.com/adrientaudiere/cati/blob/Package-cati/Documentation/vignette_Darwin_finches/vignette.pdf>.

Acknowledgements – We are grateful to Cecile Albert, Lisa P. Bentley, Maud Bernard-Verdier, Francesco de Bello, Olivier Flores, Claire Fortunel, Catherine Hulshof, Nathan Kraft, Fabien Laroche, Jessy Loranger, Julie Messier, François Munoz, Timothy Paine, Andrew Siefert and Sébastien Villéger, for thoughtful comments on the cati package and on early versions of this manuscript. We also thank Marc Coudel and Rescript.Co.NZ for language correction. This study was supported by a Marie Curie International Outgoing Fellowship within the 7th European Community Framework Program (DiversiTraits project, no. 221060), the European Research Council (ERC) Starting Grant Project 'Ecophysiological and biophysical constraints on domestication in crop plants' (Grant ERC-StG-2014-639706-CONSTRAINTS), the Agence National de la Recherche (PRAISE project, ANR-13-BIOADAPT-0015).

References

Adler, P. B. et al. 2013. Trait-based tests of coexistence mechanisms. – Ecol. Lett. 16: 1294–1306.

Aiba, M. et al. 2013. Robustness of trait distribution metrics for community assembly studies under the uncertainties of assembly processes. – Ecology 94: 2873–2885.

Albert, C. H. et al. 2010. A multi–trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits. – Funct. Ecol. 24: 1192–1201.

Anderson, M. J. et al. 2011. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. – Ecol. Lett. 14: 19–28.

Araújo, M. S. and Gonzaga, M. O. 2007. Individual specialization in the hunting wasp *Trypoxylon* (Trypargilum) *albonigrum* (Hymenoptera, Crabronidae). – Behav. Ecol. Sociobiol. 61: 1855–1863.

Bernard-Verdier, M. et al. 2012. Community assembly along a soil depth gradient: contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. – J. Ecol. 100: 1422–1433.

Blanck, A. and Lamouroux, N. 2007. Large-scale intraspecific variation in life-history traits of European freshwater fish. – J. Biogeogr. 34: 862–875.

Blonder, B. et al. 2014. The n-dimensional hypervolume. – Global Ecol. Biogeogr. 23: 595–609.

Bolnick, D. I. et al. 2003. The ecology of individuals: incidence and implications of individual specialization. – Am. Nat. 161: 1–28.

Bolnick, D. I. et al. 2011. Why intraspecific trait variation matters in community ecology. – Trends Ecol. Evol. 26: 183–192.

Cadotte, M. W. et al. 2011. Beyond species: functional diversity and the maintenance of ecological processes and services. – J. Appl. Ecol. 48: 1079–1087.

Cavender-Bares, J. et al. 2009. The merging of community ecology and phylogenetic biology. – Ecol. Lett. 12: 693–715.

Chalmandrier, L. et al. 2013. A family of null models to distinguish between environmental filtering and biotic interactions in functional diversity patterns. – J. Veg. Sci. 24: 853–864.

Cornwell, W. K. et al. 2006. A trait-based test for habitat filtering: convex hull volume. – Ecology 87: 1465–1471.

Darwin, C. 1839. Journal of researches into the geology and natural history of the various countries visited by H. M. S. Beagle, under the command of Captain FitzRoy, R. N., from 1832 to 1836. – H. Colburn, London, pp. 404–405.

de Bello, F. 2012. The quest for trait convergence and divergence in community assembly: are null-models the magic wand? – Global Ecol. Biogeogr. 21: 312–317.

de Bello, F. et al. 2011. Quantifying the relevance of intraspecific trait variability for functional diversity. – Methods Ecol. Evol. 2: 163–174.

Dray, S. and Dufour, A. B. 2007. The ade4 package: implementing the duality diagram for ecologists. – J. Stat. Softw. 22: 1–20.

Eastman, J. et al. 2013. spacodiR: spatial and phylogenetic analysis of community diversity. – R package ver. 0.13.

Enquist, B. J. et al. 2015. Scaling from traits to ecosystems: developing a general trait driver theory via integrating trait-based and metabolic scaling theories. – Adv. Ecol. Res. 52: 169–199.

Estes, J. A. et al. 2003. Individual variation in prey selection by sea otters: patterns, causes and implications. – J. Anim. Ecol. 72: 144–155.

Gotelli, N. J. and McCabe, D. J. 2002. Species co-occurrence: a meta-analysis of JM Diamond's assembly rules model. – Ecology 83: 2091–2096.

Grant, P. R. et al. 1985. Variation in the size and shape of Darwin's finches. – Biol. J. Linn. Soc. 25: 1–39.

Grime, J. P. 1979. Plant strategies and vegetation processes. – Wiley.

Grime, J. P. 2006. Trait convergence and trait divergence in herbaceous plant communities: mechanisms and consequences. – J. Veg. Sci. 17: 255–260.

Gross, N. et al. 2009. Effects of land-use change on productivity depend on small-scale plant species diversity. – Basic Appl. Ecol. 10: 687–696.

Hardy, O. J. and Senterre, B. 2007. Characterizing the phylogenetic structure of communities by an additive partitioning of phylogenetic diversity. – J. Ecol. 95: 493–506.

Hulshof, C. M. et al. 2013. Intra-specific and inter-specific variation in specific leaf area reveal the importance of abiotic and biotic drivers of species diversity across elevation and latitude. – J. Veg. Sci. 24: 921–931.

Jung, V. et al. 2010. Intraspecific variability and trait-based community assembly. – J. Ecol. 98: 1134–1140.

Kattge, J. et al. 2011. TRY – a global database of plant traits. – Global Change Biol. 17: 2905–2935.

Kembel, S. W. et al. 2010. Picante: R tools for integrating phylogenies and ecology. – Bioinformatics 26: 1463–1464.

Kraft, N. et al. 2008. Functional traits and niche-based tree community assembly in an Amazonian forest. – Science 322: 580–582.

Kraft, N. J. B. et al. 2014. Community assembly, coexistence, and the environmental filtering metaphor. – Funct. Ecol. doi: 10.1111/1365-2435.12345

Laliberté, E. and Shipley, B. 2011. FD: measuring functional diversity from multiple traits, and other tools for functional ecology. – R package ver. 1.0-11.

Lamanna, C. et al. 2014. Functional trait space and the latitudinal diversity gradient. – Proc. Natl Acad. Sci. USA 111: 13745–13750.

Laughlin, D. C. 2014. The intrinsic dimensionality of plant traits and its relevance to community assembly. – J. Ecol. 102: 186–193.

Laughlin, D. C. et al. 2012. A predictive model of community assembly that incorporates intraspecific trait variation. – Ecol. Lett. 15: 1291–1299.

Le Bagousse-Pinguet, Y. et al. 2014. Species richness of limestone grasslands increases with trait overlap: evidence from within- and between-species functional diversity partitioning. – J. Ecol. 102: 466–474.

Leps, J. et al. 2011. Community trait response to environment: disentangling species turnover vs intraspecific trait variability effects. – Ecography 34: 856–863.

Lessard, J. P. et al. 2012. Inferring local ecological processes amid species pool influences. – Trends Ecol. Evol. 27: 600–607.

MacArthur, R. H. and Levins, R. 1967. The limiting similarity, convergence and divergence of coexisting species. – Am. Nat. 101: 377–385.

Marcon, E. and Herault, B. 2013. Entropart, an R package to partition diversity. – <http://CRAN.R-project.org/package=entropart>.

Mason, N. W. H. and de Bello, F. 2013. Functional diversity: a tool for answering challenging ecological questions. – J. Veg. Sci. 24: 777–780.

Mason, N. W. H. et al. 2013. A guide for using functional diversity indices to reveal changes in assembly processes along ecological gradients. – J. Veg. Sci. 24: 794–806.

Mayfield, M. M. and Levine, J. M. 2010. Opposing effects of competitive exclusion on the phylogenetic structure of communities. – Ecol. Lett. 13: 1085–1093.

Supplementary material (Appendix ECOG-01433 at < www.ecography.org/readers/appendix >). Appendix 1–4.
10-EV

McGill, B. J. et al. 2006. Rebuilding community ecology from functional traits. – Trends Ecol. Evol. 21: 178–185.

Messier, J. et al. 2010. How do traits vary across ecological scales? A case for trait-based ecology. – Ecol. Lett. 13: 838–848.

Mouchet, M. et al. 2010. Functional diversity measures: an overview of their redundancy and their ability to discriminate community assembly rules. – Funct. Ecol. 24: 867–876.

Mouillot, D. et al. 2013. A functional approach reveals community responses to disturbances. – Trends Ecol. Evol. 28: 167–177.

Oksanen, J. et al. 2013. Vegan: community ecology package. – R package ver. 2.0-10.

Paine, C. et al. 2011. Functional traits of individual trees reveal ecological constraints on community assembly in tropical rain forests. – Oikos 120: 720–727.

Paradis, E. et al. 2004. APE: analyses of phylogenetics and evolution in R language. – Bioinformatics 20: 289–290.

Pavoine, S. and Bonsall, M. B. 2010. Measuring biodiversity to explain community assembly: a unified approach. – Biol. Rev. 86: 792–812.

Petchey, O. L. and Gaston, K. L. 2002. Functional diversity (FD), species richness and community composition. – Ecol. Lett. 5: 402–411.

Pinheiro, J. et al. 2014. nlme: linear and nonlinear mixed effects models. – R package ver. 3.1-117.

Rao, C. R. 1982. Diversity and dissimilarity coefficients: a unified approach. – Theoret. Popul. Biol. 21: 24–43.

Schleuter, D. et al. 2010. A user's guide to functional diversity indices. – Ecol. Monogr. 80: 469–484.

Schreiber, S. J. et al. 2011. The community effects of phenotypic and genetic variation within a predator population. – Ecology 92: 1582–1593.

Shipley, B. 2007. Comparative plant ecology as a tool for integrating across scales. – Ann. Bot. 99: 965–966.

Siefert, A. 2012. Incorporating intraspecific variation in tests of trait-based community assembly. – Oecologia 170: 767–775.

Swenson, N. G. 2014. Functional and phylogenetic ecology in R. Springer UseR! – Springer.

Tilman, D. 2001. Functional diversity. – In: Levin, S. (ed.), Encyclopaedia of biodiversity. Academic Press, pp. 109–120.

Villéger, S. et al. 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. – Ecology 89: 2290–2301.

Villéger, S. et al. 2012. Low functional β-diversity despite high taxonomic β–diversity among tropical estuarine fish communities. – PLoS One 7: e40679.

Violle, C. et al. 2007. Let the concept of trait be functional! – Oikos 116: 882–892.

Violle, C. et al. 2012. The return of the variance: intraspecific variability in community ecology. – Trends Ecol. Evol. 27: 244–252.

Weiher, E. 2010. A primer of trait and functional diversity. – In: Magurran, A. E. and McGill, P. J. (eds), Biological diversity: frontiers in measurement and assessment. Oxford Univ. Press, pp. 175–193.

Weiher, E. and Keddy, P. A. 1995. Assembly rules, null models, and trait dispersion: new questions from old patterns. – Oikos 74: 159–164.

Weiher, E. et al. 1998. Community assembly rules, morphological dispersion, and the coexistence of plant species. – Oikos 81: 309–322.

Weiher, E. et al. 1999. Challenging Theophrastus: a common core list of plant traits for functional ecology. – J. Veg. Sci. 10: 609–620.

Weiher, E. et al. 2011. Advances, challenges and a developing synthesis of ecological community assembly theory. – Phil. Trans. R. Soc. B 366: 2403–2413.

Zaccarelli, N. et al. 2013. RInSp: an R package for the analysis of individual specialization in resource use. – Methods Ecol. Evol. 4: 1018–1023.

Manuscrit L : "A global meta-analysis of the relative extent of intraspecific trait variation in plant communities"

ECOLOGY LETTERS

Ecology Letters, (2015) doi: 10.1111/ele.12508

REVIEW AND SYNTHESIS

A global meta-analysis of the relative extent of intraspecific trait variation in plant communities

Andrew Siefert,[1]* Cyrille Violle,[2] Loïc Chalmandrier,[3,4] Cécile H. Albert,[5] Adrien Taudiere,[2] Alex Fajardo,[6] Lonnie W. Aarssen,[7] Christopher Baraloto,[8,9] Marcos B. Carlucci,[10,11,12] Marcus V. Cianciaruso,[13] Vinícius de L. Dantas,[14] Francesco de Bello,[15,16] Leandro D. S. Duarte,[10] Carlos R. Fonseca,[17] Grégoire T. Freschet,[2,18] Stéphanie Gaucherand,[19] Nicolas Gross,[20,21] Kouki Hikosaka,[22] Benjamin Jackson,[23] Vincent Jung,[24] Chiho Kamiyama,[25] Masatoshi Katabuchi,[26] Steven W. Kembel,[27] Emilie Kichenin,[18] Nathan J. B. Kraft,[28] Anna Lagerström,[18] Yoann Le Bagousse-Pinguet,[16] Yuanzhi Li,[29] Norman Mason,[30] Julie Messier,[31] Tohru Nakashizuka,[32] Jacob McC. Overton,[30] Duane A. Peltzer,[33] I. M. Pérez-Ramos,[34] Valério D. Pillar,[10,35] Honor C. Prentice,[36] Sarah Richardson,[33] Takehiro Sasaki,[37] Brandon S. Schamp,[38] Christian Schöb,[39] Bill Shipley,[29] Maja Sundqvist,[40,41] Martin T. Sykes,[42] Marie Vandewalle[42] and David A. Wardle[18]

Abstract

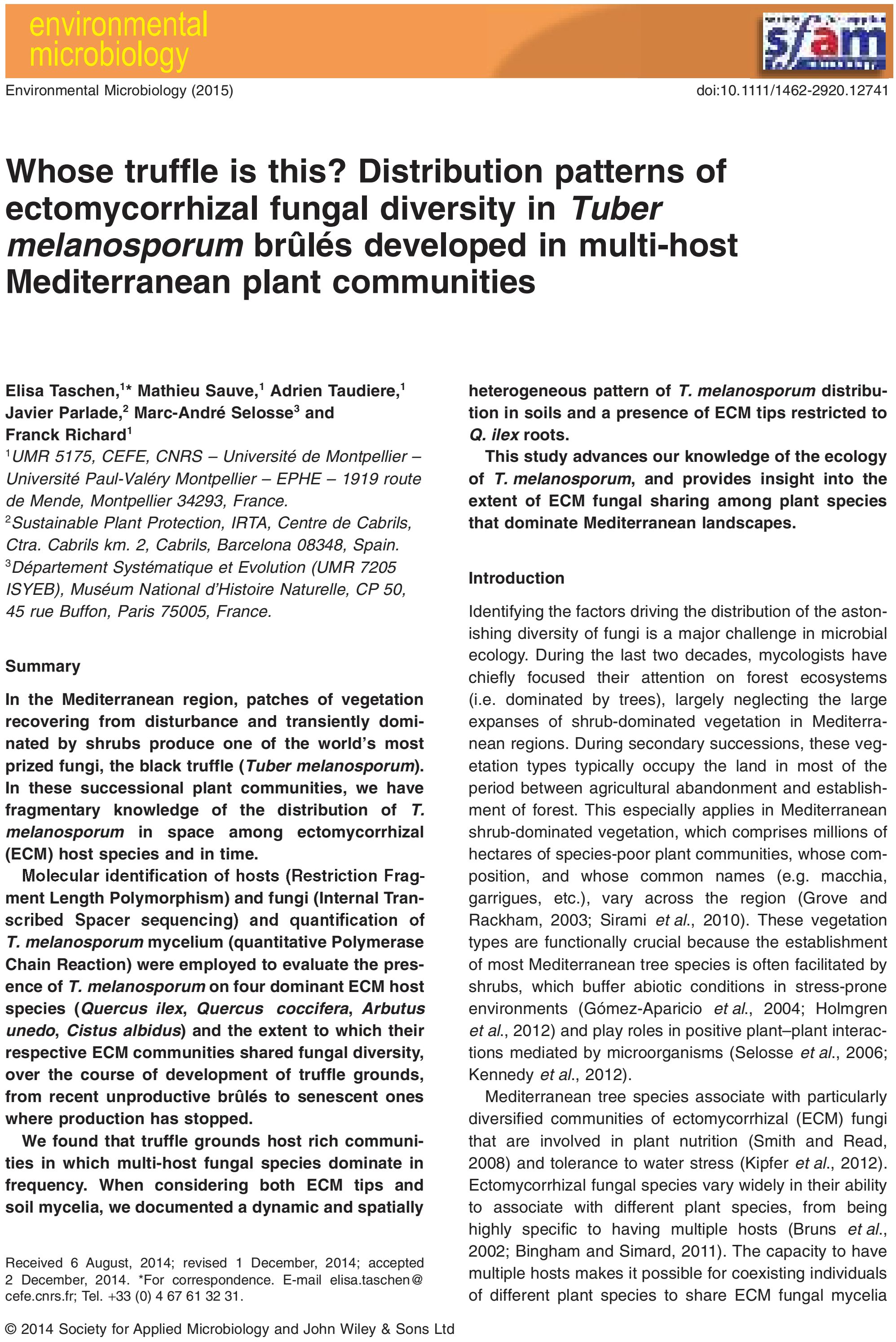
Recent studies have shown that accounting for intraspecific trait variation (ITV) may better address major questions in community ecology. However, a general picture of the relative extent of ITV compared to interspecific trait variation in plant communities is still missing. Here, we conducted a meta-analysis of the relative extent of ITV within and among plant communities worldwide, using a data set encompassing 629 communities (plots) and 36 functional traits. Overall, ITV accounted for 25% of the total trait variation within communities and 32% of the total trait variation among communities on average. The relative extent of ITV tended to be greater for whole-plant (e.g. plant height) vs. organ-level traits and for leaf chemical (e.g. leaf N and P concentration) vs. leaf morphological (e.g. leaf area and thickness) traits. The relative amount of ITV decreased with increasing species richness and spatial extent, but did not vary with plant growth form or climate. These results highlight global patterns in the relative importance of ITV in plant communities, providing practical guidelines for when researchers should include ITV in trait-based community and ecosystem studies.

Keywords

community ecology, functional diversity, interspecific variation, intraspecific variability, leaf trait, plant functional trait, trait-based ecology.

Ecology Letters (2015)

Manuscrit M : "Whose truffle is this? Distribution patterns of ECM fungal diversity in *Tuber melanosporum* brûlés developed in multi-host Mediterranean plant communities"



environmental microbiology

Environmental Microbiology (2015) doi:10.1111/1462-2920.12741

Whose truffle is this? Distribution patterns of ectomycorrhizal fungal diversity in *Tuber melanosporum* brûlés developed in multi-host Mediterranean plant communities

Elisa Taschen,[1]* Mathieu Sauve,[1] Adrien Taudiere,[1] Javier Parlade,[2] Marc-André Selosse[3] and Franck Richard[1]

[1] *UMR 5175, CEFE, CNRS – Université de Montpellier – Université Paul-Valéry Montpellier – EPHE – 1919 route de Mende, Montpellier 34293, France.*

[2] *Sustainable Plant Protection, IRTA, Centre de Cabrils, Ctra. Cabrils km. 2, Cabrils, Barcelona 08348, Spain.*

[3] *Département Systématique et Evolution (UMR 7205 ISYEB), Muséum National d'Histoire Naturelle, CP 50, 45 rue Buffon, Paris 75005, France.*

Summary

In the Mediterranean region, patches of vegetation recovering from disturbance and transiently dominated by shrubs produce one of the world's most prized fungi, the black truffle (*Tuber melanosporum*). In these successional plant communities, we have fragmentary knowledge of the distribution of *T. melanosporum* in space among ectomycorrhizal (ECM) host species and in time.

Molecular identification of hosts (Restriction Fragment Length Polymorphism) and fungi (Internal Transcribed Spacer sequencing) and quantification of *T. melanosporum* mycelium (quantitative Polymerase Chain Reaction) were employed to evaluate the presence of *T. melanosporum* on four dominant ECM host species (*Quercus ilex*, *Quercus coccifera*, *Arbutus unedo*, *Cistus albidus*) and the extent to which their respective ECM communities shared fungal diversity, over the course of development of truffle grounds, from recent unproductive brûlés to senescent ones where production has stopped.

We found that truffle grounds host rich communities in which multi-host fungal species dominate in frequency. When considering both ECM tips and soil mycelia, we documented a dynamic and spatially heterogeneous pattern of *T. melanosporum* distribution in soils and a presence of ECM tips restricted to *Q. ilex* roots.

This study advances our knowledge of the ecology of *T. melanosporum*, and provides insight into the extent of ECM fungal sharing among plant species that dominate Mediterranean landscapes.

Introduction

Identifying the factors driving the distribution of the astonishing diversity of fungi is a major challenge in microbial ecology. During the last two decades, mycologists have chiefly focused their attention on forest ecosystems (i.e. dominated by trees), largely neglecting the large expanses of shrub-dominated vegetation in Mediterranean regions. During secondary successions, these vegetation types typically occupy the land in most of the period between agricultural abandonment and establishment of forest. This especially applies in Mediterranean shrub-dominated vegetation, which comprises millions of hectares of species-poor plant communities, whose composition, and whose common names (e.g. macchia, garrigues, etc.), vary across the region (Grove and Rackham, 2003; Sirami *et al.*, 2010). These vegetation types are functionally crucial because the establishment of most Mediterranean tree species is often facilitated by shrubs, which buffer abiotic conditions in stress-prone environments (Gómez-Aparicio *et al.*, 2004; Holmgren *et al.*, 2012) and play roles in positive plant–plant interactions mediated by microorganisms (Selosse *et al.*, 2006; Kennedy *et al.*, 2012).

Mediterranean tree species associate with particularly diversified communities of ectomycorrhizal (ECM) fungi that are involved in plant nutrition (Smith and Read, 2008) and tolerance to water stress (Kipfer *et al.*, 2012). Ectomycorrhizal fungal species vary widely in their ability to associate with different plant species, from being highly specific to having multiple hosts (Bruns *et al.*, 2002; Bingham and Simard, 2011). The capacity to have multiple hosts makes it possible for coexisting individuals of different plant species to share ECM fungal mycelia

Received 6 August, 2014; revised 1 December, 2014; accepted 2 December, 2014. *For correspondence. E-mail elisa.taschen@cefe.cnrs.fr; Tel. +33 (0) 4 67 61 32 31.

Manuscrit N : "Into the functional ecology of ectomycorrhizal communities: environmental filtering of enzymatic activities"

Journal of Ecology

British Ecological Society

Journal of Ecology doi: 10.1111/1365-2745.12633

Into the functional ecology of ectomycorrhizal communities: environmental filtering of enzymatic activities

Courty Pierre-Emmanuel[1*†], Munoz François[2,3†], Selosse Marc-André[4], Duchemin Myriam[5], Criquet Stéven[6], Ziarelli Fabio[7], Buée Marc[8], Plassard Claude[9], Taudière Adrien[5], Garbaye Jean[8] and Richard Franck[5]

[1]Zurich-Basel Plant Science Center, Department of Environmental Sciences, Botany, University of Basel, CH-4056 Basel, Switzerland; [2]Equipe Diversité des Plantes et des Communautés Végétales, UMR AMAP, Université de Montpellier, Bd de la Lironde, TA A-51/PS2, 34 398 Montpellier Cedex 5, France; [3]French Institute of Pondicherry, 11 Saint Louis Street, Pondicherry 605001, India; [4]Institut de Systématique, Évolution, Biodiversité (ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE), Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, CP50, 75005 Paris, France; [5]UMR 5175 CEFE – Université Montpellier 2 – Campus CNRS, 1919 Route de Mende, 34293 Montpellier, France; [6]Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale, IMBE, UMR 7263 – IRD 237, Faculté des Sciences et Techniques de Saint-Jérôme, Aix-Marseille Université, PO Box 421, 13397 Marseille Cedex 20, France; [7]Faculté des Sciences et Techniques de Saint-Jérôme, Aix-Marseille Université, Spectropole, PO Box 512, 13397 Marseille Cedex 20, France; [8]UMR 1136 INRA/Nancy Université Interactions Arbres/Micro-organismes, INRA Nancy, 54280 Champenoux, France; and [9]INRA, UMR 1222 Eco&Sols, 2 Place Viala, F-34060 Montpellier cedex 2, France

Summary

1. Characterizing the ecological processes driving the assembly and functional composition of ectomycorrhizal (ECM) fungal communities is an area of active research.

2. Here, we applied a trait-based framework to address whether and how environmental filtering and niche differentiation influence the diversity of ECM fungal enzymatic activities in two oak-dominated forest ecosystems. We hypothesized that fungal enzymatic activities and ECM community assembly are influenced by the availability of targeted resources in the surrounding soil.

3. We pinpointed a major influence of environmental filtering on ECM fungal taxonomic and functional composition. Contrasted taxonomic composition among forest ecosystems was linked to broad interspecific trait variation and entailed contrasted functional responses at community level. However, intraspecific trait variation contributed to community response within ecosystems. We characterized a strong relationship between enzymatic activities and targeted resource availability in surrounding soil, suggesting a functional role of the enzymes for the acquisition of these resources. Conversely, we did not find a significant influence of niche differentiation in ECM community assembly.

4. *Synthesis*. Heterogeneous distribution of soil resources drives a community-level functional response and determines the functional and taxonomic mosaic of ECM communities in forest ecosystems.

Key-words: community assembly, ectomycorrhizal fungi (ECM), environmental filtering, functional diversity, niche differentiation, RLQ analysis

Journal of Ecology
British Ecological Society

Journal of Ecology doi: 10.1111/1365-2745.12633

Into the functional ecology of ectomycorrhizal communities: environmental filtering of enzymatic activities

Courty Pierre-Emmanuel[1*†], Munoz François[2,3†], Selosse Marc-André[4], Duchemin Myriam[5], Criquet Stéven[6], Ziarelli Fabio[7], Buée Marc[8], Plassard Claude[9], Taudière Adrien[5], Garbaye Jean[8] and Richard Franck[5]

[1]Zurich-Basel Plant Science Center, Department of Environmental Sciences, Botany, University of Basel, CH-4056 Basel, Switzerland; [2]Equipe Diversité des Plantes et des Communautés Végétales, UMR AMAP, Université de Montpellier, Bd de la Lironde, TA A-51/PS2, 34 398 Montpellier Cedex 5, France; [3]French Institute of Pondicherry, 11 Saint Louis Street, Pondicherry 605001, India; [4]Institut de Systématique, Évolution, Biodiversité (ISYEB – UMR 7205 – CNRS, MNHN, UPMC, EPHE), Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, CP50, 75005 Paris, France; [5]UMR 5175 CEFE – Université Montpellier 2 – Campus CNRS, 1919 Route de Mende, 34293 Montpellier, France; [6]Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale, IMBE, UMR 7263 – IRD 237, Faculté des Sciences et Techniques de Saint-Jérôme, Aix-Marseille Université, PO Box 421, 13397 Marseille Cedex 20, France; [7]Faculté des Sciences et Techniques de Saint-Jérôme, Aix-Marseille Université, Spectropole, PO Box 512, 13397 Marseille Cedex 20, France; [8]UMR 1136 INRA/Nancy Université Interactions Arbres/Micro-organismes, INRA Nancy, 54280 Champenoux, France; and [9]INRA, UMR 1222 Eco&Sols, 2 Place Viala, F-34060 Montpellier cedex 2, France

Summary

1. Characterizing the ecological processes driving the assembly and functional composition of ectomycorrhizal (ECM) fungal communities is an area of active research.

2. Here, we applied a trait-based framework to address whether and how environmental filtering and niche differentiation influence the diversity of ECM fungal enzymatic activities in two oak-dominated forest ecosystems. We hypothesized that fungal enzymatic activities and ECM community assembly are influenced by the availability of targeted resources in the surrounding soil.

3. We pinpointed a major influence of environmental filtering on ECM fungal taxonomic and functional composition. Contrasted taxonomic composition among forest ecosystems was linked to broad interspecific trait variation and entailed contrasted functional responses at community level. However, intraspecific trait variation contributed to community response within ecosystems. We characterized a strong relationship between enzymatic activities and targeted resource availability in surrounding soil, suggesting a functional role of the enzymes for the acquisition of these resources. Conversely, we did not find a significant influence of niche differentiation in ECM community assembly.

4. *Synthesis*. Heterogeneous distribution of soil resources drives a community-level functional response and determines the functional and taxonomic mosaic of ECM communities in forest ecosystems.

Key-words: community assembly, ectomycorrhizal fungi (ECM), environmental filtering, functional diversity, niche differentiation, RLQ analysis

Introduction

Ectomycorrhizal (ECM) symbioses between fungi and plant roots are essential to forest ecosystem functioning (van der Heijden *et al.* 2015). Previous work has shown that abiotic and biotic factors drive variations in the taxonomic composition of ECM fungal communities (Koide *et al.* 2005; Genney,

---

*Correspondence author. Department of Biology, University of Fribourg, 3 rue Albert Gockel, CH-1700 Fribourg, Switzerland. E-mail: pierre-emmanuel.courty@unifr.ch

†These authors contributed equally to this work.

Anderson & Alexander 2006; Pickles *et al.* 2010; Tedersoo *et al.* 2012; Anderson, Genney & Alexander 2014). In addition, rarer studies have addressed the signature of assembly processes on the functional composition of ECM communities (Koide, Fernandez & Malcolm 2014), and in particular, the relative influence of small- and large-scale environmental heterogeneities on ECM fungal community assembly (Bahram *et al.* 2013; Crowther *et al.* 2013; Talbot *et al.* 2014; Bahram, Peay & Tedersoo 2015). Previous works showed that enzymatic activities of ECM fungi are linked to resource availability at both specific and community scales (Burke *et al.* 2011; Talbot *et al.* 2013; Walker *et al.* 2014). Recent studies further evidenced a variation in enzymatic activities between taxa reflecting their ability to exploit different substrates (Baldrian *et al.* 2012; Phillips, Ward & Jones 2014). However, the way community assembly processes affect variation in functional composition within and among ECM fungal communities, over a hierarchy of environmental constraints, remains to be addressed.

Distinct signatures of community assembly processes can be detected from patterns of functional composition (Jung *et al.* 2010). Environmental filtering is a process whereby ecological constraints yield functional variation *among* communities along a gradient (Lepš *et al.* 2011) and/or limits the range and functional variation in traits *within* communities (functional convergence; Grime 2006). By contrast, niche differentiation is a process whereby the coexistence of species with similar trait values is prevented, thus leading to increased trait divergence (Kraft, Valencia & Ackerly 2008) and/or increased evenness (Stubbs & Wilson 2004) within communities. Biotic interactions such as competition (Violle *et al.* 2011) and facilitation (Valiente-Banuet & Verdu 2007) can yield niche differentiation.

Resource acquisition is a major component of plant (Westoby 1998) and fungal (McGuire *et al.* 2010) ecology. Unlike litter-decomposing fungi, ECM fungi mostly depend on their plant hosts for carbon (C) supply. However, most ECM fungal lineages produce some extracellular and cell wall-bound hydrolytic and oxidative enzymes that degrade carbon (C)-, nitrogen (N)- and phosphorus (P)-containing compounds in soil organic matter (SOM; Courty *et al.* 2010; Rineau *et al.* 2012). Enzymatic activities targeting distinct resources form a set of functional traits (Cullings & Courty 2009; Rineau & Courty 2011) that are directly involved in nutrient cycling within ecosystems (Courty *et al.* 2005; Pritsch *et al.* 2011). Enzymatic activities can be measured on excised ECM root tips, and this approach has revealed that coexisting ECM operational taxonomic units (OTU) vary widely in their ability to mobilize SOM nutrients (Courty *et al.* 2010). These variations are related to temporal dynamics of resource acquisition (Courty *et al.* 2010), to competitive interactions for resource acquisition (Koide *et al.* 2005) and to a range of abiotic and biotic variables (Courty *et al.* 2010; Jones *et al.* 2010; Walker *et al.* 2014). Although these results support the role of niche-based processes in the assembly of ECM fungal communities, how the availability of soil resources influences the functional diversity of ECM fungal communities is still little addressed.

Here, we test whether environmental filtering and niche differentiation constrain the distribution of enzymatic activities among and within ECM fungal communities (see Table 1 for the definitions of the key concepts and objectives of the present work). We investigated the functional composition of ECM fungal communities in two sites representing distinct oak-dominated forest ecosystems with highly diverse and patchily distributed ECM fungal communities (Richard *et al.* 2005; Courty *et al.* 2008). We characterized the enzymatic activities and taxonomy of ECM root tips making up the ECM fungal community found in a sampled soil core. We also analysed the physicochemical characteristics of each soil core volume. We designed a hierarchical sampling scheme to examine the nature of functional variation between ECM soil core communities within and among the two sites (Fig. 1). First, we analysed the signature of environmental filtering yielding a variation in enzymatic activities among ECM soil core communities and identified the contribution of interspecific and intraspecific trait variation to such functional variation. Secondly, we characterized the influence of functional convergence and niche differentiation on the distribution of ECM enzymatic activities within communities, by comparing it to appropriate null models (e.g. Kraft, Valencia & Ackerly 2008). Then, we addressed whether changing availability of alternative sources of C-, N- and P-containing compounds in surrounding soil organic matter influenced ECM enzymatic activities in communities, using a multitable ordination method (i.e. RLQ analysis; Doledec *et al.* 1996;). Our basic hypothesis was that the distinct forest ecosystems and the variation in available resources across soil cores constitute environmental filters driving the variation in ECM enzymatic activities *among* communities, while local functional convergence and competitive interactions for shared resources should shape the distribution of these activities *within* communities (Fig. 1a). In addition, we addressed the enzymatic activities of other organisms in soil apart from the ECM root tips. We expected similar responses between soil and ECM activities (i) if the taxa present on root tips mostly contributed to enzymatic activities in surrounding soil, or (ii) if environmental filtering for resource acquisition applied to all the co-occurring organisms in a similar way. Alternatively, we expected niche differentiation to yield distinctive patterns of enzymatic expression among coexisting organisms.

Materials and methods

STUDY SYSTEM

The research was performed in two 60-year-old oak forests located in north-east (Champenoux, 48°75′ N, 6°35′ E) and south (Puechabon, 43°44′ N, 3°35′ E) France (see Appendix S1 in Supporting Information). Champenoux is a temperate site where two deciduous oak species (*Quercus petraea* Liebl. and *Quercus robur* L.) co-dominate on luvic cambisols. Puechabon is a Mediterranean site where a single sclerophyllous oak (*Q. ilex* L.) grows on karstic soils. These sites

Table 1. Glossary

Ectomycorrhizal root tip	Symbiotic structure linking soil filamentous fungi to short roots of most trees, including oaks, and involving diverse Basidiomycota and Ascomycota (Courty *et al.* 2010; van der Heijden *et al.* 2015). Their morpho-anatomy is variable, especially in the extension of the hyphal network surrounding root tips (=extramatrical mycelium; Agerer 2001).
Enzymatic activity	As other fungi, ectomycorrhizal (ECM) fungi release extracellular enzymes to break down macromolecules—for example, nucleic acids, phenols and chitin. Activity of several enzymes can be directly measured on ECM root tips using standardized assays (Pritsch *et al.* 2011).
Functional trait	Functional traits are measurable properties (i.e. morphological, physiological characters), which define the performances of the organisms (i.e. growth, reproduction, survival; Violle *et al.* 2007). Functional traits are usually measured at the individual level and used comparatively across species (McGill *et al.* 2006).
Environmental filtering	Represents the fact that only species that are ecologically adapted to the environment can be found in a given site, while other species cannot (Keddy 1992; Lortie *et al.* 2004). It thus predicts a reduction in the range of functional traits related to this adaptation (Grime 2006).
Niche differentiation	Posits that species with identical or very similar niches cannot coexist due to biotic interactions. It thus predicts an underrepresentation of species with similar niches and similar functional traits in the community (McArthur & Levins 1967; Abrams 1983).
OTU: Operational Taxonomic Unit	A proxy for delineating fungal species obtained using DNA-based methods applied on ectomycorrhizal root tips. By convention, fungal OTUs are defined for most ECM lineages according to ITS sequence dissimilarity at 3% cut-off value (Courty *et al.* 2010).
RLQ analysis	This statistical analysis is based on separate multivariate analyses of three tables (Doledec *et al.* 1996), where the rows of table R (here the SOM compounds) are related to rows of table L (the community composition), and columns of table L are related to rows of table Q (the enzymatic expressions). The objective of the RLQ analysis is to relate tables Q and R through their indirect link in L. It represents in the present study the functional response of enzymatic expressions to variable SOM composition. The analysis provides several axes that are independent and sorted in decreasing order of importance.

thereby represent contrasted abiotic and biotic contexts for ECM community dynamics.

HIERARCHICAL SAMPLING SCHEME

Sampling was performed in September 2008 at Champenoux and November 2008 at Puechabon. The sampling periods correspond to acorn fall (a major phenological trait) and to the fruiting peak of ECM fungi in these forests. At each site, five 25 × 25 m plots of same age and same forest management history were selected at least 200 m apart for soil sampling. Four sampling locations were selected 5 m from the centre of each plot in the four cardinal directions (Fig. 1a). At each location, we sampled a soil core of 2 cm in diameter and 5 cm deep (15.7 cm^3). The 20 cores per site (4 cardinal points × 5 plots) were stored separately at 4 °C and processed within 5 days after sampling (Pritsch *et al.* 2011).

ANALYSIS OF ECM ROOT TIPS

Enzymatic activities of ECM root tips

Oak roots were soaked in tap water for 15 min before being gently washed. Root tips were observed in water under a stereomicroscope (×40), and the total number of ECM root tips was recorded in each soil core sample. ECM fungal communities made of more than two ECM root tips were detected from 34 of the 40 soil cores. Each ECM root tip was collected and analysed using high-throughput microplate assays as in Pritsch *et al.* (2011). Eight enzymatic activities (referred here as functional traits; See Appendix S2) were measured successively on each tip and considered as proxies of the ability of ECM fungi to exploit soil resources: phosphorus (acid phosphatase, PHO), chitin (N-acetylhexosaminidase, NAG), soil proteins (leucine aminopeptidase, LEU), various carbon hydrates including cellulose, hemicellulose and cellobiose (cellobiohydrolase, CEL; β-xylosidase, XYL; β-glucosidase, GLU; β-glucuronidase, GLR), and phenolic substrates (LAC). Enzymatic activities were expressed per unit time and per unit area to take into account tip size (projected area of the ECM root tip, in pmol mm^{-2} min^{-1}) as described in Pritsch *et al.* (2011) and were log-transformed to obtain an approximately Gaussian distribution.

Identification of ECM fungi

After measurement of enzymatic activities, each ECM root tip was kept individually at −20 °C. Subsequently, fungal symbionts were identified by sequencing the ITS region of fungal ribosomal DNA. DNA was extracted using the REDExtract-N-Amp Plant PCR Kit (XNAP, Sigma, St. Quentin Fallavier, France), and the ITS was amplified as in Courty *et al.* (2008). Fungal ITS sequences were edited with SEQUENCHER 4.9, corrected and identified using the BLAST algorithm and the UNITE data base (http://unite.ut.ee/). Sequences with more than 97% similarity were ascribed to the same operational taxonomic unit (OTU), named after the nearest sequence recovered by BLAST. After identification, we averaged in each soil core community the eight enzymatic activities of all root tips belonging to the same taxon. We then analysed data on enzymatic activities per OTU and per soil core (Fig. 1a).

ANALYSIS OF THE SOIL SURROUNDING ROOT TIPS

Soil enzymatic activity

Enzymes are also produced by the organisms living in the soil apart from ECM root tips, including saprotrophic fungal mycelia, bacteria

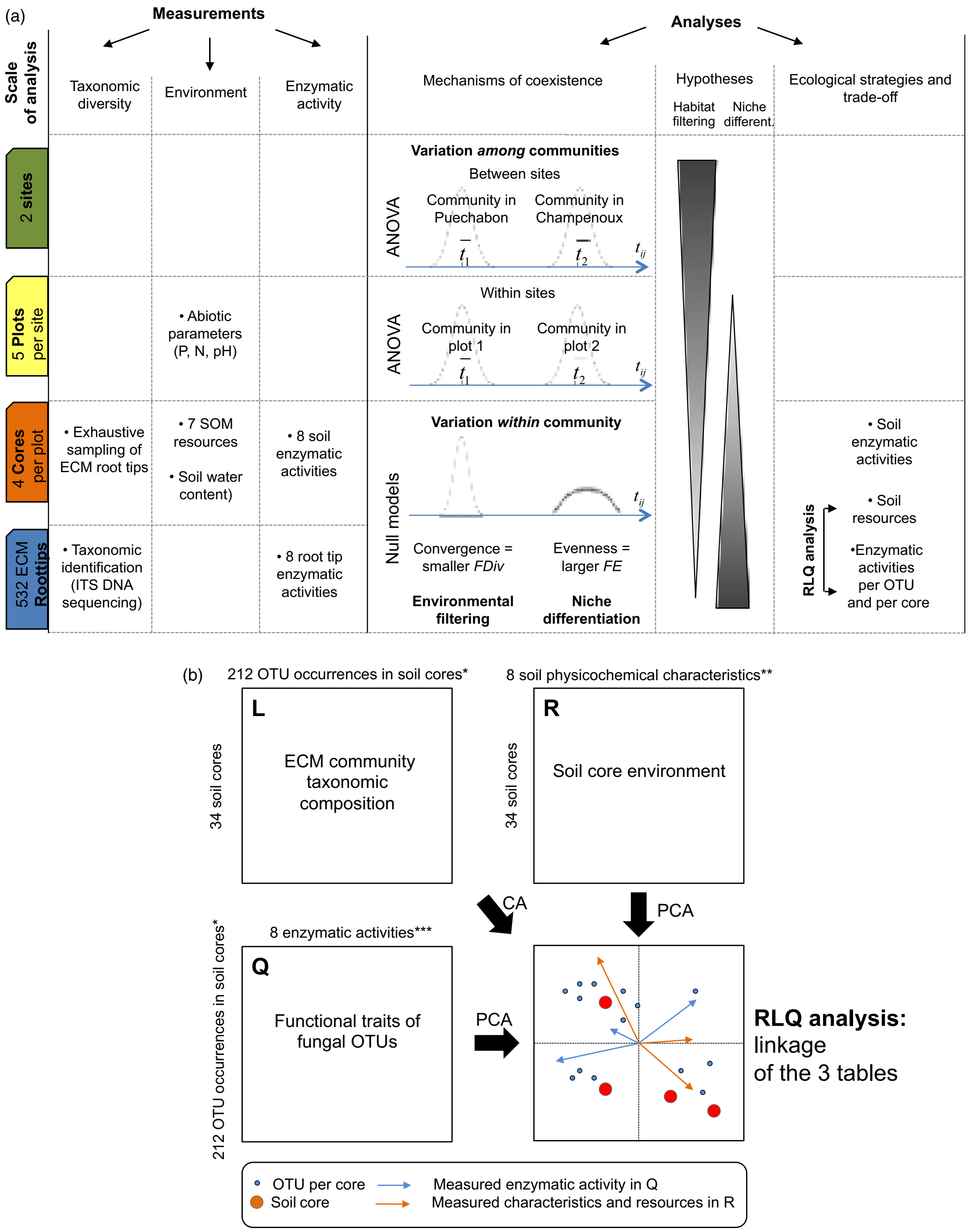


Fig. 1. (a) Diagrammatic representation of the sampling design and of the related multiscale methodology. Colours indicate the four hierarchical sampling levels (site, plot, core, root tip). (b) Schematic presentation of the RLQ analysis used to address the link between OTU traits (Q table) and local environment (R table) through the presence–absence of OTUs in local communities. Multivariate analysis of each table is performed prior to RLQ analysis (PCA, Principal Component Analysis; CA, Correspondence Analysis). *Operational Taxonomic Units (OTUs) identified by a BLAST analysis of ITS sequences. **Soil resources are concentrations of four categories of recalcitrant phenolic compounds (methoxyl C (Methoxyl.C), aromatic C (Arom.C), phenolic C (Phen.C) and carboxyl-C (Carbox.C)), CH_3 groups of lipids, waxes and cutins (alkyl C: Alk.C), polysaccharides including cellulose and hemicellulose (O-alkyl C: O.Alk.C), chitin (glucosamine: Chitin) and soil water content. ***The enzymatic activities measured on ECM root tips (Myc) are as follows: β-xylosidase (XYL), β-glucuronidase (GLR), cellobiohydrolase (CEL), β-glucosidase (GLU), N-acetylhexosaminidase (NAG), acid phosphatase (PHO), leucine aminopeptidase (LEU) and laccase (LAC).

and ECM extramatrical mycelia. We hypothesized that this set of organisms contributes to soil resource recycling and thus should likewise respond and interact depending on resource availability. To test this hypothesis, we characterized and compared the enzymatic activities of ECM root tips and of the organisms in the surrounding soil. We therefore measured the previous eight enzymatic activities in the soil volume surrounding root tips for comparison with enzymatic activities of ECM root tips (see Appendix S3). They were also log-transformed to obtain an approximately Gaussian distribution.

Soil resources and physical context

Basic soil characteristics (i.e. pH, P and N contents) were measured at plot level (Fig. 1a). Furthermore, we applied the ^{13}C CPMAS NMR spectroscopy procedure (see Appendix S4) to measure the concentration of resources related to enzyme activities in each core (Fig. 1a): (i) polysaccharides, including cellulose and hemicellulose (O.Alk.C), (ii) lignin and recalcitrant C compounds (Methoxyl.C, Arom.C, Phen.C and Carbox.C), (iii) alkyl compounds of lipids, waxes and cutins (Alk.C). A soil subsample was used to assess the concentration of glucosamine as a proxy of chitin concentration. Soil water content at the sampling date of each soil core was also measured based on soil weight difference before and after drying in an oven at 105 °C for 12 h.

STATISTICAL ANALYSES

Functional variation in ECM fungal communities between and within sites

We measured the mean enzymatic activity of each ECM soil core community as $\bar{t}_j = \frac{1}{N}\sum_{i=1}^{N} t_{ij}$, where t_{ij} was the value of enzymatic activity j for OTU i, and N was the number of ECM fungal OTUs in the soil core community. $\bar{t}_j$ was therefore an index of the community-level functional response of fungi, which could vary across communities because of changing taxonomic composition and of intraspecific trait variation within taxa (Fig. 2). We performed the ANOVA of $\bar{t}_j$ between the two sites following the method of Lepš *et al.* (2011), which allows separating the intraspecific (including both genotypic and phenotypic components) and interspecific sources of functional variation among sites. A first ANOVA was performed on $\bar{t}_j$ values to represent the overall change in functional composition between communities of the two sites ('total' component). A second ANOVA was performed on $\bar{t}_j^{fix}$ values calculated from the mean enzymatic activity t_s of each OTU s over all cores, that is, $\bar{t}_j^{fix} = \sum_{s=!}^{S} n_{sj} t_s$, where n_{sj} is the relative frequency of OTU s, and t_s is its mean enzymatic value of OTU s. $\bar{t}_j^{fix}$ could only vary between communities of the two sites if the species composition changed (see Fig. 2c), and the second ANOVA thus represented the contribution of interspecific trait variation to changing functional composition between communities of the two sites ('interspecific component'). Finally, a third ANOVA was performed on $\bar{t}_j - \bar{t}_j^{fix}$ to represent departure from $\bar{t}_j^{fix}$ due to intraspecific variation ('intraspecific component'). If significant, this ANOVA showed the contribution of intraspecific trait variation to changing community composition. Figure 2 illustrates basic cases with different patterns of these three statistics between communities. In addition, we included in the ANOVAS a nested effect of the variation in communities between plots within each site, to further assess the functional variation in the assemblages within each forest ecosystem.

A subsequent objective was to assess the functional response of soil organisms to environmental variation, apart from the response of root tips expressed in $\bar{t}_j$. We therefore performed the nested ANOVA of soil enzymatic activities as for root tip activities. We then analysed the paired correlations of $\bar{t}_j$ and of the corresponding enzymatic activity in soil, to characterize consistent or divergent trends in ECM and soil functional responses among cores.

Functional variation within ECM soil core communities

We then assessed the functional structure of ECM communities using two indexes of functional divergence $\mathrm{FDiv}_j = \frac{1}{N}\sum_{i=1}^{N}(t_{ij} - \bar{t}_j)^2$, and functional evenness $\mathrm{FE}_j = \overline{\mathrm{NND}_j}/\sigma_{\mathrm{NND}_j}$, where $\overline{\mathrm{NND}_j}$ was the mean nearest neighbour distances (NND_j) of co-occurring OTUs for enzymatic activity j, and σ_{NND_j} was the corresponding standard deviation. FDiv is a primary component of functional diversity (Mason *et al.* 2005), which is expected to be smaller than random in communities when some environmental constraint limits the range of traits around some optimal value (functional convergence). Conversely, niche differentiation yields a more even distribution of traits than random when OTUs with more similar trait values are less likely to coexist (Stubbs & Wilson 2004; Mason *et al.* 2005), which results in higher values of FE_j. We analysed FDiv_j and FE_j for each enzymatic activity. We compared observed values of FDiv_j and FE_j to those of a null model of community assembly (Fig. 1a, bottom), in order to detect the signatures of environmental filtering and niche differentiation within communities. These null models were designed to acknowledge the hierarchical structure of environmental variation in the sampling scheme (see Appendix S5). The P-values derived from this analysis were adjusted to take into account multiple testing (False Discovery Rate method, Pike 2011). All statistical analyses were carried out using the R software (R Development Core Team 2010).

Environmental drivers of functional community composition

We performed a RLQ analysis (Doledec *et al.* 1996; *rlq* function in R package ade4) to investigate the relationship between ECM enzymatic activities and resource concentrations in surrounding soil (Fig. 1b). The relationship was analysed through the presence–absence of ECM fungal OTUs per soil core community. The three-table approach thus integrated taxonomic, functional and environmental variation to identify synthetic components of environmental filtering among communities. We assessed the statistical significance of the link between traits and soil resources using the procedure *randtest.rlq* based on 999 permutations (Monte Carlo procedure; Dray & Legendre 2008). If the test was significant, the axes of the RLQ analysis represented functional syndromes of enzymatic activities depending on the availability of soil resources.

Results

The average number of ECM root tips per soil core was significantly higher at Champenoux than at Puechabon (20.5 vs. 6.2; See Table S1). The 532 ECM root tips successfully sequenced were ascribed to 104 unique OTUs (49 at Champenoux and 58 at Puechabon, three being present in both sites; see Tables S1 and S2), and we found 212 occurrences of the OTUs in the 34 selected soil cores (Fig. 1b). The richness of OTUs (rarefied to a standardized sample size of 121 root tips) was 1.8-fold lower at Champenoux, while the average number

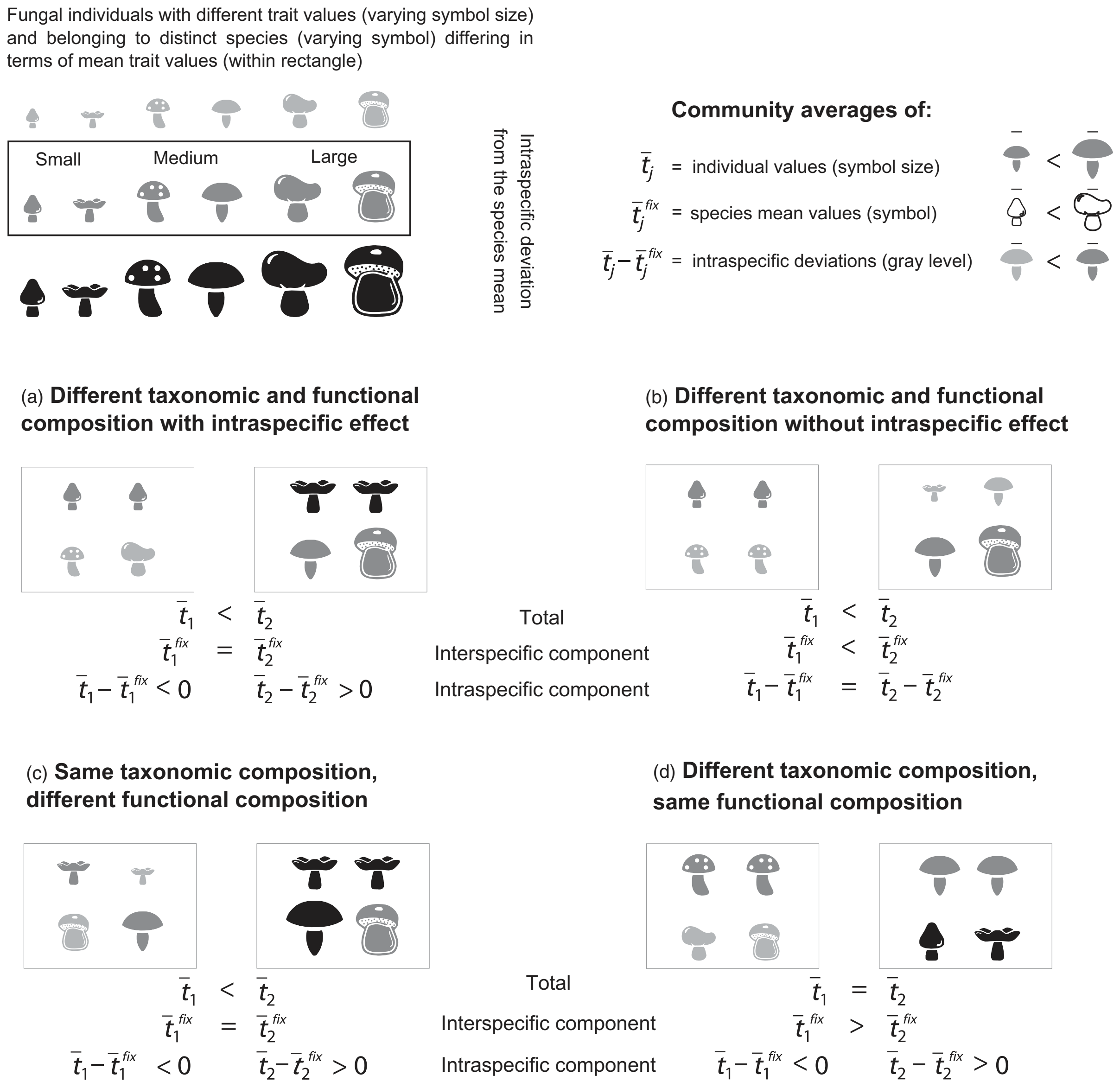


Fig. 2. Analysis of the variation in functional composition among communities, using the method of Lepš *et al.* (2011). The panels exemplify different cases with functional and/or taxonomic changes among two communities, and the consequences on $\bar{t}_j$ and $\bar{t}_j^{fix}$ statistics. Different symbols represent distinct species, and symbol size represents the trait value of an individual observed in a community. The colour represents the extent of intraspecific trait variation: black colour means that the observed individual value is greater than the mean species value, dark grey that it is equal to the mean and light grey that it is smaller than the mean. Mean values vary across species (three size groups each including two species). In (a), both taxonomic and functional compositions are different (different and smaller symbols in left community). Functional change is not due to different mean species values (as $\bar{t}_1^{fix} = \bar{t}_2^{fix}$) but to intraspecific variation (different $\bar{t}_j - \bar{t}_j^{fix}$). In (b), functional and taxonomic compositions are also different, but the intraspecific component is the same; that is, in both cases, we have the same colour composition in communities ($\bar{t}_1 - \bar{t}_1^{fix} = \bar{t}_2 - \bar{t}_2^{fix}$). Therefore, only the variation in mean species values (interspecific variation) contributes to functional change in this case ($\bar{t}_1^{fix} \neq \bar{t}_2^{fix}$). In (c), the taxonomic composition is the same (same symbols), but the functional composition differs because of intraspecific variation (different $\bar{t}_j - \bar{t}_j^{fix}$). In (d), the two communities have the same functional composition (same symbol sizes), but changing taxonomic composition with interspecific variation in traits (different symbols and ($\bar{t}_1^{fix} \neq \bar{t}_2^{fix}$)). However, intraspecific variation (different $\bar{t}_j - \bar{t}_j^{fix}$) maintains identical functional composition despite the changing mean species values.

of OTUs per soil core was 2.6-fold higher than at Puechabon (see Table S1). Three OTUs present at both sites represented 2.9% of all ECM fungal root tips (*Cenococcum geophilum*, *Tomentella atramentaria* and *T. badia*), but were not in all soil cores. *C. geophilum* was the most widespread species at both sites (present in 42% of the soil cores, i.e. 11 cores at Champenoux and five at Puechabon). Thelephoraceae and Russulaceae dominated communities of both sites (66% and 50% of total OTU number at Champenoux and Puechabon, respectively).

FUNCTIONAL VARIATION IN ECM FUNGAL COMMUNITIES AMONG AND WITHIN SITES

The ANOVA showed that mean community enzymatic activities $\bar{t}_j$ per soil core were significantly higher at Champenoux, except for CEL (Table 2a, 'Total' column). In any significant case, the variation was related to changing taxonomic composition ($P < 0.01$, 'interspecific component'), and the intraspecific component of the variation was not significant ($P > 0.05$, 'intraspecific component'), except for NAG ($P = 0.02$). However, we specifically analysed enzymatic activities of *C. geophilum*, the most widespread species at both sites, and found a significant decrease of enzymatic activities of *C. geophilum* between Champenoux and Puechabon, except for GLR, CEL and LAC (Fig. 3). This tendency was consistent with the whole community variation expressed by $\bar{t}_j$.

We found significant functional variation among plots within sites, for four enzymatic activities (XYL, CEL, GLU, NAG; $P < 0.05$ by ANOVA; Table 2a). Equivalent amount of variation was accounted among sites and among plots within sites (30.73% with 17% SD and 29.53% with 14% SD, respectively). Intraspecific trait variation contributed significantly to the total variation in these four activities ($P < 0.01$), while interspecific variation also contributed to the total variation for NAG and CEL ($P < 0.05$ but $P > 0.01$; Table 2a). We further performed separate analyses of the among-plot variation at Champenoux and Puechabon (Table S3) and found that the functional variation was only significant among plots at Champenoux.

RELATIONSHIP BETWEEN ECM AND SOIL ENZYMATIC ACTIVITIES

Soil enzymatic activities differed between soil cores of Puechabon and Champenoux, except for GLR ($P < 0.05$ by ANOVA; Table 2b). Remarkably though, three soil enzymatic activities (XYL, PHO and LEU) were significantly higher at Champenoux and four were significantly higher at Puechabon (CEL, GLU, NAG and LAC). Some soil activities (NAG, GLU) were negatively correlated with soil water content (Table S4).

Table 2. Variation in the functional composition of ECM fungal communities (a) and of the organisms found in surrounding soil (b)

(a) Change in community-level ECM enzymatic activities between Puechabon and Champenoux, and among plots within sites

	Variation between sites				Variation between plots within sites		
Enzymatic activities	Interspecific component	Intraspecific component	Total	Contrast Pu-Ch	Interspecific component	Intraspecific component	Total
XYL	**0.007**	0.26	**0.021**	−0.36	0.15	**< 0.001**	**0.013**
GLR	**< 0.001**	0.68	**< 0.001**	−0.47	0.74	0.93	0.82
CEL	0.04	0.92	0.10	−0.80	**0.03**	**0.008**	**0.007**
GLU	**< 0.001**	0.10	**< 0.001**	−1.12	0.09	**< 0.001**	**0.009**
NAG	**< 0.001**	**0.02**	**< 0.001**	−1.71	**0.01**	**< 0.001**	**< 0.001**
PHO	**0.001**	0.20	**0.002**	−1.03	0.10	0.05	0.09
LEU	**0.001**	0.15	**0.001**	−0.32	0.92	0.03	0.39
LAC	**< 0.001**	0.64	**< 0.001**	−1.03	0.20	0.13	0.26

(b) Change in soil enzymatic activities between Puechabon and Champenoux, and among plots within sites

	Variation between sites		Variation between plots within sites
Enzymatic activities	*P*-value	Contrast	*P*-value
XYL	**< 0.001**	−1.28	0.32
GLR	0.31	−0.14	0.52
CEL	**0.01**	0.03	0.25
GLU	**< 0.001**	0.77	0.30
NAG	**< 0.001**	1.09	0.85
PHO	**0.01**	−0.42	0.14
LEU	**< 0.001**	−2.38	**0.01**
LAC	**< 0.001**	0.04	**0.003**

Nested ANOVAS were performed to represent the variation in average enzymatic activities in communities between the Champenoux (Ch) and Puechabon (Pu) sites, as well as between plots within sites (nested predictor). The method of Lepš *et al.* (2011) was used in (a) to assess the relative contribution of interspecific and intraspecific trait variation to the functional changes among communities (see Fig. 2). Table (a) thereby includes *P*-values of these contributions and of the total variation between communities. The difference of enzymatic activities between sites is also provided (contrast Pu-Ch). Table (b) includes the *P*-values of the variation in enzymatic activities in soil between sites as well as between plots within sites. The difference of enzymatic activities between sites is also provided. Abbreviations: β-xylosidase (XYL), β-glucuronidase (GLR), cellobiohydrolase (CEL), β-glucosidase (GLU), N-acetylhexosaminidase (NAG), acid phosphatase (PHO), leucine aminopeptidase (LEU) and laccase (LAC). Enzymatic activities are expressed in pmol min^{-1} per gram of soil, and in pmol min^{-1} and per mm^2 of fungal sheath of ECM root tips. They are log-transformed and averaged in communities. Bold values are significant ($P < 0.05$).

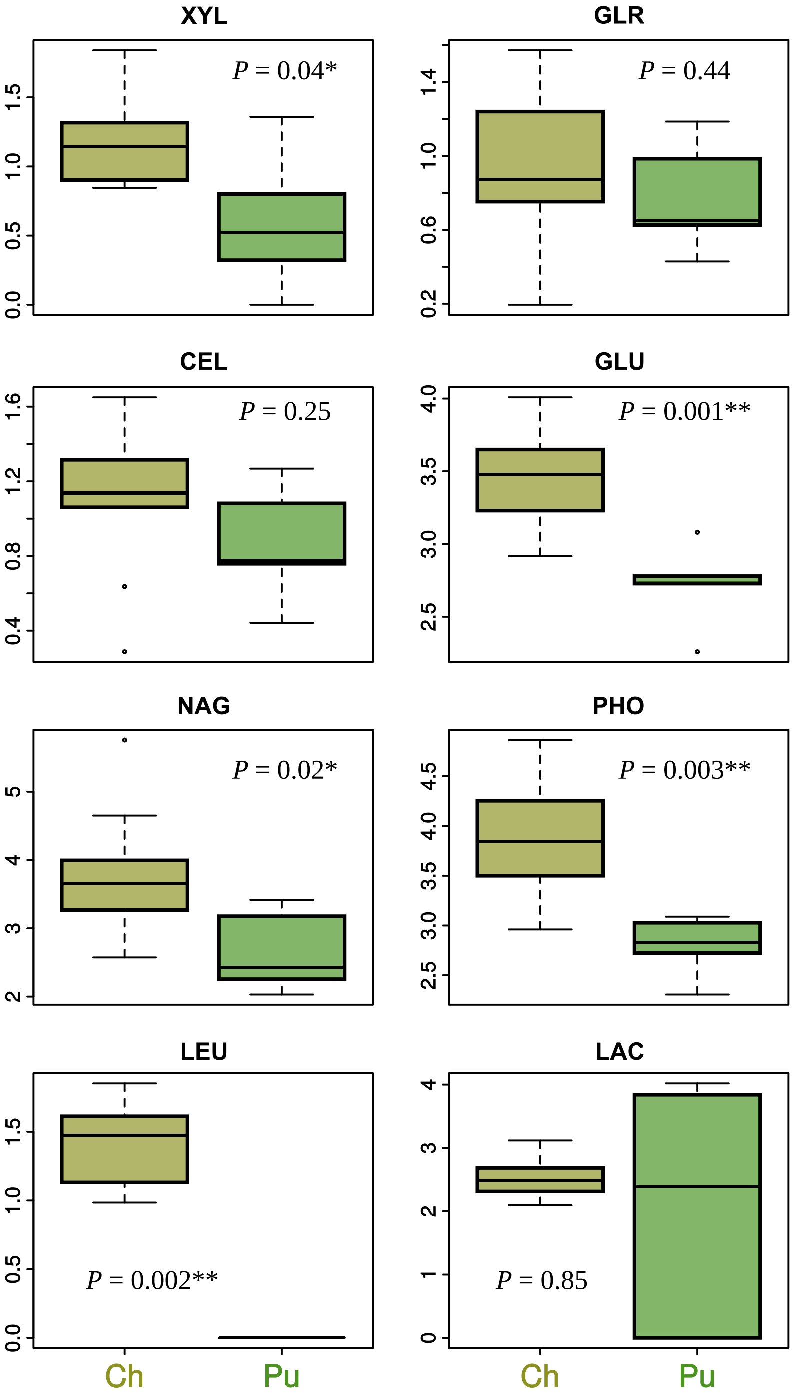


Fig. 3. Variation in enzymatic activities of the most common fungus, *Cenococcum geophilum*, between Champenoux (Ch) and Puechabon (Pu) sites, with *P*-values (P) of the unpaired Wilcoxon test. The enzymatic activities are β-xylosidase (XYL), β-glucuronidase (GLR), cellobiohydrolase (CEL), β-glucosidase (GLU), N-acetylhexosaminidase (NAG), acid phosphatase (PHO), leucine aminopeptidase (LEU) and laccase (LAC).

The activity of three soil enzymes (GLU, NAG and LAC; Table S4) positively correlated with SOM content. More variation was found among sites than among plots within sites (44.28% with 29.6% SD and 10.2% with 17.6% SD, respectively).

Overall, we found significant correlation between ECM and soil enzymatic activities (Table 3 and Fig. 1b) for all the activities except CEL, but the correlations were negative for GLU, NAG and LAC. Therefore, there was no overall congruence of ECM and soil enzymatic activities.

FUNCTIONAL VARIATION WITHIN ECM FUNGAL COMMUNITIES

Functional divergence (FDiv) was calculated for each soil core community and for each enzymatic activity. Based on the null model performed separately at Puechabon and Champenoux (see Appendix S5), we found FDiv to be significantly smaller (adjusted $P \leq 0.05$) than random in 8.8% of the combinations [soil core × enzymatic activity]. Environmental filtering constrained the variation in at least one

Table 3. Correlation between soil enzymatic activities and average ECM activities in soil cores

	XYL	GLR	CEL	GLU	NAG	PHO	LEU	LAC
ρ	0.47	0.37	0.09	−0.35	−0.65	0.38	0.45	−0.33
P-value	**0.005**	**0.03**	0.60	**0.04**	**< 0.001**	**0.03**	**0.007**	0.052

The Spearman ρ coefficient and corresponding *P*-value are provided for each activity. Bold values are significant ($P < 0.05$).

of the eight enzymatic activities in 29.4% of the soil cores, and more often at Champenoux than at Puechabon (40% vs. 14.3%). This environmental filtering primarily concerned the exploitation of carbohydrates and chitin (CEL, GLR, LEU, NAG and XYL for which more than 9% of soil cores displayed environmental filtering). Separable signatures of environmental filtering are observable on Fig. 4 at Puechabon (filled circles) and Champenoux (empty circles). Even though enzymatic activities were lower overall at Puechabon (Fig. 4, *x*-coordinate), environmental filtering was less pronounced there than in communities featuring comparable mean enzymatic activities at Champenoux (Fig. 4, *y*-coordinate). This result is consistent with the ANOVA that showed no significant variation among plots at Puechabon, while there was significant variation at Champenoux (see Table S3). When the ECM OTUs were randomized at plot level, only 1.29% of the [soil core × trait] combinations yielded *FE* significantly higher (adjusted $P \leq 0.05$) than random. Therefore, we found less influence of niche differentiation than of environmental filtering on the functional composition of ECM communities.

RELATIONSHIP BETWEEN SOIL RESOURCES AND ECM ENZYMATIC ACTIVITIES

Champenoux soil was significantly richer in lignin and recalcitrant C compounds (Methoxyl.C, Arom.C, Phen.C and Carbox.C), and in alkyl compounds (Alk.C; $P < 0.001$; Mann–Whitney U-test), while Puechabon soil was significantly richer in chitin ($P < 0.001$; Mann–Whitney U-test). Concentration of polysaccharides (O.Alk.C) did not differ between sites. The RLQ analysis evidenced an overall significant relationship between soil resources and ECM fungal enzymatic activities (randomization test, $P < 0.001$). The first and the second RLQ axes, respectively, accounted for 90.95% and 7.53% of the overall relationship and thereby represented most of the overall variation. Each axis represented how syndromes of traits co-varied along an environmental gradient. Based on community scores, the first axis depicted the taxonomic, environmental and functional contrast among Puechabon and Champenoux communities, while the second axis represented remaining variation among communities within sites (Fig. 5a). The first axis was also related to the contrasted physical soil characteristics between sites (pH and SOM Spearman ρ = −0.85 and −0.79, respectively, $P < 0.001$). In addition, both axes depicted a gradient of phosphorus in soil cores (Spearman ρ = 0.76 and 0.59, respectively, $P < 0.001$). The eight ECM enzymatic traits were positively related with axis 1 over a gradient of soil water content (Fig. 5b, abscissa). The driest plots (Mediterranean context, Puechabon) were the richest in chitin (Chitin = −0.77) and in alkyl compounds (Alk.C = −0.70, Fig. 5b, orange arrows) compared to the moister soils, which were conversely enriched in recalcitrant C pools (Methoxyl.C = +0.84; Phen.C = +0.86). On axis 2 (Fig. 5b, ordinates), the ability to hydrolyse proteins (LEU = +0.29) and chitin (NAG = +0.14) was inversely related to the ability to hydrolyse polysaccharides (CEL = −0.39). The former strategy was favoured in soil cores rich in recalcitrant C compounds (Carbox.C = +0.14; Arom.C = +0.33), while the latter was favoured in soil cores more humid and richer in chitin, polysaccharides and alkyl compounds. It is noteworthy that on both axes 1 and 2, the ability to hydrolyse chitin by ECM fungi (NAG) was negatively related to the soil chitin reservoir. Contrastingly, polyphenol concentration was positively correlated with laccase activity (LAC).

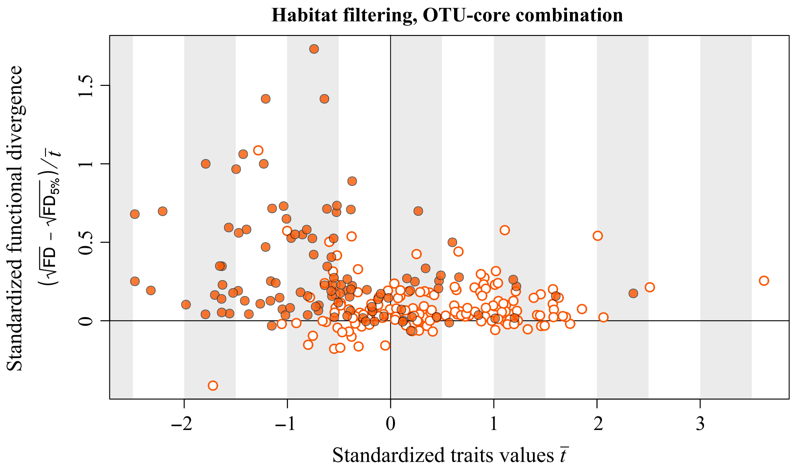


Fig. 4. Analysis of environmental filtering within ECM communities, based on the community-level variance (FDiv) of ECM enzymatic activities, at the Champenoux (red empty dots) and Puechabon (red filled dots) sites. Each dot represents an ECM fungal community (in a soil core). Mean trait values on the abscissa are standardized according to the mean and the standard deviation of each of the eight enzymatic activities, while the FDiv statistics on ordinates are standardized according to the 5% quantile of the null model and the mean enzymatic activity. Points under zero on ordinates are therefore lower than the 5% quantile.

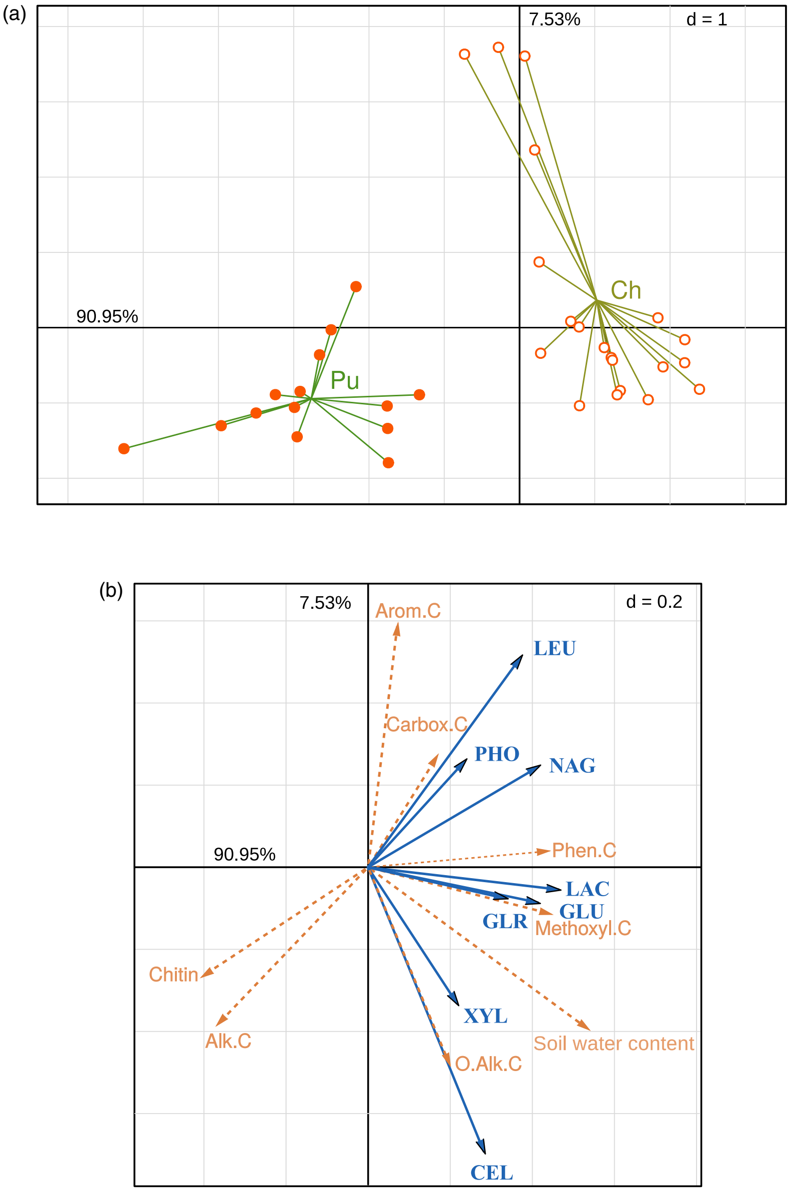


Fig. 5. Scores of (a) soil cores and (b) ECM enzymatic activities (continuous lines) and soil resources (dotted lines) on the two-first axes of the RLQ analysis relating ECM enzymatic to soil resources. Enzymatic activities are β-xylosidase (XYL), β-glucuronidase (GLR), cellobiohydrolase (CEL), β-glucosidase (GLU), N-acetylhexosaminidase (NAG), acid phosphatase (PHO), leucine aminopeptidase (LEU) and laccase (LAC). Soil resources are concentrations of four categories of recalcitrant phenolic compounds (Methoxyl.C = methoxyl C, Arom.C = aromatic C, Phen.C = phenolic C and Carbox.C = carboxyl-C), CH_3 groups of lipids, waxes and cutins (Alk.C = alkyl C), polysaccharides including cellulose and hemicellulose (O.Alk.C = O-alkyl C), chitin (Chitin = glucosamine) and soil water content.

Discussion

We observed evidence of functional and taxonomic variation among and within communities in response to environmental heterogeneity in temperate and Mediterranean ecosystems. Our study provides two main insights into how and why enzymatic activities vary between and within ECM fungal communities. First, functional variation among the two sites was related to a broad change in taxonomic composition, while the functional variation among plots in the Champenoux site was mostly related to intraspecific variation in enzymatic activities. Secondly, the functional variation in enzymatic activities depended on resource concentrations in surrounding soil, supporting the hypothesis that the functional response of ECM communities relates to resource availability (Fig. 5).

ENVIRONMENTAL CONSTRAINTS PRIMARILY DRIVE FUNCTIONAL VARIATION WITHIN AND BETWEEN ECM COMMUNITIES

The two sites (Champenoux and Puechabon) represented contrasted forest ecosystems in terms of biotic and abiotic environment, so that variations in ECM enzymatic activities were expected in response to the variable environment. We also expected that local-scale assembly processes influenced the distribution of enzymatic activity within soil core communities (Fig. 1a, bottom). Significant differences in community-level enzymatic activities among and within sites (Table 2a) indeed showed that ECM fungal communities were structured by environmental heterogeneity at both spatial scales. We note here that the functional contrast between Puechabon and Champenoux was related to strong taxonomic change, as very few species were shared between Champenoux and Puechabon (Fig. 3). Such large difference in taxonomic composition was expected in contrasted bioclimatic and pedoclimatic contexts, because different host species generally shelter distinct ECM fungal communities (Morris *et al.* 2009). Different taxonomic composition still does not necessarily imply changing functional composition (e.g. Fig. 2a). Here, the result means that different species identity is related to different functional properties, which suggests that fungi with different ecological niches are found at Champenoux and Puechabon. Conversely, variable functional properties of populations within species (intraspecific component) contributed more to the functional changes among plots at Champenoux. Therefore, we found a hierarchy of ecological processes where functional variation among ECM fungal communities was related to different taxonomic composition among contrasted forest ecosystems, and mostly to intraspecific variation at a more local scale (Tables 2a and S3). Intraspecific functional variation has been recently recognized to be a key component of community dynamics (Bolnick *et al.* 2011), which has motivated much research in plant ecology (Albert *et al.* 2011), but the contribution of individual variation in ECM symbioses remains neglected (Johnson *et al.* 2012): to our best knowledge, this is the first evidence of how the nested effects of inter- and intraspecific variation influence fungal community ecology. Although the approach does not distinguish genotypic and phenotypic variations within OTUs, it basically shows that the functional composition of ECM root tips communities is influenced by variable environment.

A third level of the ecological hierarchy concerns functional trait variation *within* ECM fungal communities. We detected functional convergence for all the enzymatic activities, thereby revealing a constraint on the variation in enzymatic activities within communities. However, functional convergence was less frequent at Puechabon than at Champenoux. The Mediterranean climatic context of Puechabon, with typical summer drought, is expected to favour more stress-tolerant strategies than the temperate context of Champenoux. It may result in an overall environmental filtering of Puechabon communities, with functional convergence operating not only in local communities but also at a regional Mediterranean scale. Conversely, a release of the regional constraint can allow a variation in environmental filtering across communities depending on soil quality, as found at Champenoux (Fig. 4). In addition, we found a far weaker signal of niche differentiation based on the analysis of functional evenness. Our results thereby suggest that environmental filtering primarily influences variations in enzymatic activities both among and within ECM fungal communities.

ENVIRONMENTAL DRIVERS OF ECM ECOLOGICAL STRATEGIES

Whenever significant functional variation was found among and within ECM communities regarding $\bar{t}_j$, FDiv$_j$ and/or FE$_j$, a subsequent issue was to identify the factors underlying such variation. We hypothesized that such community-level variation was primarily related to the availability of resources in surrounding soil. We then identified the environmental drivers underlying the functional variation in ECM fungal communities by performing a RLQ analysis relating functional trait variation to environmental variation through the changing community taxonomic composition (Thuiller *et al.* 2006; Raevel, Violle & Munoz 2012). We related the marked differences in functional and taxonomic composition between sites (Table 2a) to contrasted abiotic properties (soil water content, pH and phosphorus content) and soil resources (RLQ axis 1 on Fig. 4). The finding of a functional response of ECM fungal communities to resource availability is congruent with previous studies. Sinsabaugh, Hill & Follstad Shah (2009) and Schneider *et al.* (2012) indeed showed that the litter nutrient content and the stoichiometry of C:N:P affect activities of enzymes involved in SOM nutrient acquisition (GLU, NAG, LEU and PHO). We also found (Table S4) a negative correlation between soil water content and enzymatic activity regarding chitin (NAG) and cellulose (GLU but not XYL and CEL as in Brockett, Prescott & Grayston 2012). The activity of three soil enzymes (GLU, CEL and NAG; Table S4) positively correlates with SOM content (Sinsabaugh *et al.* 2008), as in our study. Our study reveals a contrast between Champenoux and Puechabon regarding both phosphorus availability and PHO activity. Large spatial variations in PHO activities are indeed reported in temperate forest soils (Jones *et al.* 2010). Previous studies have also identified abiotic factors such as soil pH as primary environmental variables driving soil microbial activities (e.g. Kivlin & Treseder 2014); here, the high XYL and PHO at Champenoux and the low GLU and NAG activities at Puechabon can be indirectly related to the differences in pH (4.6 vs. 7.4 at Champenoux and Puechabon, respectively). The functional variation between the two sites was therefore likely to be an ecological response to a large-scale environmental contrast in soil water content, and in the nature and content of SOM. We further found that ECM fungal community assembly was functionally constrained within sites according to soil resources, especially at Champenoux (see Table S3a and RLQ axis 2 on Fig. 5). The spread of Champenoux communities on RLQ axis 2 reflected a trade-off between the ability to hydrolyse proteins and chitin on one hand, and the ability to oxidize phenols on the other hand, in response to variable SOM resources in soil. We found that intraspecific trait variation mostly contributed to the change in functional composition among communities at Champenoux (Table S3a), which underlines that functional plasticity can play a stronger role than taxonomic variation in the functional response of ECM communities at a local scale. Indeed, the idea that functional and taxonomic diversity are not synonymous in microbial communities is now gaining momentum (e.g. Yuste *et al.* 2014).

RELATIONSHIP BETWEEN ECM ENZYMATIC ACTIVITIES IN ROOT TIPS AND SOIL ENZYMATIC ACTIVITIES

Previous experiments suggested that ECM fungi and other fungi as saprobes are main actors of the SOM degradation in the ectomycorrhizosphere (Uroz *et al.* 2013). Enzymatic activities in the ectomycorrhizosphere in soil cores encompass all microbes potentially exploiting common resources. We found that environmental heterogeneity influences differently variations in ECM enzymatic activities in root tips and of soil enzymatic activities (Table 2a vs. b). Furthermore, although the correlation between ECM and soil enzymatic activities was sometimes positive and supported partial congruence as shown by Phillips, Ward & Jones (2014), the significant negative relationships for GLU, NAG and LAC (Table 3 and see Fig. S1) suggest that ECM fungi only partly contribute to soil enzymatic activities. Other organisms (or even the extramatrical ECM mycelium) can therefore respond differently. In this regard, the negative correlations we found between ECM root tip and soil activities can reflect niche differentiation between organisms (or even between separate parts of ECM individuals). While niche differentiation was not found at the level of root tip ECM fungal communities, it could occur at a broader ecosystem level. Talbot *et al.* (2013) also reported that enzyme activity of ECM root tips and of bulk soil from the same soil cores did not correlate, suggesting that the ECM extramatrical mycelium contributes in a complementary way to nutrient cycling. Our results thus open perspectives towards enlarging the analysis of community assembly beyond the role of ECM fungi, by addressing the relative enzymatic activities of the other coexisting organisms and ECM fungus components (Talbot *et al.* 2014). However, we also note that using occurrences of OTUs in soil cores could not allow identifying their relative importance in surrounding soil. Further investigation of their relative volumes in soil would thus be needed to investigate the issue in greater details. Such methodological refinements will help better identify the functions of ECM fungi in a comprehensive functional landscape of fungi in soils (Anderson, Genney & Alexander 2014).

Conclusion

Our study provides evidence of the central role played by abiotic factors, particularly SOM composition, in the functional ecology of ECM communities. Among-community variation and local functional convergence in enzymatic activities were found within and among sites. We suggest that the assembly of ECM fungi on roots is constrained by soil resources and that there are trade-offs in enzymatic activities both within and among OTUs, depending on variations in resource availability. Moreover, while we suggest that niche differentiation is not the primary mechanism of ECM community assembly, our results support functional differentiation of ECM root tips with other organisms in the surrounding soil. Finally, these results aggregate the fungal kingdom into the current and promising attempts to revisit community ecology using a functional, trait-based approach (McGill *et al.* 2006). This opens novel perspectives for understanding the dynamics of coexistence within ECM fungal and soil microbial communities.

Acknowledgements

This study was conducted as part of the FUNDIV project (ANR606-BDIV-06). We are grateful to Dr. J.M. Ourcival, who helped us collect field data, V. Raevel and K. Pritsch for stimulating discussions. P.E. Courty gratefully acknowledges support from the Swiss National Science Foundation (PZ00P3_136651). We are grateful to the Office National des Forêts for permitting sampling at Champenoux and Puechabon State Forests.

Data accessibility

All data are present in the paper and its supporting information.

References

Abrams, P. (1983) The theory of limiting similarity. *Annual Review of Ecology and Systematics*, **14**, 359–376.

Agerer, R. (2001) Exploration types of ectomycorrhizae – a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza*, **11**, 107–114.

Albert, C.H., Grassein, F., Schurr, F.M., Vieilledent, G. & Violle, C. (2011) When and how should intraspecific variability be considered in trait-based plant ecology? *Perspectives in Plant Ecology, Evolution and Systematics*, **13**, 217–225.

Anderson, I.C., Genney, D.R. & Alexander, I.J. (2014) Fine-scale diversity and distribution of ectomycorrhizal fungal mycelium in a Scots pine forest. *New Phytologist*, **201**, 1423–1430.

Bahram, M., Peay, K.G. & Tedersoo, L. (2015) Local-scale biogeography and spatiotemporal variability in communities of mycorrhizal fungi. *New Phytologist*, **205**, 1454–1463.

Bahram, M., Koljalg, U., Courty, P.E., Diedhiou, A.G., Kjøller, R., Polme, S., Ryberg, M., Veldre, V. & Tedersoo, L. (2013) The distance decay of similarity in communities of ectomycorrhizal fungi in different ecosystems and scales. *Journal of Ecology*, **101**, 1335–1344.

Baldrian, P., Kolarik, M., Stursova, M., Kopecky, J., Valaskova, V., Vetrovsky, T., Zifcakova, L., Snajdr, J., Ridl, J., Vlcek, C. & Voriskova, J. (2012) Active and total microbial communities in forest are largely different and highly stratified during decomposition. *ISME Journal*, **6**, 248–258.

Bolnick, D.I., Amarasekare, P., Araujo, M.S., Burger, R., Levine, J.M., Novak, M. *et al.* (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution*, **26**, 183–192.

Brockett, B.F.T., Prescott, C.E. & Grayston, S.J. (2012) Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biology and Biochemistry*, **44**, 9–20.

Burke, D.J., Weintraub, M.N., Hewins, C.R. & Kalisz, S. (2011) Relationship between enzyme activities, nutrient cycling and soil fungal communities in a northern hardwood forest. *Soil Biology and Biochemistry*, **43**, 795–803.

Courty, P.E., Pritsch, K., Schloter, M., Hartmann, A. & Garbaye, J. (2005) Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. *New Phytologist*, **167**, 309–319.

Courty, P.E., Franc, A., Pierrat, J.C. & Garbaye, J. (2008) Temporal changes in the ectomycorrhizal community in two soil horizons of a temperate oak forest. *Applied and Environmental Microbiology*, **74**, 5792–5801.

Courty, P.E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F. *et al.* (2010) The role of ectomycorrhizal communities in forest ecosystem processes: new perspectives and emerging concepts. *Soil Biology and Biochemistry*, **42**, 679–798.

Crowther, T.W., Stanton, D.W.G., Thomas, S.M., A'Bear, A.D., Hiscox, J., Jones, T.H., Voříšková, J., Baldrian, P. & Boddy, L. (2013) Top-down control of soil fungal community composition by a globally distributed keystone consumer. *Ecology*, **94**, 2518–2528.

Cullings, K. & Courty, P.E. (2009) Saprotrophic capabilities as functional traits to study functional diversity and resilience of ectomycorrhizal community. *Oecologia*, **161**, 661–664.

Doledec, S., Chessel, D., terBraak, C.J.F. & Champely, S. (1996) Matching species traits to environmental variables: a new three-table ordination method. *Environmental and Ecological Statistics*, **3**, 143–166.

Dray, S. & Legendre, P. (2008) Testing the species traits-environment relationships: the fourth-corner problem revisited. *Ecology*, **89**, 3400–3412.

Genney, D.R., Anderson, I.C. & Alexander, I.J. (2006) Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. *New Phytologist*, **170**, 381–390.

Grime, J.P. (2006) Trait convergence and trait divergence in herbaceous plant communities: mechanisms and consequences. *Journal of Vegetation Science*, **17**, 255–260.

van der Heijden, M.G.A., Martin, F., Selosse, M.A. & Sanders, I.R. (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist*, **205**, 1406–1423.

Johnson, D., Martin, F., Cairney, J.W.G. & Anderson, I.C. (2012) The importance of individuals: intraspecific diversity of mycorrhizal plants and fungi in ecosystems. *New Phytologist*, **194**, 614–628.

Jones, M.D., Twieg, B.D., Ward, V., Barker, J., Durall, D.N. & Simard, S.W. (2010) Functional complementarity of Douglas-fir ectomycorrhizas for extracellular enzyme activity after wildfire or clearcut logging. *Functional Ecology*, **24**, 1139–1151.

Jung, V., Violle, C., Mondy, C., Hoffmann, L. & Muller, S. (2010) Intraspecific variability and trait-based community assembly. *Journal of Ecology*, **98**, 1134–1140.

Keddy, P.A. (1992) Assembly and response rules – 2 goals for predictive community ecology. *Journal of Vegetation Science*, **3**, 157–164.

Kivlin, S.N. & Treseder, K.K. (2014) Soil extracellular enzyme activities correspond with abiotic factors more than fungal community composition. *Biogeochemistry*, **117**, 23–37.

Koide, R.T., Fernandez, F. & Malcolm, G. (2014) Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. *New Phytologist*, **201**, 433–439.

Koide, R.T., Xu, B., Sharda, J., Lekberg, Y. & Ostiguy, N. (2005) Evidence of species interactions within an ectomycorrhizal fungal community. *New Phytologist*, **165**, 305–316.

Kraft, N.J.B., Valencia, R. & Ackerly, D.D. (2008) Functional traits and niche-based tree community assembly in an Amazonian forest. *Science*, **322**, 580–582.

Lepš, J., de Bello, F., Šmilauer, P. & Doležal, J. (2011) Community trait response to environment: disentangling species turnover vs intraspecific trait variability effects. *Ecography*, **34**, 856–863.

Lortie, C.J., Brooker, R.W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F.I. *et al.* (2004) Rethinking plant community theory. *Oikos*, **107**, 433–438.

Mason, N.W.H., Mouillot, D., Lee, W.G. & Wilson, J.B. (2005) Functional richness, functional evenness and functional divergence: the primary components of functional diversity. *Oikos*, **111**, 112–118.

McArthur, R. & Levins, R. (1967) The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist*, **101**, 377–385.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006) Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, **21**, 178–185.

McGuire, K.L., Bent, E., Borneman, J., Majumder, A., Allison, S.D. & Treseder, K.K. (2010) Functional diversity in resource use by fungi. *Ecology*, **91**, 2324–2332.

Morris, M.H., Perez-Perez, M.A., Smith, M.E. & Bledsoe, C.S. (2009) Influence of host species on ectomycorrhizal communities associated with two co-occurring oaks (*Quercus* spp.) in a tropical cloud forest. *FEMS Microbiology Ecology*, **69**, 274–287.

Phillips, L.A., Ward, V. & Jones, M.D. (2014) Ectomycorrhizal fungi contribute to soil organic matter cycling in sub-boreal forests. *ISME Journal*, **8**, 699–713.

Pickles, B.J., Genney, D.R., Potts, J., Lennon, J., Anderson, I.C. & Alexander, I.J. (2010) Spatial and temporal ecology of Scots pine ectomycorrhizas. *New Phytologist*, **186**, 755–768.

Pike, N. (2011) Using false discovery rates for multiple comparisons in ecology and evolution. *Methods in Ecology and Evolution*, **2**, 278–282.

Pritsch, K., Courty, P.E., Churin, J.L., Cloutier-Hurteau, B., Ali, M.A., Damon, C. *et al.* (2011) Optimized assay and storage conditions for enzymatic activity profiling of ectomycorrhizae. *Mycorrhiza*, **21**, 589–600.

R Development Core Team (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Raevel, V., Violle, C. & Munoz, F. (2012) Mechanisms of ecological succession: insights from plant functional strategies. *Oikos*, **121**, 1761–1770.

Richard, F., Millot, S., Gardes, M. & Selosse, M.A. (2005) Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*. *New Phytologist*, **166**, 1011–1023.

Rineau, F. & Courty, P.E. (2011) Secreted enzymatic activities of ectomycorrhizal fungi as a case study of functional diversity and functional redundancy. *Annals of Forest Science*, **68**, 69–80.

Rineau, F., Roth, D., Shah, F., Smits, M., Johansson, T., Canbäck, B. *et al.* (2012) The ectomycorrhizal fungus *Paxillus involutus* converts organic matter in plant litter using a trimmed brown-rot mechanism involving Fenton chemistry. *Environmental Microbiology*, **14**, 1477–1487.

Schneider, T., Keiblinger, K.M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B. *et al.* (2012) Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *ISME Journal*, **6**, 1749–1762.

Sinsabaugh, R.L., Hill, B.H. & Follstad Shah, J.J. (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature*, **462**, 795–799.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C. *et al.* (2008) Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, **11**, 1252–1264.

Stubbs, W.J. & Wilson, J.B. (2004) Evidence for limiting similarity in a sand dune community. *Journal of Ecology*, **92**, 557–567.

Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S. *et al.* (2013) Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. *Soil Biology and Biochemistry*, **57**, 282–291.

Talbot, J.M., Bruns, T.D., Taylor, J.W., Smith, D.P., Branco, S., Glassman, S.I. *et al.* (2014) Endemism and functional convergence across the North American soil mycobiome. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 6341–6346.

Tedersoo, L., Naadel, T., Bahram, M., Pritsch, K., Buegger, F., Leal, M. *et al.* (2012) Enzymatic activities and stable isotope patterns of ectomycorrhizal fungi in relation to phylogeny and exploration types in an afrotropical rain forest. *New Phytologist*, **195**, 832–843.

Thuiller, W., Richardson, D.M., Rouget, M., Procheş, S. & Wilson, J.R.U. (2006) Interactions between environment, species traits, and human uses describe patterns of plant invasions. *Ecology*, **87**, 1755–1769.

Uroz, S., Courty, P.E., Pierrat, J.C., Peter, M., Buée, M., Turpault, M.P. *et al.* (2013) Functional profiling and distribution of the forest soil bacterial communities along the soil mycorrhizosphere continuum. *Microbial Ecology*, **66**, 404–415.

Valiente-Banuet, A. & Verdu, M. (2007) Facilitation can increase the phylogenetic diversity of plant communities. *Ecology Letters*, **10**, 1029–1036.

Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. & Garnier, E. (2007) Let the concept of trait be functional!. *Oikos*, **116**, 882–892.

Violle, C., Nemergut, D.R., Pu, Z. & Jiang, L. (2011) Phylogenetic limiting similarity and competitive exclusion. *Ecology Letters*, **14**, 782–878.

Walker, J.K.M., Cohen, H., Higgins, L.M. & Kennedy, P.G. (2014) Testing the link between community structure and function for ectomycorrhizal fungi involved in a global tripartite symbiosis. *New Phytologist*, **202**, 287–296.

Westoby, M. (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil*, **199**, 213–227.

Yuste, J.C., Fernandez-Gonzalez, A.J., Fernandez-Lopez, M., Ogaya, R., Penuelas, J. & Lloret, F. (2014) Functional diversification within bacterial lineages promotes wide functional overlapping between taxonomic groups in a Mediterranean forest soil. *FEMS Microbiology Ecology*, **90**, 54–67.

Received 14 September 2015; accepted 8 June 2016
Handling Editor: Nina Wurzburger

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Relationship between mean enzymatic activities of ECM communities (abscissa) and enzymatic activities of the surrounding soil, for each of the eight targeted activities as in Figure 3.

Table S1. Number of root tips and of OTUs in ECM communities at Champenoux (Ch) and Puechabon (Pu).

Table S2. Abundance (number of ECM root tips) of the 104 ECM operational taxonomic units (OTUs) found at Champenoux (Ch) and Puechabon (Pu) sites.

Table S3. Variation of the functional composition of ECM fungal communities among plots within each site.

Table S4. Spearman correlation (*P*-values corrected for false discovery rate, with *p.adjust*) between soil characteristics and soil enzyme activities.

Appendix S1. Study system.

Appendix S2. Measurements of functional traits of ECM apices.

Appendix S3. Measurement of soil enzymatic activity.

Appendix S4. Soil nutrients analysis.

Appendix S5. Null models of community assembly.

Manuscrit O : "Ectomycorrhizal fungi are shared between seedlings and adults in a monodominant *Gilbertiodendron dewevrei* rainforest in Cameroon"

Ectomycorrhizal Fungi are Shared between Seedlings and Adults in a Monodominant *Gilbertiodendron dewevrei* Rainforest in Cameroon

Helvyne C. Michaella Ebenye[1,2,3,8,9,10*], **Adrien Taudière**[3], **Nogaye Niang**[1], **Cheikh Ndiaye**[1], **Mathieu Sauve**[3], **Onguene N. Awana**[4], **Mieke Verbeken**[5], **André De Kesel**[6], **Seynabou Séne**[1], **Abdala G. Diédhiou**[1], **Violette Sarda**[3], **Omar Sadio**[7], **Maïmouna Cissoko**[1], **Ibrahima Ndoye**[1], **Marc-André Selosse**[2,8,*], and **Amadou M. Bâ**[9,10,*]

[1] Laboratoire Commun de Microbiologie, IRD/UCAD/ISRA, BP 1386 Dakar, Sénégal

[2] Institut de Systématique, Évolution, Biodiversité (ISYEB – UMR 7205 –CNRS, MNHN, UPMC, EPHE), Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, CP50, 75005, Paris, France

[3] UMR 5175, CEFE – CNRS – Université de Montpellier – Université Paul Valéry Montpellier – EPHE, Montpellier, France

[4] Institute of Agriculture Research for Development, Soil, Water and Atmosphere Department, BP. 2123, Yaoundé, Cameroon

[5] Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

[6] Botanic Garden Meise, Nieuwelaan 38, BE-1860 Meise, Belgium

[7] IRD, UMR 195 LEMAR (UBO/CNRS/IRD/Ifremer), BP 1386, CP 18524, Dakar, Senegal

[8] Department of Plant Taxonomy and Nature Conservation, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland

[9] Laboratoire des Symbioses Tropicales et Méditerranéennes, UMR113- INRA/AGRO-M/CIRAD/IRD/UM2-TA10/J, Campus International de Baillarguet, 34398 Montpellier cedex 5, France

[10] Laboratoire de Biologie et Physiologie Végétales, Faculté des Sciences Exactes et Naturelles, Université des Antilles, BP 592, 97159, Pointe-à-Pitre, Guadeloupe, France

[9,10] Corresponding author; email: amadou.ba@ird.fr

* These authors equally contributed to this work

Accepted in Biotropica

Abstract. Ectomycorrhizal networks may facilitate the establishment and survival of seedlings regenerating under the canopies of tropical forests. Thus, ectomycorrhizal networks are often invoked as a potential contributor to monodominance. Here, we identified ectomycorrhizal fungi in a monodominant *Gilbertiodendron dewevrei* (Fabaceae) rainforest in Cameroon, using sporocarps and ectomycorrhizae of three age categories (seedlings, intermediate trees and large trees), and tentatively revealed nutrient transfer through ectomycorrhizal networks by measuring spontaneous isotopic (13C and 15N) abundances in seedlings. Sporocarp surveys revealed fewer ectomycorrhizal fungal taxa (59 species from 1 030 sporocarps) than molecular barcoding of ectomycorrhizal roots (75 operational taxonomic units from 828 ectomycorrhizas). Our observations suggest that ectomycorrhizal fungal diversity is similar to that in other mixed tropical forests. This study reports for the first time the *Tuber-Helvella* lineage in a tropical forest. Despite some differences, all age categories of *G. dewevrei* had overlapping ectomycorrhizal fungal communities, with families belonging to Thelephoraceae, Russulaceae, Sebacinaceae, Boletaceae and Clavulinaceae. Of the 49 OTUs and species from ectomycorrhizas and sporocarps, respectively, shared by the three age categories (65.3% of the ectomycorrhizal fungal community), 19 were the most abundant on root tips of all categories (38.7% of the shared taxa), supporting the likelihood of ectomycorrhizal networks. Yet no evidence for nutrient transfer from trees to seedlings was detected based on analyses conducted here. We discuss the composition of the ectomycorrhizal fungal community among the *G. dewevrei* age categories and the possible role of common ectomycorrhizal networks in this rainforest.

Key words: Fabaceae subfamily Caesalpinioideae; monodominant forest; common ectomycorrhizal network; 13C; ectomycorrhiza; Internal Transcribed Spacer; 15N; sporocarps.

Résumé. Les réseaux mycorhiziens pourraient faciliter l'établissement et la survie des semis sous la canopée des forêts tropicales, en particulier dans les forêts monodominantes. Pour étudier ce mécanisme, des champignons ont été identifiés à partir de sporophores et d'ectomycorhizes sur trois catégories d'âge (semis, arbres intermédiaires, arbres adultes) et leur rôle potentiel dans le transfert de nutriments au bénéfice des semis a été testé par des mesures de teneurs isotopiques spontanées (13C et 15N) dans une forêt monodominante à *Gilbertiodendron dewevrei* (Fabaceae) du Cameroun. Les sporophores ont été moins diversifiés (59 espèces parmi 1 030 sporophores) que les ectomycorhizes (75 unités taxinomiques opérationnelles parmi 828 ectomycorhizes). La diversité fongique ne diffère pas de celle des forêts tropicales mixtes. Nous décrivons la lignée *Tuber-Helvella* pour la première fois en forêt tropicale. Malgré des différences, les catégories d'âge, *G. dewevrei*, partagent des espèces de Thelephoraceae, Russulaceae, Sebacinaceae, Boletaceae et Clavulinaceae. Parmi les 49 taxons fongiques d'ectomycorhizes et de sporophores (65,3% de la communauté fongique) des trois catégories d'âge, 19 d'entre eux (38,7% de la communauté fongique partagée) formeraient un réseau mycorhizien commun entre les arbres et les semis. Toutefois, les approches isotopiques n'ont pas permis de détecter de transfert de nutriments des arbres aux semis. Nous discutons la composition et le rôle des communautés fongiques des trois catégories d'âge de *G. dewevrei* dans cette forêt tropicale humide.

Mots clés : Caesalpiniacées ; forêt monodominante ; réseau mycorhizien commun ; 13C ; ectomycorhizes ; espaceur interne transcrit ; 15N ; sporophores.

Rapport de stage d'Ananda Christophe : "Effets du feu et de la coexistence entre *Pinus pinaster*, *Pinus nigra* subsp. *laricio* et *Fagus sylvatica* sur les propriétés du sol dans les forêts montagnardes de Corse"

Col de Rospa Sorba 15 ans après le feu. On distingue une forêt mixte hêtre-pin laricio dans le fond.

Université des Sciences et Techniques du Languedoc
Master « *Sciences et technologies* » Mention "*Ecologie Biodiversite*"
Spécialité « *Biodiversité Evolution* »
Parcours « Ecosystèmes »

Stage de M1 Recherche :

Effets du feu et de la coexistence entre *Pinus pinaster*, *Pinus nigra subsp. laricio* et *Fagus sylvatica* sur les propriétés du sol dans les forêts montagnardes de Corse

Ananda CHRISTOPHE

Feu d'octobre 2014

Réalisé sous la direction de

Franck RICHARD - Maître de Conférences de Montpellier II -
& sous la co-direction de : Adrien TAUDIERE -Doctorant
UMR 5175 CEFE
Equipe Interactions Biotiques

Tutelles institutionnelles : CNRS et Université de Montpellier. Tutelles secondaires : Université Paul Valery, SupAgro Montpellier, Ecole Pratique des Hautes Etudes (EPHE), CIRAD, Institut de Recherche pour le Développement (IRD) et de Institut National de Recherche Agronomique (INRA ; USC 1338)

Table des matières

Les mots présents dans le glossaire en Annexe sont suivis d'un astérisque.

Introduction

La compréhension du fonctionnement des écosystèmes nécessite de prendre en compte la dynamique des espèces et de leurs interactions, notamment en lien avec les perturbations.

Les feux ont des effets directs sur le sol : le parcours de l'incendie génère des phénomènes érosifs par ruissellement (Certini, 2005). En modifiant les paramètres environnementaux, le feu induit des dynamiques de réponses variables selon les espèces (Karst et al., 2014) définissant des stratégies écologiques (Westoby, 1998), et pourrait également modifier les interactions intra- et interspécifiques.

La symbiose ectomycorhiziennes* est une interaction obligatoire entre la majorité des arbres non tropicaux et des champignons ectomycorhiziens (cECM). De nombreux travaux ont montré l'importance des cECM dans l'établissement des plantes au cours des successions écologiques (*e.g.* Richard et al. 2009). Ainsi la présence d'un des partenaires permet la présence de l'autre.

Le feu est une perturbation qui peut induire des changements profonds dans la composition et la structure. Karst et al., (2014) ont par exemple montré que l'effet de l'incendie sur les peuplements de pins est en mesure d'affecter la dynamique des populations et des communautés de cECM, et de manière indirecte le fonctionnement de l'écosystème. Ainsi la destruction des peuplements consécutive au passage du feu diminue l'abondance de cECM et résulte en un changement de richesse spécifique, dont l'ampleur est positivement corrélée avec la sévérité de l'incendie (Karst et al., 2014). Le feu augmente aussi la perte de matière organique dans le sol et augmente le lessivage, dont l'exportation de l'azote et du phosphore minéral (Giovannini et al., 1988).

Mon projet de recherche s'intègre dans le cadre de la thèse d'Adrien Taudiere dont les travaux de terrain se déroulent en Corse, dans les forêts de pin laricio de Corse (*Pinus nigra* subsp. *laricio*), sous espèce endémique de la Corse. Les peuplements de cette essence emblématique sont considérés comme prioritaires au sens de la Directive Habitats (CEE 92/43, 1992), mais sont frappés de manière régulière par le feu (Souleres, 2000). Le pin laricio de Corse domine les paysages aux étages supraméditerranéens (*cf.* Annexe 1 Fig.2) et surtout montagnards, où il constitue des peuplements purs, mais aussi des peuplements mélangés dans lesquels coexistent d'autres espèces d'arbres ECM, comme le pin maritime (*Pinus pinaster*) et le hêtre (*Fagus sylvatica*). L'objectif final de ce travail de thèse est d'explorer (i) les communautés de cECM réalisées avec les racines des trois espèces d'arbres précités et (ii) la réponse des communautés propres au pin laricio à différents régimes de feux (feu parcourant *vs.* feu destructeur).

Pour répondre à ces problématiques, nous devons comprendre le rôle du sol dans la structuration des communautés de cECM, mais aussi sa réponse chimique au passage du feu (Horton et al., 1999). La distribution des nutriments dans le sol (carbone organique, azote total et phosphore) sont ici analysées afin de comprendre les impacts des feux et de la coexistence sur le sol. Ce rapport explore successivement ainsi trois aspects fondamentaux du système étudié.

(i) Dans un premier temps, à partir d'un jeu de données initialement généré par A. Taudiere et al. (2012), une analyse des places respectivement occupées par le pin laricio, le pin maritime et le hêtre dans le réseau d'interactions ECM global est réalisée. Cette vision globale du réseau d'interaction permet de mettre en valeur les partages de cortège potentiel entre ces essences et de connaitre l'importance du pin laricio dans la structure du réseau.

(ii) Dans un second temps, à partir de l'analyse de prélèvements de sol réalisée à l'automne 2014 dans des peuplements purs de pin laricio parcourus par des incendies plus ou moins anciens, la composition chimique du sol est mesurée à différentes profondeurs et analysées en lien avec l'intensité du feu. Nous testons ici la persistance temporelle de l'impact de l'incendie sur la composition chimique du sol, à l'aide d'une approche synchronique comparant des feux déclarés entre 1970 et 2008. Notre hypothèse est qu'un feu récent est davantage perceptible dans les concentrations en nutriments du sol qu'un feu plus ancien. L'horizon A étant plus en contact avec la chaleur et les cendres produites par le feu, on s'attend à un impact du feu plus important dans l'horizon A (holo-organique) que dans l'horizon B (organo-minéral),

(iii) Enfin, à partir de l'analyse des mêmes prélèvements de sol, effectués dans des peuplements purs et mélangés, le lien entre la composition du peuplement forestier et les caractéristiques physico-chimiques du sol est analysé. Dans cette partie du travail, un typage moléculaire des racines de plantes a été réalisé par RFLP afin de vérifier l'appartenance taxonomique des racines (pin laricio *vs.* pin maritime) prélevées sur le terrain. Les niches biotiques des trois espèces sont en parties structurées par leur cECM associés. L'analyse RFLP permet aussi de tester si le recouvrement de niche potentiel observé dans le réseau (partie (i)) est également observé lors de la coexistence des essences. Contrairement au pin maritime (situé en altitude plus basse que le pin laricio), le pin laricio pousse lentement, mais survit plus facilement à des perturbations comme le feu. Le pin maritime arrivera plus facilement à se régénérer que le pin laricio si les feux sont récurrents. Cependant en absence de feux récents dans certains écosystèmes, le hêtre devient plus compétitif que le pin laricio. Le risque est donc que le pin maritime (en cas de feux intenses et fréquents) ou le hêtre (en l'absence de feu) supplante le pin laricio.

1 Matériels et Méthodes

1.1 Analyse du réseau d'interactions plantes champignons ectomycorhiziens.

L'analyse des réseaux des pins et du hêtre s'appuie sur une base de données comprenant 16 espèces d'arbres et arbustes ECM, et 411 espèces de champignons ECM de Corse. Nous sommes dans le cas d'un réseau d'interactions bipartite*. Le calcul de la modularité est une fonction permettant de quantifier la structuration d'un réseau en détectant la présence de groupes d'espèces (modules) caractérisées par une densité de connexions entre elles plus élevée que la moyenne. Ainsi, les modules sont caractérisés par une plus grande densité de liens intra-modules que de liens inter-modules. La détection des modules a été effectuée grâce à l'algorithme de « recuit simulé » du logiciel NETCARTO. On peut calculer deux valeurs par nœud en utilisant cette notion de modules (Guimerà et Amaral, 2005): (i) le coefficient de participation* (liens du taxon entre les modules/influence du taxon sur la connexion des modules) quantifie l'importance des espèces dans les liens entre modules et (ii) le degré relatif intra-module* quantifie l'importance des espèces dans la structure de leur module. On peut ainsi faire un focus sur une espèce dite clé de voûte du réseau (Mills et al., 1993) et sur l'impact de sa disparition éventuelle du réseau. Ensuite, l'imbrication* est une propriété communément analysée dans les réseaux mutualistes (Thebault et Fontaine, 2010). C'est une mesure qui permet de connaitre le niveau d'emboitement des partenaires des espèces spécialistes parmi les partenaires des espèces généralistes. L'imbrication du réseau a été calculée au moyen de la NODF (Almeida-Neto et al., 2008), testée avec un modèle nul qui contraint la somme des lignes et des colonnes, et testée par 1000 permutations.

1.2 Matériels biologiques et méthodes pour l'analyse des impacts du feu et de la coexistence sur la composition chimique du sol

Les échantillons de racines et de sols ont été prélevés en Corse dans les stations de Ghisoni (Ghi), Manganello (Man), Restonica (Res), Rospa Sorba (Ros), Tattone (Tato), Verghellu (Ver) et Vizzavona (Viz) (Annexe 1 Fig.1). La Corse est une île avec une altitude pouvant aller de 0 à 2750m. Les prélèvements ont été effectués entre le 6/11/2014 et le 17/11/2014.

Le pin laricio, reconnaissable par son tronc clair, est un arbre qui peut vivre des centaines d'années (ONF) (*cf.* Photo 1a et 1b). Il se retrouve à une position altitudinale intermédiaire entre celle du pin maritime (plus bas) et celle du hêtre (plus haut) (Gamisans, 1991 ; *cf.* Annexe 1 Fig.2). Pour les échantillons de sol je dispose de 178 échantillons prélevés sur le terrain, mais seulement 32 ont été analysés lors de cette phase exploratoire (*cf.* Annexe 4). Pour chaque échantillon, 15g de sol a été tamisés à 2mm. Ensuite, 10g sur les 15g tamisés ont été broyés en particules de 250µm grâce au broyeur à billes* disponible sur la plateforme de la PACE (Plateforme d' Analyses Chimiques en Ecologie) au CEFE. Le phosphore (en g/100g), le carbone organique (en g/kg) et l'azote total (en g/kg) ont été analysés par le laboratoire d'analyse des sols de l'INRA situé à Arras.

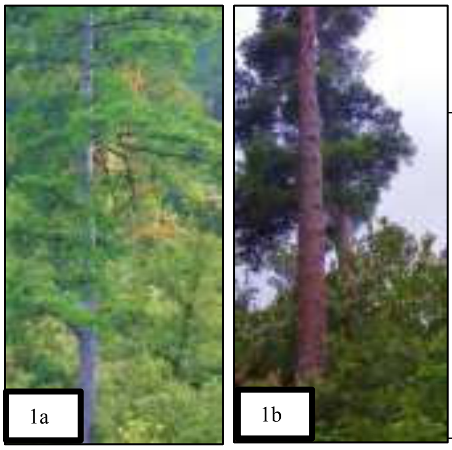


Photo 1a &1b – **Photos personnelles prises en Corse**
Photo 1a : Pin laricio reconnaissable grâce à son écorce claire.
Photo1b : Coexistence d'un pin laricio et d'un pin maritime (au premier plan, écorce foncée).

Des corrélations de Pearson et des tests de Wilcoxon ont été effectués entre les différentes modalités et les taux de carbone, azote et phosphate. Les échantillons datant de 2000 permettent de tester l'impact du feu en fonction de deux sévérités : feux parcourants ou destructeurs. Les échantillons de 1970, 1980, et 1993 permettent de tester la persistance de l'impact du feu au cours du temps selon les deux sévérités. Les échantillons non datés permettent de tester l'influence de la co-occurrence des plantes sur les caractéristiques chimiques du sol (*cf.* Tab A1).

1.3 Analyses moléculaires pour le typage des racines de pins échantillonnées

1.3.1 Extraction de l'ADN du pin laricio et du pin maritime

Pour extraire l'ADN de mes 85 échantillons racinaires (*cf.* Annexe 1 Tab.1 et Tab.A2), j'ai adapté un protocole spécifique pour l'extraction d'ADN d'aiguilles de pins (*cf.* A2). Cette extraction se déroule en plusieurs étapes : une extraction de l'ADN des cellules, une purification, une précipitation de l'ADN et pour finir une nouvelle étape de nettoyage.

1.3.2 Amplification de l'ADN par PCR et digestion

1.3.2.1 Amplification de l'ADN racinaires des pins

Après extraction, l'ADN a été amplifié par PCR (pour le détail du programme, *cf.* A2) à l'aide d'un thermocycleur et d'un programme nommé TouchDown, qui dure 2h30. Ce programme a la particularité de se placer le mélange réactionnel 10° au-dessus de la température d'hybridations et ainsi d'augmenter la spécificité des amorces.

Nous avons utilisé le gène RBCL (Kress et al., 2005) en suivant le protocole de Wang et al. (1999) utilisant les amorces RBCL1F et 724R. Pour des raisons pratiques nous avons choisi ce gène, car nous pouvions obtenir les 2 séquences des deux espèces de pins pour ce gène en ligne.

1.3.2.2 Digestion enzymatique : Technique des RFLP

L'étape qui suit est celle de la digestion enzymatique par une enzyme de restriction. La méthode des RFLP* ("restriction fragment lenght polymorphisme" ; en français : polymorphisme de longueur des fragments de restriction) permet d'identifier les taxons auxquels appartiennent les individus échantillonnés. Pour le choix de l'enzyme, je suis me suis appuyée sur Wang et al., (1999), et sur le site www.restriconmapper.org. Etant donné qu'il n'y a qu'un seul changement de nucléotides entre les deux gènes RBCL des deux pins, une seule enzyme permet de discriminer les deux sous-espèces. L'enzyme choisie s'appelle StuI, et coupe le gène RBCL (701pb) en deux fragments (343pb + 358pb) chez le pin laricio mais ne coupe pas le gène chez le pin maritime.

2 Résultats

2.1 Réseau d'interactions plantes-champignons ectomycorhiziens.

Les deux espèces de pins partagent beaucoup d'espèces de cECM (Fig.1 ; indice de similarité de jaccard = 0.38). En revanche, le hêtre partage peu de cECM avec chacune de ces espèces de pins (similarité de jaccard avec pin laricio = 0.13 ; avec pin maritime = 0.10). Chacune des trois espèces possède son cortège fongique d'espèces propres. Un seul cECM fait un lien avec ces trois seules espèces d'arbre. Il s'agit d'un hydne (*Hydnum albidum*).

La Fig.2 présente le degré relatif intra-module en fonction du coefficient de participation pour chaque individu. La répartition des individus (plantes et champignons) dans les secteurs R1 à R7 indique une hétérogénéité dans la répartition des champignons (principalement secteurs R1 et R3) et des plantes (principalement secteurs R5 et R6). Les trois plantes ciblées présentent un coefficient de participation relativement faible si on prend comme valeur remarquable 0.6 (Olesen et al., 2007). Elles appartiennent cependant au secteur R6 et forment donc quelques liens entre modules. Aussi le pin laricio possède le coefficient de participation le plus élevé parmi nos trois espèces (pin laricio à 0.55, pin maritime à 0.50 et hêtre à 0.40). Ces essences ont de fortes valeurs de degré relatif intra-module (>2). Elles sont donc structurantes au sein de leur propre module mais relient assez faiblement les modules entre eux. Le réseau n'est pas imbriqué (valeur de NODF = 48 et p-value = 1). La valeur de la modularité de notre modèle est de 0.458 (valeur simulée = 0.362 ; écart-type ± 0.002), notre réseau est modulaire, la répartition des modules n'est donc pas aléatoire.

La Fig.3 montre le niveau de coexistence (distance de Jaccard à partir des 8000 communautés échantillonnées en Corse, provenant de la base de données SOPHY) des paires de plantes ciblées. On remarque qu'il y a une forte coexistence entre les pins et le hêtre alors que les deux pins ne coexistent pas beaucoup.

2.2 Impacts de la coexistence et du feu sur la composition chimique du sol

Les concentrations en N total, Phosphore, C organique dans les sols des forêts incendiées ne montrent pas de différence en fonction du temps depuis le passage du dernier feu (tests de Pearson : p-value > 0.05 ; Fig.4 a, b, c, d). L'étude de la concentration de carbone et d'azote en fonction de l'horizon pédologique (horizon A *vs* horizon B ; Fig.5), révèle une différence significative de même direction pour l'azote et le carbone organique.

Les concentrations en azote et en carbone sont étroitement corrélées (cor= 0.94, p-value < 0.05), que ce soit dans les données sur les feux (deux sévérités et âge depuis le dernier feu) (Fig.6) et dans les données concernant la coexistence des trois espèces.

Pour finir, il y a que le rapport C/N qui montre une différence entre les différentes modalités de coexistence du hêtre et pin maritime, du hêtre et du pin laricio et des trois espèces en peuplement purs. Le hêtre seul à un rapport C/N faible par rapport aux pins martime et aux pins laricio seuls. En effet c'est le pin martime qui a le rapport le plus élevé. Le pin laricio est intermédiaire entre les deux autres espèces.

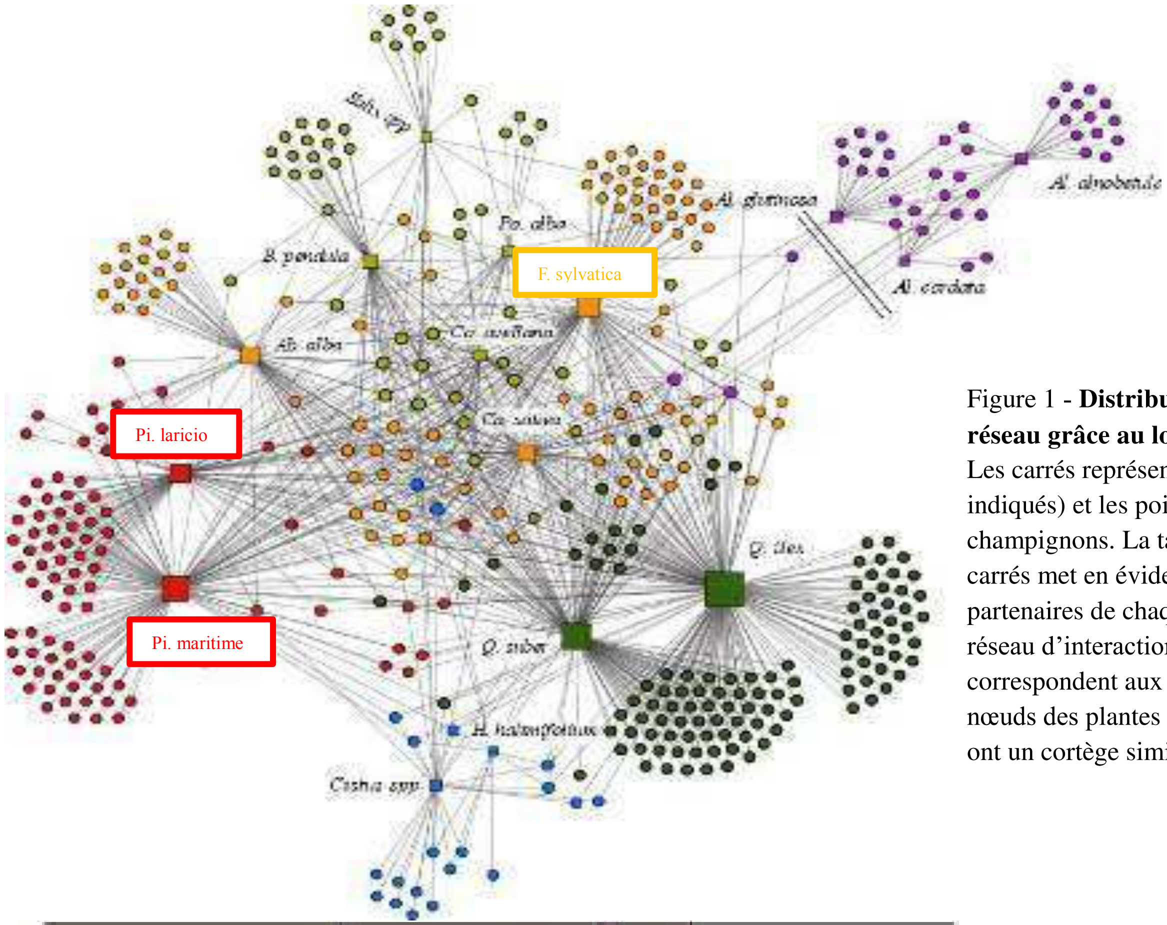


Figure 1 - **Distribution des modules du réseau grâce au logiciel NETCARTO.** Les carrés représentent les plantes (noms indiqués) et les points représentent les champignons. La taille différente des carrés met en évidence le nombre de partenaires de chaque plante dans le réseau d'interaction. Les couleurs correspondent aux modules. Plus des nœuds des plantes seront proches, plus ils ont un cortège similaire.

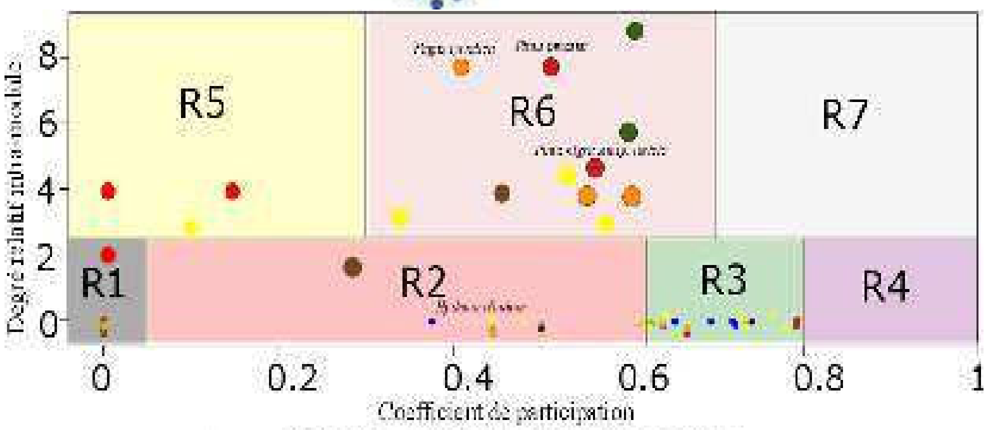


Figure 2 – **Position des taxons dans les modules.** Les gros points représentent les plantes (noms indiqués) et les petits points représentent les champignons). Les secteurs (R1-R7) sont définis d'après Guimerà and Nunes Amaral, 2005. Les couleurs correspondent aux différents modules.

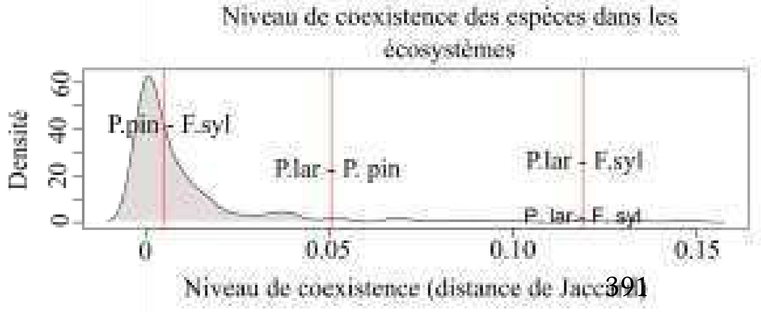


Figure 3 **– Niveau de coexistence des paires de plantes calculés avec la distance de Jaccard.**

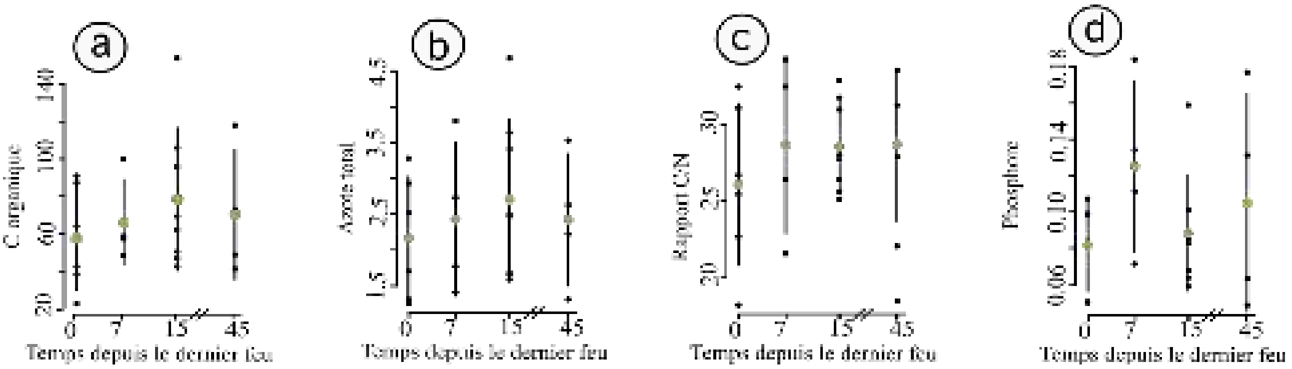


Figure 4 – **Concentration en minéraux (Carbone, azote, carbone /azote et phosphore) en fonction du temps depuis le dernier feu.** Aucune des corrélations n'est significative.

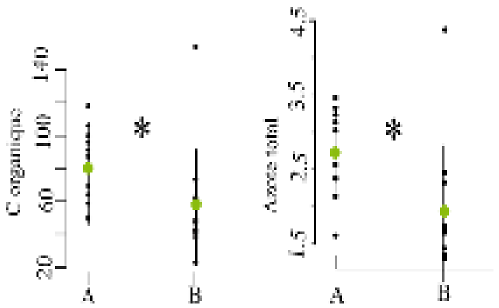


Figure 5 – **Concentration en minéraux (Carbone, et azote) en fonction des deux profondeurs de sol, toutes modalités de sévérité de feu confondues.**
* : tests de Wilcoxon avec p-value < 0.05

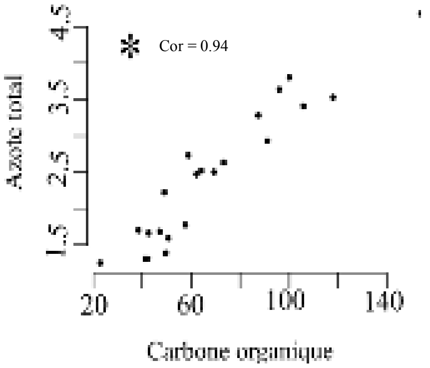


Figure 6 – **Concentration en azote en fonction de la concentration en carbone.**
* : tests de Pearson avec p-value < 0.05

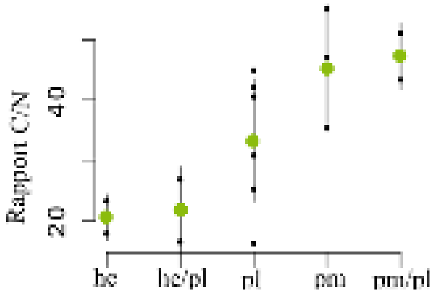


Figure 7 – **Rapport carbone/azote en fonction des modalités de coexistences.**

3 Discussion

3.1 Réseau d'interactions plantes-champignons et place des trois essences dans le réseau global

La Fig.1 permet de mettre en évidence l'importance des trois essences d'arbres étudiées (*Pinus nigra subsp. laricio*, *Pinus pinaster* et *Fagus sylvatica*) au sein de ce réseau modulaire et non imbriqué. Ces essences possèdent un degré relatif intra-module important en particulier chez le pin laricio. Elles structurent fortement leurs modules. La théorie de la niche nous incite à faire l'hypothèse que la coexistence dans la communauté devrait être plus difficile pour les paires de plantes dont les cortèges fongiques sont proches.

Les plus fortes coexistences sont observées dans les forêts mixtes (Taudiere et al., 2012), c'est-à-dire qu'il y a au moins une espèce d'Angiosperme et une espèce de Gymnosperme (*e.g.* la hêtraie-pineraie). Or parmi les trois paires analysées, (*cf.* Fig.3) c'est en effet le hêtre et le pin laricio qui coexistent le plus. Les modules sont structurés par genre/famille de plante (Fig.1). L'importante différence de cortège entre les pins et le hêtre et la forte ressemblance entre les deux pins pourrait être déterminée par la distance phylogénétique entre ces espèces (Taudiere et al., 2012).

En faisant l'hypothèse que la distance phylogénie est un proxy de la distance écologique, la coexistence en forêt mixte pourrait s'expliquer en partie par une séparation de niche des Angiospermes et des Gymnospermes. La contrainte phylogénétique forte sur l'association suggère que la niche pédologique des pins et du hêtre pourrait être une des dimensions de la niche favorisant leur coexistence. Mais malgré un sous-embranchement phylogénétique distinct, ces genres, Fagaceae et Pinaceae partagent des champignons généralistes. Il reste à savoir si en coexistence le hêtre est associé à ces champignons généralistes ou si les deux essences de ces forêts mixtes ont tendance à supporter des cortèges de cECM radicalement différents. La question est la même pour le pin laricio et le pin maritime, d'où l'importance des analyses racinaires par RFLP pour réassigner les cECM aux deux espèces de pins.

Après une perturbation comme le feu, les successions végétales vont se dérouler sous l'effet de nombreux facteurs, au sein desquels les cECM prennent place, notamment via la banque de spores disponible dans le sol (Glassman et al., 2015). Les feux de forêts ont par ailleurs un réel impact sur les communautés fongiques, notamment en modifiant la structure et la composition chimique du sol (e.g. Cairney et Bastias, 2007; Karst et al., 2014). Les feux réduisent en particulier l'abondance des taxons fongiques de fin de successions écologiques. Les forêts matures de pins laricio appartiennent à des forêts matures dans la succession, et sont largement colonisées par les champignons de fin de successions. Par exemple, *Hydnum albidum* appartient à l'ordre des Cantharellales, qui comprend des taxons particulièrement sensibles à l'incendie et présent en fin de successions (Buscardo et al., 2015). C'est également la seule espèce de champignon associée uniquement aux trois espèces cibles. Selon Buscardo et al., (2015), les changements engendrés par le feu peuvent éroder les communautés fongiques du sol surtout dans les premiers horizons du sol (Cairney et Bastias, 2007). Cette modification de la communauté des cECM entrave la régénération des pins, et par là-même diminue la résilience des forêts de pins. En effet l'une des premières causes de mortalité des champignons suite à un feu est la perte du partenaire et la mortalité directe du mycelium. Mais un cECM peut aussi survivre grâce à des structures de résistance telles que la spore ou le sclérote. Un des facteurs secondaires de mortalité des cECM est indirect. Il s'agit de la modification de la composition chimique du sol suite à un feu. Ainsi le but de ma seconde partie a été de mettre en évidence le rôle du sol sur les cECM dans un système perturbé par le feu.

3.2 Impacts de la coexistence et du feu sur la composition chimique du sol

Nos résultats sur l'impact du feu sur la composition chimique du sol montrent qu'il n'y a pas de différence de concentration en minéraux entre les forêts atteintes par le feu et les forêts mixtes. Bien que s'appuyant sur un faible nombre d'échantillons, nos résultats semblent en accord avec les travaux précédents montrant le caractère éphémère de l'impact d'un feu sur les concentrations en minéraux (Certini, 2005). Concernant l'impact du feu selon la profondeur de sol, il apparaît que les concentrations en carbone et azote, très corrélées dans notre étude (Fig.6), sont plus élevées dans l'horizon organique que dans l'horizon organo-minéral. Ce résultat logique (plus de matière organique dans la couche organique) montre surtout la structuration verticale des sols bruns acides analysés, et bien sûr indique que l'échantillonnage et l'analyse de composition chimique ont correctement fonctionné. En revanche les rapports C/N et le taux de P de nos deux couches sont similaires. Ainsi l'impact du feu n'est pas mis en évidence. Même si un plus grand nombre d'échantillons pourrait révéler des effets faibles du feu sur la chimie du sol, la répartition des minéraux dans le sol selon la profondeur est un facteur beaucoup plus structurant que l'impact du feu.

Concernant la coexistence, seul le rapport C/ N est variable selon les modalités. Les sols de peuplements purs de pin laricio se retrouvent être intermédiaires entre ceux de la hêtraie et ceux de la pinède maritime. Un C/N plus bas dans la hêtraie, différent de ceux des pinèdes (*cf.* 1.1 Matériels biologiques) reflète une activité biologique (recyclage par les décomposeurs) plus importante en forêt feuillue qu'en peuplement de conifères. Les valeurs plus élevées de C/N constatées pour le pin maritime par rapport au pin laricio pourraient signifier qu'aux altitudes échantillonnées (de 661 m à 1529 m d'altitude) on est proches des conditions de croissance optimales du pin laricio, les matériaux (aiguilles, cônes, branches) issus du pin laricio sont les plus facilement décomposés.

Conclusion

Dans cette étude, nous avons étudié la position du pin laricio, du pin maritime, et du hêtre dans le réseau que les arbres forment avec leurs champignons ectomycorhiziens. Ces données nous ont permis de resituer le contexte global d'échanges et de liens qui existent entre ces espèces. Les techniques moléculaires et l'analyse chimique du sol de ce travail sont des préalables nécessaires à l'analyse des communautés de cECM en cours.

Bibliographie

Agerer, R. (2001). Exploration types of ectomycorrhizae. Mycorrhiza *11*, 107–114.

Almeida-Neto, M., Guimaraes, P., Guimaraes Jr, P.R., Loyola, R.D., and Werner, U. (2008). A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. Oikos *117*, 1227–1239.

Barbara J. Cade-Menun, S.M.B. (2000). Phosphorus forms and related soil chemistry of Podzolic soils on northern Vancouver Island. II. The effects of clear-cutting and burning. Can. J. For. Res.-Rev. Can. Rech. For. - CAN J For. RES *30*, 1726–1741.

Bascompte, J., Jordano, P., Melián, C.J., and Olesen, J.M. (2003). The nested assembly of plant-animal mutualistic networks. Proc. Natl. Acad. Sci. U. S. A. *100*, 9383–9387.

Buscardo, E., Rodriguez-Echeverria, S., Freitas, H., De Angelis, P., Pereira, J.S., and Muller, L.A.H. (2015). Contrasting soil fungal communities in Mediterranean pine forests subjected to different wildfire frequencies. Fungal Divers. *70*, 85–99.

Cairney, J.W.G., and Bastias, B.A. (2007). Influences of fire on forest soil fungal communities. Can. J. For. Res.-Rev. Can. Rech. For. *37*, 207–215.

Certini, G. (2005). Effects of fire on properties of forest soils: a review. Oecologia *143*, 1–10.

Courty, P.-E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.-P., Uroz, S., and Garbaye, J. (2010). The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biol. Biochem. *42*, 679–698.

Gamisans, J. (1999). La végétation de la Corse (Aix-en-Provence: Édisud).

G. GIOVANNINI, S.L. (1988). Effect of Heating on Some Physical and Chemical Parameters Related to Soil Aggregation and Erodibility. Soil Sci. *146*.

Giroux, M., and Audesse, P. (2004). Comparaison de deux méthodes de détermination des teneurs en carbone organique, en azote total et du rapport C/N de divers amendements organiques et engrais de ferme. Agrosol Prod. Anim. *15*, 107–110.

Glassman, S.I., Peay, K.G., Talbot, J.M., Smith, D.P., Chung, J.A., Taylor, J.W., Vilgalys, R., and Bruns, T.D. (2015). A continental view of pine-associated ectomycorrhizal fungal spore banks: a quiescent functional guild with a strong biogeographic pattern. New Phytol. *205*, 1619–1631.

Gobat, J.-M., Aragno, M., and Matthey, W. (2010). Le sol vivant : Bases de Pédologie-Biologie des sols. (Presses polytechniques et universitaires romandes).

Grime, J.. (1974). Vegetation classification by reference to strategies. Nature 26–31.

Guimerà, R., and Nunes Amaral, L.A. (2005). Functional cartography of complex metabolic networks. Nature *433*, 895–900.

Horton, T.R., Bruns, T.D., and Parker, V.T. (1999). Ectomycorrhizal fungi associated with Arctostaphylos contribute to Pseudotsuga menziesii establishment. Can. J. Bot. *77*, 93–102.

Kalinka, A.T., and Tomancak, P. (2011). linkcomm: an R package for the generation, visualization, and analysis of link communities in networks of arbitrary size and type. Bioinforma. Oxf. Engl. *27*, 2011–2012.

Karst, J., Randall, M., and Gehring, C. (2014). Consequences for ectomycorrhizal fungi of the selective loss or gain of pine across landscapes. Botany *92*.

Kress, W.J., Wurdack, K.J., Zimmer, E.A., Weigt, L.A., and Janzen, D.H. (2005). Use of DNA barcodes to identify flowering plants. Proc. Natl. Acad. Sci. U. S. A. *102*, 8369–8374.

Mills, L.S., Soulé, M.E., and Doak, D.F. (1993). The Keystone-Species Concept in Ecology and Conservation. BioScience *43*, 219–224.

Olesen, J.M., Bascompte, J., Dupont, Y.L., and Jordano, P. (2007). The modularity of pollination networks. Proc. Natl. Acad. Sci. *104*, 19891–19896.

Öpik, M., and Moora, M. (2012). Missing nodes and links in mycorrhizal networks. New Phytol. *194*, 304–306.

Richard, F., Moreau, P.-A., Selosse, M.-A., and Gardes, M. (2004). Diversity and fruiting patterns of ectomycorrhizal and saprobic fungi in an old-growth Mediterranean forest dominated by Quercus ilex L. Can. J. Bot. *82*, 1711–1729.

Richard, F., Selosse, M.-A., and Gardes, M. (2009). Facilitated establishment of Quercus ilex in shrub-dominated communities within a Mediterranean ecosystem: do mycorrhizal partners matter? FEMS Microbiol. Ecol. *68*, 14–24.

Souleres, O. (2000). Forest fires in upper Corsica. 401–405.

Taudiere, A., Richard, F., Munoz, F., and Lesne A. (2012). Stage de M2: Analyse des réseaux d'interactions plantes-champignons ectomycorhiziens.

Thebault, E., and Fontaine, C. (2010). Stability of Ecological Communities and the Architecture of Mutualistic and Trophic Networks. Science *329*, 853–856.

Wang, X.R., Tsumura, Y., Yoshimaru, H., Nagasaka, K., and Szmidt, A.E. (1999). Phylogenetic relationships of Eurasian pines (Pinus, Pinaceae) based on chloroplast rbcL, MATK, RPL20-RPS18 spacer, and TRNV intron sequences. Am. J. Bot. *86*, 1742–1753.

Westoby, M. (1998). A leaf-height-seed (LHS) plant ecology strategy scheme. Plant Soil 213–227.

Webographie

CEE 92/43 (1992) : Directive CEE 92/43 du Conseil du 21 mai 1992 concernant la conservation des habitats naturels ainsi que de la faune et de la flore sauvages.

ncbi.gov. National Center for Biotechnology Information.

Onf.fr. Les résineux: Emblématique de la Corse, je suis le pin laricio de Corse.

Restriconmapper.org: Maps sites for restriction enzymes, a.k.a. restriction endonucleases, in DNA sequences. Also does virtual digestion.

Fructification de *Russula sanguinea.* ©Erich Cramer

Table des Matières détaillée

RÉSUMÉ : L'étude de l'écologie des micro-organismes est récente malgré son importance pratique et théorique intrinsèque mais également son rôle central dans la niche des macro-organismes. Les interactions plantes-champignons, de par leur importance socio-écologique et de leur diversité – du mutualisme au parasitisme en passant par le commensalisme –, offrent un modèle judicieux pour étudier l'écologie des communautés de micro-organismes en interaction avec des macro-organismes. À l'aide de techniques de séquençage à haut débit (NGS) et d'analyse des réseaux, nous explorons certains déterminants de la structure des champignons des forêts corses à travers trois guildes : les champignons ectomycorhiziens, endophytiques et saprotrophes. Ce travail considère les processus de dispersion, les perturbations (feux et chablis), les facteurs environnementaux (par ex. la profondeur du sol) et les contraintes dérivées de l'interaction avec les hôtes (par ex. taxinomie). Les assemblages des communautés des différentes guildes présentent des patrons communs qui pourraient être issus de mécanismes identiques. Ainsi, l'ensemble des guildes étudiées présentent des variations fortes à l'échelle des micro-régions de Corse et entre forêts ayant des histoires de feux différentes. En revanche, l'importance des différents processus d'assemblage et les échelles spatiales auxquelles ils s'appliquent varient selon les guildes. Nous discutons des implications que suscitent ce travail pour les écologues des communautés et pour les gestionnaires d'espaces naturels.

Mots clés : champignons ; mycorhizes ; régime de feu ; écologie des communautés ; chablis ; décomposition ; *Pinus nigra* subsp. *laricio* ; forêt mixte

Title: **Drivers of fungal community composition in Corsican forests: role of perturbations and vegetation composition**

ABSTRACT: Study of micro-organisms ecology arose recently despite its intrinsic importance – both practical and theoretical –, but also despite its overriding role in the niches of macro-organisms. Plant-fungi interactions offer a relevant model to study the ecology of micro-organisms interacting with macro-organisms because of their considerable ecological and economical values in addition to their high taxonomic and ecological diversity. Using next-generation sequencing (NGS) and network analysis, we explore some drivers of fungal community composition in Corsica, at various scales and through three guilds: ectomycorrhizal, endophytic and saprotrophic fungi. We investigate the effect of disturbance (e.g. fire and treefall), environmental variables (e.g. soil depth), constraints due to the interacting plants (e.g. taxonomy) and dispersion on fungal communities. Some community assembly rules are similar across guilds. For instance, forests in different micro-regions of Corsica harbor dissimilar fungal communities. However relative importance of processes and the scales at which they occur vary across guilds. In Corsican pine forests, fifteen years after fire occurrence, soil fungal diversity is close to the level of diversity in unburnt stand. Despite the absence of effect on diversity, fire induces marked shifts in soil fungal community composition, in particular for soil saprobic fungi. We discuss the implication of this work for ecologists – both plant and fungal ecologist – and stakeholders.

Keywords: fungi; mycorrhiza; fire regime; community ecology; canopy gaps; decomposition; *Pinus nigra* subsp. *laricio*; mixed forest