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Comparative transcriptomics of fungal endophytes in co-culture with their moss host *Dicranum scoparium* reveals fungal trophic lability and moss unchanged to slightly increased growth rates

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Summary

• Mosses harbor fungi whose interactions within their hosts remain largely unexplored. Trophic ranges of fungal endophytes from the moss *Dicranum scoparium* were hypothesized to encompass saprotrophism. This moss is an ideal host to study fungal trophic lability because of its natural senescence gradient, and because it can be grown axenically.

• *Dicranum scoparium* was co-cultured with each of eight endophytic fungi isolated from naturally occurring *D. scoparium*. Moss growth rates, and gene expression levels (RNA sequencing) of fungi and *D. scoparium*, were compared between axenic and co-culture treatments. Functional lability of two fungal endophytes was tested by comparing their RNA expression levels when colonizing living vs dead gametophytes.

• Growth rates of *D. scoparium* were unchanged, or increased, when in co-culture. One fungal isolate (Hyaloscyphaceae sp.) that promoted moss growth was associated with differential expression of auxin-related genes. When grown with living vs dead gametophytes, *Coniochaeta* sp. switched from having upregulated carbohydrate transporter activity to upregulated oxidation-based degradation, suggesting an endophytism to saprotrophism transition. However, no such transition was detected for Hyaloscyphaceae sp.

• Individually, fungal endophytes did not negatively impact growth rates of *D. scoparium*. Our results support the long-standing hypothesis that some fungal endophytes can switch to saprotrophism.

Introduction

Fungal endophytes are defined as fungi living inside plants without causing obvious symptoms (Rodriguez *et al.*, 2009). As a phylogenetically and functionally diverse group, fungal endophytes can be involved in a wide array of interactions with plants, ranging from parasitic to commensal and mutualistic, depending on the physiological condition of the host, the presence of co-occurring microbes, and environmental conditions (Carroll, 1988; Rodriguez *et al.*, 2009). Phylogenetic studies have revealed close affinities of many fungal endophytes with saprotrophic fungi (e.g. Promputtha *et al.*, 2007; Arnold *et al.*, 2009). The most studied endophytic fungi capable of engaging in both endophytic and saprotrophic lifestyles are root-associated fungi. For example, *Piriformospora indica* (Sebacinales, Basidiomycota) can promote plant growth when the plant is alive and colonize plant roots after host death (Zuccaro *et al.*, 2011; Zhou *et al.*, 2018).

Many endophytes associated with aboveground plant tissues can also colonize dead plant material (Rodriguez *et al.*, 2009), suggesting that several endophytic fungi might be latent saprotrophs, i.e., colonizing living plants asymptomatically and having the opportunity to be first to feed on senescing plant tissues, which is believed to enable completion of their life cycle (Osono & Hirose, 2011; U'Ren & Arnold, 2016; Hirose *et al.*, 2017). However, the genetic basis of the functional switch between endophytism and saprotrophism in aboveground endophytes is still unknown.

The trophic outcomes of plant–fungus interactions are the results of molecular communication between the two partners (Yi & Valent, 2013; Liao *et al.*, 2016; Van't Padje *et al.*, 2016). A core set of genes related to mutual recognition and immune system response of the plant is usually involved in plant-fungus symbiotic (including pathogenic) interactions (Eaton *et al.*, 2014; Liao *et al.*, 2016). Fungi first detect chemicals on the plant

surface and initiate growth of hyphae or specialized structures to penetrate host tissue (Yi & Valent, 2013; Evangelisti *et al.*, 2014). Subsequently, fungi release effectors, which usually are small secreted proteins that often manipulate the plant immune response (e.g. jasmonic acid, ethylene-related, and/or salicylic acid pathways) (Lo Presti *et al.*, 2015). Notably, effector-like proteins also regulate degradation activity of saprotrophic fungi (Feldman *et al.*, 2017). As a result of infection, fungal microbe-associated molecular patterns are detected by plant receptors, triggering a plant immune response (Yi & Valent, 2013; Evangelisti *et al.*, 2014). Genetic events differ for various plant–fungus interactions (Bonfante & Genre, 2010; Behie & Bidochka, 2014; Liao *et al.*, 2016). For example, some leaf endophytes can reprogram host plant physiology, favoring secondary metabolism and defense mechanisms at the cost of decreasing primary metabolism and photosynthesis (Mejía *et al.*, 2014; Dupont *et al.*, 2015).

Because of their gametophyte-dominated life cycle and relatively simple morphology, traits thought to be shared with the most recent common ancestor of land plants, mosses (phylum Bryophyta) represent a unique plant lineage (McDaniel, 2021). Therefore, studying moss–fungus associations is essential for a comprehensive understanding of plant–fungus interactions (Field *et al.*, 2015; Delavaux *et al.*, 2019). Primarily based on phenotypic features, it has been reported that bryophyte-associated fungi have various trophic interactions ranging from pathogenic and parasitic to saprotrophic or commensal (Martínez-Abaigar *et al.*, 2005; Davey & Currah, 2006; Davey *et al.*, 2010; Stenroos *et al.*, 2010; Korotkin *et al.*, 2018; Raudabaugh *et al.*, 2021). Though there is rising awareness of the functional importance of the plant microbiome, and the large number of endophytic fungi that inhabit mosses (Kauserud *et al.*, 2008; U'Ren *et al.*, 2012, 2019; Davey *et al.*, 2013a; Yu *et al.*, 2014; Chen *et al.*, 2018, 2019), the functions of moss-associated fungal endophytes are mostly unknown. At the molecular level, moss–fungus interactions have been mostly examined in the model species *Physcomitrella patens*. Comparative genomics revealed that *P. patens* has the genetic toolkit to form mycorrhizae with fungi (Delaux *et al.*, 2013). Recent studies focusing on *P. patens* revealed that reactive oxygen species and a hypersensitive response were triggered by fungal pathogens, which are mechanisms used by other land plants (e.g. angiosperms and gymnosperms) in response to fungal infection (Sarris *et al.*, 2016; Ponce de León & Montesano, 2017). Most of these studies used pathogenic fungi from other plant hosts (Bressendorff *et al.*, 2016). Therefore, how naturally occurring fungal isolates from mosses interact at the molecular level with their original hosts remains unexplored.

Dicranum scoparium is a perennial, acrocarpous moss that has a world-wide distribution (Lang & Stech, 2014). The erect growth of its gametophyte forms a senescence gradient from a photosynthetic (top) to a decomposing (bottom) layer via a senescing (middle) layer (Fig. 1a). Previous studies have shown that *D. scoparium* is associated with diverse fungal communities (Davey *et al.*, 2013a,b; Chen *et al.*, 2018). By investigating the metabolic activity defined by the relative abundance of transcribed ribosomal RNAs (rRNAs) in metatranscriptomes, fungal communities were shown to be structured by the senescence gradient of *D. scoparium* (Chen *et al.*, 2018). However, many fungi are able to colonize all three layers, suggesting that these fungi could undergo functional switches associated with occurrence in living vs dead tissue (Chen *et al.*, 2018, 2019). *Dicranum scoparium* can be cultivated axenically from spores obtained from unopened capsules (Vujičić *et al.*, 2009) and has been studied previously via culture-dependent and culture-free methods with respect to the diverse fungi it harbors (e.g. Davey *et al.*, 2012, 2013a; Chen *et al.*, 2018). The aforementioned features of *D. scoparium* allowed us to link environmental sampling to a tractable *in vitro* system to ask the following questions: (1) Do fungal strains representing different taxonomic groups and different activities across *D. scoparium* senescence layers have different effects on *D. scoparium* growth? (2) How does *D. scoparium* respond to growth-promoting endophytes at the transcriptomic level? (3) When a focal endophyte infects living and dead moss tissues, does it alter expression of genes associated with different trophic states (biotrophic vs saprotrophic)? To answer these questions, we used co-culture experiments to characterize the growth of *D. scoparium* (Fig. 1b) in response to fungal colonization. Based on growth rate results, we selected endophytes that stimulated *D. scoparium* growth and obtained metatranscriptomic data for the moss with and without fungal inoculation (Fig. 1c). To understand potential functional switches of fungi, we compared fungal transcriptomes obtained from living vs dead tissue of *D. scoparium* (Fig. 1c). By investigating moss–fungus interactions reflecting natural assemblies detected in the environment, this study elucidates moss–fungus interactions and the trophic lability of fungal endophytes.

Materials and Methods

Fungal strain selection

The fungal strains used for this study were obtained from a previous study (Chen *et al.*, 2018). In that study, naturally occurring gametophytes of *D. scoparium* Hedw. were collected from a forest environment (Duke Forest, NC, USA, 2014). A total of 398 fungal strains were then isolated from 900 surface-sterilized pieces of *D. scoparium* across three levels of senescence (300 pieces per layer) (Fig. 1a: top, green and actively growing layer; middle, intermediate layer; bottom, brown partially decomposed layer) (Chen *et al.*, 2018). These fungal endophytes represent a broad phylogenetic diversity and three types of activity, inferred by using abundance of rRNA reads as a proxy for metabolic activity in different layers of *D. scoparium*: (1) high activity in the photosynthetic (top) layer, (2) high activity in the decomposing (bottom) layer, and (3) low activity throughout gametophytes in nature but frequently isolated from gametophytes in their natural habitat (Chen *et al.*, 2018) (Fig. 1a). Among the 398 isolates, eight fungal strains (1M05 (Hyaloscyphaceae sp.), 1M12 (*Pholiota castanea*), 3B14 (*Cladophialophora* sp.), 2T69 (*Coniochaeta* sp.), 1T38 (*Hypoxylon* sp.), 3T12 (*Umbelopsis* sp.), 3M03 (*Exophiala dermatitidis*), and 1M06 (*Pithya* sp.)), representing different activity types and taxonomic groups according to Chen

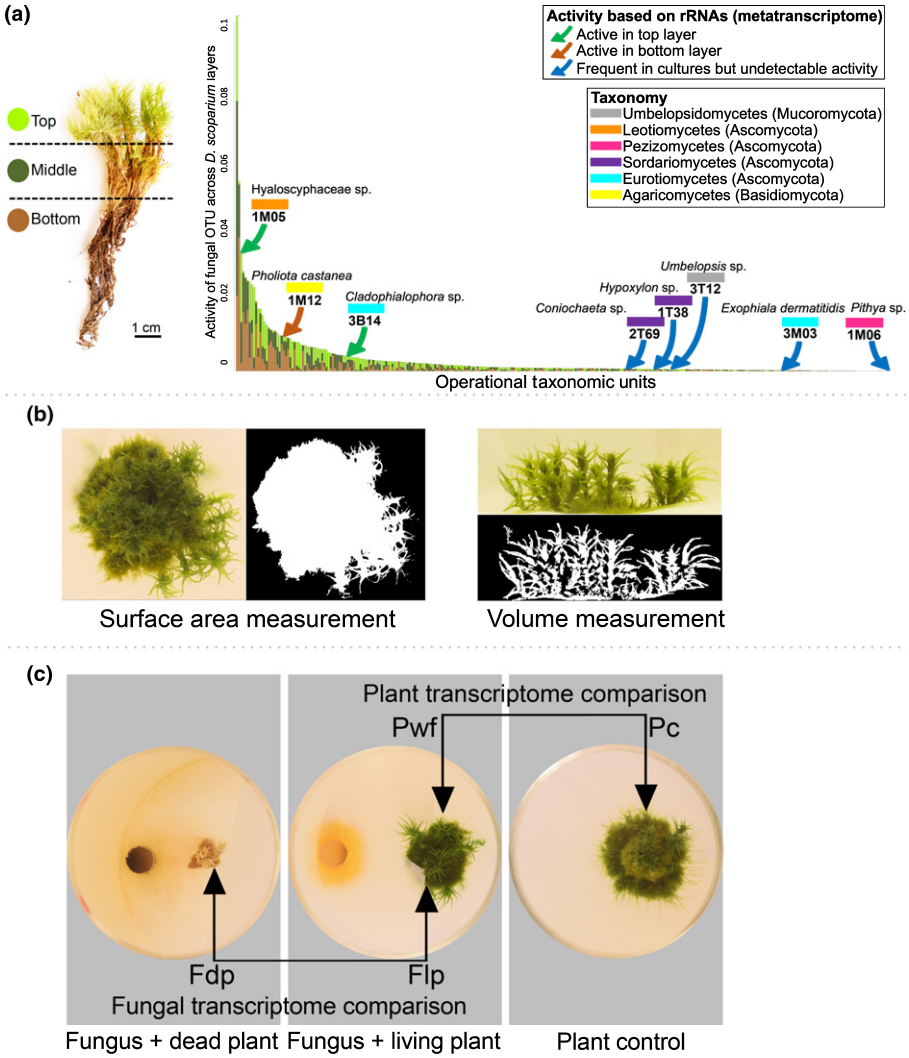

Fig. 1 Fungal strain selection and co-culture experimental design with *Dicranum scoparium*. (a) Fungal cultures isolated from *D. scoparium* in nature and selected based on previous study (Chen *et al*., 2018). Each bar in the histogram corresponds to one operational taxonomic unit (OTU) defined at 97% similarity of the nuclear large ribosomal subunit. Short reads from metatranscriptomic data were mapped to each OTU. Colors in each bar correspond to the ratio of reads from the top, middle, or bottom layer of *D. scoparium* gametophytes in nature (Chen *et al*., 2018). (b) *In vitro* co-culture growth assessment of *D. scoparium*. Examples of pictures taken for surface area and volume measurements and for resulting automated contrast enhancement. (c) Experimental setup for comparisons of RNA sequencing data between *D. scoparium* in pure culture (plant control (Pc)) vs in co-culture with one fungal strain (plant with fungus (Pwf)) from panel (a), and between a selected fungal strain (panel (a)) growing in living *D. scoparium* (fungus with living plant (Flp)) vs growing in dead tissue of *D. scoparium* (fungus with dead plant (Fdp)).

et al. (2018), were selected for the inoculation experiment of the present study (Fig. 1a; Supporting Information Table S1; Methods S1).

Axenic *D. scoparium* culture and preparation of endophytes for experiment

Axenic cultures of *D. scoparium* were obtained from spores of unopened capsules (collected at Tellico Plains, TN, USA, on 7 July 2009; TENN-B-0102884). First, the capsules were sterilized with 10% sodium hypochlorite for 30 s before they were cut open. Spores were then distributed on Murashige & Skoog solid media (Murashige & Skoog, 1962) with 10% agarose and an addition of 1.5% of sucrose (Vujičić *et al.*, 2009). Spores were germinated in a growth chamber (CU32L; Percival Scientific Inc., Boone, IA, USA) set at 18°C with an 18 h : 6 h day : night cycle and a daily light intensity of 40–70 µmol $m^{-2}s^{-1}$. *Dicranum scoparium* cultures used for this study were prepared by transferring *c.* 5 mm^2 blocks of tissue to newly prepared 80 ml solid media in glass jars. After the transfer, we waited 1.5 months for the moss to grow well before initiating the experiment.

All eight fungal strains were grown on malt extract agar (MEA) before being transferred to new plates amended with antibiotics (final concentration: penicillin, 100 µg ml^{-1}; streptomycin, 48 µg ml^{-1}) and subcultured for use in the co-culture experiments (Fig. 1c). Blocks of MEA containing actively growing mycelium (*c.* 6 mm diameter) were placed on sterile nylon filter paper (47 mm, 0.22 µm pore; Magna Nylon Membrane Filters, Sanford, ME, USA) to assist subsequent collection of mycelia for RNA extraction and placed *c.* 2 cm away from the moss gametophyte to establish co-cultures (Fig. 1c).

To prepare the dead plant tissue, gametophytes from axenic cultures were submerged in liquid nitrogen (N_2) for at least 2 min before being placed on top of the solid medium (Fig. 1c). The experiment, including interactions between live symbionts, a moss-only control, and the fungal strains grown with dead tissue from *D. scoparium* (Fig. 1c), was initiated in September 2016.

Dicranum scoparium growth and physiological measurements

Growth of gametophytes was first examined every 3 d. The ratios of either gametophyte surface area or volume between day 60 and

day 1 were calculated as the 'growth of gametophytes', with day 1 defined as the day when the fungal mycelium of each sample physically touched the moss. Pictures were taken through the lid for plant surface areas and from the sides for plant volume using a Nikon D3500 camera (Nikon Inc., Tokyo, Japan; Fig. 1b). The surface areas of plants were measured using PHOTOSHOP (Adobe Inc., San Jose, CA, USA). To get the three-dimensional measurements for the volume of each moss gametophyte, the glass jar was placed on a turntable and 10 pictures were taken around each glass jar using the software CAMERA CONTROL PRO 2 (Nikon Inc.). Subsequently, the images were processed with GIA ROOTS (Galkovskyi *et al.*, 2012) to extract the plant portions of these images (Fig. 1b) using a color threshold detection filter. All measurements were based on three to five replicates, and the jars remained unopened throughout the entire experimental period. Morphological modifications resulting from the moss–fungus interactions were monitored using a stereomicroscope (Leica MZ125, Heerbrugg, Switzerland) (Fig. 2a). In addition to growth, chlorophyll (Chl) content and electrolyte leakage were monitored as described in Methods S2.

Statistics for fungal effect on growth rate, Chlorophyll, and electrolyte leakage of *D. scoparium*

We evaluated variation in the ratios of surface area and volume as a function of treatment via ANOVA following confirmation that the ratio data for each measure were distributed normally. We used Dunnett's tests against controls to detect promotion or suppression of growth in response to each fungal co-culture. Multiple comparisons in Dunnett's test were corrected by false discovery rate (FDR). Bean plots were used to show the spread of these data points (Fig. 2b).

Strains selected for RNA extraction and RNA sequencing

Two fungi, representing two different classes, significantly promoted plant growth (Fig. 2b): *Coniochaeta* sp. 2T69 (Sordariomycetes) and Hyaloscyphaceae sp. 1M05 (Leotiomycetes). These two strains were selected for the transcriptomic study.

The RNA sequencing (RNAseq) assessment was designed to unveil the molecular interaction between the moss and its endophytes. We compared gene expression of *D. scoparium* when growing axenically (i.e. plant control (Pc)) vs when inoculated with a fungus (plant with fungus (Pwf)) (Fig. 1c). We also compared reads derived from fungi growing on or inside dead *D. scoparium* tissues (fungus with dead plant (Fdp)) with reads from fungi growing on or inside living gametophytes (fungus with living plant (Flp)) (Fig. 1c).

Sixty days after the mycelium initiated physical contact with the moss, gametophytes (dead (Fdp) and alive (Pwf and Flp)) above the culture medium were collected and immediately submerged and ground with a pestle and mortar in liquid N_2. The plant-only controls were harvested on the last harvest date of moss–fungus pairs. Lysis buffer from the RNAqueous® Kit (ThermoFisher Scientific, Waltham, MA, USA) was added to the tubes immediately after grinding to stop potential RNAase activity, and samples were stored in a −80°C freezer until the rest of the RNA extraction steps were performed. Lysates (ground plant tissue with remaining nucleic acids) left from the RNA extraction steps were used to confirm inoculation success by detecting the presence of the fungus in the moss via PCR (primer set: ITS1F, LR3) (Vilgalys & Hester, 1990; Gardes & Bruns, 1993), followed by visualization of amplicons with electrophoresis and Sanger sequencing. Only samples with expected outcomes (i.e. control plant: without fungal DNA amplified; infected living and dead plant: with fungal DNA amplified and the identity of the original inoculated fungus confirmed using Sanger sequencing) were used to proceed for library preparation and RNAseq.

RNAseq library preparation and sequencing

The RNAseq libraries were prepared and barcoded with the Illumina TruSeq Stranded mRNA Libraries Preparation Kit (Illumina Inc., San Diego, CA, USA), which enriched for mature poly-A transcripts produced by eukaryotes, and sequenced on an Illumina HiSeq 4000 system at the Duke Center for Genomic and Computational Biology. The barcoded RNAseq libraries of fungal and plant samples were pooled and sequenced in separate lanes. See Table S2 for read numbers and quality.

Reference transcriptome assembly

The procedure to obtain a reference transcriptome from axenic cultures of *D. scoparium* was described in Chen *et al.* (2019). Reference transcriptomes for the targeted fungi were prepared from pure culture growing in liquid malt extract (Table S2). The RNAseq data from pure cultures were assembled using TRINITY (Haas *et al.*, 2013). RNAseq reads from all samples were mapped onto the moss and fungal reference transcriptomes with BOWTIE 2 (Langmead & Salzberg, 2012). To account for the problem of not having reference genomes, which also include genes expressed only under symbiotic conditions (i.e. not expressed in the moss without the fungal partner, and not expressed in the fungus in pure culture), the reads not mapped to the plant and fungal reference transcriptomes were collected for *de novo* assembly (Liao *et al.*, 2016) (see Fig. S1 for workflow). Annotations were performed with the TRINOTATE (Haas *et al.*, 2013) pipeline using the National Center for Biotechnology Information (NCBI) Genbank and UniProt databases, and MEGAN (Huson *et al.*, 2007) to assign taxonomy. Differential gene expression tests were conducted with DESEQ2 (Love *et al.*, 2014). Unless stated otherwise, FDR < 0.05 and absolute $\log_2$(fold change) > 2 were the criteria for significantly expressed genes. GOSEQ (Young *et al.*, 2010) was used to detect enriched Gene Ontology (GO) terms. Owing to the large difference between the number of mapped reads between Flp and Fdp colonized by *Coniochaeta* sp. 2T69, we randomly subsampled 10 000 000 reads without replacement from these samples. The same mapping and differential expression tests were applied on the subsampled datasets. The results obtained with subsampling overall agree with those obtained from the original datasets. Therefore, we decided to present the results without subsampling. We further investigated the

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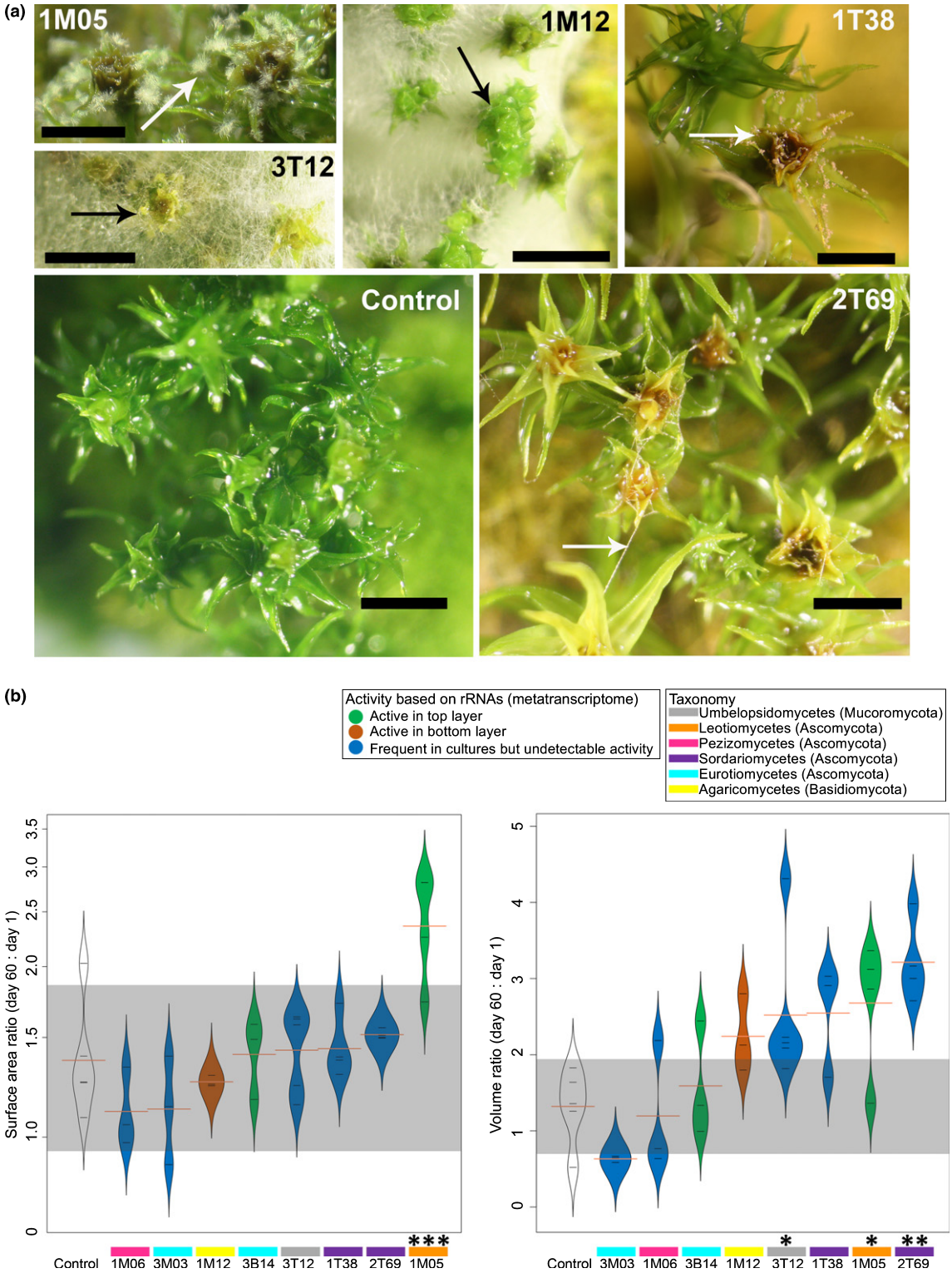

Fig. 2 (a) Phenotypes of *Dicranum scoparium* infected with endophytic fungi in co-culture experiments. Bar, 1 mm. All pictures were taken 73 d after inoculation. Arrows: 1M05 and 1T38, fungal sporulating structures; 3T12 and 1M12, compact *D. scoparium* gametophytes; 2T69, fungal hypha. (b) Ratio (day 60 : day 1) for surface area (left) and volume (right) of gametophytes of *D. scoparium,* as a function of treatment. Day 1 was determined as the date a fungal mycelium reached the gametophyte edge. The *x*-axis corresponds to strains used in co-culture experiments. Color bars above each strain name along the *x*-axis indicate the taxonomic classes of the strains. The colors of the plots correspond to the activity type detected in nature across *D. scoparium*'s senescence gradient (Chen *et al*., 2018) (Fig. 1a). Orange lines correspond to the mean for each group. Short black lines indicate values for individual replicates. The gray shade corresponds to 95% confidence interval calculated for the control group using *t*-distribution with four degrees of freedom (*, FDR < 0.05; **, FDR < 0.01; ***, FDR < 0.001). FDR, false discovery rate; rRNA, ribosomal RNA.

expression of genes that code for enzymes central for the decomposition of cellulose, one major cell wall component of mosses (Roberts *et al.*, 2012). More specifically, three enzyme families of glycoside hydrolases (GHs: GH5, GH6, GH7) (Drula *et al.*, 2022) were investigated. The total expression per GH family was calculated by summing up the counts (variance stabilizing transformation (VST)) per sample. Finally, we examined the small-secreted proteins for fungal isolates colonizing living (Flp) and dead (Fdp) plant materials. The predicted protein sequences of differentially expressed genes with signaling peptide (predicted by SignalP 3.0) (Petersen *et al.*, 2011) were imported into EffectorP 3.0 (Sperschneider & Dodds, 2022) to determine their effector probability. All raw reads were submitted to the Sequence Read Archive (NCBI Genbank) with the accession no. PRJNA754834.

Examination of fungus-associated bacteria

The plant and fungal cultures were apparently axenic, with no visible bacterial growth on the cultures, moss tissues, or in the growth media at the time of the experiment. However, bacterial reads were detected unexpectedly in the RNAseq data (Table S2). Recent evidence that *Coniochaeta*, diverse Pezizomycotina, and various Mucoromycota frequently host facultative endohyphal bacteria (EHB; see Heydari *et al.*, 2021; Muszewska *et al.*, 2021) led us to investigate source cultures for evidence of bacterial endosymbionts and to rule out the possibility that contamination had biased interpretation of our results. Although some previous studies have eradicated EHB from fungal cultures via antibiotic treatment, it is plausible that EHB could have survived the antibiotic treatment used here, which was less stringent than that used in previous studies (Hoffman *et al.*, 2013; Arendt *et al.*, 2016). To examine fungal mycelia for EHB and to rule out contamination, we amplified part of the 16S (Hoffman & Arnold, 2010) and used live/dead stain to visualize and confirm the viability of bacteria and hyphae as per Arendt *et al.* (2016) in *Coniochaeta* sp. 2T69 (Methods S3).

Results

Growth and phenotype responses of *D. scoparium* in co-culture with individual fungal endophytes

The eight selected fungal endophytic strains had a wide range of effects on plant growth. None resulted in mortality of *D. scoparium* in the *in vitro* experiment, nor reduced its growth rate significantly during our co-culture experiments. Overall, the results from surface area and volume measurements are congruent (Fig. 2b), and both showed significant differences in terms of responses to different fungal strains (Table S3). According to both surface area and volume measurements, the fungal strain Hyaloscyphaceae sp. 1M05 significantly promoted plant growth (Fig. 2b; Table S3). *Coniochaeta* sp. 2T69 and *Umbelopsis* sp. 3T12 also demonstrated potential plant growth promotion (supported only by volume measurements; Fig. 2b; Table S3). The remaining five fungal strains did not significantly impact plant growth (Fig. 2b). Chl content and electrolyte leakage were not significantly different among different plants inoculated with different fungi or between inoculated and control plants (Figs S2, S3).

These growth responses of inoculated moss individuals cannot be explained readily by the pattern of their metabolic activity across their senescence gradient in nature, nor by the taxonomic affiliation of the fungal inocula (Fig. 1a). Both the pezizomycete *Pithya* sp. (1M06) and eurotiomycete *Exophiala dermatitidis* (3M03) had no effect on moss growth rate. Both fungi had relatively lower metabolic activity than other endophytes in *D. scoparium* in nature, based on metatranscriptomic data (Chen *et al.*, 2018). One sordariomycete, one fungus from the family umbelopsidaceae (Mucoromycota) (both with low activity in *D. scoparium* in nature), and one leotiomycete (with high activity detected in the top photosynthetic layer of *D. scoparium*) increased gametophytic growth (Figs 1a, 2b). The agaricomycetous strain, with high activity in the decomposing (bottom) layer of *D. scoparium* in nature, did not affect the growth rate of the gametophyte.

Two fungi (*Hypoxylon* sp. 1T38 and Hyaloscyphaceae sp. 1M05) produced sporulating structures on *D. scoparium* gametophytes in co-culture experiments (Fig. 2a). Fungal hyphae of *Coniochaeta* sp. 2T69 were observed on the gametophyte *in vitro*, but no sporulating structures were observed (Fig. 2a). We observed gametophyte yellowing (chlorosis) or browning in at least one replicate of all isolates tested here (Figs 2a, S4). Though these fungi were isolated as endophytes in nature, *Umbelopsis* sp. 3T12 formed a dense, easily visible, mycelial net on the surface of gametophytes and induced thickened, denser and smaller gametophytes (Figs 2a, S5). To demonstrate that growth measured for samples covered by fungal mycelia was valid, examples of captured images and the portion measured are provided in Fig. S6.

Comparisons of Hyaloscyphaceae sp. 1M05 and *Coniochaeta* sp. 2T69 functions between living and dead moss tissues

RNAseq analyses focused on two strains that promoted growth in *D. scoparium*. Differentially expressed genes were detected for both fungal strains in living vs dead gametophytes, suggesting that a physiological switch occurred for both strains (Tables 1, S4–S7). Overall, Hyaloscyphaceae sp. 1M05 had far more genes that were differentially transcribed *in planta* vs in dead plant tissue compared with *Coniochaeta* sp. 2T69. Compared with the counts of fungal genes significantly upregulated in Fdp (474), more fungal genes (1282) were upregulated in Flp for 1M05.

Despite the high number of upregulated genes detected for Hyaloscyphaceae sp. 1M05 (Table 1), goseq analyses, which identify enriched GO terms under conditions compared, did not detect any GO term (FDR < 0.05) enriched for either *in planta* or in dead plant tissue. However, *Coniochaeta* sp. 2T69 has 71 and 1 GO term enriched (FDR < 0.05) for Flp and Fdp, respectively (Fig. S7; Table S8). Oxidation–reduction processes (GO:0055114) were upregulated when *Coniochaeta* sp. 2T69 was growing with dead moss tissues. Fourteen of these

Table 1 Numbers of genes differentially expressed by the plant *Dicranum scoparium*, and fungi *Coniochaeta* sp. 2T69 and Hyaloscyphaceae sp. 1M05.

Comparison	Plant/fungal transcriptome	1M05	2T69
	Fungal ref. transcriptome	**1M05**	**2T69**
Flp vs Fdp	Upregulated in Flp	1282	344
Flp vs Fdp	Upregulated in Fdp	474	438
Flp vs Fdp	Nondifferentially expressed	26 603	29 470
	Plant ref. transcriptome	***D. scoparium***	***D. scoparium***
Pwf vs Pc	Upregulated in Pwf	239	32
Pwf vs Pc	Downregulated in Pwf	86	7
Pwf vs Pc	Nondifferentially expressed	22 610	22 896

Plant gene expression was compared between *D. scoparium* inoculated with *Coniochaeta* sp. 2T69 or Hyaloscyphaceae sp. 1M05 (i.e. plant with fungus (Pwf)) and the same moss growing in axenic culture (plant control (Pc)) (Fig. 1c). Fungal expression was compared between the fungus growing with living plant tissue (fungus with living plant (Flp)) and growing with dead plant tissue (fungus with dead plant (Fdp)) (Fig. 1c). False discovery rate < 0.05 and absolute $\log_2$(fold change) > 2.

genes were cytochrome P450 monooxygenases (Fig. 3). Interestingly, one dye-decolorizing peroxidase and four multicopper oxidase genes (including one annotated as Laccase abr2) that are involved in the decomposition of polyphenolic compounds had higher expression when associated with dead plants, suggesting oxidation-based decomposition activity might have been triggered when the fungus is associated with dead plant tissues. Fungal genes with higher expression *in planta* were enriched for GO terms associated with various functions related to carbohydrates, including carbohydrate transport, carbohydrate metabolism, and hydrolysis (Fig. S7; Table S8). Whereas Hyaloscyphaceae sp. 1M05 did not have GO terms enriched in either Fdp or Flp, one GH gene (*GH7*) involved in cellulose decomposition was significantly more expressed when the fungus was with living plants (Flp) (Fig. S8). *Coniochaeta* sp. 2T69 had more transcribed gene families coding for cellulolytic enzymes (GH5, GH6, GH7) compared with Hyaloscyphaceae sp. 1M05 (Fig. S8). Although none of these families were significantly differentially expressed, more genes had slightly higher expression levels in Fdp, and the total expressions (i.e. summed up VST count) of GH7 was marginally higher ($P = 0.07$) when *Coniochaeta* sp. 2T69 was growing with dead plant material (Fdp) (Fig. S8).

When categorizing differentially expressed genes that are relevant to plant–microbe interactions (Fig. 4a), we found that Hyaloscyphaceae sp. 1M05 had more hydrolases, cytochrome P450, and transcription factor genes upregulated in Flp compared to Fdp, which was not the case for *Coniochaeta* sp. 2T69 (Fig. 4a). Notably, Fdp of *Coniochaeta* sp. 2T69 had more polyketide and Cytochrome P450 genes differentially expressed compared to Fdp and Flp of Hyaloscyphaceae sp. 1M05 (Fig. 4a). Regarding predicted effector-like small secreted proteins, Hyaloscyphaceae sp. 1M05 had more effector-like proteins producing genes up-regulated in Flp than in Fdp (Fig. 4b). In contrast, *Coniochaeta* sp. 2T69 had more effector-like protein producing genes up-regulated in Fdp (Fig. 4b).

Metatranscriptomic analysis of *D. scoparium* in co-culture with Hyaloscyphaceae sp. 1M05 or *Coniochaeta* sp. 2T69

Gene expression of *D. scoparium* changed drastically when in co-culture with Hyaloscyphaceae sp. 1M05, with 325 differentially expressed genes (FDR < 0.05) compared with axenic culture (Pc) (Tables 1, S9, S10). By contrast, only 39 differentially expressed genes were detected in *D. scoparium* when in co-culture with *Coniochaeta* sp. 2T69 (FDR < 0.05) compared with Pc (Table 1). Cupin genes were up or downregulated in *D. scoparium* when inoculated with either fungus (Fig. 5). Of the 32 *D. scoparium* genes that had higher expression when in co-culture with *Coniochaeta* sp. 2T69 (Table 1), 17 also were upregulated in *D. scoparium* colonized by Hyaloscyphaceae sp. 1M05 (Fig. 6a). These shared *D. scoparium* gene sets include genes with hydrolysis activity (e.g. endoglucanase and pectinesterase) as well as receptor kinase ability (leucine-rich repeat (LRR) and lectin-domain-containing receptor-like kinase) (Fig. 6b).

Two of the seven genes of *D. scoparium* having lower expression when in co-culture with *Coniochaeta* sp. 2T69 also had suppressed expression when in co-culture with Hyaloscyphaceae sp. 1M05 (Fig. 6a). These two genes encode for cupin (oxalate oxidase) and mitochondrial phosphate carrier protein (Fig. 6b). Of the *D. scoparium* genes only detected to be differentially expressed (222 upregulated, 84 downregulated) when in co-culture with Hyaloscyphaceae sp. 1M05 (Fig. 6a), some are related to defense, such as the ferric reductase and LURP-one-related protein genes (Fig. 5). Hyalosycphaceae sp. 1M05 also up and downregulated many growth-related genes, including auxin response and auxin transporter genes (Fig. 5). Other genes related to growth, such as the repetitive proline rich cell wall protein relevant to cell wall organization, and one leucine-rich repeat receptor related to gametophyte development, were also highly expressed in *D. scoparium* in co-culture with Hyaloscyphaceae sp. 1M05 (Fig. 5).

Fungus-associated bacteria

BLAST analyses of bacterial 16S rRNA amplified from fungal cultures suggested the presence of *Paenibacillus* (Paenibacillaceae, Firmicutes) in all eight fungal isolates (Table S11). Phylogenetic analysis revealed that the 16S rRNA sequences obtained from four focal fungi (Fig. S9) are part of a well-supported lineage of EHB detected from isolates obtained about one decade earlier in surveys of endophytes in North Carolina and Arizona (Clade A). These EHB occurred in diverse fungi, including *Coniochaeta* and members of the Pezizomycotina lineages included here (other Sordariomycetes; Dothideomycetes) (Shaffer *et al.*, 2016). A second clade, without strong support, also includes *Paenibacillus* from this study (Clade B, Fig. S9). When examining hyphae of *Coniochaeta* sp. 2T69, we detected living, rod-shaped, bacteria in association with living hyphae (Fig. S10). Imaging suggested that the bacteria often occurred outside of the hyphae, consistent with our observation that facultative EHB of *Coniochaeta* spp. often emerge from hyphae and live extrahyphally as mycelia age or experience stress (see also Heydari *et al.*, 2021). Overall, our evaluation suggests that there was no laboratory contamination by

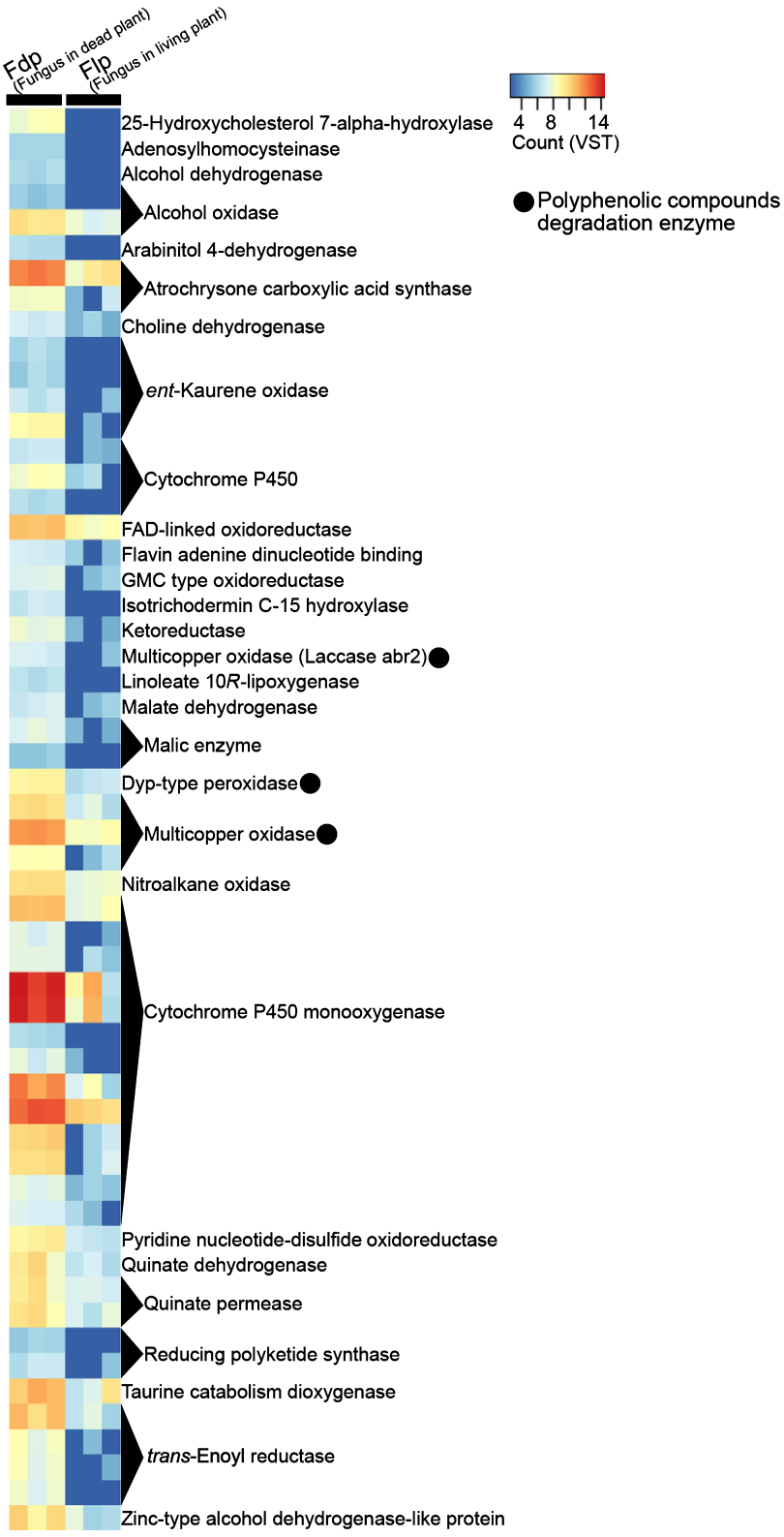

Fig. 3 Heatmap of the significantly (FDR < 0.05) differentially expressed genes belonging to the top enriched (FDR < 0.05) Gene Ontology term (GO:0055114, oxidation–reduction processes) detected in *Coniochaeta* sp. 2T69 in association with dead *Dicranum scoparium* (Fdp, fungus with dead plant). Names of genes are shown only when known. Dyp, dye-decolorizing peroxidase; FAD, flavin adenine dinucleotide; FDR, false discovery rate; GMC, glucose–methanol–choline; VST, variance stabilizing transformation.

bacteria. Therefore, bacterial reads detected with RNAseq likely represent facultative EHB, which we assume are part of the extended phenotype intrinsic to the fungal treatments. Because prokaryotic transcripts were selected against, the detected bacterial reads likely resulted from leakage and, therefore, not suitable for further analysis.

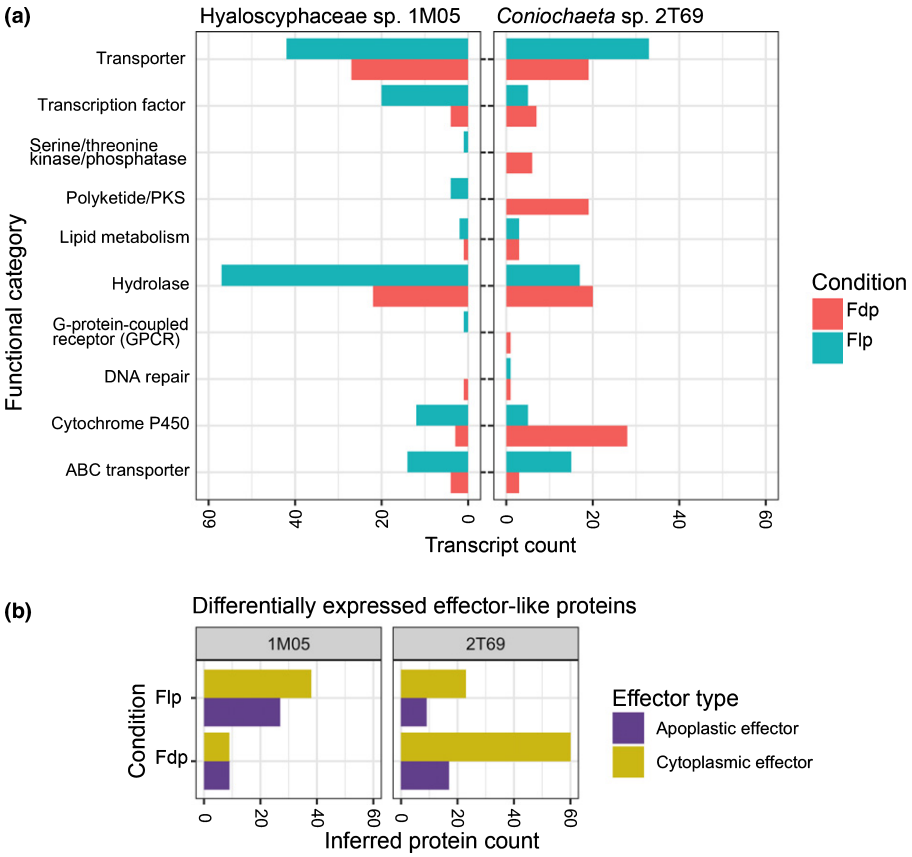

Fig. 4 Differentially expressed fungal genes assigned to functional category. (a) The number of genes in different functional categories. (b) The number of proteins predicted to be cytoplasmic and apoplastic effector-like proteins (EffectorP 3.0, probably > 50%). Fdp, fungus with dead plant; Flp, fungus with living plant; PKS, polyketide synthase.

Discussion

This study on naturally occurring fungal endophytes of *D. scoparium* in individual co-culture (*in vitro*) experiments with the same moss species revealed that neither the activity of these endophytic fungi in nature nor their taxonomic affiliation was predictive of their effect on plant growth *in vitro*. However, the endophytic fungal isolate that had the highest metabolic activity compared with the other tested isolates in gametophytes in nature (Hyaloscyphaceae sp. 1M05, based on rRNA expression levels; Fig. 1a) invoked a larger transcriptomic response in the host. The other fungal strain that was common in growing tissue in nature, but had low metabolic activity under natural conditions (*Coniochaeta* sp. 2T69), revealed an onset of oxidation-based decomposition mechanisms when the fungus was cultivated on dead plant material, consistent with the long-standing hypothesis that aboveground fungal endophytes can switch to saprotrophism when the host is dead (Promputtha *et al.*, 2007; Kuo *et al.*, 2014; Zhou *et al.*, 2018). In addition to occurring as endophytes and saprotrophs, some species of *Coniochaeta* are associated with wood necrosis (Damm *et al.*, 2010). Our study further highlights the ecological versatility of that lineage.

Endophyte-mediated effects on plant phenotypes

Fungal endophytes can have a variety of effects on plant hosts, ranging from beneficial to detrimental (Rodriguez *et al.*, 2009). Such interactions are better known in selected vascular plants but have been demonstrated recently for the liverwort *Marchantia polymorpha* (Nelson *et al.*, 2018). None of the eight fungal isolates tested killed the moss *D. scoparium* by the end of the experiment (i.e. 60 d after the initial contact of the fungus with the plant), consistent with their isolation from asymptomatic tissue of this plant species in nature (Chen *et al.*, 2018). *Umbelopsis* sp. 3T12 caused *D. scoparium* gametophytes to become thick and dense (Figs 2, S5), which might be similar to a phenotypic change caused by drought (Rowntree *et al.*, 2007; Roberts *et al.*, 2012; Berry *et al.*, 2016) or may reflect production of plant hormones associated with growth (Hoffman *et al.*, 2013). Some inoculated gametophytes showed signs of chlorosis (Fig. 2). Despite the unchanged to slightly increased growth rate of the moss after inoculation, these fungi might have triggered immune system responses such as oxalate oxidase (encoded by cupin genes) (Hu *et al.*, 2003), or chlorophyll degradation (e.g. caused by chlorophyllase) (Fig. 5) (Hu *et al.*, 2015; Shen *et al.*, 2017; Kretschmer *et al.*, 2019), which could cause chlorosis in plants. However, unaltered chlorophyll content and negligible electrolyte leakage (Figs S2, S3) suggest limited effects of these fungi on photosynthesis and minimal physical damages to the moss (Demidchik *et al.*, 2014).

The Sordariomycetes included in this study (*Coniochaeta* sp. 2T69 and *Hypoxylon* sp. 1T38) had low metabolic activities in naturally occurring *D. scoparium* (Chen *et al.*, 2018). However, in our *in vitro* experiment, we detected major stimulatory

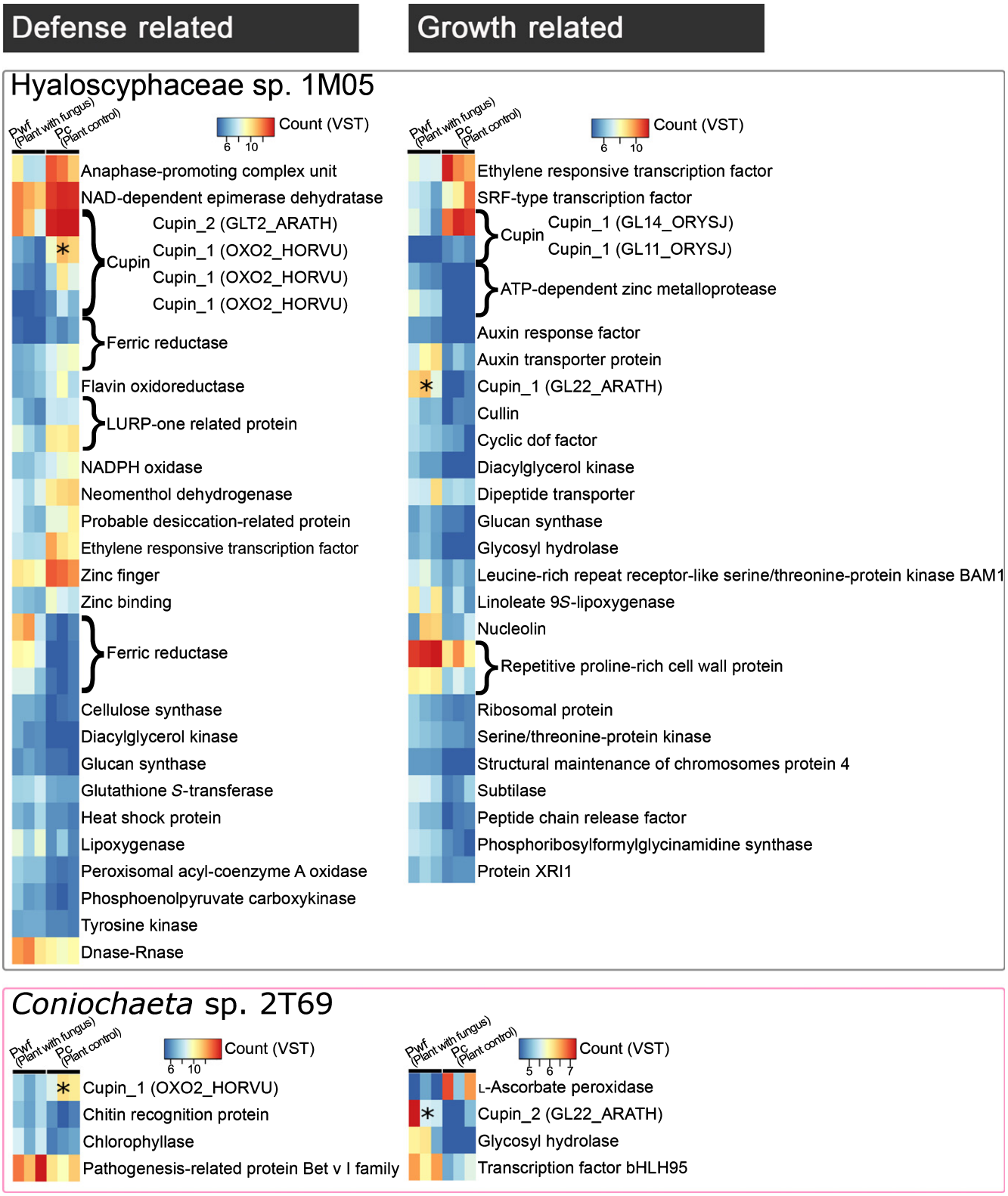

Fig. 5 Heatmaps of significantly differentially expressed *Dicranum scoparium* genes associated with defense or growth when grown in co-culture with Hyaloscyphaceae sp. 1M05 or *Coniochaeta* sp. 2T69 (Pwf, plant with fungus) vs when grown axenically (Pc, plant control). Asterisks indicate cupin (oxalate oxidase), which was detected for both functions and both co-culture experiments. VST, variance stabilizing transformation.

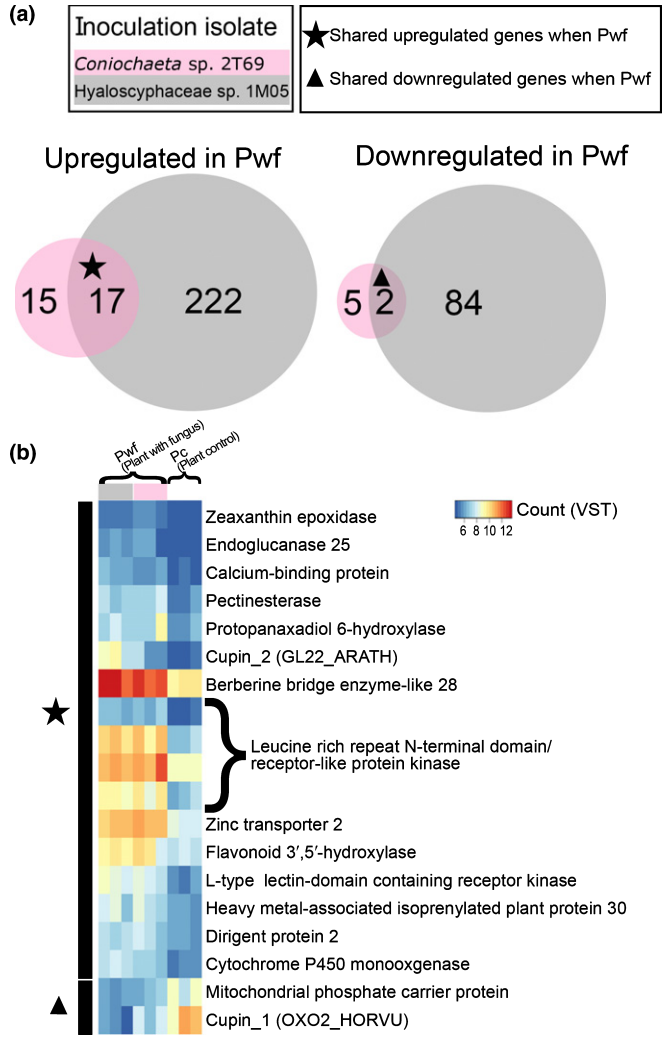

Fig. 6 (a) Venn diagrams showing the number of differentially expressed genes in *Dicranum scoparium* shared when in co-culture with two different fungal strains (1M05 and 2T69). (b) Heatmap showing the 19 differentially expressed genes in *D. scoparium* shared when in co-culture with two different fungal strains (1M05 and 2T69). VST, variance stabilizing transformation.

effects of these fungi on the growth of *D. scoparium*. It is plausible that growth induction by these fungi was promoted by the experimental design, or that the timeframe of this experiment captured growth promotion that could not be observed in our previous study (Chen *et al.*, 2018). More broadly, Sordariomycetes, and genera within that lineage, such as *Coniochaeta*, are commonly isolated from various hosts in temperate and boreal zones (U'Ren *et al.*, 2010, 2012; Rosa *et al.*, 2013). Some strains of *Coniochaeta* have been observed to promote plant growth in other systems, such as in grass (Challacombe *et al.*, 2019; Vázquez de Aldana *et al.*, 2021). Our results provide evidence that the growth-promoting ability of *Coniochaeta* could be widespread among plants, including mosses. Our result suggests that these fungal endophytes with low levels of rRNA expression in nature can also have a major effect on moss growth rates *in vitro* (Fig. 2).

Production of fungal sporulating structures on *D. scoparium*

An inoculation experiment conducted by Davey *et al.* (2010) revealed that *Coniochaeta velutina* produced sporulating structures on the moss *Funaria hygrometrica*. The ability to form sporulating structures on mosses, observed here for *Hypoxylon* sp. 1T38 and Hyaloscyphaceae sp. 1M05 (Fig. 2a), is consistent with the capacity of some fungal endophytes to sporulate on plants under particular environmental conditions, thus completing their life cycle as endophytes; that is, not requiring a transition to saprotrophism. Because bryophytes host species-rich endophytic fungal communities (U'Ren *et al.*, 2010), it has been hypothesized that mosses are reservoirs for fungal endophytes of other plants or lichens (Davey & Currah, 2006; Davey *et al.*, 2010). For example, it is now well established that mosses and lichens share many fungal endophytes (U'Ren *et al.*, 2010, 2012, 2019).

Fungal transcriptomes of Hyaloscyphaceae sp. 1M05 and *Coniochaeta* sp. 2T69 on living vs dead gametophytes

Significant differences in gene expression were found when the same fungus was colonizing dead vs living plant gametophytes (Table 1). This was true for both isolates tested here, suggesting that these endophytic fungi have the necessary genetic makeup to switch between endophytism and saprotrophism.

More Hyaloscyphaceae sp. 1M05 genes were upregulated *in planta* (Fig. 4; Table 1), reflecting that this fungus was more active in the top, photosynthetic proportion of naturally occurring *D. scoparium* (Fig. 1). *Coniochaeta* sp. 2T69 activated oxidation-related genes, such as cytochrome P450 monooxygenase (Fig. 3), when in association with dead plant material. Cytochrome P450 monooxygenases have diverse functions and could be involved in toxin production or degradation, but could also be involved in active decomposition of dead plant material for nutrients (Suzuki *et al.*, 2012; Shah, 2014). Notably, genes involved in oxidative decomposition mechanisms related to lignin degradation had higher expression in that fungal strain when colonizing dead moss tissues. Though mosses do not have lignin, lignin-like compounds and a phenol-rich cuticle are present (Roberts *et al.*, 2012; Renault *et al.*, 2017). Therefore, the activated peroxidase and multicopper oxidase suggested the onset of specialized oxidation-based decomposition mechanisms that are likely able to degrade tough, nonhydrolyzable plant biopolymers.

When associated with a living plant, carbohydrate-related GO terms, including carbohydrate transporters, were highly expressed in *Coniochaeta* sp. 2T69 (Fig. S7), suggesting that the fungus is taking up photosynthates from the plant as a source of carbon. Several hydrolases were also expressed at higher levels in the endophytic strain when growing in association with living plants *in vitro* (Flp) (Fig. 4). Previous studies on mycorrhizal fungi also revealed hydrolase activities, which allow modification of plant cell development at the interface of mycorrhizal formation (Kohler *et al.*, 2015). Endophytes generally do not have specialized structures within plants other than hyphae. Hydrolase

activity might be related to hyphal extension inside plant tissues or involved in degradation of plant tissues and facilitate nutrient release. In *Coniochaeta* sp. 2T69, though the *in planta* gene expression indicated hydrolysis activity, the dead plant tissues seemed to stimulate more vigorous decomposition activity via oxidation-based mechanisms. Our results highlight the different strategies and interactions endophytic fungi engage in when associated with the same plant but in two different physiological states; that is, living vs dead (Fig. 7). Enzyme assays may provide additional information regarding the enzymatic activity of fungi in association with living vs dead plant tissues (Talbot *et al.*, 2014; Borstlap *et al.*, 2019). Interestingly, *Coniochaeta* sp. 2T69 had more effector-like proteins upregulated in Fdp compared with Hyaloscyphaceae sp. 1M05 (Fig. 4b). The greater upregulation of effector-like proteins in Fdp is in line with previous studies that necrotic fungi encode for higher proportions of effectors (Kim *et al.*, 2016) and that effector-like proteins can be involved in wood degradation (Feldman *et al.*, 2020). Together with the upregulated oxidation-based degradation activity of *Coniochaeta* sp. 2T69 in association with dead plant tissue (Fdp) (Figs 3, S7), the higher expression of effector-like proteins in Fdp further supports its trophic transition to saprotrophism. Instead of producing apoplastic effectors (Fig. 4), which are proteins that act at the interface between plant host and fungi, the high number of cytoplasmic effectors in *Coniochaeta* sp. 2T69 Fdp may target different plant compartments (Wang *et al.*, 2017). The importance of cytoplasmic effectors in saprotrophic activity is yet to be elucidated.

This experimental co-culture system was different from the natural occurring *D. scoparium* in several aspects. First, whereas all fungal isolates were isolated as endophytes from *D. scoparium* in nature, we did not visually confirm their ability to grow inside moss tissues *in vitro*. Moreover, in co-culture, the presence of the fungus was often visible on the moss and caused visible phenotypic symptoms (Fig. 2), which goes against the asymptomatic core principle defining fungal endophytes. Second, the dead moss materials were prepared using a flash freezing treatment. Though such treatment killed the moss, the chemical component of the dead tissues was likely more similar to living mosses than to naturally senescing or decomposing parts of the gametophytes. Finally, unlike in nature, the co-culture system only included one fungus rather than multiple microorganisms simultaneously interacting with the host.

Plant transcriptomic response to co-culture with Hyaloscyphaceae sp. 1M05 or *Coniochaeta* sp. 2T69

Compared with *D. scoparium* in co-culture with Hyaloscyphaceae sp. 1M05, the same moss inoculated with *Coniochaeta* sp. 2T69 showed an inconspicuous transcriptomic response. This might reflect the nature of the two isolates in the environment. When these two strains were isolated and detected in the naturally occurring *D. scoparium* gametophytes, *Coniochaeta* sp. 2T69 had almost no activity detectable at the DNA/RNA level (Fig. 1a) but was isolated multiple times when using a culture-based method (Chen *et al.*, 2018). By contrast, Hyaloscyphaceae sp. 1M05 was one of the highly biologically active fungi detected in *D. scoparium* gametophytes (Fig. 1a).

Despite the drastically different transcriptional response of *D. scoparium* to these two fungal isolates, some of the differentially regulated genes were the same when inoculated with different fungal strains. Upregulation of cupin genes in the presence of fungi (Pwf) can be explained by their functions in plant defense or development (Dunwell *et al.*, 2004). The downregulated genes with (Pwf) and without fungi (Pc) might be explained by response to stress (Dunwell *et al.*, 2004). The diverse cupins detected here surely reflect their importance in moss responses to fungi. Given the broad array of functions different cupin genes can have collectively, more research on cupin genes is needed to better understand moss–fungus interactions (Nakata *et al.*, 2004).

Several LRR receptors were upregulated exclusively in the inoculated moss (Pwf) (Fig. 5). Certain LRR genes are absent from algal genomes, suggesting that they might have played important roles in helping early embryophytes to defend

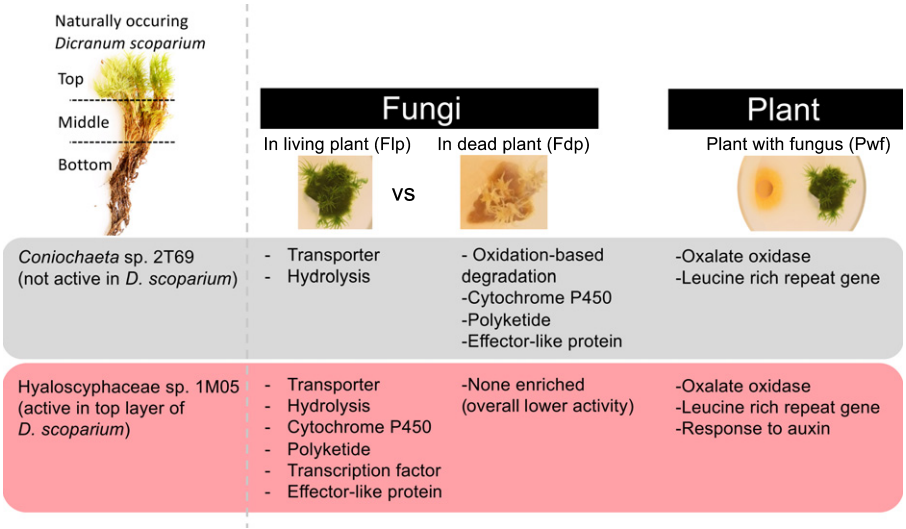

Fig. 7 Schematic summary of the interaction of *Dicranum scoparium* with two endophytic fungi (*Coniochaeta* sp. 2T69 and Hyaloscyphaceae sp. 1M05). Fdp, fungus with dead plant; Flp, fungus with living plant.

themselves from terrestrial pathogens (Sarris *et al.*, 2016). Many LRRs are also involved in the control of plant growth (Diévart & Clark, 2004). The upregulation of LRR genes in *D. scoparium* in the presence of fungal endophytes might be relevant for their growth-promoting ability.

Several genes related to defense were only upregulated in the *Coniochaeta* sp. 2T69 inoculated plants, including a chitin recognition protein and a chlorophyllase, suggesting a common defense mechanism utilized by mosses (Hu *et al.*, 2015; Ali *et al.*, 2018). In the Hyaloscyphaceae sp. 1M05 inoculated moss, several ferric-reductase genes relevant to defense were likely triggered by microbial siderophores (Fig. 5) (Aznar *et al.*, 2014).

Paenibacillus associated with moss inhabiting fungal endophytes

Through the live/dead stain technique, we demonstrated that *Paenibacillus* was externally associated with the hyphae of *Coniochaeta* sp. 2T69 and were likely co-isolated along with the fungus during fungal culture isolation (Fig. S10). A detailed study using fluorescence *in situ* hybridization would enhance our understanding of these fungal-associated bacteria (Bertaux *et al.*, 2003). More strains need to be examined to confirm the physical association of members of this clade with fungal hyphae. Sequences of different loci for these *Paenibacillus* will be required to confirm the phylogenetic placement of these fungal endophyte-associated isolates. The plant response observed here might reflect combined effects of endophytic fungi and bacteria (Hoffman & Arnold, 2010), consistent with the observation that various *Paenibacillus* occur frequently as facultative symbionts within hyphae of endophytic fungi (Hoffman & Arnold, 2010; Shaffer *et al.*, 2016; Heydari *et al.*, 2021). With specific antibiotic treatments, these bacteria associated with, or living within, fungal endophytes could be removed, creating opportunities for future experiments that can distinguish fungal and bacterial effects on plants (Arendt *et al.*, 2016; Uehling *et al.*, 2017).

Conclusion

This study demonstrates the advantages of using fungi originally isolated from the same moss species (*D. scoparium*) for *in vitro* experiments deriving phenotypic and transcriptomic data to better understand fungus–plant interactions. Most moss–fungus interactions examined to date have focused on parasitic, pathogenic, or saprotrophic lifestyles (Redhead, 1981; Davey & Currah, 2006; Field *et al.*, 2015), yet bryophytes host diverse communities of fungal endophytes (commensals or mutualists; U'Ren *et al.*, 2010, 2012, 2019). One fungus from this study seems to take up carbohydrates from *D. scoparium* (Fig. S7), yet these fungal isolates caused unchanged to slightly increased plant growth rates. The upregulation of genes that are a part of the plant defense system might aid in plant defense from pathogens, but such effect awaits experimental investigation. The inclusion of potential plant pathogens, as well as the manipulation of nutrients in the media of co-culture experiments, and inclusion/exclusion of fungal endophyte-associated bacteria will be critical in determining moss–fungus interactions more comprehensively.

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Author contributions

K-HC, FL, H-LL designed the experiment. K-HC, SHW and AEA performed analyses. HBK and PBM established the axenic *D. scoparium* culture. K-HC and FL wrote the manuscript. All authors contributed to editing the manuscript.

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Data availability

Short read data generated in this study were deposited in the NCBI Sequence Read Archive with the accession no. PRJNA754834. A total of 33 sequences generated by Sanger sequencing were submitted to GenBank: OM758169–OM758180, OM669555–OM669564, OM574624–OM574629, and OM570291–OM570295.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Reference library preparation workflow for *Dicranum scoparium* and the fungal strains 2T69 and 1M05 in pure culture.

Fig. S2 Chl content of control and inoculated (*Coniochaeta* sp. 2T69 and Hyaloscyphaceae sp. 1M05) *Dicranum scoparium*.

Fig. S3 Electrolyte leakage measured on day 10 and day 17 after inoculation.

Fig. S4 Top-down view of moss inoculated by the eight selected endophytic strains.

Fig. S5 Dense mycelial net and thickened gametophytes observed in *Dicranum scoparium* inoculated with *Umbelopsis* sp. 3T12.

Fig. S6 Demonstration of image capture for moss samples overgrown with fungal mycelia.

Fig. S7 Significantly (FDR < 0.05) enriched GO terms in *Coniochaeta* sp. 2T69 (Fdp vs Flp).

Fig. S8 Gene expressions of glycoside hydrolase (GH) family 5, 6, and 7 found in Hyaloscyphaceae sp. 1M05 and *Coniochaeta* sp. 2T69.

Fig. S9 Phylogenetic placements of endohyphal bacteria (EHB) detected in all eight fungal isolates used for this study.

Fig. S10 Live/dead stain shows free-living bacteria among hyphae of *Coniochaeta* sp. 2T69.

Methods S1 Identification of fungal cultures.

Methods S2 Measurement of Chl content and electrolyte leakage.

Methods S3 Examination of fungus-associated bacteria.

Table S1 Sequence information for the eight isolates chosen for this study.

Table S2 Sequencing report of RNAseq results across samples.

Table S3 ANOVA table for surface area and volume fold change comparison.

Table S4 Information and sequences of the differentially expressed genes of Hyaloscyphaceae sp. 1M05 between fungus on dead plant (Fdp) and fungus on living plant (Flp).

Table S5 Annotation of the differentially expressed genes of Hyaloscyphaceae sp. 1M05 between fungus on dead plant (Fdp) and fungus on living plant (Flp).

Table S6 Information and sequences of the differentially expressed genes of *Coniochaeta* sp. 2T69 between fungus on dead plant (Fdp) and fungus on living plant (Flp).

Table S7 Annotation of the differentially expressed genes of *Coniochaeta* sp. 2T69 between fungus on dead plant (Fdp) and fungus on living plant (Flp).

Table S8 GOSEQ enrichment for fungus on living plant (Flp)/fungus on dead plant (Fdp) comparison.

Table S9 Information and sequences of the differentially expressed genes of the moss *Dicranum scoparium* between inoculated vs control plants.

Table S10 Annotation of the differentially expressed genes of the moss *Dicranum scoparium* between inoculated vs control plants.

Table S11 BLAST results of *Paenibacillus* amplified from focal endophytic fungi.

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