***New Phytologist* Supporting Information**

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

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The following Supporting Information is available for this article:

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

Fig. S2 Chlorophyll 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.

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Fig. S6 Demonstration of image capture for moss samples overgrown with fungal mycelia (Example sample = 3T12_6).

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

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Supplementary Figures:

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

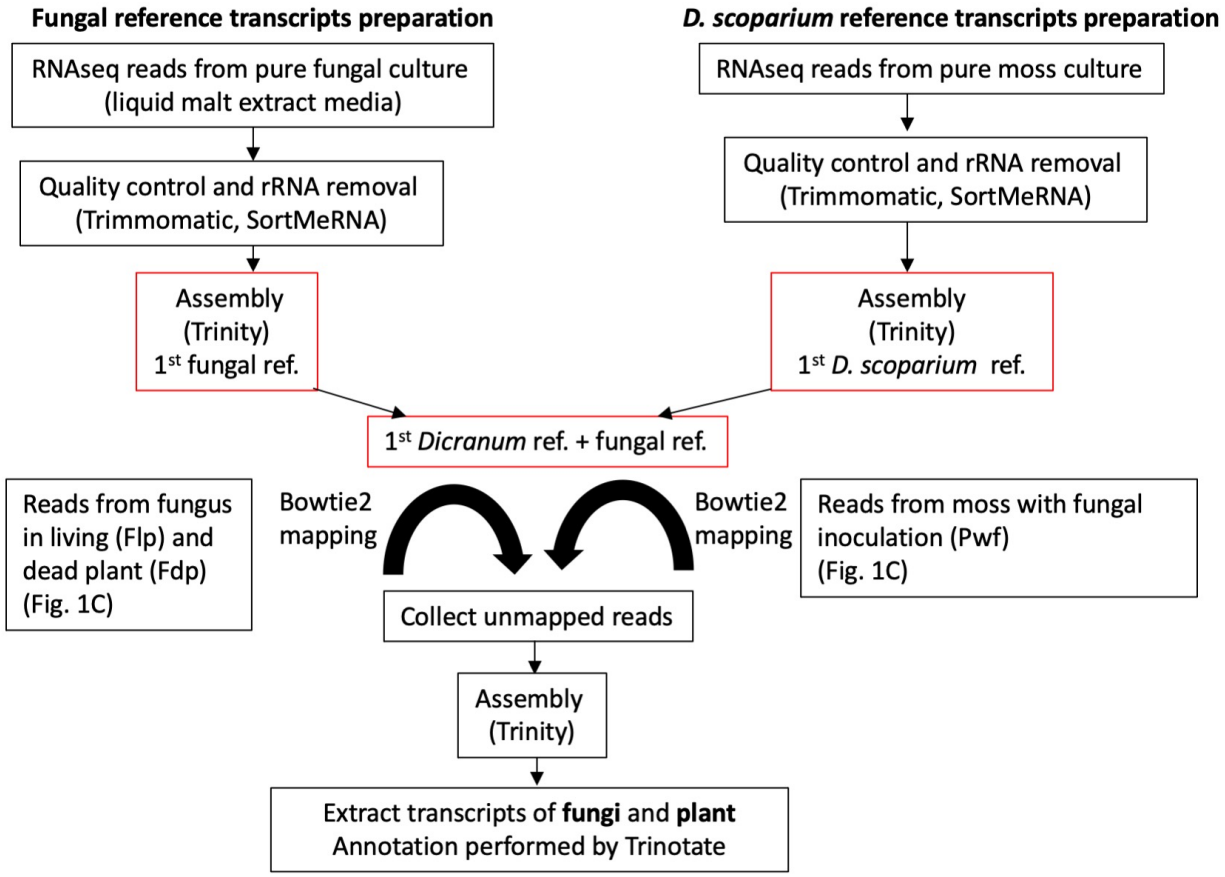


Fig. S2. Chlorophyll content of control and inoculated (*Coniochaeta* sp. 2T69 or Hyaloscyphaceae sp. 1M05) *Dicranum scoparium.* Chlorophyll data were collected at a weekly interval over 16 weeks. One control plant showed signs of contamination at week 9 and was not measured for the remaining weeks. Due to the small sample size, these time points are binned into 4 categories. Each line corresponds to one plant-fungal co-culture sample. Two-way ANOVA was performed on two sub-datasets, and none of the factors showed significant results. Number after the underscore following the strain name indicates the replication code, which corresponds to an independent *D. scoparium* plant in a separate jar.

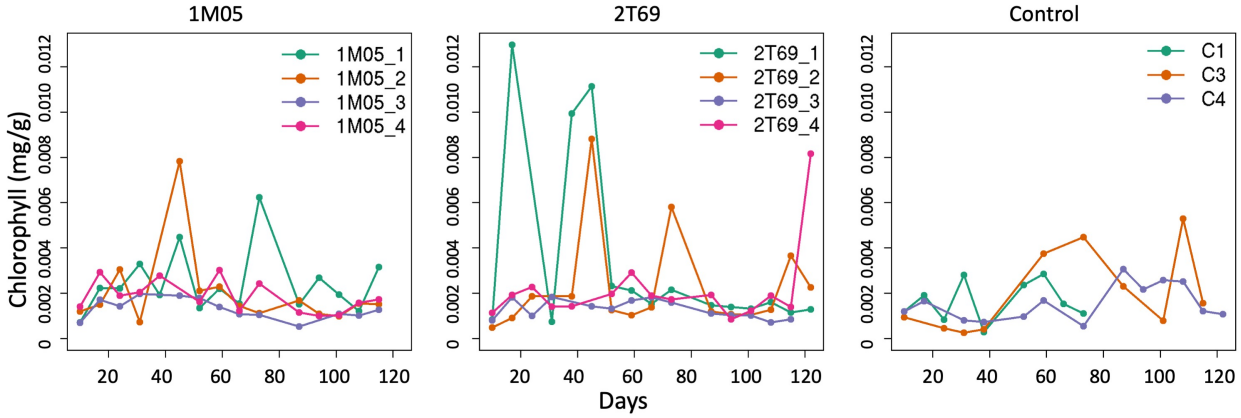


Fig. S3. Electrolyte leakage measured on Day 10 and Day 17 after inoculation. IC = Initial conductivity, TC = Total conductivity. No significant differences were observed at Day 10 or Day 17 (Dunnett's tests for comparisons of the control with each treatment, $p>0.05$). Treatment C = control. The thick line represents the median value in the dataset, the grey box includes both upper and lower quartile. The whisker extended to the maximum and minimum value in the dataset within 1.5 times the inter-quartile range from the box. Circles represent individual data points.

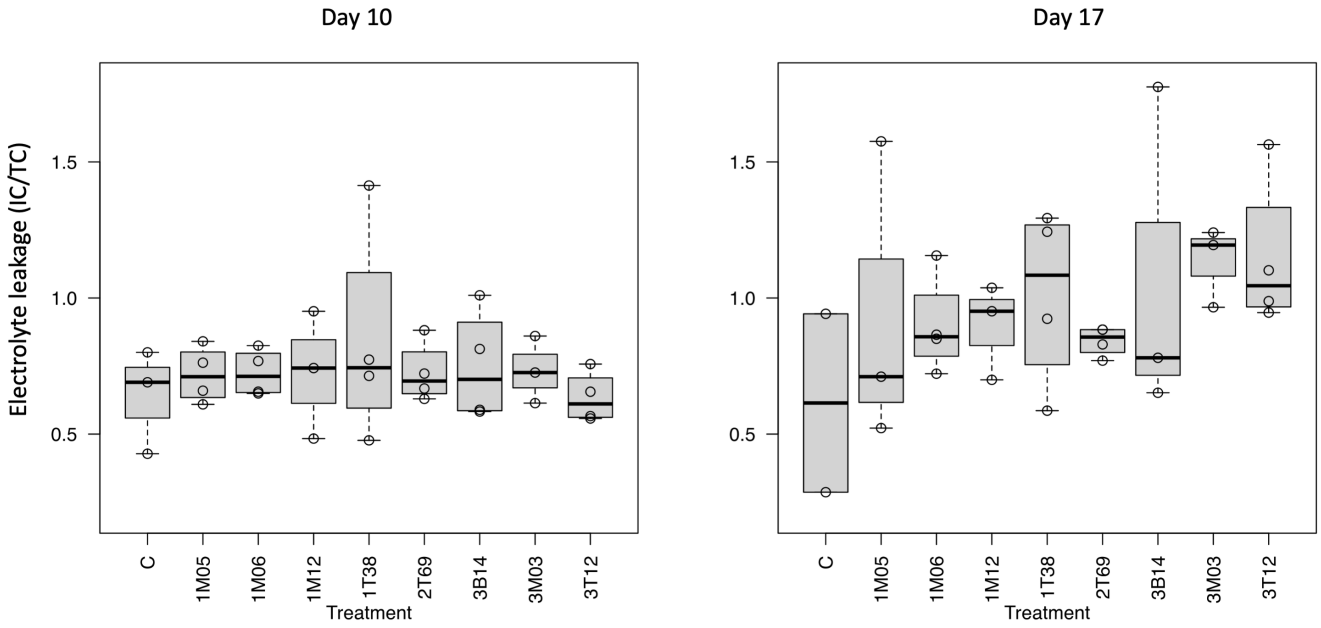


Fig. S4. Top-down view of moss inoculated by the eight selected endophytic strains. Only one replicate is shown per treatment. Day 1 was defined as the day when the fungal mycelium physically touched the moss. Number after the underscore following the strain name indicates the replication code, which corresponds to an independent *D. scoparium* plant in a separate jar.

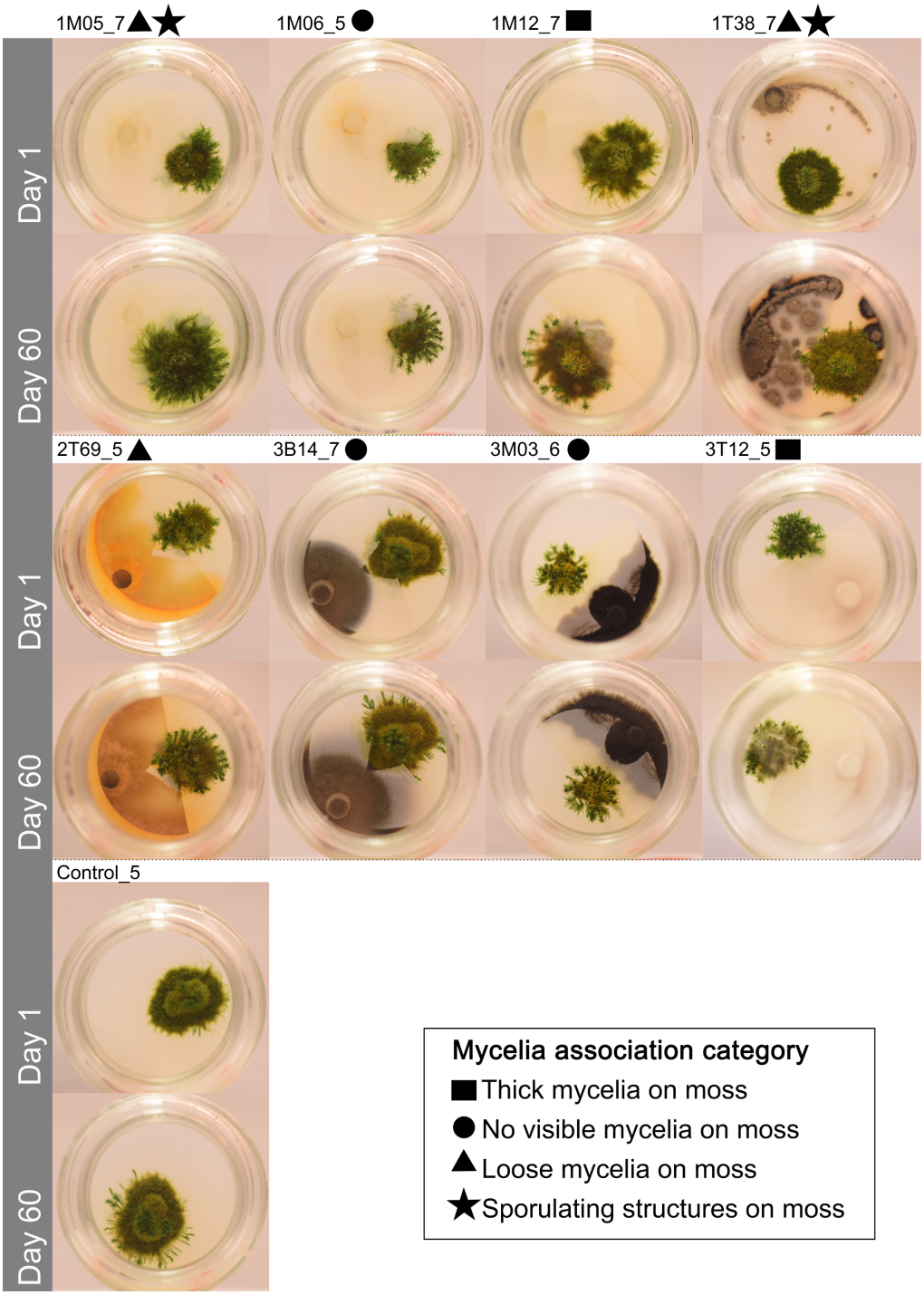


Fig. S5. Dense mycelial net and thickened gametophytes observed in *D. scoparium* inoculated with *Umbelopsis* sp. 3T12. Number after the underscore indicates the replication code, which corresponds to an independent *D. scoparium* plant in a separate jar. The pictures were taken 60 days after the mycelium reached the plant. Black arrow: dense, thickened gametophyte.

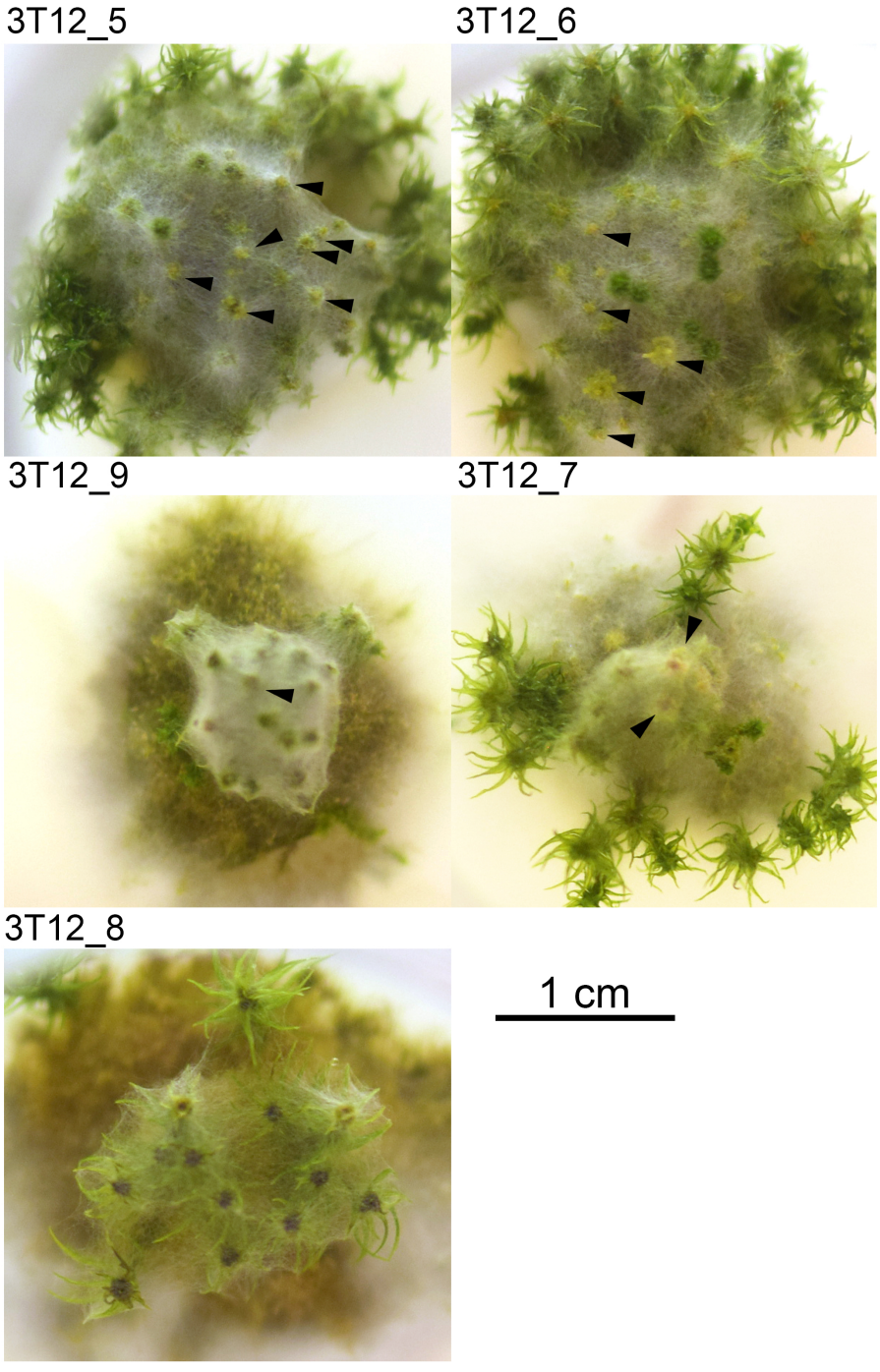


Fig. S6. Demonstration of image capture for moss samples overgrown with fungal mycelia (Example sample = 3T12_6). (**a**) Top-down view of moss area selected with magic wand in Adobe Photoshop. The surface area was measured by Adobe Photoshop. (**b**) Volume captured despite the overgrown fungal mycelium (showing one of ten pictures taken on turntable). This demo picture was captured automatically with DIRT (Das *et al.*, 2015), a successor program of GiARoot, which was used for this study. No significant difference was detected between these two programs when measuring volumes of *Dicranum* gametophytes.

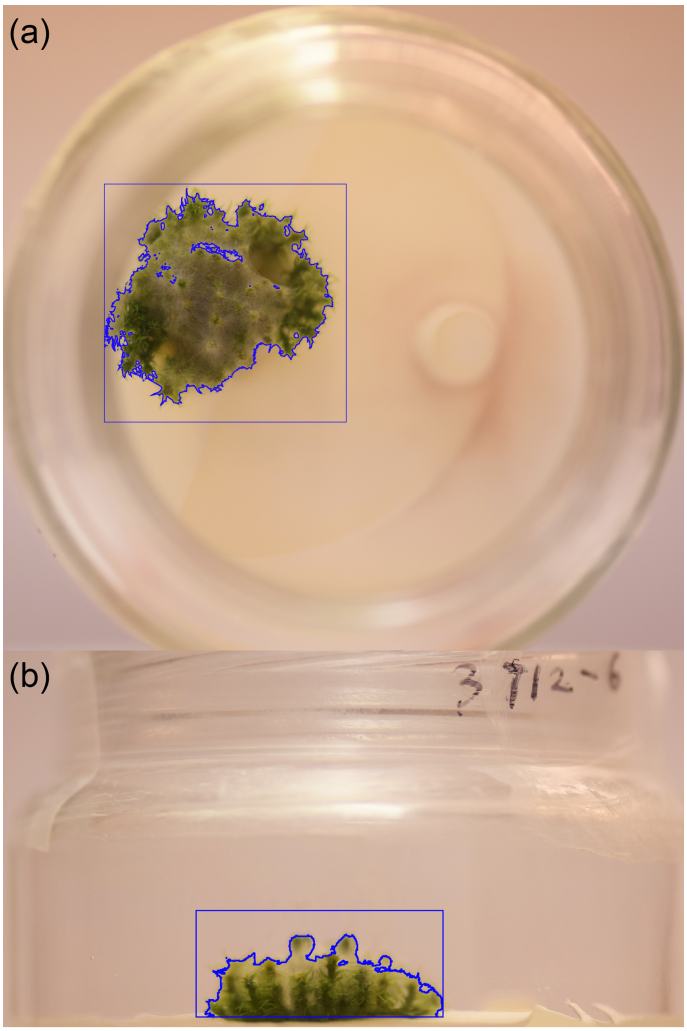


Fig. S7. Significantly (FDR < 0.05) enriched GO terms in *Coniochaeta* sp. 2T69 (Fdp vs. Flp). Fdp = Fungus on dead plant; Flp = Fungus on living plant.

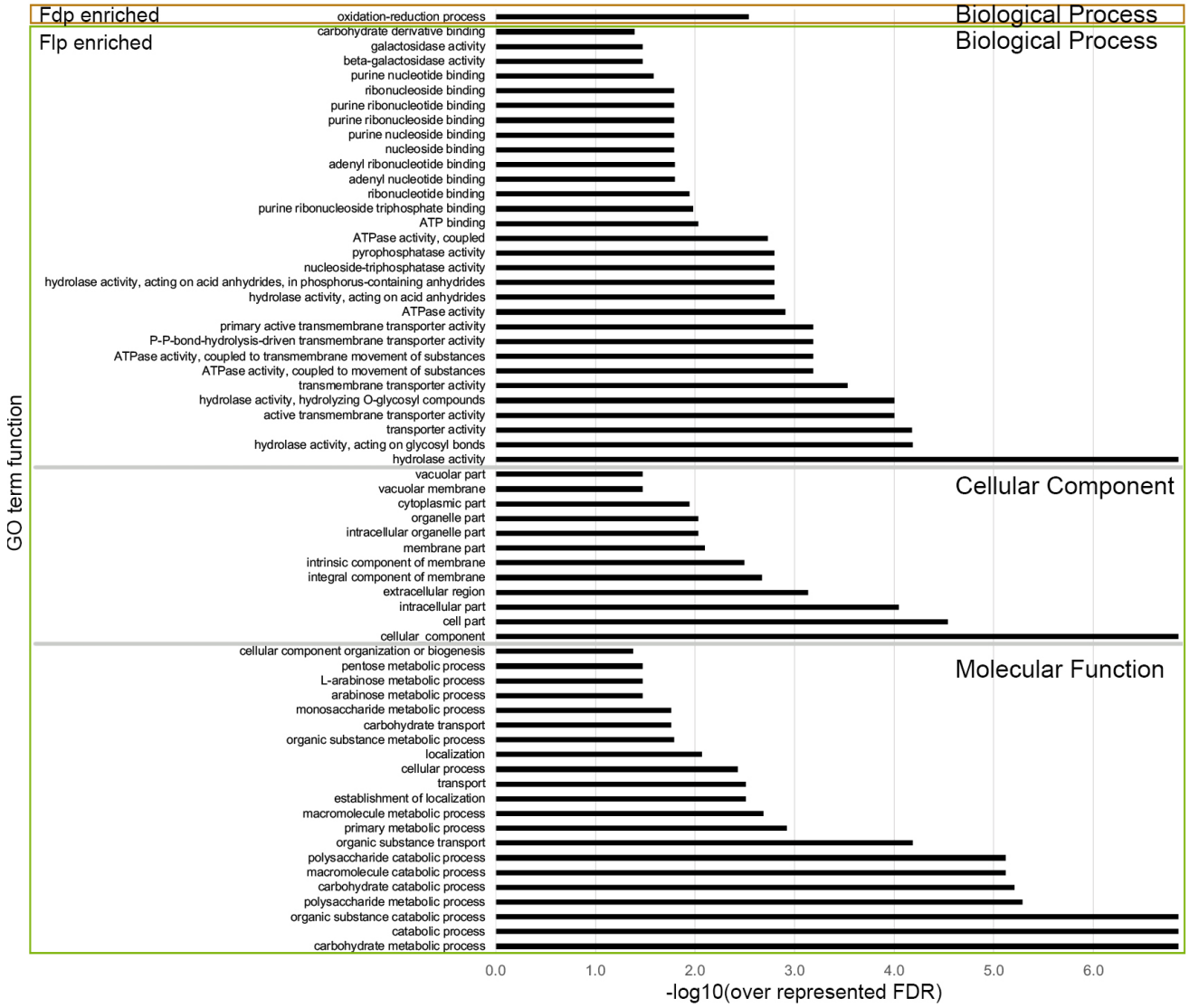


Fig. S8. Gene expressions of Glycoside Hydrolase (GH) family 5, 6, and 7 found in Hyaloscyphaceae sp. 1M05 and *Coniochaeta* sp. 2T69. (**a, c**) Volcano plot of gene expression. n = number of genes. Fdp = Fungus with dead plant; Flp = Fungus with living plant. (**b, d**) Summed count (VST=Variance Stabilizing Transformation) for each GH family per sample. The thick line represents the median value in the dataset, the box includes both upper and lower quartile. The whisker extended to the maximum and minimum value in the dataset within 1.5 times the inter-quartile range from the box.

* = p < 0.05, • = 0.1 (Welch's two-sample t-test).

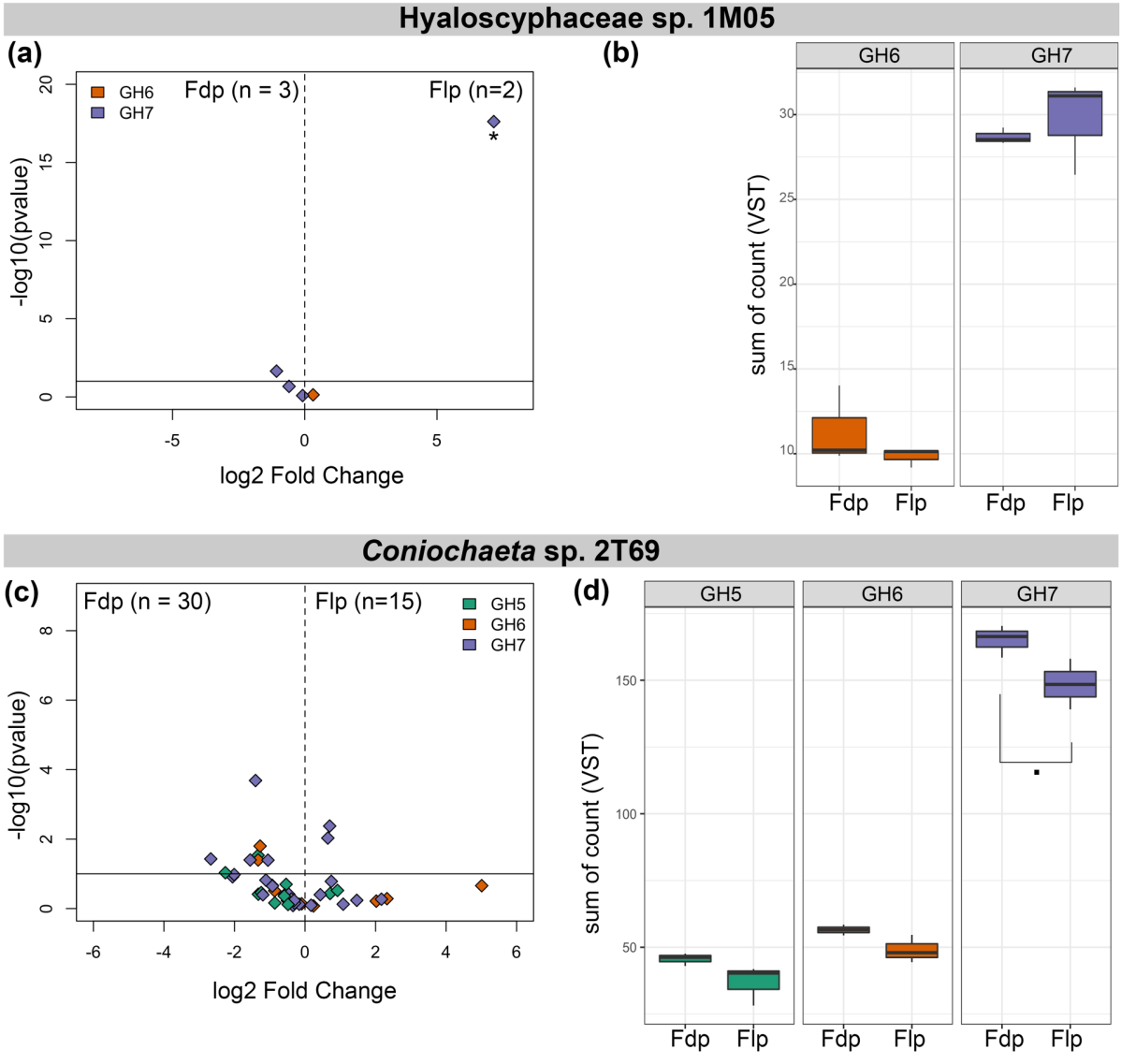


Fig. S9. Phylogenetic placement of endohyphal bacteria (EHB) detected in all eight fungal isolates used for this study. Numbers associated with internodes are bootstrap values obtained with Maximum Likelihood. Only bootstrap support > 70 is shown. Bold = strain name.

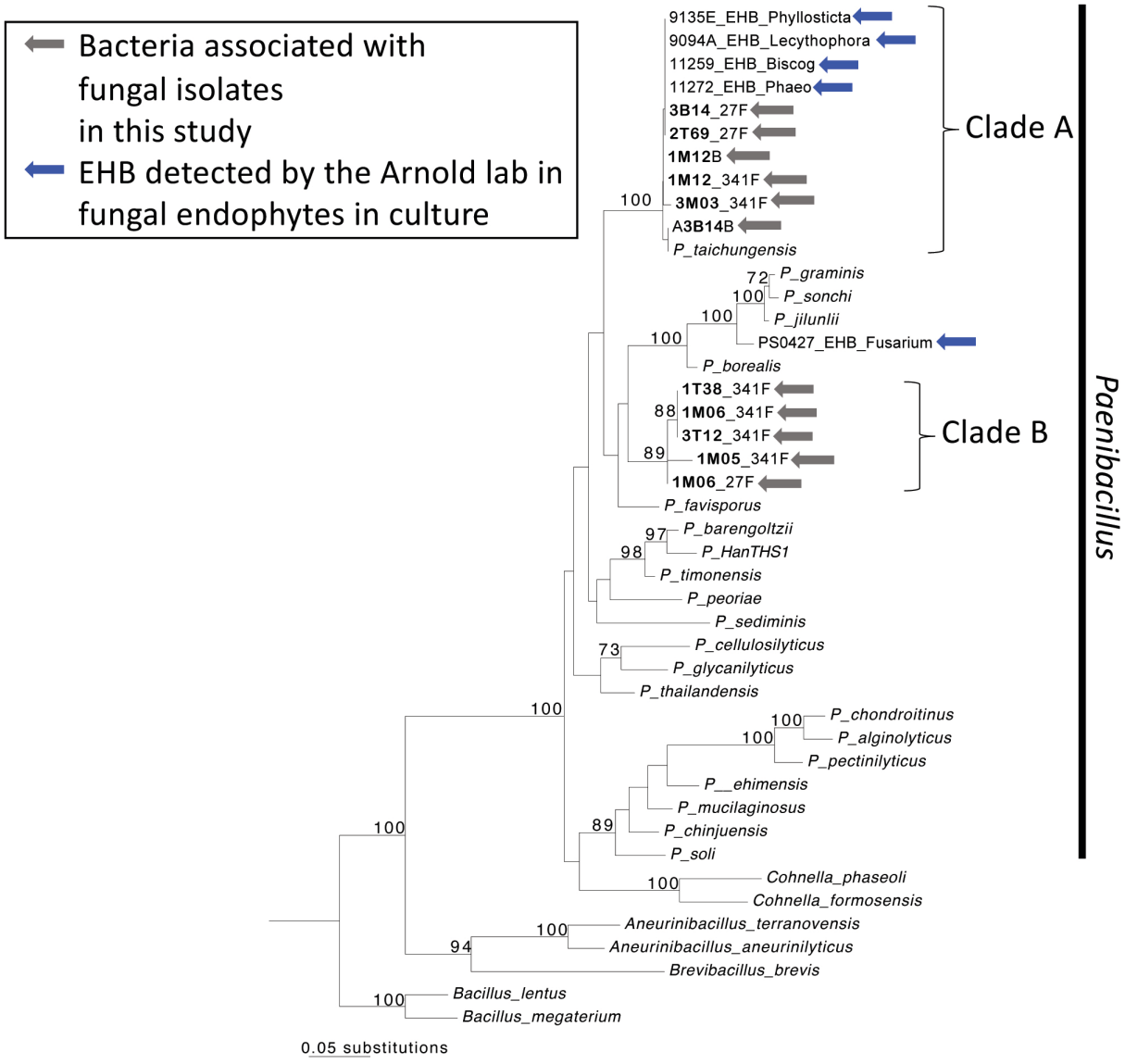
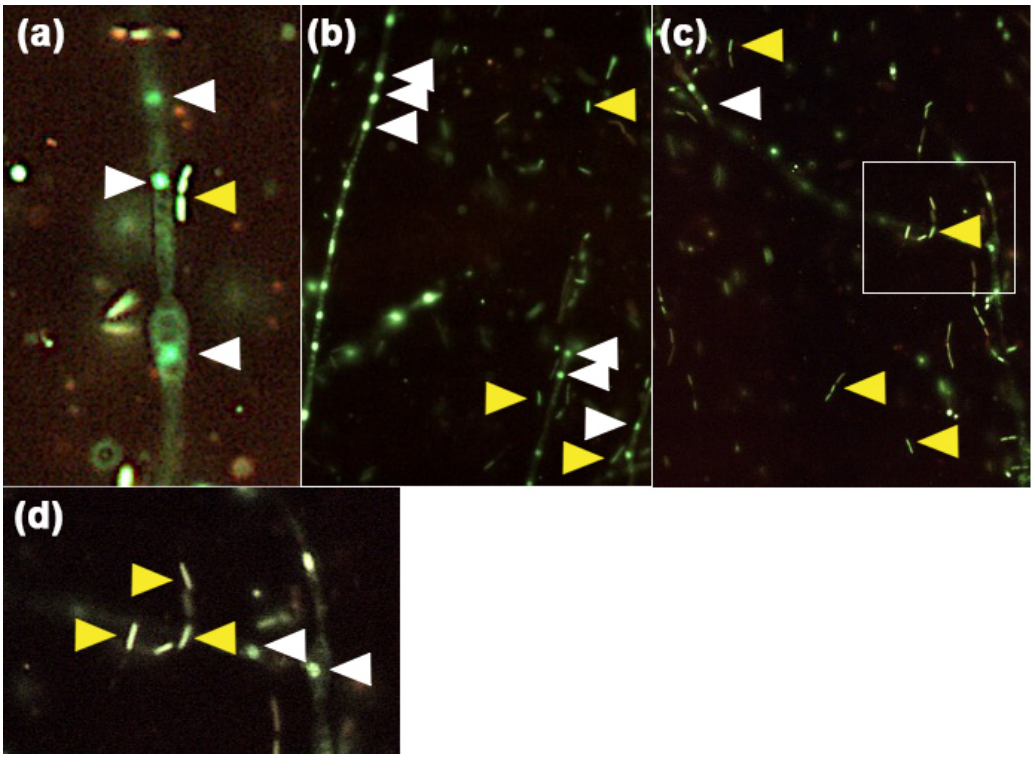


Fig. S10. Live/Dead stain shows free-living bacteria among hyphae of *Coniochaeta* sp. 2T69. (**a-c**) Examples of hyphae prepared from cultures. (**d**) Enlargement of area shown in middle right of Panel c. All pictures are taken at 400X, with panel a and panel d enlarged for display. White triangles = fungal nuclei. Yellow triangles = living bacteria.



Reference:

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Supplementary Methods:

Method S1: Identification of fungal cultures

The taxonomic identity of these fungi was inferred from their nuclear internal transcribed spacer and partial large subunit (nrITS-nrLSU) sequences, obtained with the primer set ITS1F-LR3 (see U'Ren *et al.*, 2010). To further confirm the taxonomic identity of the eight fungal strains, we sequenced at least one of five additional loci (nuclear small subunit ribosomal DNA [nrSSU], nuclear large subunit ribosomal DNA [nrLSU], RNA polymerase II 2nd largest subunit [*RPB2*], RNA polymerase II largest subunit [*RPB1*], translation elongation factor 1α [*EFT1α*], Supplementary Table S1). These sequences were used as queries for BLAST searches against GenBank to compare the top hits. A taxonomic name was accepted only when the top hit for at least two loci converged on the same taxonomic assignment. The resulting identities of these eight strains are: 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.). Higher taxonomic ranks for these strains are shown in Fig. 1a.

We selected two isolates (2T69 and 1M05) for transcriptomic analysis. Based on the result of a BLAST search against GenBank, strain *Coniochaeta* sp. 2T69 has a nrITS sequence consistent with that of strains isolated from lichens and gymnosperms (U'Ren *et al.*, 2010, 2019; U'Ren & Arnold, 2016). This fungal strain is one of the 16 strains of a common operational taxonomic unit (95%) isolated by Chen *et al.* (2018) from *D. scoparium* in North Carolina. Hyaloscyphaceae sp. 1M05 was isolated only once from *D. scoparium* in Chen *et al.* (2018). The most similar nrITS sequence in GenBank was 98.6% similar to 1M05.

Method S2: Measurement of chlorophyll content and electrolyte leakage

We set up additional plant-fungal co-culture jars to measure chlorophyll content (for *Coniochaeta* sp. 2T69 and Hyaloscyphaceae sp. 1M05) and electrolyte leakage (for all eight fungal strains). After 10 days for fungal establishment, chlorophyll content of *D. scoparium* was measured for plants every 7 days. One gametophyte shoot was cut per jar, weighed, and submerged in 65°C Dimethyl sulfoxide (DMSO) for 1 hr. The absorbance at 663 nm and 645 nm were measured with a LabQuest®2 spectrophotometer. Total chlorophyll content was calculated using the formula presented in Alpert (1984). Electrolyte leakage (initial conductivity [IC]/total conductivity [TC]) measurement was modified from Alfrez et al. (2006) on day 10 and day 17 after inoculation. Briefly, a fresh shoot of the gametophyte was submerged in 500 µl of ddH_20 and shaken at 40 rpm in a 1.5mL microcentrifuge tube. After 15 mins, the initial conductivity (IC) was measured with an Orion Star series meter

(Thermo Scientific). The Eppendorf was then placed in a -80 freezer. Total conductivity (TC) was measured after 12 hrs.

Method S3: Examination of fungus-associated bacteria

To examine representative fungal mycelia for endohyphal bacteria (EHB), and to rule out contamination, we used three approaches. First, total genomic DNA was extracted from three representative cultures that were apparently axenic (1M12, representing Agaricomycetes; 3B14, representing Eurotiomycetes; and 2T69, representing Sordariomycetes). We amplified 16S rRNA with primers 27F-1492R (Acinas *et al.*, 2004) and followed the methods of Hoffman and Arnold (2010) for PCR and sequencing to determine whether bacterial DNA was present in these cultures. Second, to rule out the potential for local contamination by laboratory materials at the University of Arizona, where the three DNA extractions were processed and sequenced, we also sequenced 16S rRNA as above from archived DNA extractions of the eight focal fungal isolates that had been retained at Duke University in 2016. All sequences were deposited in NCBI Genbank (Supplementary Table S11). Sequences were integrated into the alignment of Shaffer *et al.* (2016), with additional taxon sampling based on Hoffman and Arnold (2010). The resulting alignment was adjusted manually and then was analyzed with the GTRCAT model using maximum likelihood as the optimization criterion in RAxML-HPC2 on XSEDE (Stamatakis, 2014). Finally, we verified that the bacteria could occur endohyphally within living hyphae of the focal fungi. For this we focused on strain *Coniochaeta* sp. 2T69, using Live/Dead stain to visualize and confirm the viability of bacteria and hyphae per Arendt *et al.* (2016).

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