

The American Grape Terroir: Influence of agricultural management on the microbiome

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Concord sample collected by Thoreau



Acknowledgements

- Hatch-USDA NIFA MAS00583 NE2022: Multi-state Coordinated Evaluation of Winegrape Cultivars and Clones
- Students in the lab:
 - Patrick Ewick
 - Hayley Kozikowski
 - Abby Basen
 - Elsie Murphy
 - Julietta Mascitelli





Elsa Petit

Teaching, research and Extension Massachusetts Grapes

- Teaching grape production and plant diseases courses at the University of Massachusetts, Amherst
- Massachusetts grape Extension:
 - Twilight meetings/field days
 - Newsletter Grape Notes
- Research on sustainable grape production

Research on sustainable grape production



1. Cold protection of European grapes in MA (SARE Farmers grant)
2. Organic biopesticide trials against downy mildew (Marrone)
3. Resistance to diseases in hybrid grapes
4. Grape microbiomes

Agriculture
facing two
main issues

Decreasing
biodiversity

Climate change



Agriculture a solution: Agroecology



Agriculture a
solution:
Agroecology

Microbiomes
represent an
impressive
amount of
diversity



Microbiomes changes correlated with grapevine health

Grapevine decline is associated with difference in soil microbial composition and activity



Romain Darriaut¹, Guilherme Martins^{2,4}, Coralie Dewasme¹, Séverine Mary³, Guillaume Darrieutort³, Patricia Ballestra², Elisa Marguerit¹, Philippe Vivin¹, Nathalie Ollat¹, Isabelle Masneuf-Pomarède^{2,4}, and Virginie Lauvergeat^{1*}

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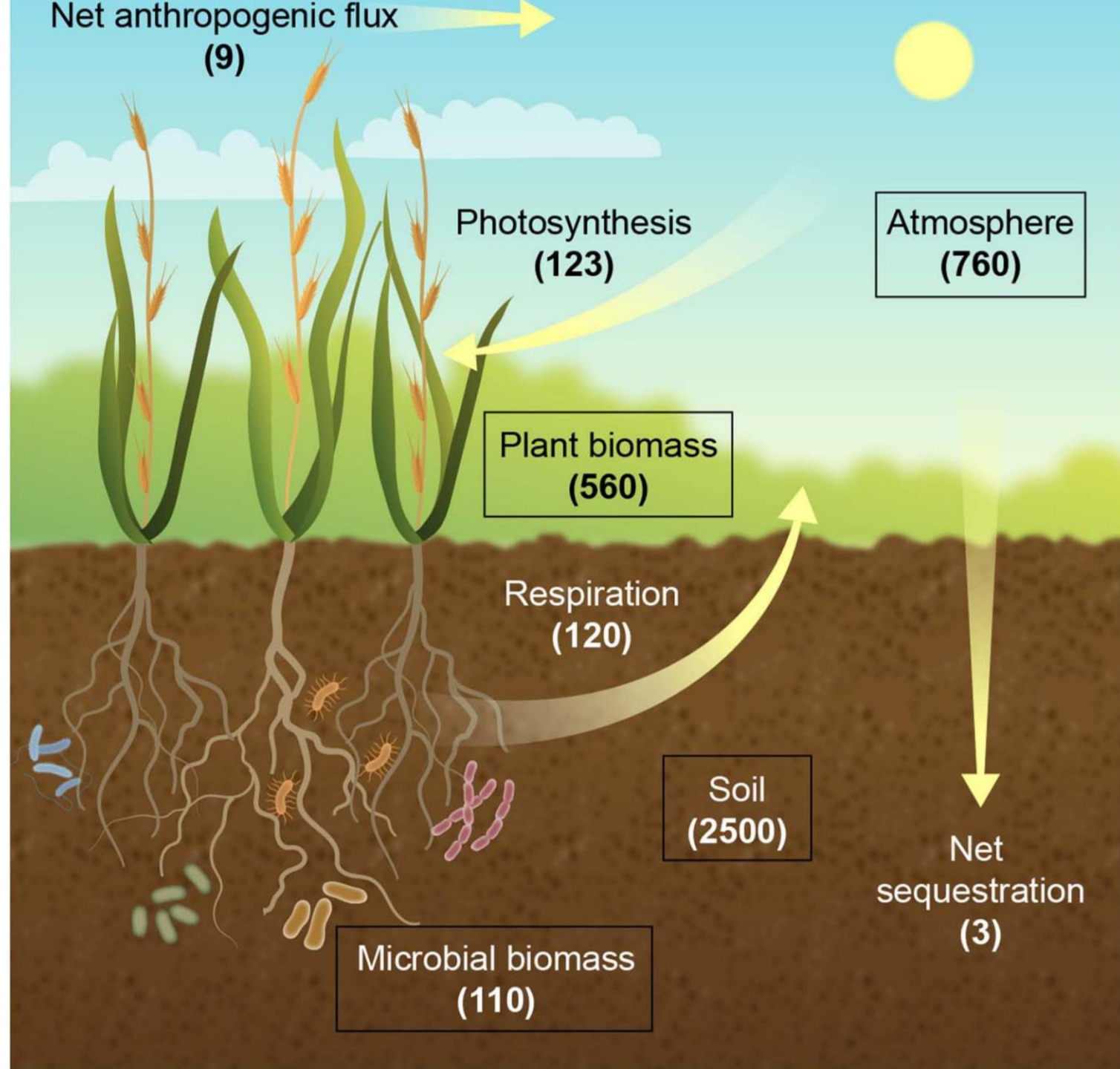
³Université de Bordeaux, Vitinnov, Bordeaux Sciences Agro, ISVV, 1 cours du Général de Gaulle, 33170 Gradignan, France

⁴Bordeaux Sciences Agro, 1 cours du Général de Gaulle, 33170 Gradignan, France

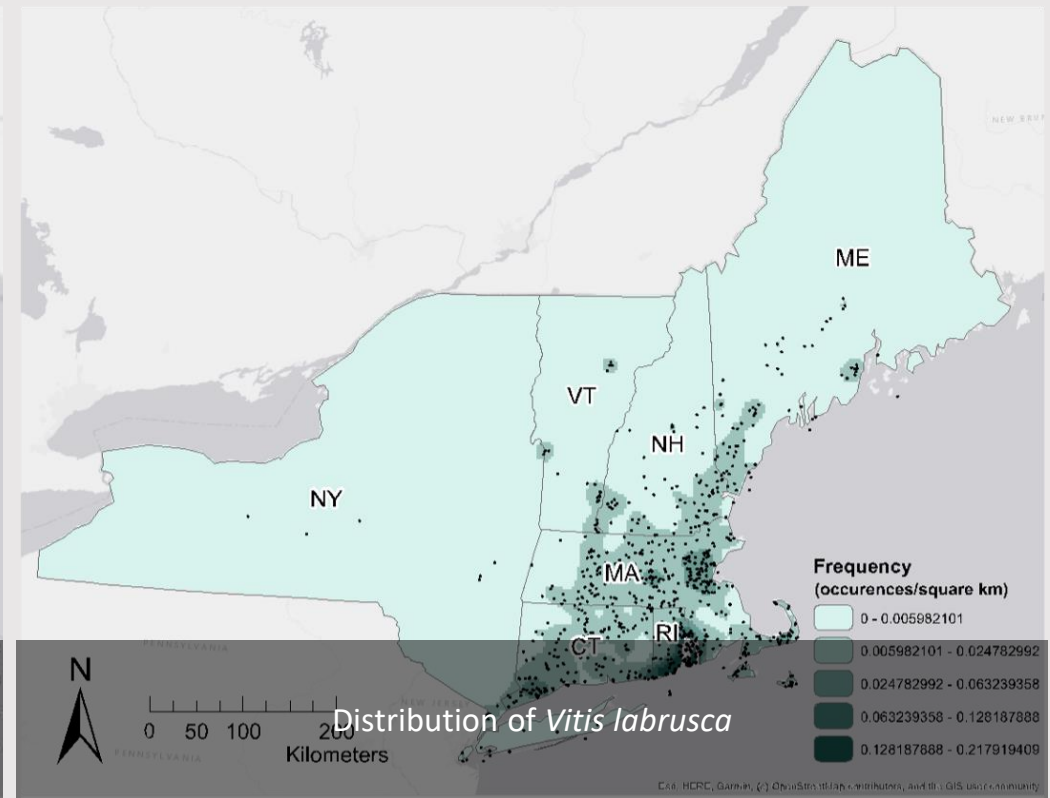
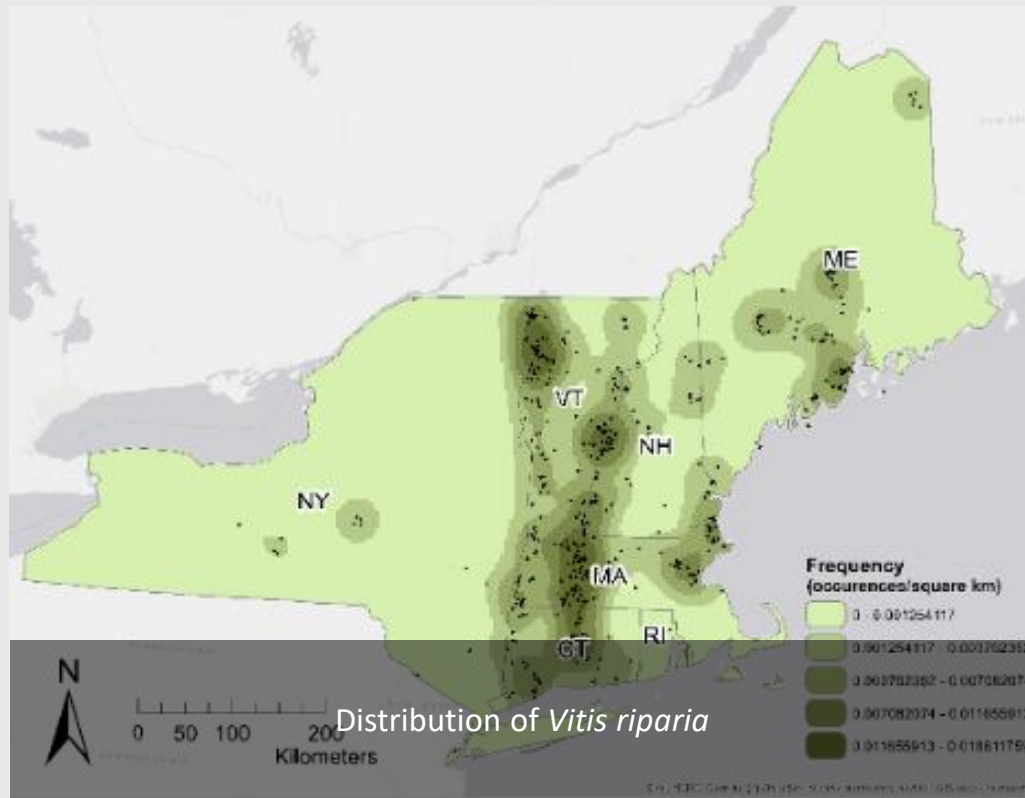
Microbiomes are key in climate change

The terrestrial carbon (C) cycle. Carbon stocks (boxes) are shown as gigatons (GT), and fluxes (arrows) are shown in GT per year. Respiration refers to accumulated plant and microbial respiration.

<https://doi.org/10.3389/fpls.2021.636709>



Distribution of wild native grapes





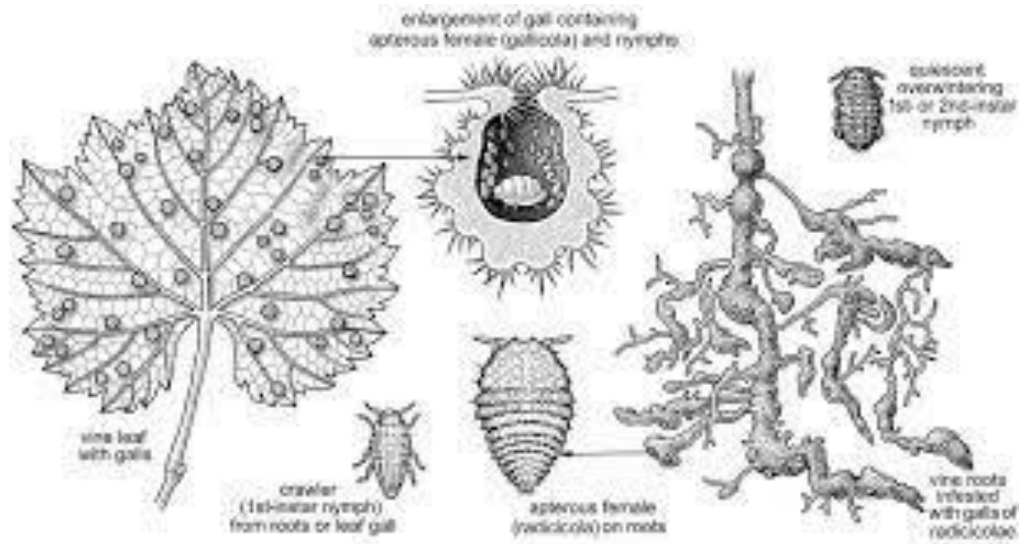
Model system: Wild native grapes with domesticated counterparts in their surroundings

1. Wild native grapes with domesticated counterpart in their surroundings
2. Biodiverse host coupled with biodiversity of microbes
3. American grape only recently domesticated



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Model system: Wild native grapes with domesticated counterparts in their surroundings

1. Wild native grapes with domesticated counterpart in their surroundings
2. Biodiverse host coupled with biodiversity of microbes
3. American grape only recently domesticated
4. American rootstocks used worldwide



Images:

Top: Phylloxera and damages to grapes, Doug Walsh

Bottom: Scion plant material grafted onto rootstock. (Washington State University Extension.)

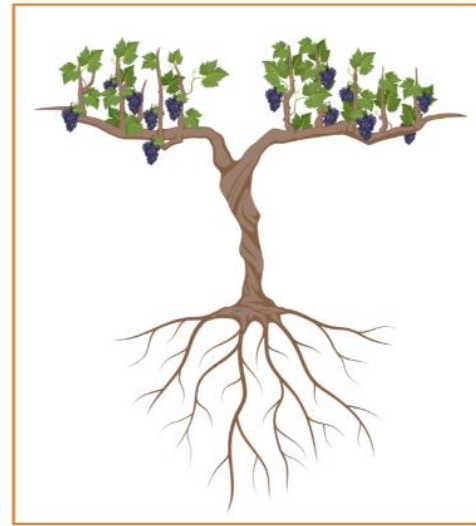


Project: Comparing wild to managed grapes
Sampling domesticated and wild counterparts in the same location



Vitis riparia

Frontenac
Marquette



Vitis labrusca

Concord

Genetics of host:
Sampling
different wild
species and their
domesticated
counterparts

Berries



Leaves



Roots

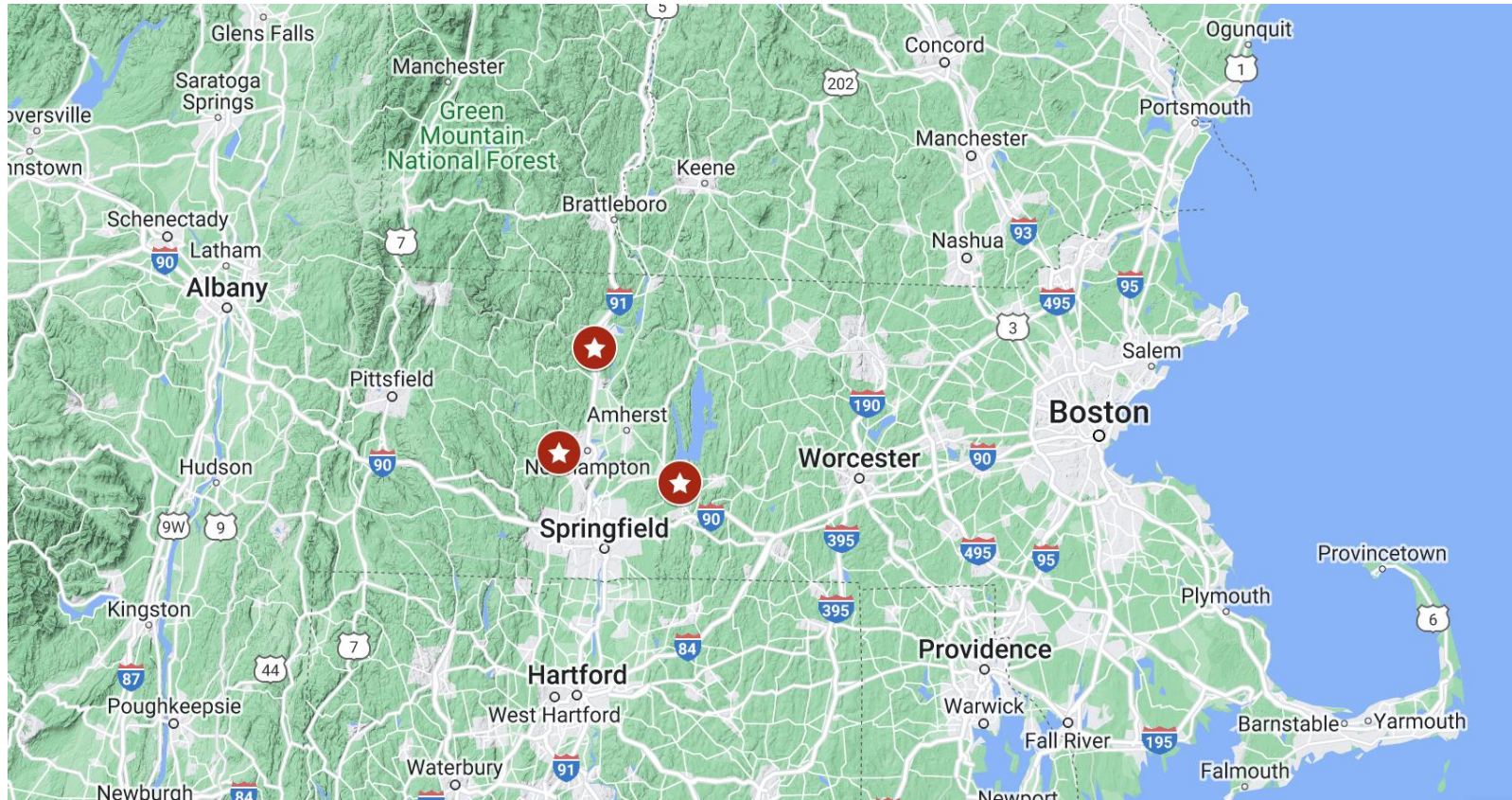


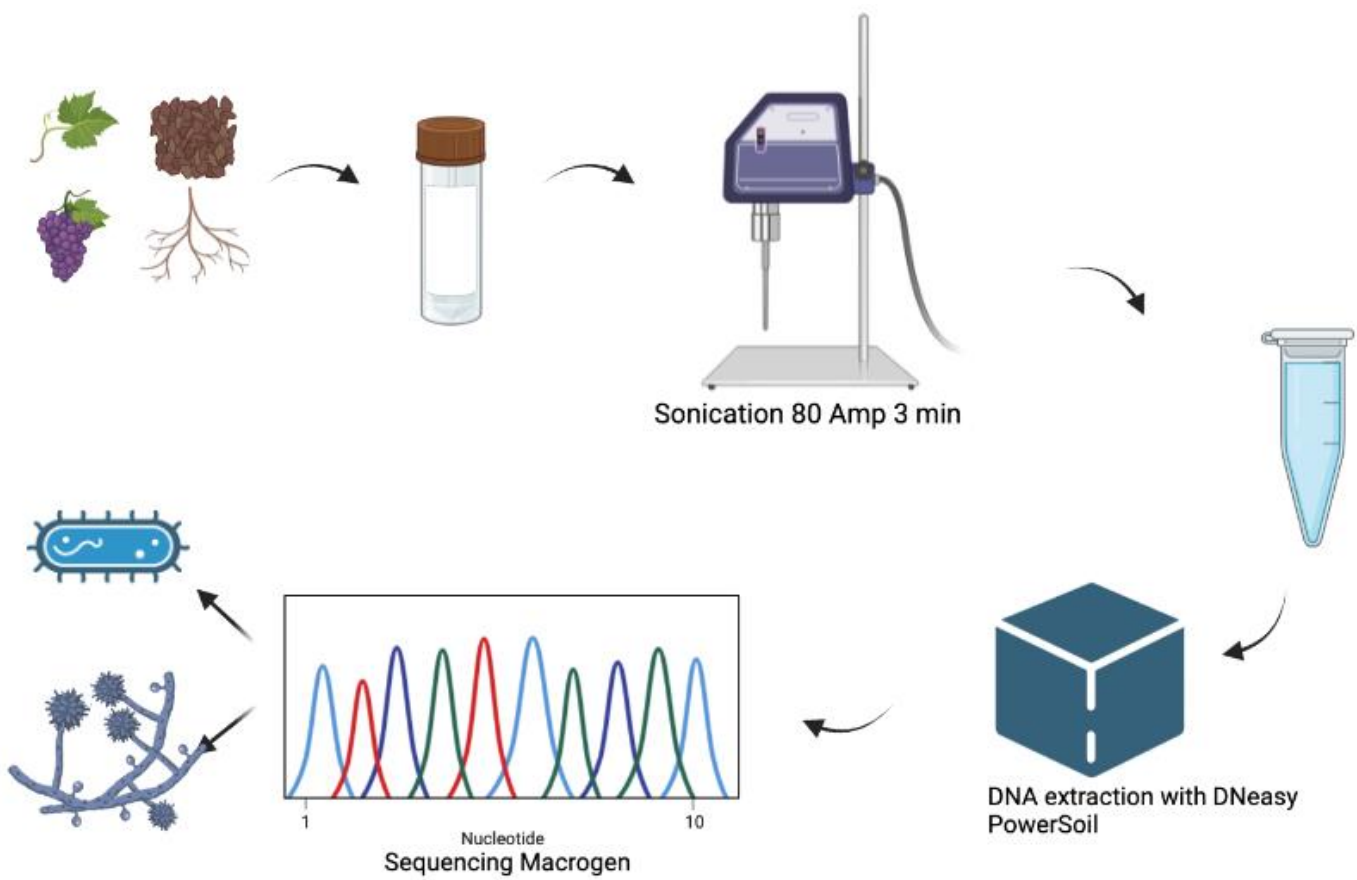
Soil



Sampling
compartments
of the grapes

Geographic locations of sampling Massachusetts, August 15, 2016





DNA extraction protocol

Preprocessing
&
Clustering

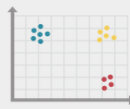


Raw Data



ATGC

Preprocessing

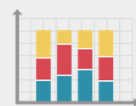


Clustering

Taxonomic assignment



Alignment



Taxonomy

Diversity statistics



Diversity Index



Rarefaction



PCoA

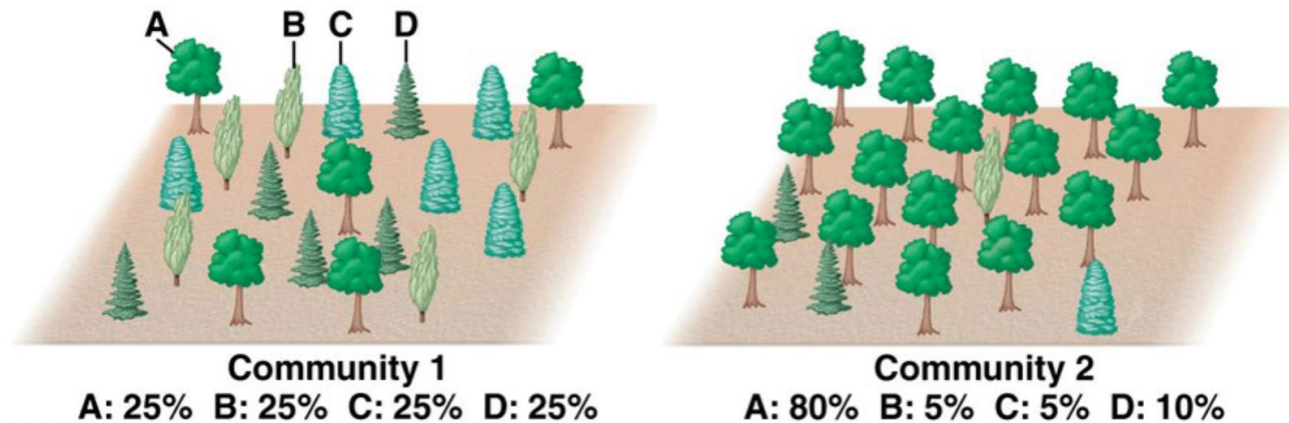


Dendrogram

Analysis of
the
sequences

Diversity: Difference between richness and evenness

Alpha-diversity is within sample diversity. It is how many different species (OTUs) are in each sample (richness) and how evenly they are distributed (evenness), which together are diversity. Each sample has one value for each metric.

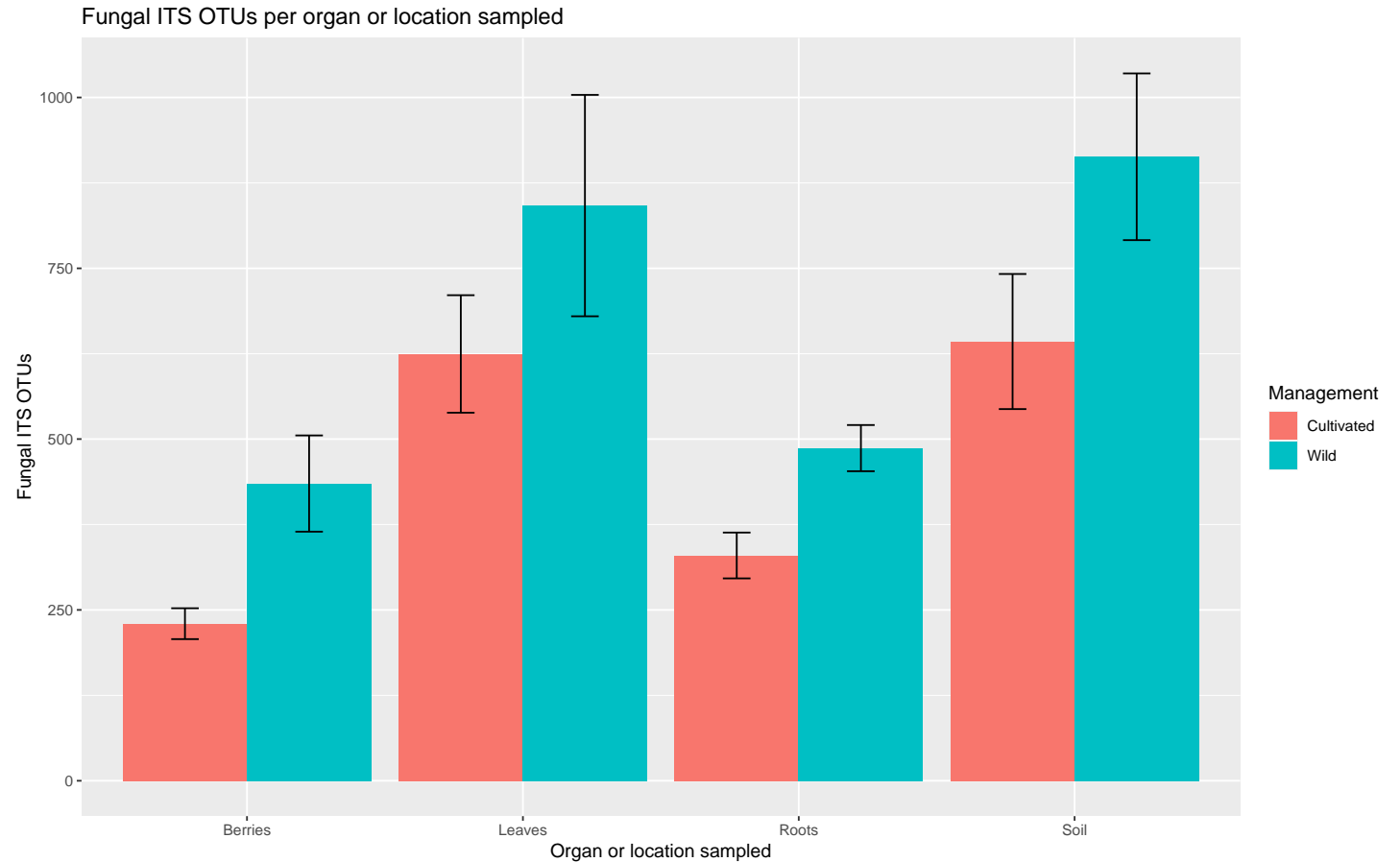


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This image illustrates richness vs. diversity. Both forests have the same richness (4 tree species) but Community 1 has much more even distribution of the 4 species while Community 2 is dominated by tree species A. This makes Community 1 more diverse than Community 2.

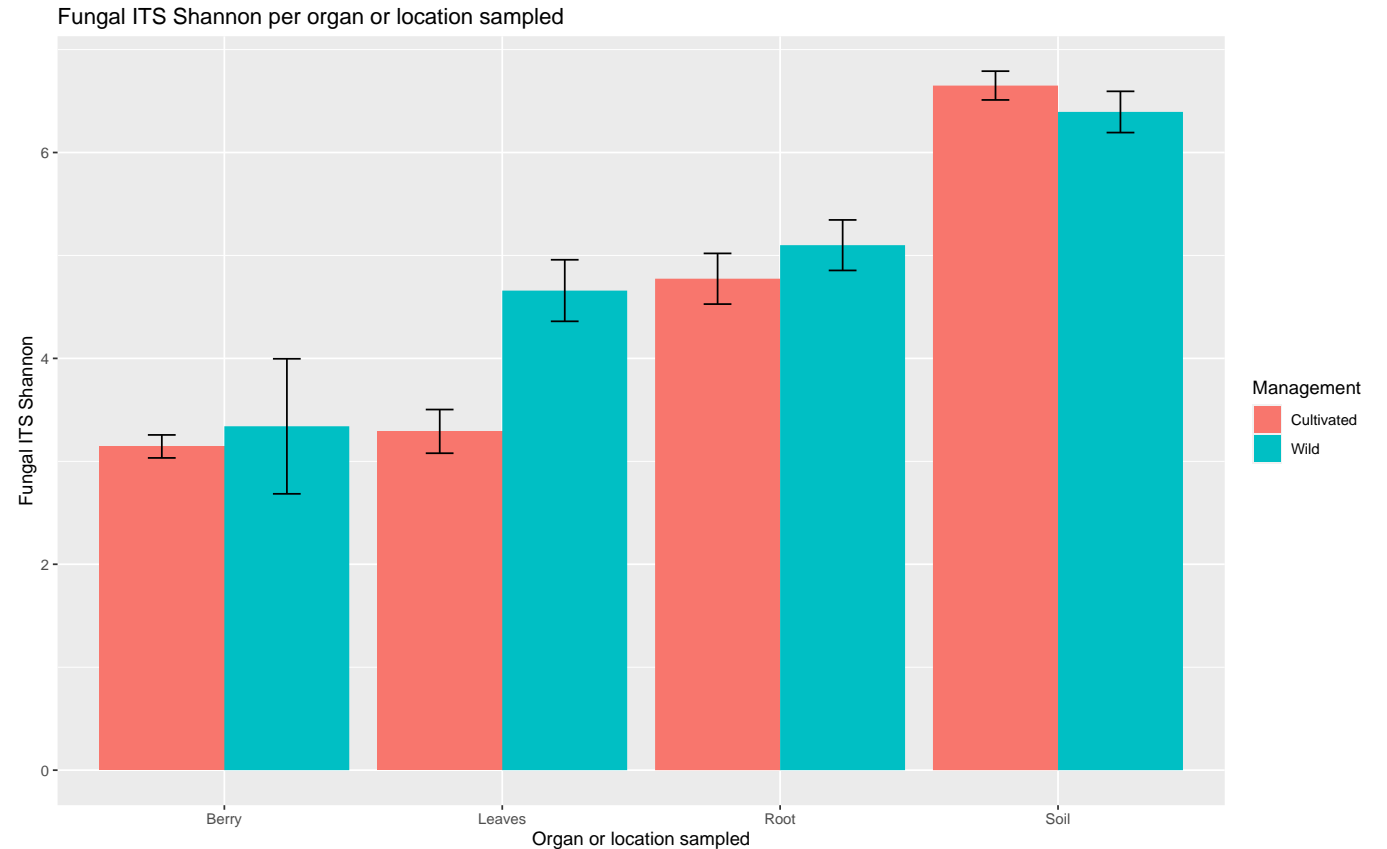
Fungal ITS richness: Number of OTUs

- OTU = Operational taxonomic units
- Wild always higher diversity than managed
- Ranked compartments by OTU number:
 1. Soil
 2. Leaves
 3. Roots/Berries
- In each compartment, OTUs are higher – statistically significant – in wild than managed, except for leaves.



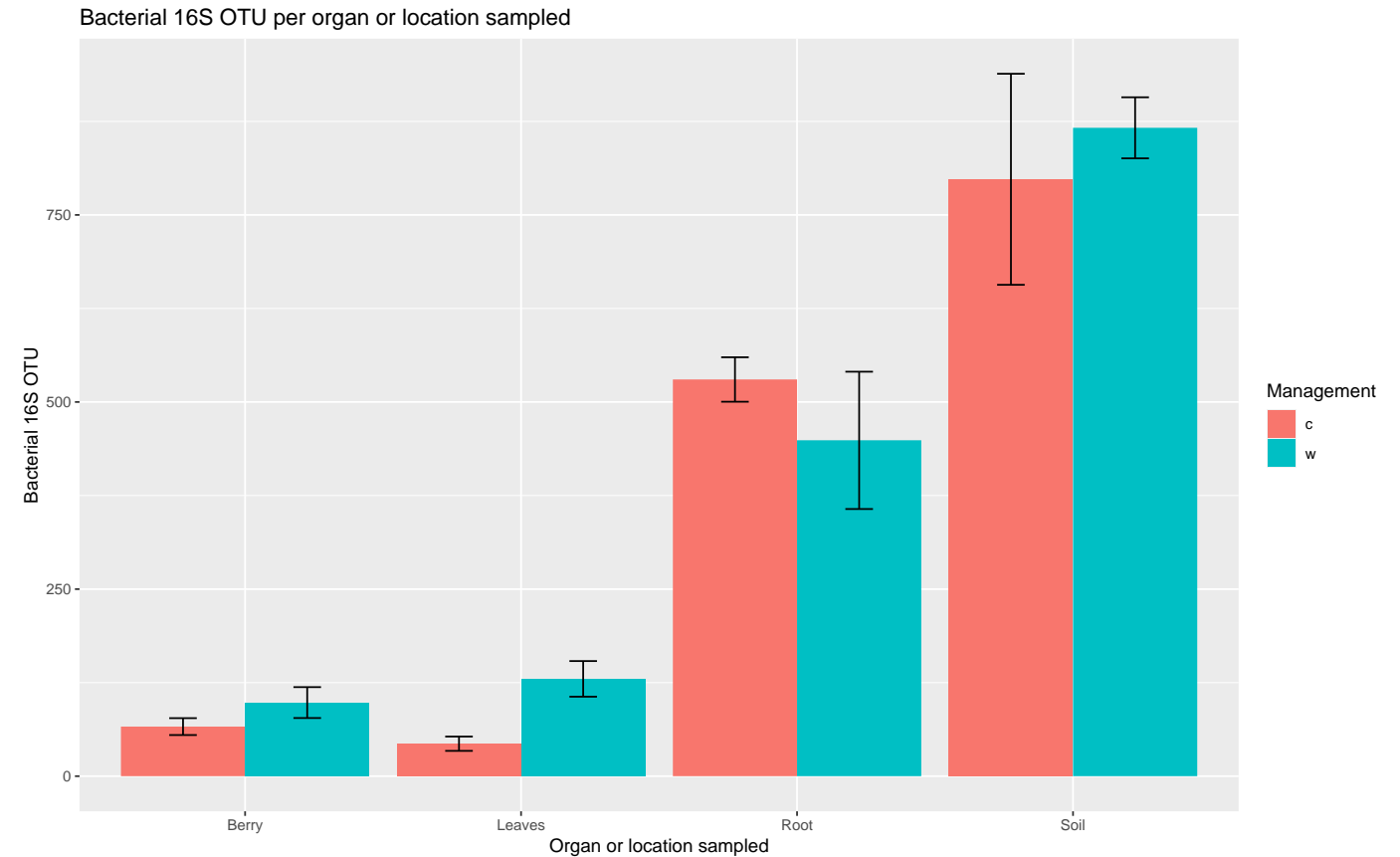
Fungal ITS evenness: Shannon

- Shannon = Measures diversity
- Ranked compartments by Shannon index:
 1. Soil
 2. Roots
 3. Leaves
 4. Fruits
- Only for leaves, the diversity is higher – statistically significant – in wild than managed.



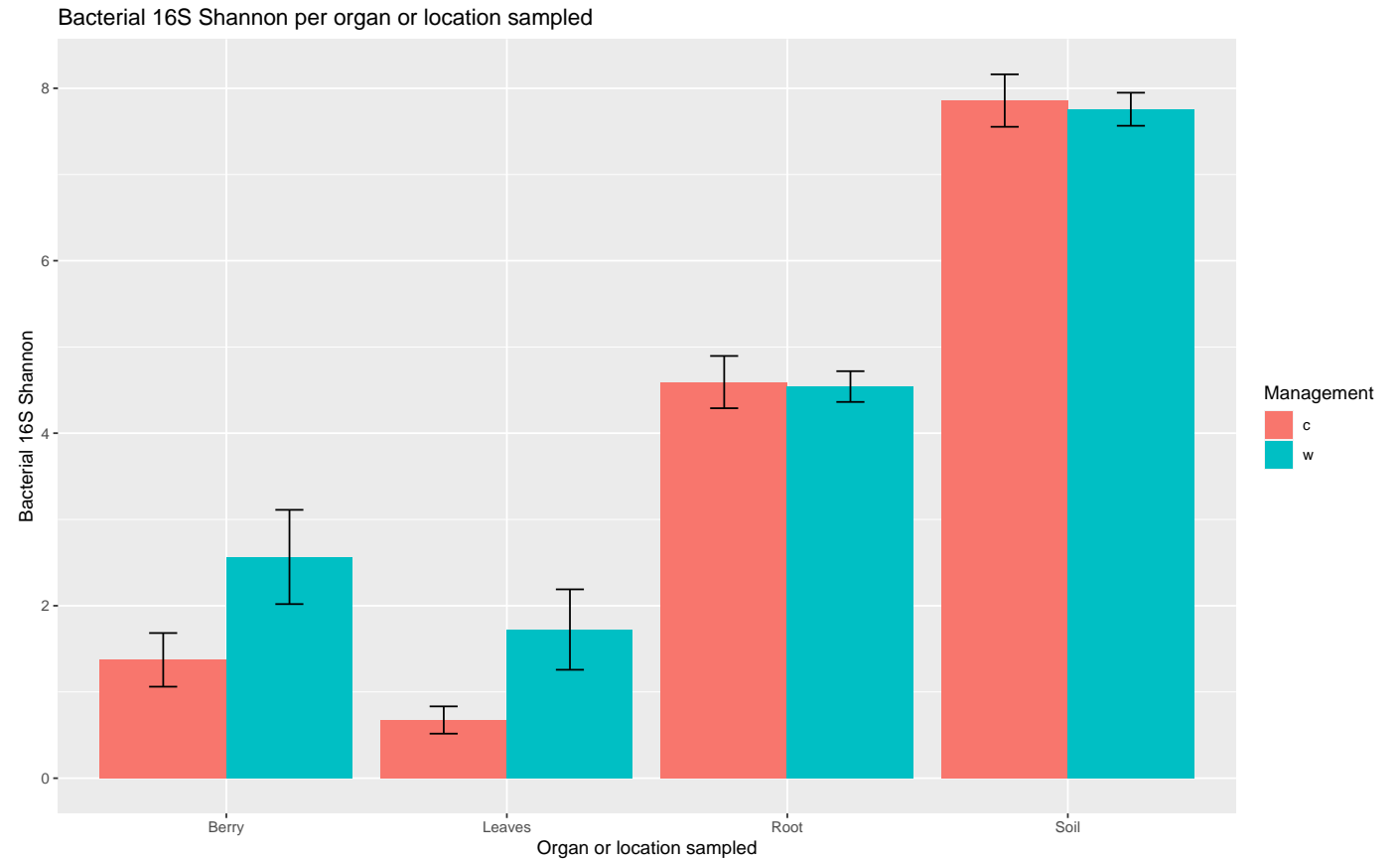
Bacterial 16S richness: Number of OTUs

- OTU = Operational taxonomic units
- Ranked compartments by OTU number:
 1. Soil
 2. Roots
 3. Leaves/Berries
- Only for leaves, the OTUs number is higher – statistically significant- in wild than managed.



Bacterial 16S evenness: Shannon

- Shannon = Measures diversity
- Ranked compartments by Shannon index:
 1. Soil
 2. Roots
 3. Leaves/Fruits
- For leaves and berries, the diversity is higher – statistically significant- in wild than managed.

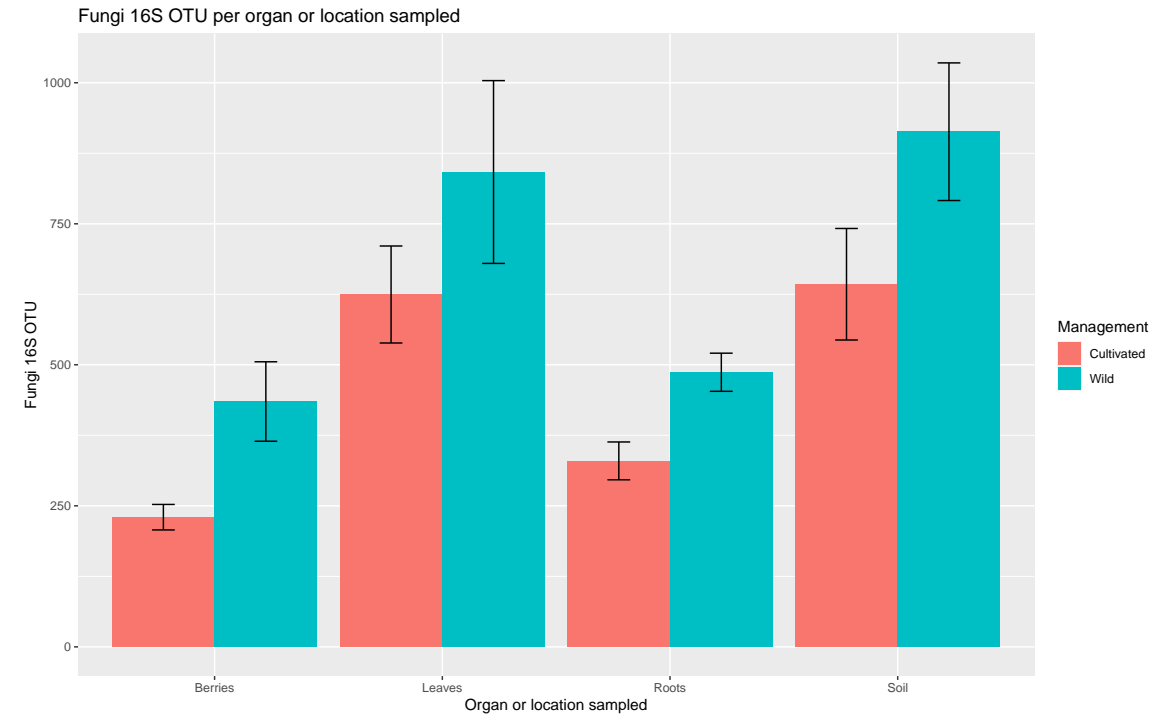
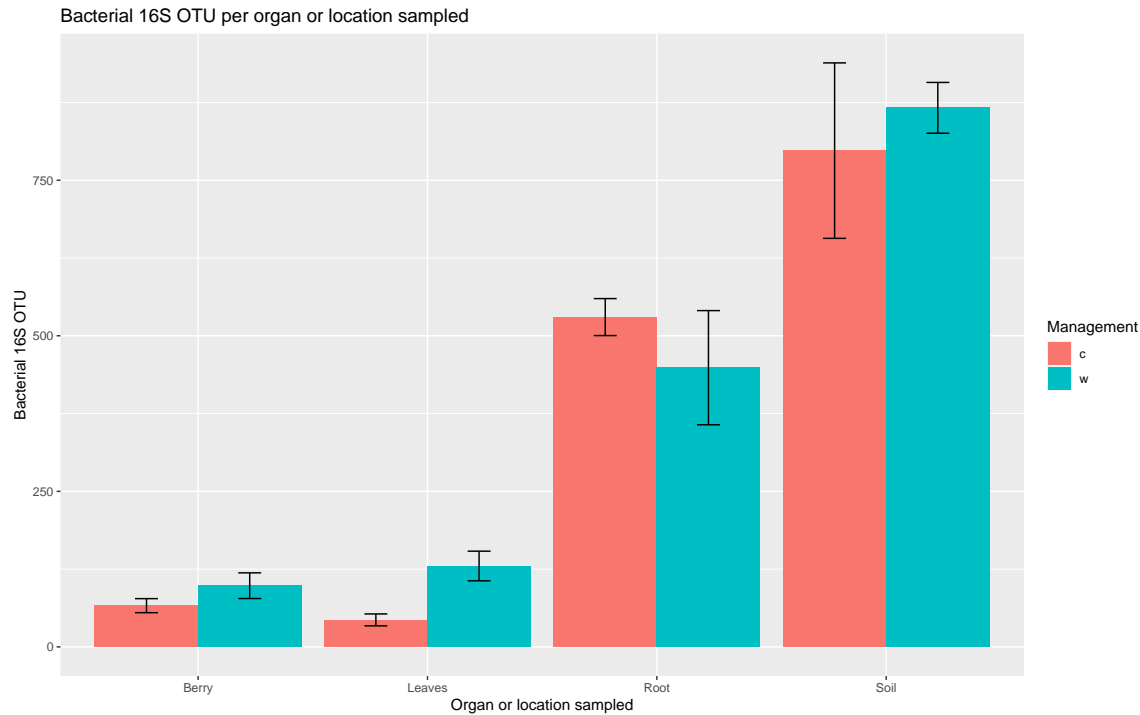




Conclusions on diversity

1. Significant difference in diversity between wild and managed systems
2. Overall higher diversity in wild systems
3. Fungal diversity in all compartments (soil, roots, leaves and berries) is reduced in managed systems
4. Bacterial diversity is reduced only in berries and leaves in managed systems, soil and roots are not affected.

Soils and Roots: Management affects fungal but not bacterial richness



Ecology and Evolution

Open Access

Comparison of soil microbial communities inhabiting vineyards and native sclerophyllous forests in central Chile

Luis E. Castañeda^{1,2}, Karina Godoy^{1,2}, Marlene Manzano^{2,3}, Pablo A. Marquet^{2,3,4,5,6} & Olga Barbosa^{1,2}

¹Facultad de Ciencias, Instituto de Ciencias Ambientales y Evolutivas, Campus Isla Teja, Universidad Austral de Chile, Valdivia, Chile

²Instituto de Ecología & Biodiversidad (IEB-Chile), Casilla 653, Santiago, Chile

³Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile

⁴The Santa Fe Institute, Santa Fe, New Mexico 87501

⁵Laboratorio Internacional de Cambio Global, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile

⁶Centro Cambio Global UC, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile

sampled growing areas; however, the fungal community did not differ. At the local scale, our findings show that fungal communities differed between habitats because fungi species might be more sensitive to land-use change compared to bacterial species, as bacterial communities did not change between forests and vineyards. We discuss these findings based on the sensitivity of microbial com-



Fungal taxons

Soil Fungi unique to the wild grape environment

- Ascomycota
 1. Cordycipitaceae (undefined within Family)
 2. Chaetosphaeria
 3. Dothideales (Undefined within Order)
- Basidiomycota
 1. Trechispora

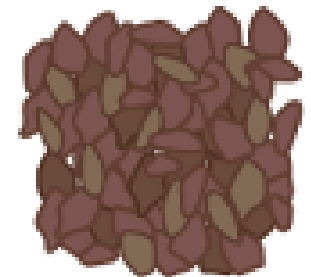
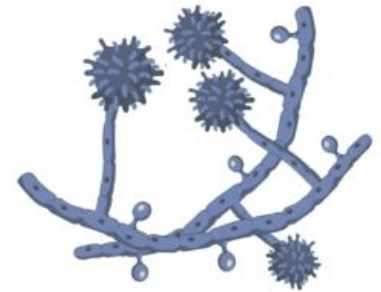
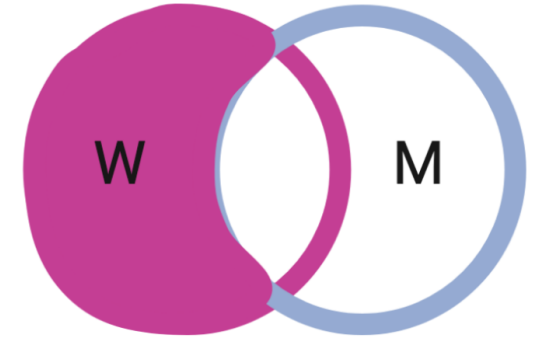


A. *Cordyceps bassiana* on coleopteran larva.

B. *Torrubiella hemipterigena* on planthopper.

Source: Vega et al. 2012, *Insect Pathology*. DOI: 10.1016/B978-0-12-384984-7.00006-3

Entomopathogens



Soil Fungi unique to the wild grape environment

- Ascomycota
 1. Cordycipitaceae (undefined within Family)
 2. Chaetosphaeria
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 1. Trechispora

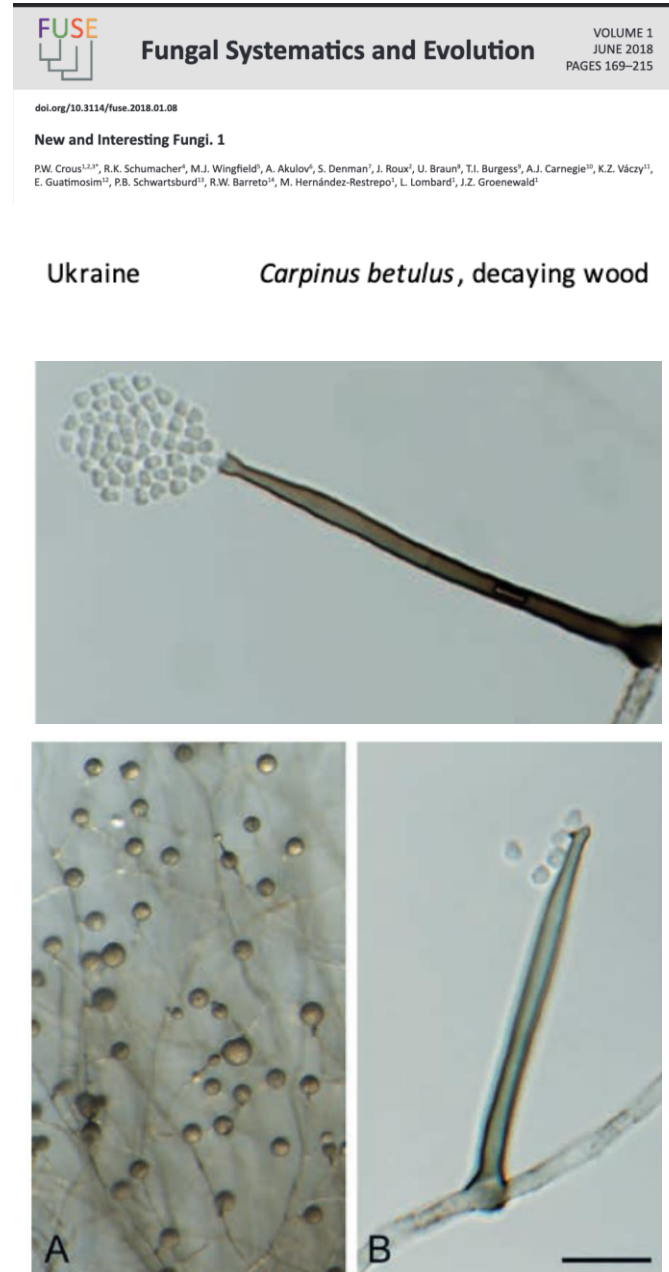
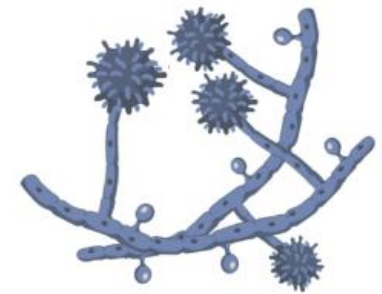
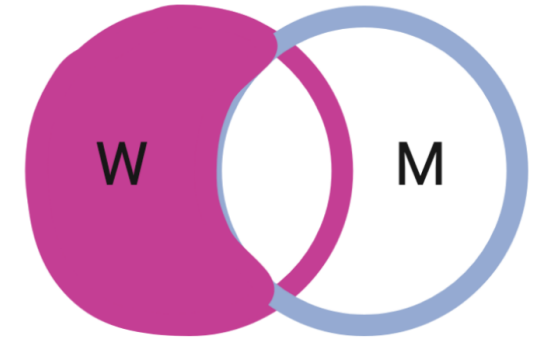


Fig. 9. *Chaetosphaeria myriocarpa* (CBS 143389). A. Conidi

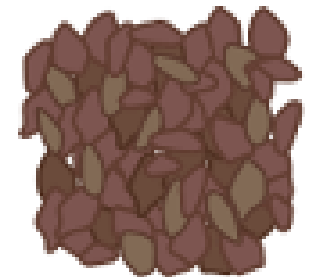
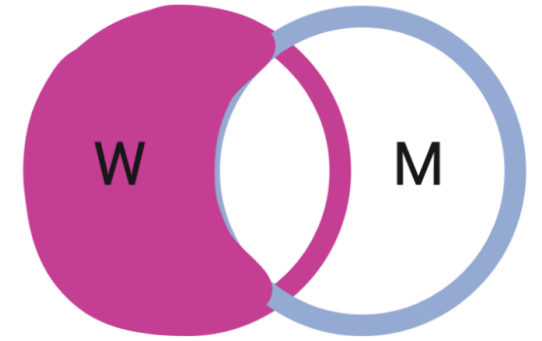


Soil Fungi unique to the wild grape environment

- Ascomycota
 1. Cordycipitaceae (undefined within Family)
 2. Chaetosphaeria
 3. Dothideales (Undefined within Order)
- Basidiomycota
 1. Trechispora



Trechispora, an important genus of wood-inhabiting fungi that have the ability to decompose rotten wood in the forest ecosystem.



Root Fungi unique to the wild grapes are a subset of the unique soil Fungi

- Ascomycota
 1. Cordycipitaceae (undefined within Family)
 2. Dothideomycetes (Undefined within Order)

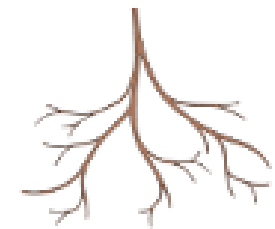
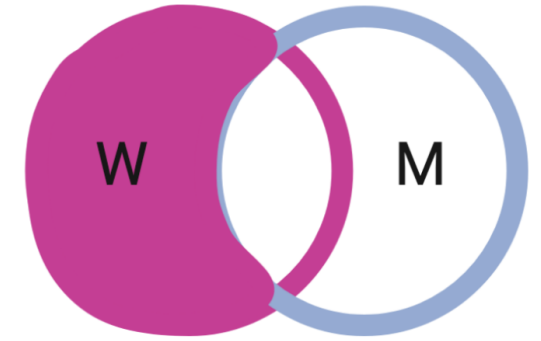


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B. *Torrubiella hemipterigena* on planthopper.

Source: Vega et al. 2012, *Insect Pathology*. DOI: 10.1016/B978-0-12-384984-7.00006-3

Entomopathogens



Soil Fungi unique to the managed systems

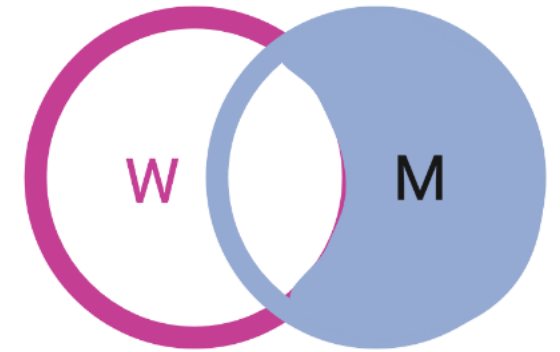
- Ascomycota:
 1. *Cylindrocarpon* (trunk disease)
 2. *Emericellopsis*
 3. *Stachybotrys* (mold)
- Basidiomycota:
 1. *Ceratobasidium*
- Chytridiomycota
 1. *Rhizophlyctis rosea*



A, black discoloration and necrosis of wood tissue which develops from the base of the rootstock, characteristic of black-foot disease; B, longitudinal section of a rootstock showing dark-brown to black discoloration; C, Un-sprouted grapevine propagation material in a grapevine nursery; D, grapevine plants showing stunted growth, reduced vigour and retarded sprouting in a young plantation.

Source: Agustí-Brisachet *al.* 2013, *Phytopathologia Mediterranea* DOI: 10.14601

Black foot disease, a trunk disease



Root Fungi unique to the managed systems

A subset of interesting ones

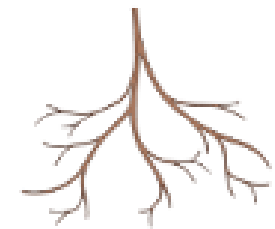
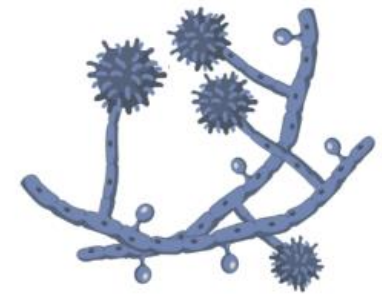
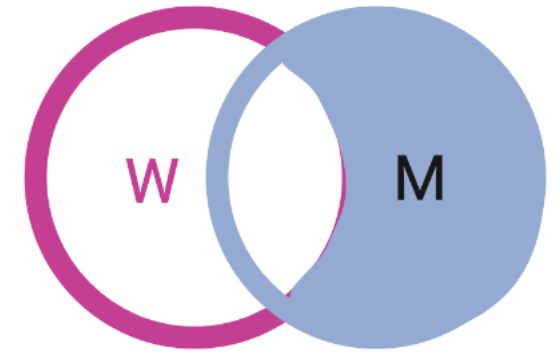
1. Ascomycota:
 1. *Cylindrocarpon* (trunk disease)
2. Chytridiomycota
 1. *Rhizophlyctis rosea*
3. Glomeromycota
 1. *Gigaspora* (Vesicular Arbuscular Mycorrhizae)



A, black discoloration and necrosis of wood tissue which develops from the base of the rootstock, characteristic of black-foot disease; B, longitudinal section of a rootstock showing dark-brown to black discoloration; C, Un-sprouted grapevine propagation material in a grapevine nursery; D, grapevine plants showing stunted growth, reduced vigour and retarded sprouting in a young plantation.

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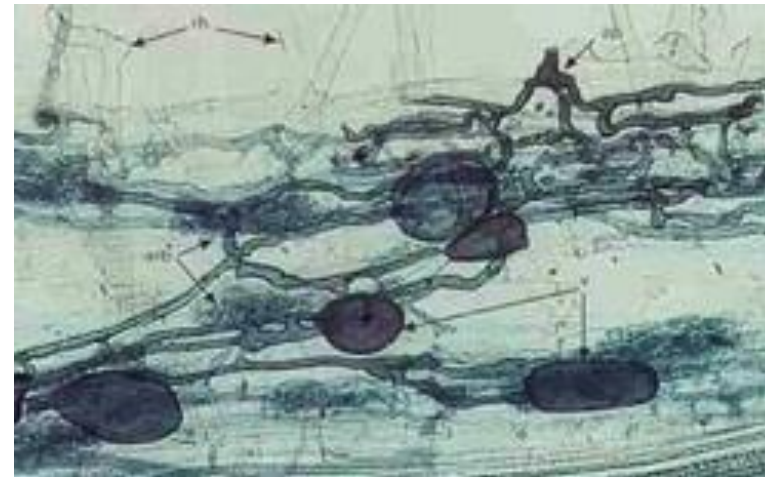
Black foot disease, a trunk disease



Root Fungi unique to the managed systems

A subset of interesting ones

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 1. *Cylindrocarpon* (trunk disease)
2. Chytridiomycota
 1. *Rhizophlyctis rosea*
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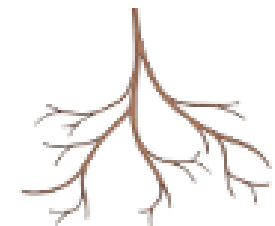
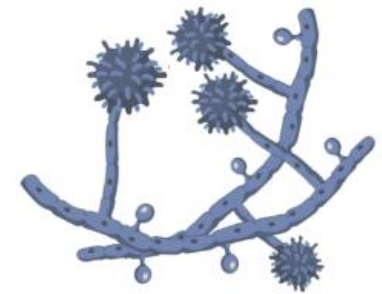
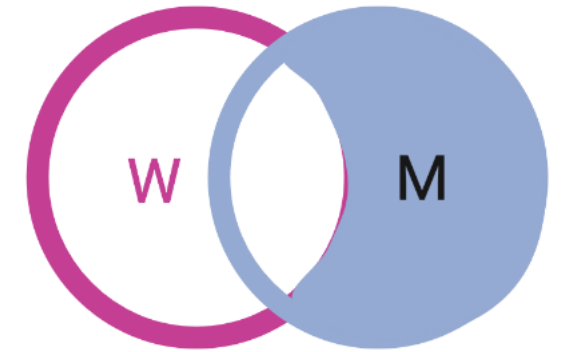


Top: Extracted population of *Gigaspora gigantea*

Bottom: AM fungi in plant roots

Source: INVAM Texas

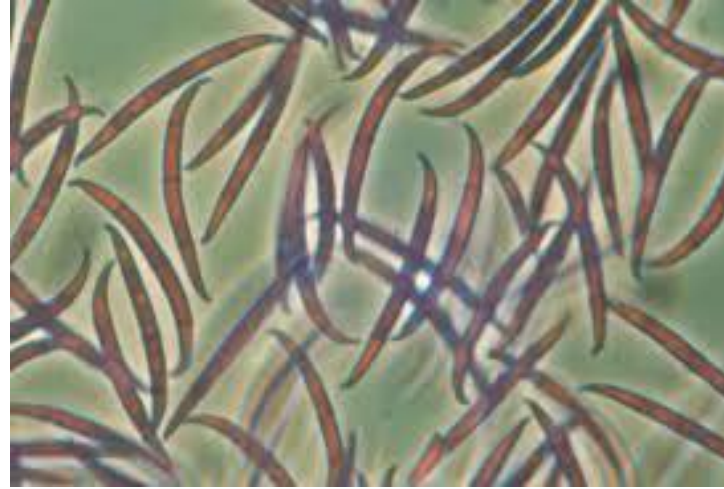
Vesicular Arbuscular Mycorrhizae



Soil Fungi present in all samples Top 10 Ranked by abundance

Ascomycota:

1. Fusarium
2. Penicillium
3. Epicoccum
4. Cladosporium
5. Acremonium
6. Pseudogymnoascus
7. Metarhizium (Biological Control)
8. Trichoderma (Biological Control)
9. Clonostachys rosea (Biological control)
10. Neonectria



Top: *Fusarium*

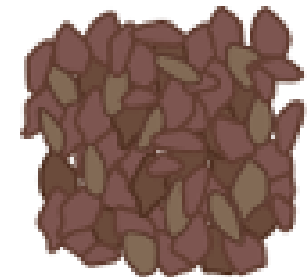
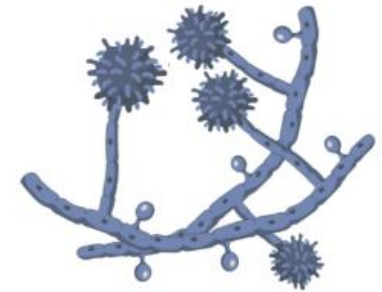
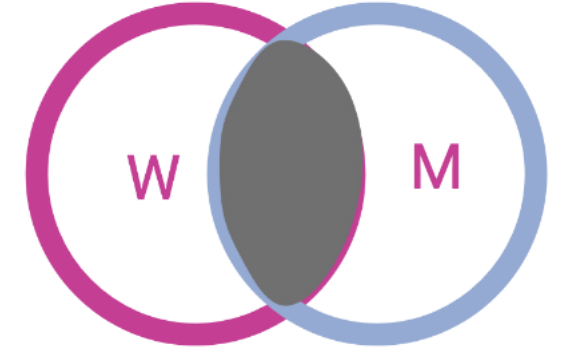
Source:

<https://www.adelaide.edu.au/mycology/>

Bottom: *Penicillium* on oranges

Source:

<https://en.wikipedia.org/wiki/Penicillium>

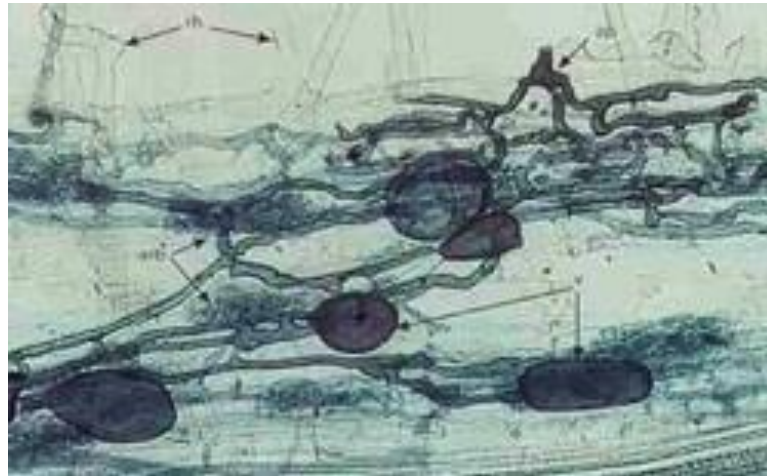
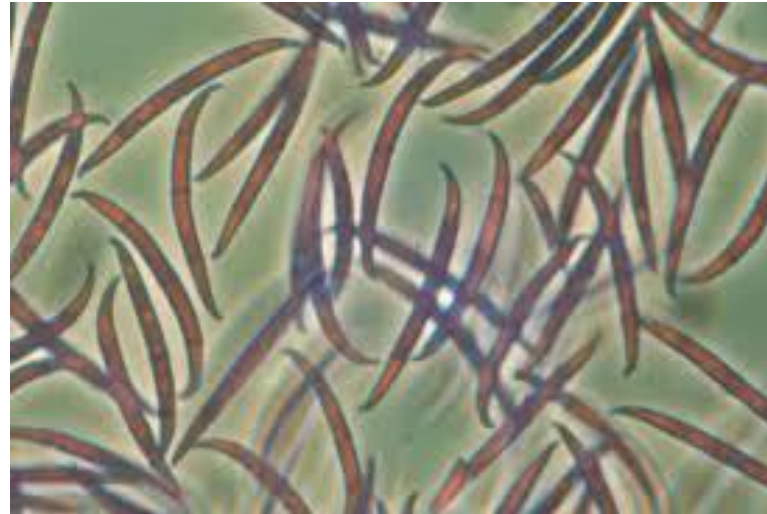


Root Fungi present in all samples

Top 10

Ranked by abundance

1. Ascomycota:
 1. Fusarium
 2. Neonectria
 3. Ilyonectria
 4. Clonostachys
 5. Leptodontidium
 6. Penicillium
 7. Epicoccum
 8. Cladosporium
 9. Lophiostoma
 10. Trichoderma
2. Glomeromycota (Vesicular Arbuscular Mycorrhizae)
 1. Glomeraceae – Undefined Genus

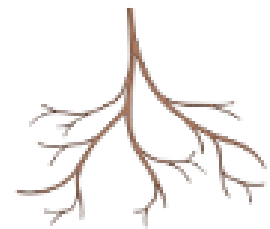
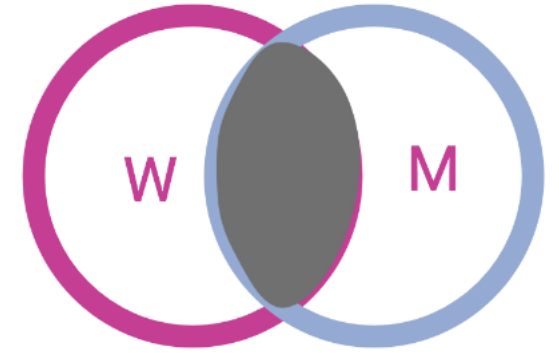


Top: *Fusarium*

Source:

<https://www.adelaide.edu.au/mycology/>

Bottom: Bottom: AM fungi in plant roots



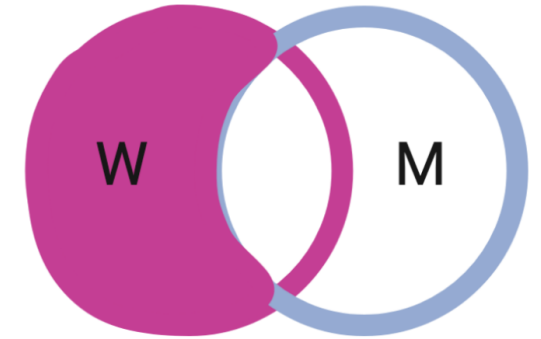
Leaves Fungi unique to the wild grape environment

1. Ascomycota:
 1. Hyaloscyphaceae (Family)
 2. Muriphaeosphaeria
 3. Phaeomoniellales (Order)
 4. Rhytismatales (Order)
2. Basidiomycota
 1. Auriculoscypha
 2. Cystobasidiaceae (Family)
 3. Erythrobasidium

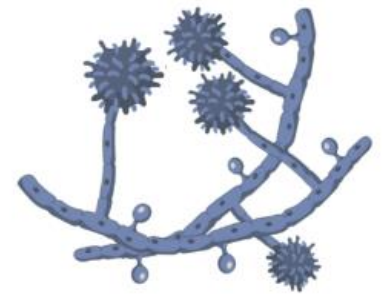
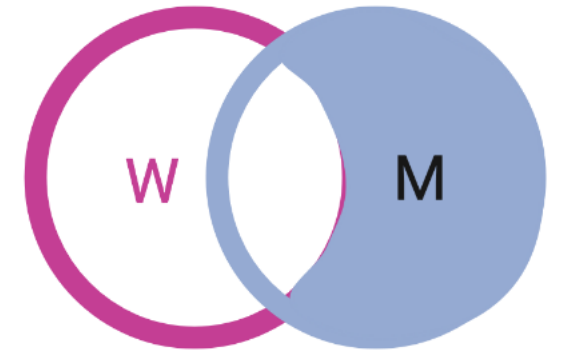
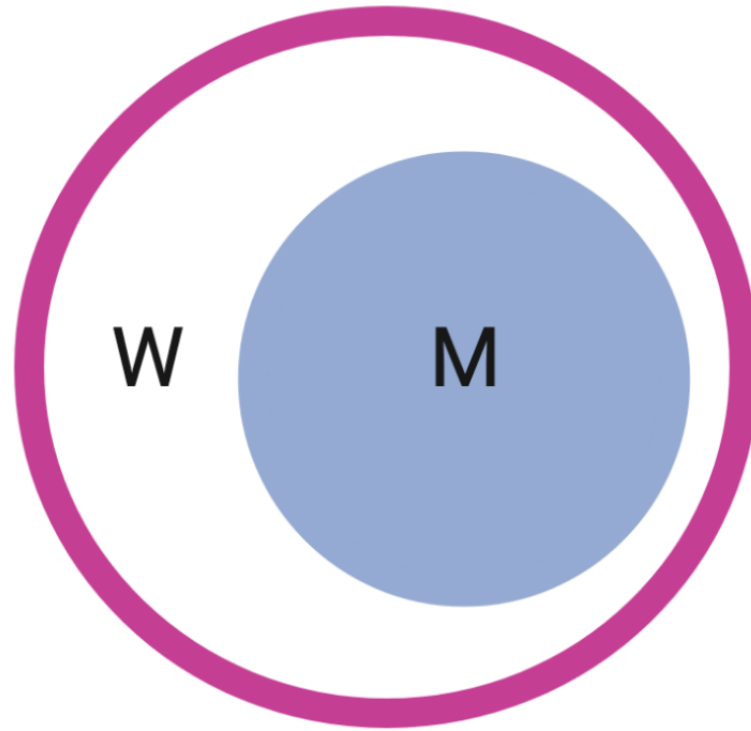


Top: Maple spot, Rhytismatales
Source: <https://eol.org/pages/18617>

Bottom: *Erythrobasidium hasegawianum*
Source: <https://mycocosm.jgi.doe.gov>



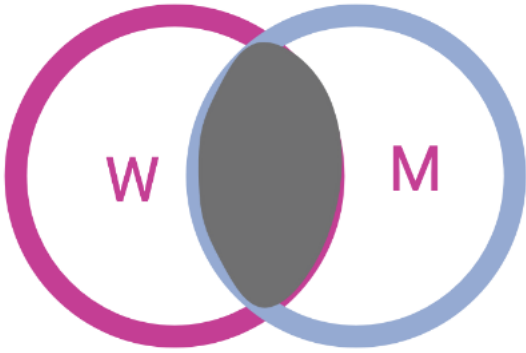
No leaves
Fungi unique
to the
managed
grape
environment!



Leaves Fungi present
in all samples
Top 10
Ranked by abundance

1. Aureobasidium*
 2. Sporidiobolus
 3. Epicoccum*
 4. Cladosporium*
 5. Filobasidium
 6. Taphrina
 7. Pseudopithomyces
 8. Pestalotiopsis
 9. Vishniacozyma
 10. Mycosphaerella
 11. Botrytis
- Ascomycota
Basidiomycota

Alternaria
Aureobasidium
Cladosporium
Guehomyces
Epicoccum
Mucor
Pandora
Rhizopus
Sporormiella



Top: Majority of genera associated with leaves

Source: Bettenfeld et al. 2022 DOI:10.1016



Berry Fungi unique to the wild grape environment

Top 10 in abundance

1. Ascomycota
 1. Cyphellophora
 2. Muriphaeosphaeria
 3. Peltaster
 4. Strelitziana
 5. Zymoseptoria
2. Basidiomycota
 1. Bulleribasidium
 2. Cystofilobasidiaceae (undefined Genus)*
 3. Kondoa*
 4. Sirobasidiaceae (undefined Genus)
 5. Tremella

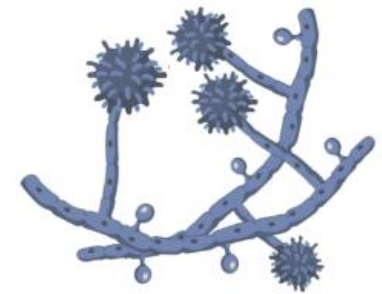
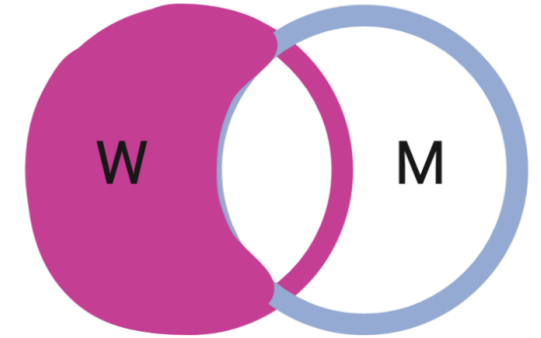
*Top 2



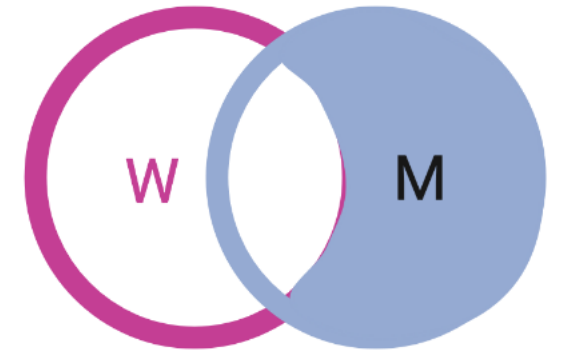
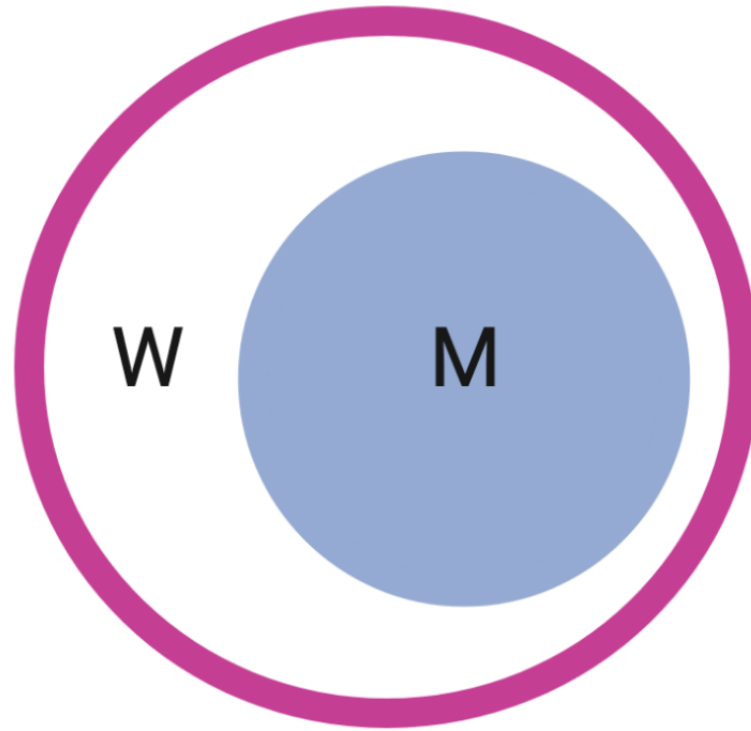
Top: *Cystofilobasidiaceae*,
Source: <https://eol.org/>

Bottom: *Kondoa yuccicola*
Source:
https://www.jcm.riken.jp/cgi-bin/jcm/jcm_number?JCM=6251

Basidiomycota yeasts



No Berry Fungi
unique to the
managed
grape
environment!

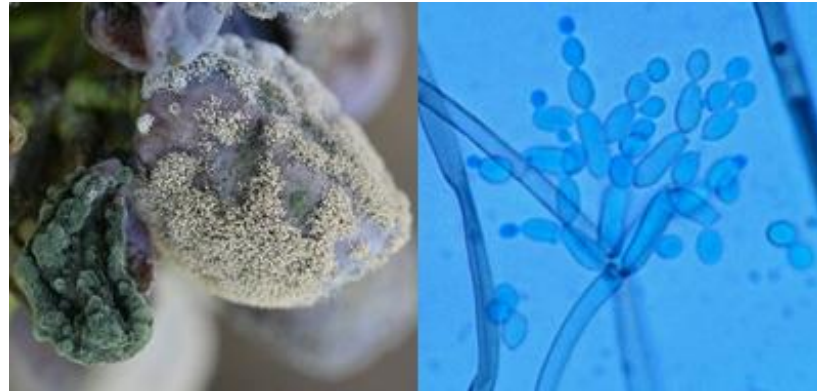


Berry Fungi present in
all samples
Top 10
Ranked by abundance

1. Cladosporium
2. Aureobasidium
3. Sporidiobolus
4. Epicoccum
5. Penicillium
6. Filobasidium
7. Auricularia
8. Botrytis (Pathogen)
9. Rhodotorula
10. Nigrospora

Ascomycota

Basidiomycota

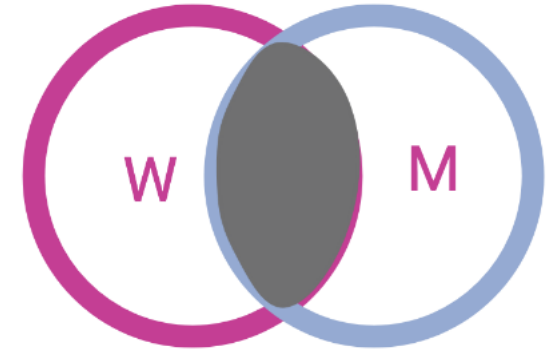


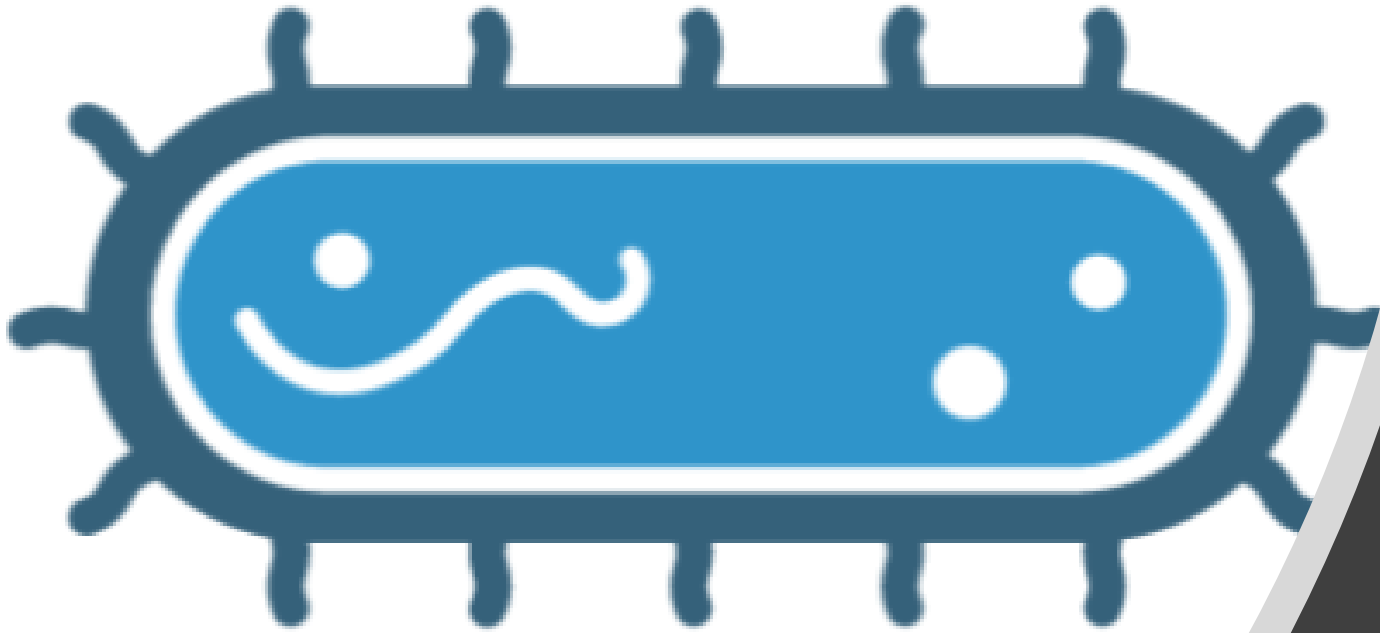
Cladosporium rot

Source:

<http://ephytia.inra.fr/en/C/6098/Grapevine-Rot-Cladosporium>

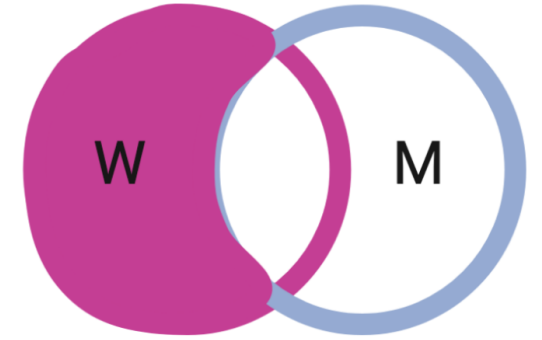
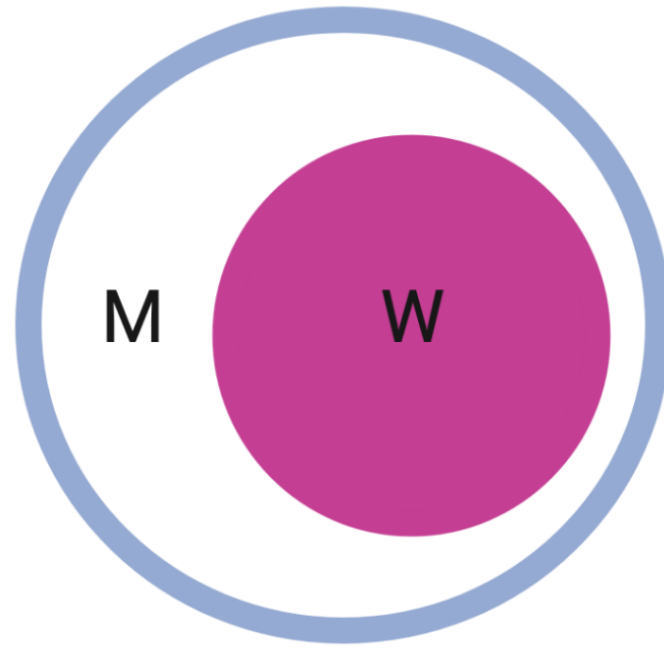
“Population levels increase as the berries mature, are responsible for olive green rot causing berries to dry out in the vineyard or during storage.”



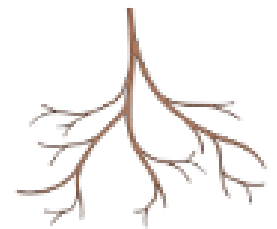
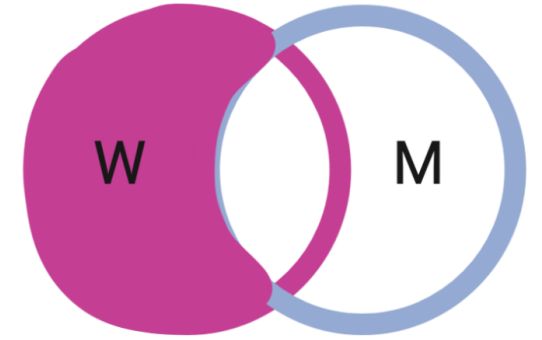
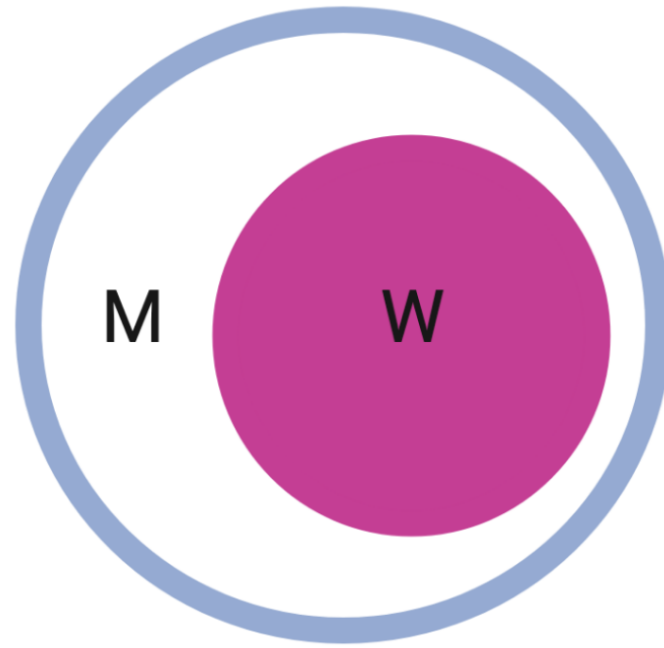


Bacterial
taxons

No soil
bacteria are
unique to the
wild grape
environment!



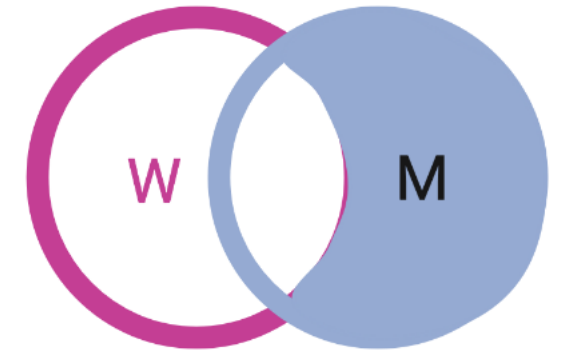
No root
bacteria are
unique to the
wild grape
environment!



Soil bacteria unique
to the managed
environment
Ranked by
abundance

1. Polaromonas
2. Adhaeribacter
3. Planifilum
4. Rhodopseudomonas
5. Dyadobacter
6. Skermanella
7. Flavisolibacter
8. Dactylosporangium

Bacillus
Blastococcus
Clostridium
Flavobacterium
Gaiella
Methylobacterium
Micrococcus
Nitrososphaera
Pseudomonas
Rhizobium
Steroidobacter
Sphingomonas



*Top: Majority of genera associated with
grape soils and roots*

Source: Bettenfeld et al. 2022 DOI:10.1016



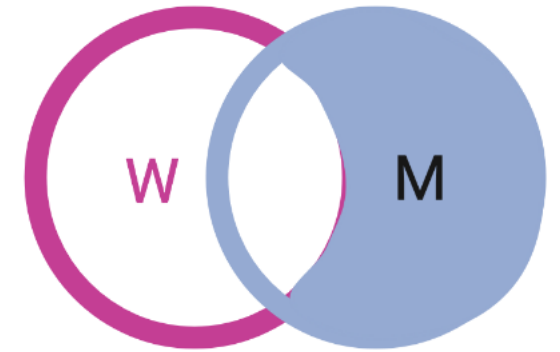
Root bacteria unique
to the managed
environment

Top 10

Ranked by abundance

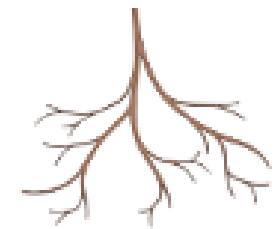
1. Dyadobacter
2. Methylothera
3. Methylophilus
4. Microvirga
5. Fluviicola
6. Geodermatophilus
7. Paenibacillus

Bacillus
Blastococcus
Clostridium
Flavobacterium
Gaiella
Methylobacterium
Micrococcus
Nitrososphaera
Pseudomonas
Rhizobium
Steroidobacter
Sphingomonas



*Top: Majority of genera associated with
grape soils and roots*

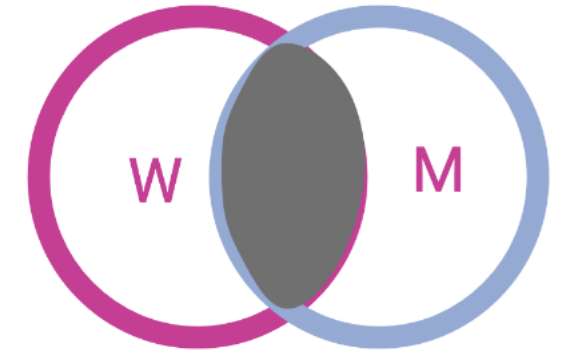
Source: Bettenfeld et al. 2022 DOI:10.1016



Soil Bacteria present
in all samples
Top 10
Ranked by abundance

1. Rhizobiales
2. Acidobacteria_Gp1*
3. Spartobacteria_genera_incer
tae_sedis
4. Acidobacteria_Gp3*
5. Alphaproteobacteria
6. Acidobacteria_Gp4*
7. Betaproteobacteria
8. Acidobacteria_Gp2*
9. Chitinophagaceae
10. Acidobacteria_Gp6*

Actinobacteria
Acidobacteria
Bacteroidetes
Chloroflexi
Firmicutes
Planctomycetes
Proteobacteria
Verrucomicrobia



*Top: Majority of phyla associated with
grape soils and roots*

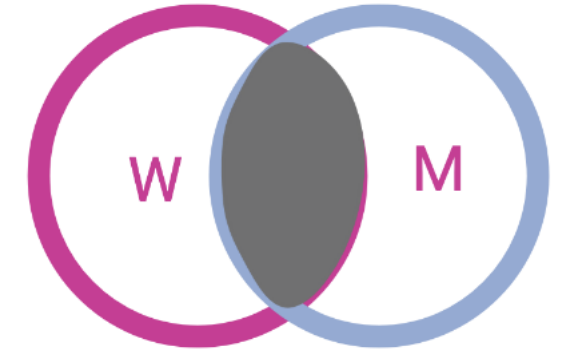
Source: Bettenfeld et al. 2022 DOI:10.1016



Root Bacteria present
in all samples
Top 10
Ranked by abundance

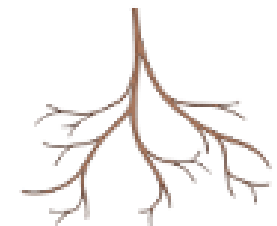
1. Okibacterium
2. Steroidobacter*
3. Burkholderia
4. Streptomyces
5. Rhizobium*
6. Pseudomonas*
7. Novosphingobium
8. Mucilaginibacter
9. Bradyrhizobium
10. Burkholderiales_incertae_sedis

Bacillus
Blastococcus
Clostridium
Flavobacterium
Gaiella
Methylobacterium
Micrococcus
Nitrososphaera
Pseudomonas
Rhizobium
Steroidobacter
Sphingomonas



*Top: Majority of genera associated with
grape soils and roots*

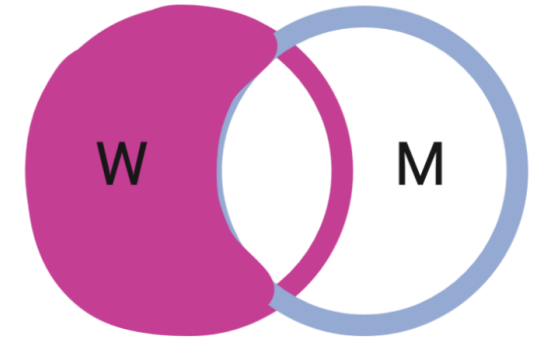
Source: Bettenfeld et al. 2022 DOI:10.1016



Leaves Bacteria
unique to the wild
grape environment
Ranked by
abundance

1. Terriglobus
 2. Bdellovibrio
 3. Acidimicrobiales
- ✓ Uncultured bacteria
 - ✓ Not reported very
common on grapes

Arthrobacter
Bacillus
Blastococcus
Curtobacterium
Enterococcus
Flavobacterium
Methylobacterium
Pantoea
Pseudomonas
Sphingomonas
Streptococcus

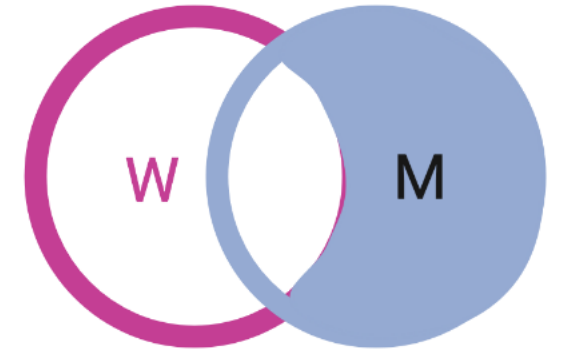
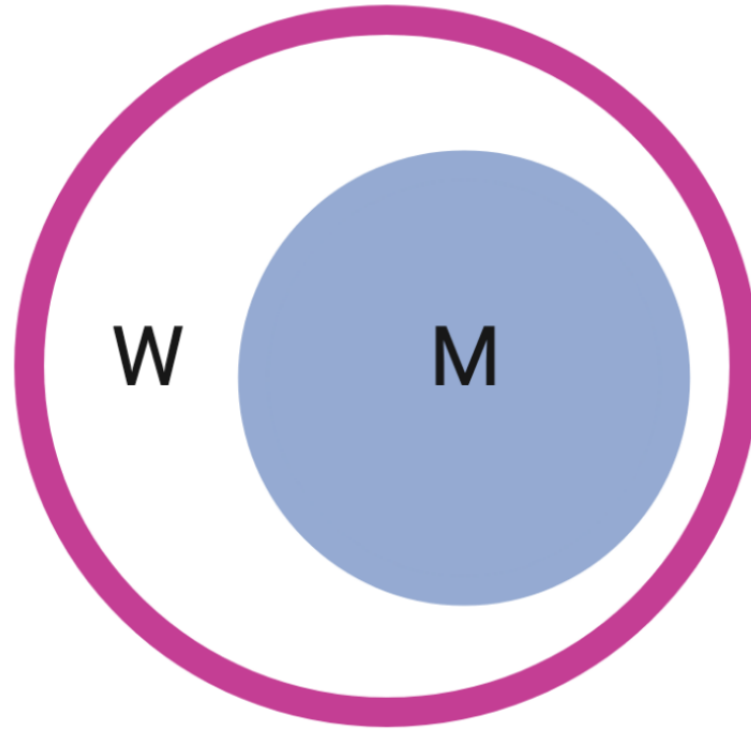


*Top: Majority of genera associated with
grape leaves*

Source: Bettenfeld et al. 2022 DOI:10.1016



No leaves
Bacteria
unique to the
managed
grape
environment!

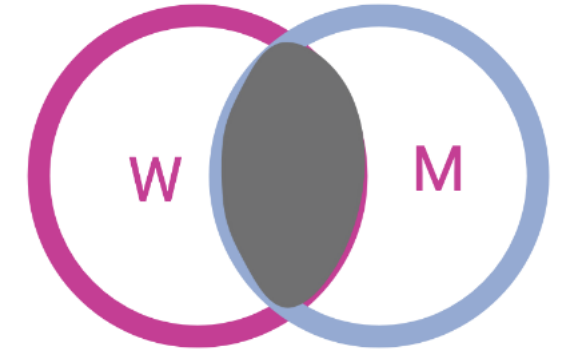


Leaves Bacteria
present in all samples

Ranked by abundance

1. Okibacterium
 2. Methylobacterium*
- ✓ Not many bacteria are present across all samples
 - ✓ Okibacterium also in the roots and berries of all samples

Arthrobacter
Bacillus
Blastococcus
Curtobacterium
Enterococcus
Flavobacterium
Methylobacterium
Pantoea
Pseudomonas
Sphingomonas
Streptococcus

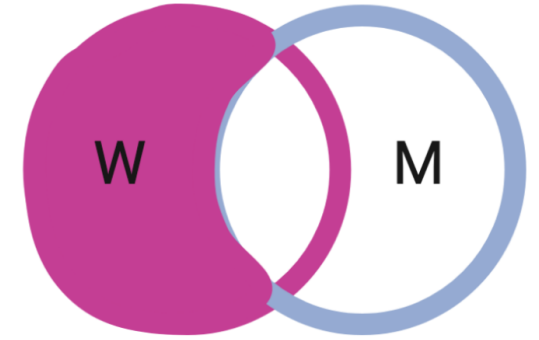
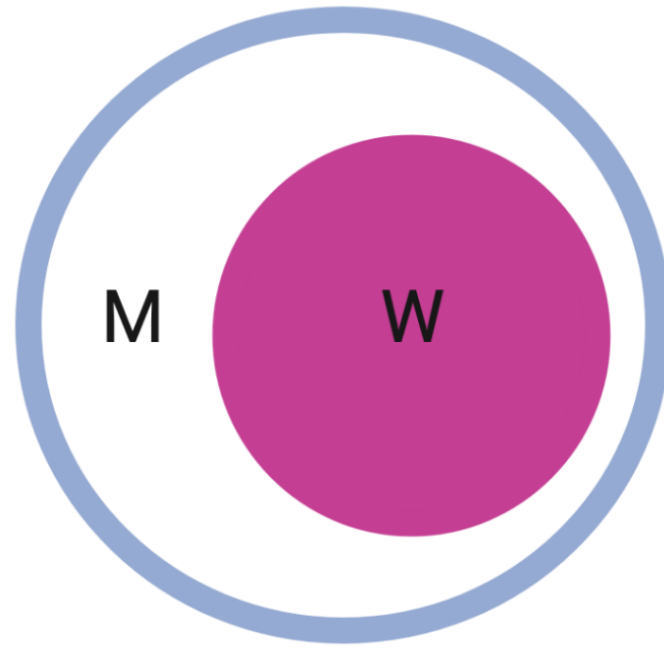


Top: Majority of genera associated with grape leaves

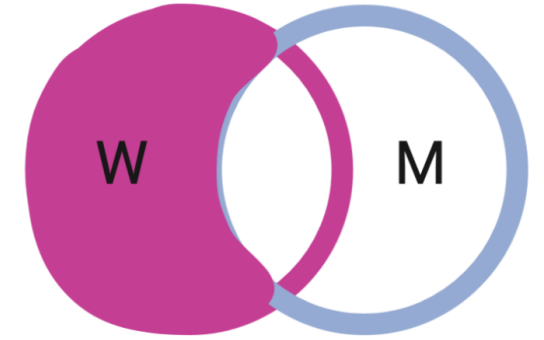
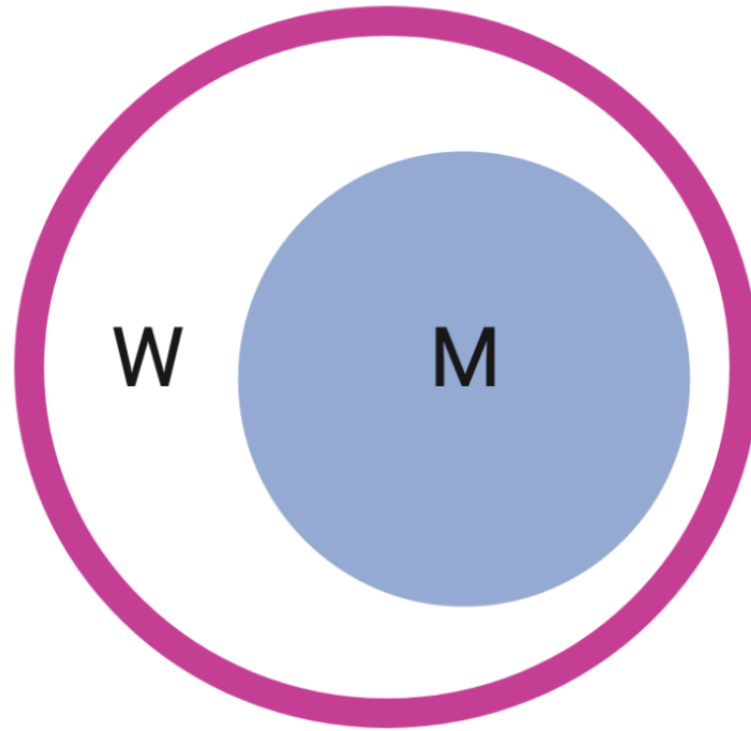
Source: Bettenfeld et al. 2022 DOI:10.1016



No berry
bacteria unique
to the wild
grape
environment!



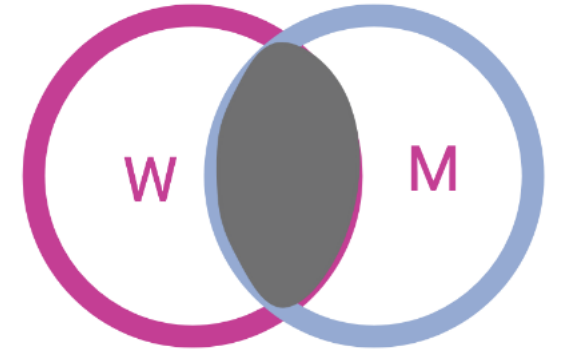
No berry
bacteria unique
to the managed
grape
environment!



Berry bacteria present in all samples

1. Actinobacteria
 1. Corynebacterium
 2. Curtobacterium
 3. Okibacterium
 4. Propionibacterium
2. Proteobacteria
 1. Massilia*
 2. Methylobacterium*
 3. Pantoea
 4. Pseudomonas
 5. Sphingomonas*
3. Firmicutes
 1. Staphylococcus

Bacillus
Blastococcus
Enterobacter
Erwinia
Gaiella
Massilia
Methylobacterium
Micrococcus
Pseudomonas
Sphingomonas



Top: Majority of genera associated with berries

Source: Bettenfeld et al. 2022 DOI:10.1016





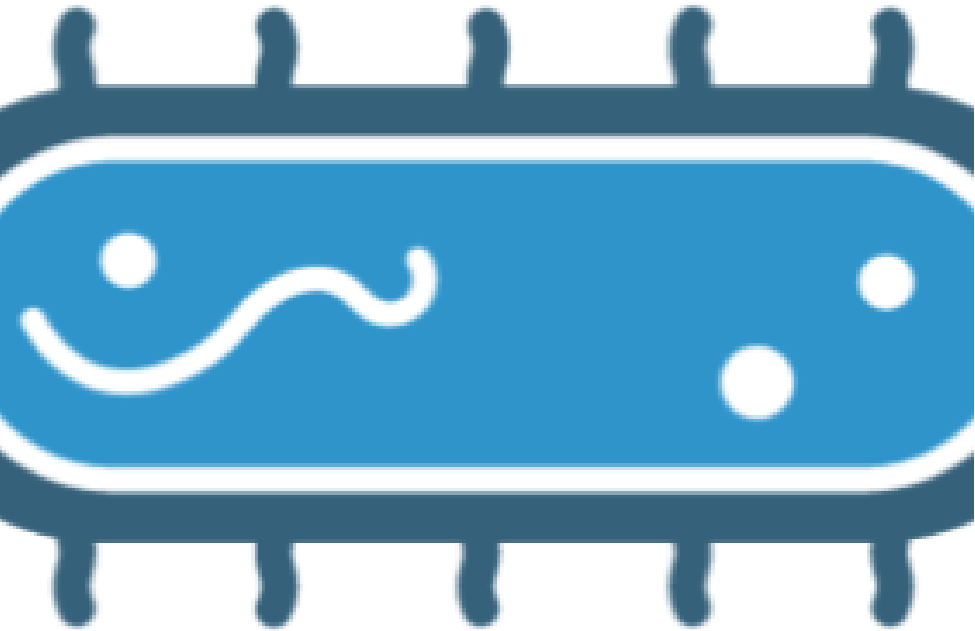
Conclusions on effect of management on fungal taxa

Soil and roots:

1. Wild soil and root fungal taxa include an entomopathogen-related fungus, absent from managed system.
2. Managed systems include the root/trunk disease causing fungus *Cylindrocarpon* but also the arbuscular mycorrhiza *Gigaspora*, absent from the wild.

Leaves and berry:

1. Leaves and berries Fungal taxa in the managed system are a subset of the wild community.



Conclusions on effect of management on bacterial taxa

1. Soil and roots bacterial taxa in the wild systems are a subset of the managed communities.
2. Leaves bacterial taxa in the managed systems are a subset of the wild communities.
3. The berry bacterial wild and managed communities have no taxa unique to either.

Questions

Contact me and Newsletter Grape Notes



My email



Newsletter
subscription form
(free in 2023)