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Diplodia tip blight (*Diplodia sapinea*) and site conditions shape Scots pine (*Pinus sylvestris*) endophytic mycobiome

Laura Brodde [a,b], Silvia Miñana-Posada [a,c,¥], Amelia Tudoran [a,*,¥], Miguel Angel Redondo [a,d], Malin Elfstrand [a], Jonás Oliva [e], Jan Stenlid [a]

[a] Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[b] Swedish Veterinary Agency (SVA), Uppsala, Sweden
[c] Department of Environmental Systems Science, ETH Zürich, Universitätstrasse 2, Zürich CH-8092, Switzerland
[d] National Bioinformatics Infrastructure Sweden (NBIS), Science for Life Laboratory, Husargatan 3, Uppsala 752 37, Sweden
[e] Dept. Crop and Forest Sciences, University of Lleida, Lleida, Spain

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ABSTRACT

Diplodia sapinea (Fr.) Fuckelis is an opportunistic pathogen of Scots pine (*Pinus sylvestris* (L.) that causes Diplodia tip blight following host stress. The factors driving its shift from endophyte to pathogen are not well understood, particularly in relation to the surrounding fungal community. The objective of the current study was to determine the association of *D. sapinea* and the composition of the general endophyte community with symptomatic and asymptomatic sites, trees, twigs and tissues in an ongoing outbreak of Diplodia tip blight. The endophytic fungal community was characterized using metabarcoding of the ribosomal ITS2 region. We found that variation in fungal community composition was most influenced by differences between sites, highlighting the importance of site-specific environmental conditions such as previous drought impact and associated crown dieback. However, the fungal communities also varied between symptomatic and asymptomatic trees indicating the significance of tree health. The fungal communities of symptomatic trees, especially in twigs with tip blight symptoms, included *D. sapinea*, *Therrya pini*, and *Lophodermium arboricola*. These results are consistent with the balanced antagonism hypothesis, suggesting that shifts in community composition under stress may facilitate the transition of *D. sapinea* from a latent endophyte to a pathogen. *D. sapinea* was found in both healthy and symptomatic twigs, with a ~60-fold increase in symptomatic tissues. Site differences accounted for 42.6 % of fungal community variation. In contrast, taxa from the order Phaothecales were more abundant in asymptomatic twigs and in healthy tissues of of symptomatic twigs - suggesting potential antagonism. Our findings provide insights into early disease detection and underscore the importance of monitoring endophyte community shifts to support Scots pine forest resilience under climate stress.

1. Introduction

Diplodia tip blight is a major disease on pines globally and over the last ten years the damages from this disease have increased in Northern Europe (Stein Åslund et al., 2024; Brodde et al., 2023; Brodde et al., 2019; Terhonen et al., 2021, Adamson et al., 2015). The pathogen causing Diplodia tip blight, *Diplodia sapinea* (Fr.) Fuckel (syn. *Diplodia pinea* (Desm.) Kickx., *Sphaeropsis sapinea* (Fr.: Fr.) Dyko & Sutton), can be present within the host mycobiome without causing disease symptoms (Maresi et al., 2007; Bihon et al., 2011; Bußkamp et al., 2020). The fungi that comprise the mycobiome—the fungal communities that inhabit surfaces and tissues of the plant are taxonomically and functionally diverse communities that strongly influence the health and productivity of their host plants (Arnold et al., 2003; Bulgarelli et al., 2013; Vandenkoornhuyse et al., 2015; Bacon and White, 2016; Koskella et al., 2017; Bullington et al., 2018; Trivedi et al., 2020; Oliva et al., 2021). Fungi in the mycobiome may exhibit various trophic strategies, such as pathogenic, mutualistic, saprotrophic, or endophytic. Endophytic fungi, in particular, are defined as metabolically active organisms that colonize healthy plant tissues without causing visible signs of

* Corresponding author.
E-mail address: Amelia.Tudoran@slu.se (A. Tudoran).
¥ These authors contributed equally to this study.

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infection (Stone et al., 2000; Schulz and Boyle, 2005). Interactions within the plant mycobiome are complex and involve competition for space and nutrients (Oliva et al., 2021). For endophytic colonization to remain asymptomatic, the interactions between the host, fungi, and other microbial community members must be balanced (Schulz et al., 2015). Any changes in the host's physiology can disrupt this balance, potentially influencing the composition and function of the endophyte community and affecting the host's health status (Bullington et al., 2018; Oliva et al., 2021). Environmental stressors can play a central role in driving such physiological shifts, and may lead to substantial changes in the endophytic community (Sherwood et al., 2015; Arnold et al., 2003; Porras-Alfaro and Bayman, 2011). As a result, site-level environmental variation is increasingly recognized as a key factor shaping the assembly and dynamics of plant-associated fungal communities. Many fungi within the plant mycobiome exhibit flexible lifestyles, shifting between endophytic, saprotrophic, and pathogenic states depending on environmental conditions and host physiology (Rodriguez et al., 2009; Schulz et al., 2015; Hassani et al., 2018). This latent pathogenicity is increasingly recognized as a key feature of many forest-associated fungal pathogens (Sieber, 2007; Terhonen et al., 2019), and transitions from endophyte to pathogen have been documented across diverse host species (Arnold et al., 2003; Bullington et al., 2018; Porras-Alfaro and Bayman, 2011). Abiotic stressors such as drought (Bachi and Peterson, 1985; Sherwood et al., 2015), mechanical damage (Swart et al., 1987; Oliva et al., 2021), and nutrient limitation (Rajala et al., 2014) can disrupt the balance between host and microbiome, enabling opportunistic fungi to colonize living tissues aggressively and cause disease. Understanding these transitions is essential in the context of climate change, as stress conditions are expected to become more frequent and may accelerate disease emergence in forest ecosystems. Changes in the environment, such as abiotic stress on the host (e.g., Swart et al., 1987; Smith et al., 2002; Sherwood et al., 2015) may disrupt the balance in the interaction between *D. sapinea* and its host or with the endophytic community in the host tissue, enabling *D. sapinea* to cause necrosis. For instance, the mechanical stress inflicted on pine trees by hail can induce Diplodia tip blight symptoms, through the release of nutrient rich metabolites in the tissues (Oliva et al., 2021). Interestingly in that study, fungi that were functionally similar to *D. sapinea* in their response traits to hail, but likely more efficient in occupying their common niche, i.e. consuming host derived stress metabolites, dominated the endophyte community in the trees that did not develop symptoms of *Diplodia* tip blight (Oliva et al., 2021), indicating that the mycobiome may modulate the development of Diplodia tip blight. To understand the mycobiome in promotion or suppression of Diplodia tip blight in the context of the balanced antagonism hypothesis (Schulz et al., 2015), a better understanding of mycobiomes in symptomatic versus apparently healthy tissues is needed.

Analyzing the composition and diversity of the mycobiome and its interaction with the host plant disease status can improve our understanding of the role of the mycobiome in promotion or suppression of disease (Ata et al., 2022). Various methods have been employed to study *D. sapinea* and its potential transition from an endophytic lifestyle to necrotizing host tissues. Qualitative methods, such as culture based or (nested-) PCR analyses, often report a widespread presence of *D. sapinea* also in apparently healthy trees or stands (e.g., Flowers et al., 2003; Maresi et al., 2007; Bihon et al., 2011; Bußkamp et al., 2020). However, these techniques make it challenging to accurately determine the change in *D. sapinea* abundance in symptomatic versus apparently healthy tissues or trees. The use of quantitative methods such as qPCR or relative abundance approaches (e.g., metabarcoding; Lindahl et al., 2013) allows for comparing the abundance of *D. sapinea* between samples. Furthermore, this approach enables profiling of the entire endophytic fungal community, facilitating the identification of taxa that co-occur with or potentially suppress *D. sapinea*—as suggested by the balanced antagonism hypothesis (Schulz et al., 2015).This study builds on recent findings by Oliva et al. (2021), who proposed that shifts in host or environmental conditions may disrupt the balance between host and latent endophytes, leading to disease. Brodde et al. (2023) demonstrated that drought stress increases host susceptibility to *D. sapinea*, but the role of co-occurring fungi in mediating this transition remains unclear, thus the objective of the current study was to determine the association of *D. sapinea* and the composition of the general endophyte community with symptomatic and asymptomatic tissues in an ongoing drought-driven outbreak of Diplodia tip blight (Brodde et al., 2023). Under the overarching hypothesis that *D. sapinea* would be present in the trees irrespective of health status, we performed a structured sampling of asymptomatic and symptomatic trees and twigs in eight stands (Fig. 1). The relative abundance of *D. sapinea* and the coexisting endophyte community were profiled using a semi-quantitative metabarcoding approach. Metabarcoding offers a powerful approach to characterize endophyte communities in a culture-independent and semi-quantitative manner, enabling a broader understanding of interactions between latent pathogens like *D. sapinea* and other fungal taxa within host tissues. In the study we tested the specific hypotheses: the endophyte communities differ between *i*) sites with and without visible Diplodia tip blight symptoms; *ii*) symptomatic and asymptomatic trees across sites; *iii*) symptomatic and asymptomatic twigs in trees with Diplodia tip blight; and iv) healthy and necrotic tissue collected from twigs with Diplodia tip blight. Sampling followed a detected outbreak during the severe 2018 drought on Gotland, characterized by mortalities and substantial crown dieback (Brodde et al., 2023).

2. Methods

2.1. Experimental sites, sampling, and sample processing

The experimental sites are described in detail in Brodde et al. (2023). In brief, they were located east to northeast of the city of Visby on Gotland Island, Sweden—an area that was strongly affected by the severe summer drought of 2018, which coincided with widespread crown dieback and symptoms of Diplodia tip blight. Drought stress conditions at the sites were characterized using crown dieback and climate data previously reported in Brodde et al. (2023). Specifically, the severity of drought impact at each site was assessed by categorizing crown dieback percentages measured during that study. We used the same eight Scots pine (*Pinus sylvestris*) stands for sampling in this study: four sites showed clear signs of crown dieback and were classified as "affected" (Affected 1–4), while four sites with healthy-looking pines were classified as "healthy" (Healthy 1–4). The average tree height at these sites ranged from 5.1 to 12.1 m (see Supplementary Table S1 for coordinates and site characteristics).

In winter 2018, after the drought, we sampled three symptomatic trees from each selected from the surveyed trees at affected site, collecting three asymptomatic and three symptomatic twigs per tree (Fig. 1). At both the affected and healthy sites, we also sampled three healthy-looking trees (also selected from he surveyed trees) for asymptomatic twigs. Healthy trees (n = 12) showed $<=$10 % crown dieback, and all but one symptomatic tree (n = 12) showed $>=$20 % crown dieback. Twigs were transported at $<$ 4℃ and stored at −20℃ within five days. From symptomatic twigs, we sampled healthy twig tissue, as well as material from the infection border, where necrotic and healthy tissues meet (Fig. 1). Asymptomatic twigs were sampled for the current and previous year's growth. Symptomatic twigs were sampled at the growth year where the infection border was localized, which occasionally reached internodes from 10 previous years. Surface sterilization was carried out according to Bußkamp (2018) with minor modifications. In brief, needles were removed, and stems were brushed under running tap water, then surface-sterilised. Twigs were surface-sterilised for one min in ethanol (70 % v/v), five min in NaOCl (3 % v/v), one min again in ethanol (70 %), and a final washing step in double distilled water for ca. 15 sec. Each sample contained a one-cm twig segment comprising bark, cambium and sapwood. The segments were collected from healthy,

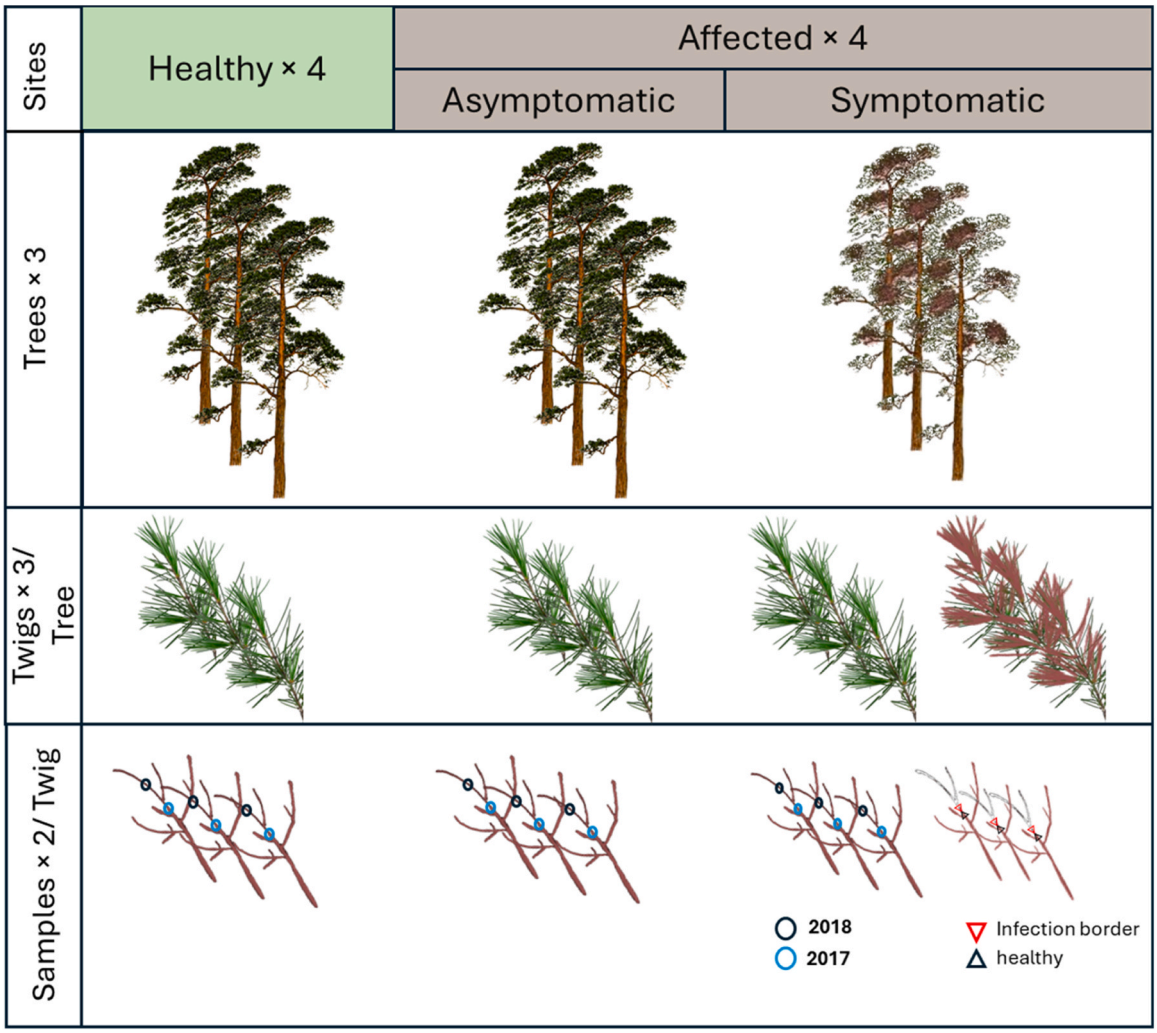

Fig. 1. Overview of sampling scheme. Trees were sampled from four affected and four healthy-looking sites. At each site, growth years 2018 and 2017 were collected from three twigs per tree across three asymptomatic trees. Additionally, at the affected sites, three symptomatic trees were sampled. For these trees, three asymptomatic twigs and three symptomatic twigs were collected. The asymptomatic twigs were sampled using the same protocol as for healthy-looking sites, while symptomatic twigs were sampled at the infection border and in the adjacent healthy-looking tissue. Tree and twig images obtained from png.tree (https://www.pngegg.com).

asymptomatic twigs, or from the border of an infection, including necrotic and healthy tissue on symptomatic twigs or from apparently healthy tissues on the same symptomatic twigs (Fig. 1). Measurements of fresh weight, dry weight, length, and diameter of each sampled segment were carried out to exclude factors influencing the endophytic community besides the presence of *D. sapinea.* Twig samples were cut into fine slices which were transferred into a 2 mL screw cap tube containing one 5-mm and two 2-mm glass beads and stored at −20℃ until lyophilization. These measurements were recorded with the intention of evaluating potential physical influences on the endophyte community. However, after initial exploration, the data were not used in subsequent analyses due to limited variation across samples and a lack of apparent effect, as such, they were excluded from the final analysis.

2.2. DNA extraction, amplification, and sequencing of the endophyte community

Freeze-dried samples were homogenized twice using the Precellys® 24 Tissue homogenizer (Bertin Instruments) for 25 sec at 5000 rpm. DNA extraction was carried out according to instructions with the E.Z.N.A ®SP Plant DNA Kit (Omega Biotek). DNA was extracted using the E.Z.N.A® SP Plant DNA Kit (Omega Biotek) according to the manufacturer's instructions. Prior to the amplification of the ITS2 region DNA quantification was performed using a NanoDropOne spectrophotometer (Thermo Scientific). Samples were diluted to a concentration of 0.5 ng/μL. Of these, 28 samples did not amplify at that dilution and were further diluted to 0.1 ng/ μl. Each sample was amplified separately using the primer pair fITS7 (5′-xxxxxxxxGTGARTCATCGAATCTTTG-3′) and ITS4 (5′-xxxxxxxxTCCTCCGCTTATTGATATGC-3′) (Ihrmark et al., 2012) containing 8-bp sample identification barcodes denoted by x, as previously published (Clemmensen et al., 2016). Each sample was amplified using unique barcode combinations. The PCR amplifications were carried out in 50 μL reactions with a final concentration of 0.025 unit/μl DreamTaq Green Polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 0.2 mM dNTPs, 2.75 mM $MgCl_2$, 300 nM tagged fITS7/ITS4 primer mix and 12.5 ng of template DNA were run with the following cycle conditions: 5 min at 94 °C, 26–35 cycles of 30 s at 95 °C, 30 s at 57 °C and 30 s 72 °C, and a final 7 min at 72 °C, blanks were run as negative controls. PCR products were verified via gel electrophoresis on 1 % agarose gels. The number of PCR cycles was optimized for each sample separately, aiming for the minimum number of cycles to achieve an amount of DNA visible on gel. This procedure follows the established protocol used in our lab for fungal community profiling in plant tissues (Clemmensen et al., 2016) and has been optimized to maximize

amplification success while minimizing biases associated with over-cycling. The resulting amplicons were purified with the AMPure kit (Beckman Coulter, Indianapolis, IN, USA). The concentration of purified PCR products was determined fluorometrically (Quantus Fluorometer) using the Qubit® ds DNA Broad Range (BR) Assay Kit (ThermoFischer). Equimolar mixes of PCR products from 368 samples, including the blanks, were pooled, creating four sample pools (each containing 84 – 88 samples and at least two PCR blank samples) that were sequenced on the PacBio Sequel platform using four SMRT cells by SciLifeLab NGI (Uppsala, Sweden). After quality filtering and removal of samples with low read counts, poor amplification and duplicates, 357 samples were retained for downstream analysis. Construction of the sequencing library and sequencing were carried out by NGI SciLifeLab (Uppsala, Sweden). The sequence read data are deposited in PRJNA1187862 BioProject. The sequence reads were quality controlled, clustered and demultiplexed using the in-house SCATA pipeline (https://scata.mykopat.slu.se/). Low read quality (average quality score of <20, base average quality of < 3), and short (<200 bp) sequences, primer dimers, and sequences missing a primer site (primer match value = 0.9) were removed. Sequences were clustered into operational taxonomic units (OTUs) based on 98 % similarity. OTUs with < 10 reads or that appeared in less than 3 samples were removed from the dataset. The dataset was tested for PCR contamination by analyzing the PCR blanks and confounding factors, such as variation between sequencing libraries.

Each of the likely fungal OTUs was taxonomically assigned using Protax software (Somervuo et al., 2016; Abarenkov et al., 2018) implemented in PlutoF (https://plutof.ut.ee) and the UNITE database using a threshold value of 0.5 (plausible classification) (Abarenkov et al., 2018, Kõljalg et al., 2013). The species hypothesis for the 20 most common OTUs, as well as indicator species were also cross referenced manually through GenBank (NCBI) database using the Blastn algorithm. The most similar match referring to a physical culture or type specimen was used to assign a taxonomic name to the OTUs. To identify OTUs belonging to plants, we performed a least common ancestor analysis with the software MEGAN (Huson et al., 2007), with a min-score of 300 and a minimum identity of 90 %, on the OTUs that could not be assigned at any phylum level by Protax. This additional step was necessary because the UNITE database is curated for fungal identification and does not reliably assign taxonomy to non-fungal sequences such as plant-derived reads. The MEGAN LCA approach enabled us to identify and exclude these plant-origin OTUs from downstream fungal community analyses.

2.3. Statistical analysis

All analyses were performed using R Statistical Software (v4.1.1; R Core Team 2021). We first tested the effect of the health status of the sites and trees on the diversity of fungal communities. For this, we aggregated the fungal communities of all the twigs within each tree to obtain one community per tree (biological replicate). Relative abundances were then transformed with Hellinger transformation and analysed with the Phyloseq (v1.38.9) and vegan (v.2.6.2) R packages. We tested the effect of the health status of the sites, and trees on the composition of the fungal communities through a Permutational multivariate analysis of variance (PERMANOVA, *adonis2* function of vegan R package), stratifying the permutations by site as trees were nested within the sites. For all subsequent analysis at twig level, samples were not aggregated. Estimating the effect of the health status of the twig tissue on the variation in fungal communities with PERMANOVA was done in a subset of the data set, including only symptomatic trees on affected sites. Here, the permutation was stratified also by tree number. All analyses were carried out with a non-rarefied OTU table. Instead, the square root of the total read number per sample was included as the first factor in all models, accounting for bias resulting from varying sequencing depth of the samples (McMurdie and Holmes, 2013; Bálint et al., 2015; Wagner et al., 2016). The variations in community compositions were visualized by principal coordinate analysis (PCoA) using the Bray-Curtis distance within the Phyloseq R package. We obtained the indicator species for symptomatic and asymptomatic tree, twig and tissue using the *multipatt* function of the *indicspecies* (De Cáceres et al., 2020) package for R. Table 1

3. Results

3.1. Sequencing overview and community composition

The sequencing of all samples generated 1383,716 reads. Of these, 721,918 (52 %) reads passed the initial quality filtering and were clustered into 1230 OTUs and 1472 singletons from 357 samples. A summary of read depth of the samples is available in Supplementary Table S2. Plant OTUs corresponded to 0.1 % of the total reads. Seventy-seven percent of the fungal OTUs could be assigned a phylum; 38 % a class, 33 % a genus, and 9 % a species. Fungi of the phylum Ascomycota dominated the endophyte community comprising 60 % of the OTUs (Fig. 2, Supplementary Table S3). The second largest group of OTUs were classified as Basidiomycota (18 %), while 22 % of the OTUs could not be assigned taxonomically. Five OTUs each could be assigned to Mucormycota and Chytridiomycota (Supplementary Table S3). The largest orders were represented by Capnodiales (8.1 %), Pleosporales (7.9 %), and Helotiales (7.1 %) (Fig. 2).

3.2. Site and tree health influence fungal community structure

Site-specific factors had a greater impact on community variation compared to the health status of the trees (Table 2). Tree health status contributed a significant 2.9 % ($p = 0.019$) to the variation in endophytic community composition, whereas 42.6 % ($p = 0.043$, Table 2) of the variation was explained by the site. The type of site (affected vs healthy) and the health status of the trees at different sites (symptomatic or asymptomatic) significantly influenced the detected endophyte communities (Fig. 5, Table 2). Specifically, the type of site (healthy or affected) and tree health status explained 8 % and 3 % of the variation in fungal community composition, respectively (Table 2). Sites displaying symptoms of Diplodia tip blight harbored more divergent communities ($R^2 = 0.08$, $p = 0.055$, Table 2) than unaffected sites. In particular, symptomatic sites 1 and 4 exhibited communities that were relatively distinct from the other six sites (Fig. 5). Our findings revealed a significantly higher (~60-fold) *D. sapinea* abundance in symptomatic trees compared to healthy trees. These results align with the known latent endophytic nature of *D. sapinea*, indicating that certain triggers, potentially related to drought severity or shifts in the fungal community composition, are involved in its transition from endophyte to pathogen.

To gain understanding of which OTUs were associated with symptomatic or asymptomatic trees, we analyzed the indicator species of three classes of sampled trees: asymptomatic trees sampled in unaffected sites and both symptomatic and asymptomatic trees sampled at affected sites (Table 3). Eight OTUs were associated with symptomatic Scots pine trees (Table 3). Consistent with the symptoms of Diplodia tip blight on these trees, *Diplodia sapinea* (sp_15) was the species most strongly associated with symptomatic trees (Table 3), showing higher relative abundances, and being present more frequently in symptomatic trees compared to asymptomatic trees. *Lophium arboricola* (sp_24) showed a nearly equally strong association with symptomatic trees. However, *L. arboricola* showed an overall lower relative abundance than *D. sapinea* (Table 1). In contrast, seven OTUs were strongly associated with asymptomatic trees. Notably, sp_3 (Phaeothecales), sp_18 (*Neophaeothecoidea proteae*) and sp_51 (*Phaeosclera* sp.) were among the most common. For instance, the OTU sp_3 (Phaeothecales) is the fifth most abundant OTU in asymptomatic trees (Table 3).

The sequencing data revealed significant variation in the fungal community composition across sites and in relation to tree health status.

Table 1
Relative abundance (%) of the 20 most common OTUs, including indicator species, sequenced from Scots pine twigs affected by Diplodia tip blight. Sampling was conducted at four levels: (1) between affected and unaffected sites, (2) between symptomatic and asymptomatic trees within affected sites, (3) between symptomatic and asymptomatic twigs within symptomatic trees, and (4) between healthy and necrotic tissues within symptomatic twigs. The final column shows the overall abundance across all samples. *Diplodia sapinea* (sp_15) is marked with (*).

Taxa	rel. Abundance [%]								
	Sites		Affected Sites		Symp. Trees		Symp. Twig		
	Healthy	**Affected**	**Tree asymp.**	**Tree symp.**	**Twig asymp.**	**Twig symp.**	**Tissue healthy**	**Tissue necrotic**	**All**
Sydowia polyspora	19.1	17.9	17.7	18.1	20.0	16.1	21.4	10.3	18.1
Ascomycota_1	18.4	16.1	18.4	13.7	12.9	14.5	12.5	16.6	16.5
Lapidomyces aloidentricola	6.6	5.7	6.4	5.0	6.7	3.4	4.6	2.1	5.9
Phaeoteca sp.	8.6	3.9	3.7	4.1	5.7	2.4	4.5	0.2	4.8
Phaeothecales	7.2	3.7	4.5	2.9	3.5	2.3	4.0	0.4	4.4
Cladosporium oxysporum	3.2	5.1	5.1	5.1	7.6	2.6	4.1	1.0	4.7
Coniothyrium	0.9	4.4	3.9	4.9	6.4	3.5	2.8	4.2	3.7
Ascomycota_2	4.0	2.8	2.6	3.1	3.5	2.8	3.5	2.0	3.1
Hyphodiscus sp.	3.9	2.3	2.4	2.3	1.3	3.3	3.3	3.3	2.7
Ceratobasidium sp.	1.4	3.2	4.0	2.3	2.6	2.0	2.5	1.3	2.8
Pestalotiopsis sp.	2.0	2.4	1.3	3.5	4.1	3.0	3.6	2.2	2.3
Coniothyrium olivaceum	0.9	2.1	1.5	2.7	2.9	2.5	1.7	3.4	1.8
Diplodia sapinea*	0.0	2.1	0.1	4.3	0.4	8.2	6.5	10.0	1.7
Ascomycota_3	0.0	2.0	3.3	0.8	0.7	0.8	0.9	0.7	1.6
Ostropales	4.4	0.8	1.0	0.5	0.7	0.4	0.6	0.2	1.5
Foliophoma	0.0	1.5	1.2	1.9	3.1	0.7	0.5	1.0	1.2
Capnodiales	1.5	0.8	1.0	0.7	0.6	0.7	0.8	0.6	1.0
Banhegyia setispora	0.1	1.1	1.3	0.9	0.2	1.5	1.0	2.1	0.9
Ascomycota_4	1.3	0.8	1.1	0.6	0.7	0.5	0.8	0.2	0.9
Neophaeothecoidea proteae	2.1	0.6	0.6	0.5	0.7	0.3	0.5	0.1	0.9
Rachicladosporium sp.	0.0	0.8	0.6	1.0	1.4	0.5	0.9	0.0	0.6
Therrya pini	0.3	0.7	0.4	0.9	0.2	1.6	0.4	2.8	0.6
Pragmopora cf. pini	0.6	0.5	0.5	0.6	0.2	1.0	1.1	0.9	0.6
Celosporium laricicola	0.8	0.5	0.8	0.1	0.2	0.1	0.1	0.0	0.6
Lophium arboricola	0.1	0.5	0.2	0.9	0.3	1.6	0.2	3.1	0.5
Ascomycota_5	0.2	0.5	0.2	0.8	0.5	1.1	0.2	2.1	0.5
Capronia sp.	0.1	0.5	0.7	0.3	0.0	0.5	0.2	0.8	0.4
Ascomycota_6	0.0	0.4	0.3	0.5	0.4	0.7	0.2	1.1	0.3
Ascomycota_7	0.0	0.4	0.4	0.3	0.3	0.4	0.2	0.7	0.3
Ascomycota	0.0	0.4	0.2	0.6	0.8	0.4	0.4	0.4	0.3
Pseudocamarosporium brabeji	0.1	0.4	0.2	0.6	0.7	0.4	0.1	0.8	0.3
Ascomycota_8	0.5	0.2	0.2	0.3	0.3	0.2	0.4	0.0	0.3
Sarea resinae	0.4	0.2	0.1	0.2	0.0	0.4	0.0	0.8	0.2

(*continued on next page*)

Table 1 (*continued*)

Taxa	rel. Abundance [%]								
	Sites		Affected Sites		Symp. Trees		Symp. Twig		
	Healthy	**Affected**	**Tree asymp.**	**Tree symp.**	**Twig asymp.**	**Twig symp.**	**Tissue healthy**	**Tissue necrotic**	**All**
Petrophila incerta	0.3	0.2	0.3	0.2	0.1	0.4	0.3	0.6	0.3
Ascomycota_9	0.1	0.2	0.1	0.4	0.3	0.4	0.5	0.3	0.2
Phaeosclera sp.	0.3	0.2	0.3	0.1	0.0	0.1	0.2	0.0	0.2
Capronia sp.	0.4	0.1	0.2	0.1	0.0	0.2	0.1	0.4	0.2
Arthoniales	0.0	0.2	0.2	0.2	0.1	0.3	0.0	0.5	0.2
Basidiomycota_1	0.5	0.1	0.1	0.1	0.1	0.2	0.1	0.3	0.2
Kabatiella sp.	0.4	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.2
Ascomycota_10	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.3	0.1
Amphosoma personiae	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.2	0.1
Myriangiales sp.	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.2	0.1
Basidiomycota_2	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1
Ascomycota_11	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Basidiomycota_3	0.0	0.1	0.0	0.1	0.2	0.0	0.0	0.0	0.1
Vishniacozyma victoriae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Basidiomycota_4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Symptomatic sites and trees displayed a distinct fungal community, with specific OTUs, such as *Diplodia sapinea* and *Lophium arboricola* being strongly associated with the symptomatic trees. Conversely, OTUs with related to Phaeothecales and *Neophaeothecoidea proteae*, were more frequently identified in asymptomatic trees.

3.3. Spatial distribution of fungal communities within trees

Next, we tested whether endophyte communities differ between symptomatic and asymptomatic twigs sampled from symptomatic trees. Then, we examined differences in endophyte communities between healthy and necrotic tissue collected from symptomatic twigs. We found that the twig and its health status influenced the detected endophyte community ($R^2 = 0.042$, $p = 0.001$, Table 4, Fig. 6a). The community varied also among the symptomatic twigs ($R^2 = 0.0547$, $p = 0.008$, Table 5, Fig. 6a). Furthermore, the communities at the infection border (symptomatic necrotic tissues) and the distal healthy, green areas (asymptomatic tissues) also showed significant differences ($R^2 = 0.0570$, $p = 0.001$, Table 5, Fig. 6b), indicating substantial spatial separation of endophyte communities in tree crowns.

To further explore within-twig patterns, we quantified alpha diversity (Shannon and Simpson indices, and species richness) in necrotic and healthy tissue. All three diversity metrics were significantly higher in necrotic tissue (Wilcoxon rank sum test: Shannon $p = 0.0025$; Simpson $p = 0.0099$; Richness $p < 0.0001$; Fig. 3). In contrast, no significant differences were detected between symptomatic and asymptomatic twigs or trees (all $p > 0.1$, Supplementary Fig. S1). These results suggest that local tissue necrosis is associated with increased fungal diversity, even when tree- or twig-level symptom status does not reflect a clear shift in diversity. Indicator species analyses identified five OTUs strongly associated with asymptomatic twigs and four OTUs associated with tissues in symptomatic trees (Table 3). Notably, sp_3 (Phaeothecales), sp_4 (Cladosporium oxysporum), sp_10 (Phaeotheca sp.), and sp_18 (Neophaeothecoidea proteae) were associated with both asymptomatic twigs and the sampled asymptomatic tissues within symptomatic twigs. The first three OTUs were also relatively abundant in asymptomatic twigs (3.5–7.6 %, Table 1) and tissues (4.0–4.5 %, Table 1). *Diplodia sapinea* was strongly associated with symptomatic twigs and was highly abundant in both healthy and necrotic tissues, with no significant difference between the tissue types. Five of the eight OTUs associated with symptomatic twigs were also strongly associated with symptomatic tissues in these twigs (Table 3), e.g., sp_23 (*Therrya pini*) and sp_24 (*Lophium arboricola*).

3.4. Co-occurrence patterns between D. sapinea and potential antagonists

To assess whether this apparent antagonism had statistical support, we conducted co-occurrence and correlation analyses between *Diplodia sapinea* and Phaeothecales taxa. Across all samples, Phaeothecales abundance was significantly negatively correlated with D. sapinea (Spearman's $\rho = -0.16$, $p = 0.0023$, Fig. 4). This negative association was strongest in symptomatic twigs ($\rho = -0.40$, $p = 0.0005$), while no significant relationship was observed in asymptomatic twigs or tissue-level subsets. Co-occurrence analysis further supported a statistically significant negative association between *D. sapinea* and Phaeothecales in symptomatic twigs ($p = 0.009$), but not in asymptomatic twigs or at the tissue level. These results provide statistical support for a potential antagonistic interaction between Phaeothecales taxa and *D. sapinea*, particularly in symptomatic tissues, consistent with the balanced antagonism hypothesis. . 5

4. Discussion

Diplodia sapinea is a latent pathogen that can exist as an endophyte within its host, and under suitable environmental conditions and in a susceptible host, it can produce symptoms (Bachi and Peterson, 1985;

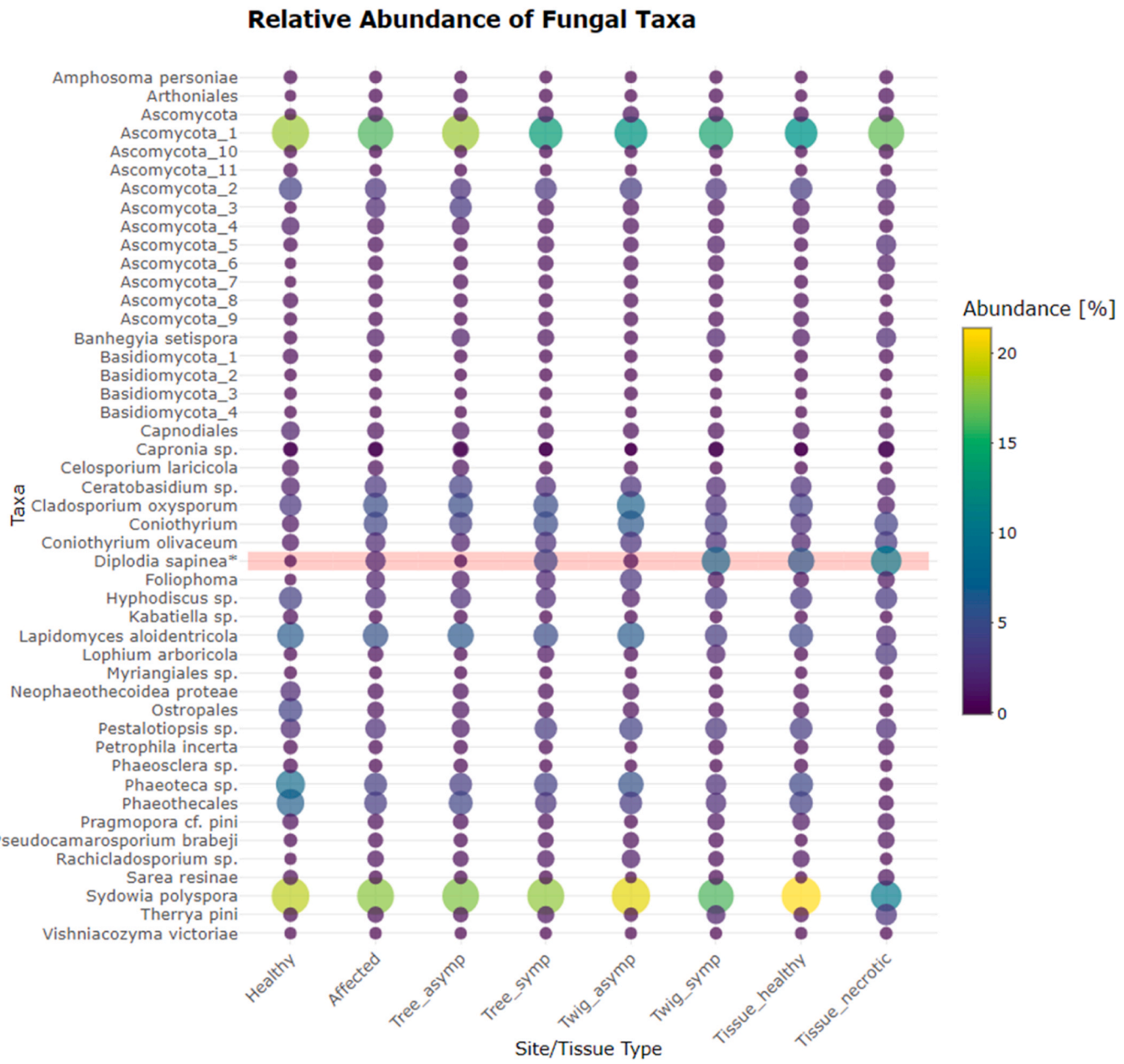

Fig. 2. Relative abundance of OTUs identified on order level. OTUs with unknown order sorted by phylum; excluded OTUs: less than 10 reads & occurred in less than 3 samples. Circle sizes represent % of relative abundance with the smaller circles being close to 0 while largest circles represent larger % of abundance.

Table 2
Permutational multivariate analysis of variance (PERMANOVA) analysis of the effects of read depth (total reads), site type (affected vs healthy), site number and tree health (symptomatic vs asymptomatic) on the endophytic fungal community of Scots pine trees in a region affected by drought and Diplodia tip blight. Significant differences ($p < 0.05$) indicated with *.

Variable	Df	SumOfSqs	R^2	F	Pr(>F)
Total reads	1	0.3685	0.086	5.821	0.035*
Site type	1	0.3257	0.076	5.145	0.055
Site no.	6	1.8308	0.426	4.820	0.043*
Tree health	1	0.1239	0.029	1.956	0.019*
Residual	26	1.6456	0.383		
Total	35	4.2944	1.000		

Sherwood et al., 2015; Oliva et al., 2021). In line with previous studies demonstrating that endophytic fungal communities in conifers are influenced by tree health status (Rajala et al., 2014; Millberg et al., 2015; Marčiulynas et al., 2022; Redondo et al., 2022), we found clear evidence that endophytic fungal communities significantly differed between affected and healthy sites as well as between symptomatic and asymptomatic trees. Notably, we observed that site-specific characteristics had a stronger impact on the fungal communities detected than the overall health status of the trees at each site or the specific health of individual trees. Fig. 6a

This aligns with current understanding of endophyte community assembly within the phyllosphere of conifers, particularly needles and young shoots, where community composition largely reflects local habitat conditions and the available spore inoculum at the site (Millberg et al., 2015; Eusemann et al., 2016; Redondo et al., 2020, 2022). Our results also confirm substantial spatial variation in endophyte

Table 3
Overview of indicator species in endophytic communities comparing symptomatic trees with symptoms of Diplodia tip blight, as well as asymptomatic and symptomatic twigs and tissues from symptomatic trees.

Taxa	Between trees		Within symptomatic trees		Within symptomatic twigs	
	asympt. tree	**sympt. tree**	**asympt. twig**	**sympt. twig**	**asympt. tissue**	**sympt. tissue**
Phaeothecales	0.840**		0.747*		0.798***	
Neophaeothecoidea proteae	0.840*		0.678***		0.665**	
Celosporium laricicola	0.875*				0.570*	
Ostropales	0.853*					
Dothideomycetes sp.	0.809*					
Phaeosclera sp.	0.890**					
Kabatiella sp.	0.849**					
Ascomycota (sp_89)	0.851*					
Cystobasidiomycetes incertae sedis	0.811*					
Cladosporium oxysporum			0.825*		0.844*	
Phaeotheca sp.			0.729***		0.774*	
Lapidomyces aloidendricola			0.810***			
Ascomycota (sp_47)					0.594*	
Lophium arboricola		0.942**		0.636***		0.833***
Diplodia sapinea		**0.992*** **		**0.784*** **		
Ascomycota (sp_35)		0.910*				0.673**
Pseudocamarosporium brabeji		0.890**				0.701*
Constantinomyces oldenburgensis		0.868*				
Ascomycota (sp_47)		0.892**				
Fungi (sp_94)		0.834*				
Vishniacozyma victoriae		0.800*				
Cladosporium sp.		0.828*			0.533*	
Therrya pini				0.825**		0.909**
Capronia sp.				0.751***		0.761**
Petrophila incerta				0.713***		0.739**
Capronia sp.				0.676***		0.727*
Exophiala nigra				0.606***		0.723*
Banhegyia setispora				0.680**		
Pragmopora cf. pini				0.671**		
Fungi (sp_34)						0.548*
Ascomycota (sp_36)						0.556*
Arthoniales sp.						0.603**
Ascomycota (sp_44)						0.61**
Basidiomycota (sp_52)						0.551*
Amphosoma sp.						0.567*
Microbotryomycetes incertae sedis						0.626**
Myriangium sp						0.611*

Table 4
Permutational multivariate analysis of variance (PERMANOVA) analysis of the effects of read depth (total reads), site number and twig status (symptomatic vs asymptomatic) on the endophytic fungal community of Scots pine trees in a region affected by drought and Diplodia tip blight. Significant differences ($p < 0.05$) indicated with *.

	Df	**SumOfSqs**	**R^2**	**F**	**Pr(>F)**
Total reads	1	0.287	0.008	1.384	0.154
Site nr	3	6.900	0.189	11.110	0.001***
Twig	1	1.548	0.042	7.478	0.001***
Residual	134	27.742	0.761		
Total	139	36.477	1.000		

Table 5
Permutational multivariate analysis of variance (PERMANOVA) analysis of the effects of read depth (total reads), twig no. and tissue status (necrotic vs healthy) on the endophytic fungal community of Scots pine trees in a region affected by drought and Diplodia tip blight. Significant differences ($p < 0.05$) indicated with *.

	Df	**SumOfSqs**	**R^2**	**F**	**Pr(>F)**
Total reads	1	0.376	0.0189	1.407	0.241
Twig nr.	3	1.090	0.0547	1.362	0.008**
Tissue	1	1.137	0.0570	4.264	0.001***
Residual	65	17.330	0.870		
Total	70	19.9319	1.00000		

communities within tree crowns, consistent with observations reported in earlier studies (Osono and Mori, 2004; Unterseher and Tal, 2006; Addison et al., 2023). Thus, our findings highlight the importance of local environmental conditions in shaping fungal community structure, which may in turn modulate interactions between *D. sapinea*, the broader tree mycobiome, and host susceptibility.

The most consistently abundant endophyte across all samples, regardless of site or symptom status was *Sydowia polyspora*. This aligns with findings from studies in conifer systems in Northern and Central Europe (e.g., Larsson et al., 2024; Jordán Muñoz-Adalia et al., 2017; Ridout and Newcombe, 2018), where *S. polyspora* is often reported as a common component of the mycobiome. Its consistent presence in this study likely reflects its generalist and environmentally resilient nature, rather than a functional role in tip blight dynamics. *Diplodia sapinea* was found to be associated with symptomatic trees in the indicator species analysis. The association of *D. sapinea* with the observed Diplodia tip blight symptoms were further supported by *D. sapinea* being an indicator species also for symptomatic twigs in trees showing Diplodia tip blight symptoms. *D. sapinea* was not the only fungus that was associated with symptomatic trees, twigs, or tissues in the indicator species analysis. For instance, both *L. arboricola* and *T. pini* were significantly associated with symptomatic twigs and tissues. However, they were both less abundant than *D. sapinea* and are not known to cause the observed shoot dieback symptoms. *Therrya pini* is considered a saprophyte known to associate with dead pine twigs, while *L. arboricola* originally was isolated from stem cankers on conifers (Bugzacki, 1972; Solheim et al., 2013). Taken together, it is evident that *D. sapinea* associates with symptomatic trees, and specifically with the symptomatic twigs in this study. Even if it was

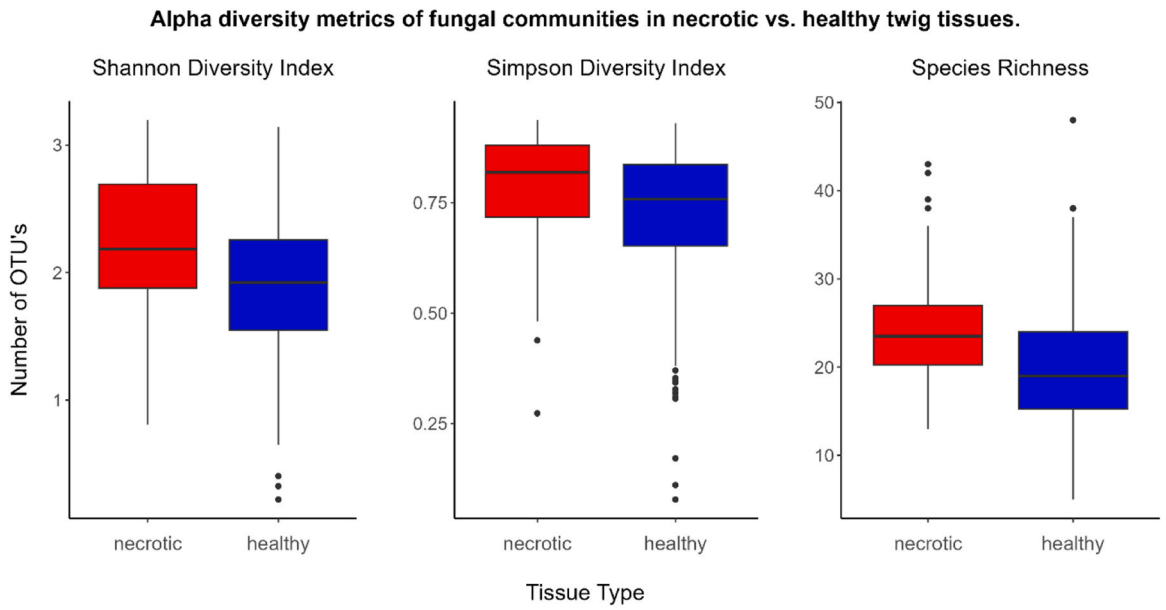

Fig. 3. Boxplots showing Shannon diversity, Simpson diversity, and species richness in necrotic and healthy tissues. All metrics indicate significantly higher diversity in necrotic tissue (Wilcoxon rank sum test: Shannon $p = 0.0025$; Simpson $p = 0.0099$; Richness $p < 0.0001$).

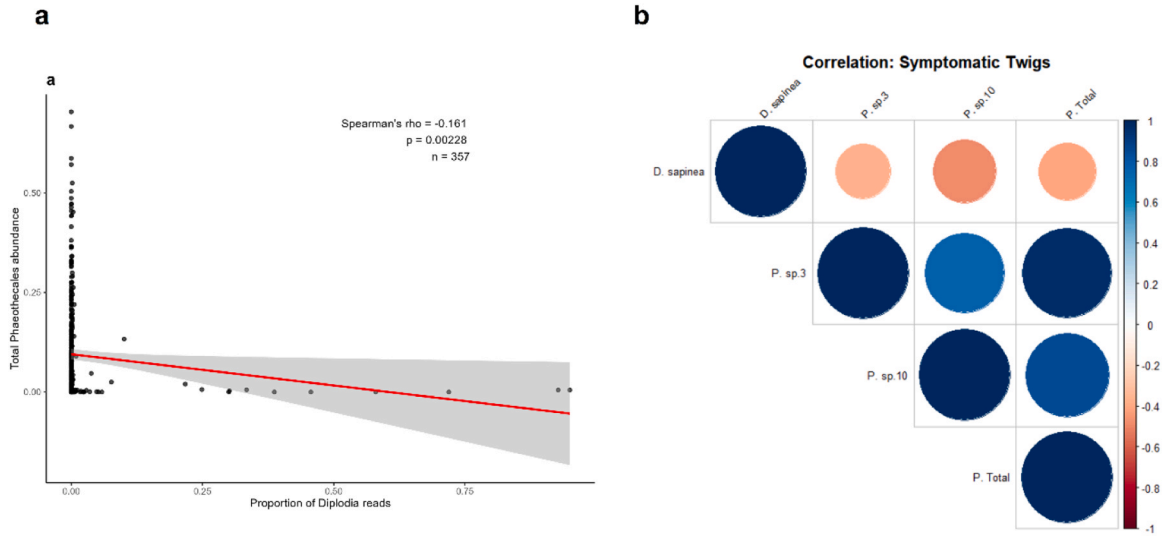

Fig. 4. Relationship between Diplodia sapinea and Phaeothecales in symptomatic twigs. (A) Scatterplot of relative abundances of Diplodia sapinea and total Phaeothecales across all samples (n = 357), showing a significant negative correlation (Spearman's $\rho = -0.161$, $p = 0.0023$); (B) Correlation matrix (Spearman's ρ) of *D. sapinea* and three abundant Phaeothecales OTUs in symptomatic twigs only (n = 71). The matrix shows consistent negative correlations (light orange) between D. sapinea and each of the Phaeothecales taxa, including total Phaeothecales abundance, supporting a potential antagonistic relationship under symptomatic conditions.

possible to detect sequence reads from *D. sapinea* in 70 % of the apparently healthy (asymptomatic) trees, the abundance of the pathogen was 60-fold larger in the communities of trees that display shoot dieback symptoms (Supplementary Table S4). These findings should be interpreted in the context of the 2018 drought event that preceded the sampling. The drought likely acted as a predisposing factor, a physiological stressor contributing to the disruption of host-mycobiome balance and potentially triggering the transition of *D. sapinea* from a latent endophyte to a pathogenic state (Bachi and Peterson, 1985; Sherwood et al., 2015; Brodde et al., 2023). This aligns with the concept that abiotic stress can modulate fungal community dynamics and increase disease risk in conifer systems. The distribution of *D. sapinea* is compatible with it being an active contributing factor to the dieback in drought-stressed pines when present locally (Blumenstein et al., 2021b). In symptomatic twigs it develops strongly together with symptoms but is also present in still green tissue adjacent to the dieback symptoms. This can be interpreted as an invasion into still healthy-looking tissue (Vilanova et al., 2024). The observations in the present study are in stark contrast to the reports by Blumenstein et al. (2021a) that *D. sapinea* were highly abundant in trees showing no symptoms of Diplodia tip blight. Instead, our findings are more in line with the work by Oliva et al. (2021) who found *D. sapinea* to be present as an endophyte in asymptomatic pines, but generally in low amounts.

Several fungi were associated with the endophytic community in asymptomatic trees, twigs, and tissues. Among these were two relatively abundant OTUs in the order Phaeothecales. These fungi were associated with both asymptomatic twigs and asymptomatic tissues in symptomatic twigs. Isolates of *Phaeoteca dimorphospora* in the order Phaeothecales have been explored for their capacity to inhibit the growth of a wide range of tree pathogens such as *Ophiostoma ulmi, Gremmeniella* spp.,

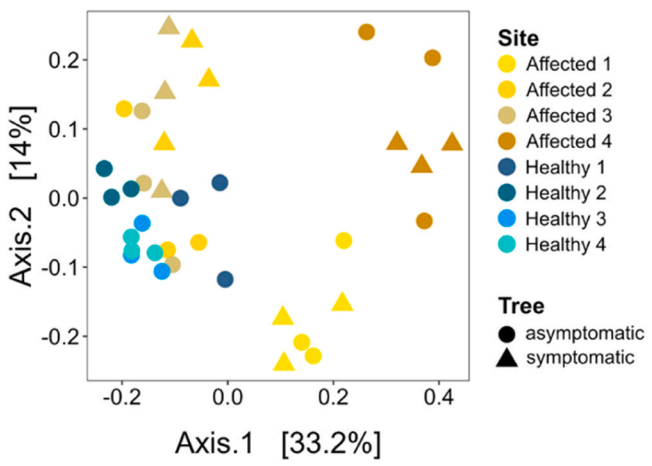

Fig. 5. Ordination plot of principal component analysis (PCoA) of the endophytic fungal community sampled from asymptomatic (●) and symptomatic Scots pines showing Diplodia tip blight (▲) on affected and healthy sites. Affected sites 1–4 in yellow/orange shades, healthy sites 1–4 in blue shades.

Sphaerulina musiva, and *Heterobasidion annosum* s.l. *in vitro* and *in planta* (Yang et al., 1994, 1995; Roy et al., 2001). *Phaeoteca dimorphospora* produces diffusible metabolites with capacities to inhibit the growth of pathogenic fungi (Yang et al., 1993; Roy et al., 2001) indicating that these fungi in Phaeothecales may use antibiosis to keep competing fungi at bay *in planta*. It tempting to speculate that the different colonization patterns of *D. sapinea* and the OTUs from Phaeothecales in this study derives from the fungi inhibiting *D. sapinea;* or priority effects that also can influence on the expression of disease symptoms (Leopold and Busby, 2020), as experimental work with fungi in Phaeothecales has indicated that prior colonization of host tissues inhibit pathogen colonization (Roy et al., 2001). However is unclear if the two fungi from Phaeothecales associated with asymptomatic tissues also produce antifungal metabolites and if such metabolites can inhibit the growth of *D. sapinea* or if prior colonization of fungi in the Phaeothecales exclude *D. sapinea* from the tissue. This would be interesting to follow up on in inoculation studies to further our understanding of fungal interactions with *D. sapinea in planta* beyond the known competition for key metabolites (e.g. proline) has previously been shown to be a mechanism of suppression of *D. sapinea* in the fungal community (Oliva et al., 2021). Another fungus that was strongly associated with asymptomatic trees, twigs and tissues was identified as *Neophaeothecoidea proteae* (sp_18). Interestingly this species was identified as one of five indicators for healthy Scots pine trees in an outbreak of Diplodia tip blight in Germany in 2018 (Blumenstein et al., 2021). However, *N. proteae* was relatively rare in the endophyte community in both the present study (<1 % of the reads) and in the work reported by Blumenstein and coworkers (2021). Our speculation is that this taxon is an example of a member of the endophyte community that is disfavoured by necrotic tissues rather than a potential antagonist to *D. sapinea,* but that too remains to be tested. Our results also demonstrate site dependent variation in fungal community composition, which is commonly observed in metabarcoding studies across sites (Ata et al., 2022, Bowman and Arnold, 2021). Bowman and Arnold (2021) argue that the dispersal of foliar endophytes are constrained by specific environmental conditions. It is likely that the dispersal and proliferation of the endophytic community in the current study also were limited by environmental variables. Site-specific differences like drought stress severity and stand characteristics including the trees vitality prior to the drought play a role in shaping these communities and influencing *D. sapinea* pathogenicity, as indicated by earlier research (Brodde et al., 2023) but this cannot be tested within this experimental design. While nature of our study may limit broad generalizations, it serves as a valuable insight into potential future climate scenario. Due to shallow soil depth, low water retention, and high exposure to drought as described in Stein Åslund (2020) the study area represents drought stress intensities that may become more widespread under a future climate.

Taken together, this study gives important insights into the dynamics of endophytic communities in relation to the emerging Diplodia tip blight disease in Northern Europe where it can still be considered an emerging disease. The results from this study show that the relative importance of *D. sapinea* in Scots pine endophyte communities from healthy trees is limited. High relative abundance of *D. sapinea* is closely associated with apparent symptoms of Diplodia tip blight, as previously reported by Brodde et al. (2023). Despite the focused scope of this study, there is no support that the pathogen currently accumulates unnoticed as an endophyte in healthy Scots pine trees in this region, as reported from continental Europe (Blumenstein et al., 2021a). The endophytic fungal community was also analyzed in order to generate an understanding of potential members of the mycobiome with potential to promote or suppress of Diplodia tip blight in the context of the balanced antagonism hypothesis (Schulz et al., 2015). The presence of other fungi, notably from the order Phaeothecales, in asymptomatic trees and tissues, raises the possibility of natural antagonistic relationships that could be exploited to inhibit accumulation of *D. sapinea* or development of Diplodia tip blight. These findings open new pathways for research into the potential antifungal mechanisms employed by these fungi and their role in maintaining tree health. Future studies should focus on

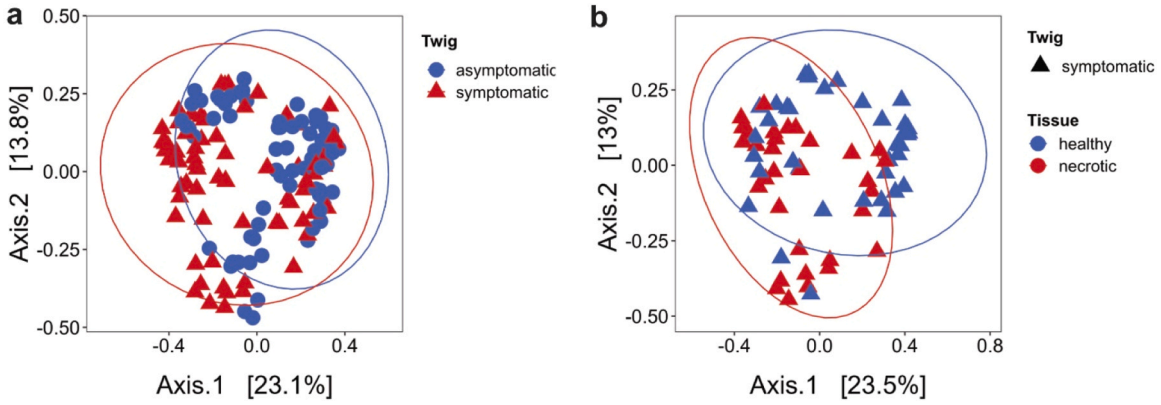

Fig. 6. Ordination plot of principal component analysis (PCoA) of the endophytic fungal community sampled from Scots pine showing Diplodia tip blight symptoms. This figure zooms in on fungal community variation within symptomatic trees, providing a more detailed comparison of the communities in different tissue conditions. a) Comparison of symptomatic (▲) and asymptomatic (●) twigs within the affected trees. b) Comparison of healthy (●) and necrotic tissues (●) within symptomatic (▲) twigs.

elucidating these interactions to enhance our understanding of fungal ecology in forest ecosystems.

CRediT authorship contribution statement

Redondo Miguel Angel: Writing – review & editing, Formal analysis, Data curation. **Tudoran Amelia Augusta:** Writing – review & editing, Visualization, Validation, Formal analysis. **Oliva Jonás:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization. **Elfstrand Malin:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Data curation, Conceptualization. **Miñana-Posada Silvia:** Writing – review & editing, Methodology, Investigation. **Brodde Laura:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Stenlid Jan:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2025.122781.

Data availability

Data will be made available on request.

References

Abarenkov, K., Somervuo, P., Nilsson, R.H., Kirk, P.M., Huotari, T., Abrego, N., Ovaskainen, O., 2018. Protax-fungi: a web-based tool for probabilistic taxonomic placement of fungal internal transcribed spacer sequences. N. Phytol. 220 (2), 517–525. https://doi.org/10.1111/nph.15301.

Adamson, K., Klavina, D., Drenkhan, R., Gaitnieks, T., Hanso, M., 2015. *Diplodia sapinea* is colonizing the native Scots pine (*Pinus sylvestris*) in the northern Baltics. Eur. J. Plant Pathol. 143 (2), 343–350. https://doi.org/10.1007/s10658-015-0686-8.

Addison, S., Armstrong, C., Wigley, K., Hartley, R., Wakelin, S., 2023. What matters most? Assessment of within-canopy factors influencing the needle microbiome of the model conifer, *Pinus radiata* (Article). Environ. Micro 18, 45. https://doi.org/10.1186/s40793-023-00507-8.

Arnold, A.E., Mejía, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins, N., Herre, E.A., 2003. Fungal endophytes limit pathogen damage in a tropical tree. Proc. Natl. Acad. Sci. 100 (26), 15649–15654. https://doi.org/10.1073/pnas.2533483100.

Ata, J.P., Ibarra Caballero, J.R., Abdo, Z., Mondo, Sj, Stewart, J.E., 2022. Transitions of foliar mycobiota community and transcriptome in response to pathogenic conifer needle interactions. Sci. Rep. 12, 7832. https://doi.org/10.1038/s41598-022-11907-0.

Bachi, P.R., Peterson, J.L., 1985. Enhancement of Sphaeropsis sapinea stem invasion of pines by water deficits. Plant Dis. 69 (9), 798–799. Retrieved from. ⟨https://www.apsnet.org/⟩.

Bacon, C.W., White, J.F., 2016. Functions, mechanisms and regulation of endophytic and epiphytic microbial communities of plants. Symbiosis 68, 87–98. https://doi.org/10.1007/s13199-015-0350-2.

Bálint, M., Bartha, L., O'Hara, R.B., Olson, M.S., Otte, J., Pfenninger, M., Robertson, A.L., Tiffin, P., Schmitt, I., 2015. Relocation, high-latitude warming, and host genetic identity shape the foliar fungal microbiome of poplars. Mol. Ecol. 24 (1), 235–248. https://doi.org/10.1111/mec.13018.

Bihon, W., Burgess, T., Slippers, B., Wingfield, M.J., Wingfield, B.D., 2011. Distribution of *Diplodia pinea* and its genotypic diversity within asymptomatic *Pinus patula* trees. Australas. Plant Pathol. https://doi.org/10.1007/s13313-011-0060-z.

Blumenstein, K., Bußkamp, J., Langer, G.J., Langer, E.J., 2021a. High abundance of Diplodia sapinea in asymptomatic Scots pine mycobiomes: Implications for forest health. *J. Mycol. Prog.*, 18, 320–335. https://doi.org/10.3390/jof7080607.

Blumenstein, K., Bußkamp, J., Langer, G.J., Schlößer, R., Parra Rojas, N.M., Terhonen, E., 2021. *Sphaeropsis sapinea* and associated endophytes in Scots pine: Interactions and effect on the host under variable water content. Front. For. Glob. Change 4, 655769. https://doi.org/10.3389/ffgc.2021.655769.

Bowman, E.A., Arnold, A.E., 2021. Drivers and implications of distance decay differ for ectomycorrhizal and foliar endophytic fungi across an anciently fragmented landscape. ISME J. 15 (12), 3437–3454. https://doi.org/10.1038/s41396-021-01006-9.

Brodde, L., Adamson, K., Camarero, J.J., Castaño, C., Drenkhan, R., Lehtijärvi, A., Luchi, N., Migliorini, D., Sánchez-Miranda, Á., Stenlid, J., Özdağ, Ş., Oliva, J., 2019. Diplodia tip blight on its way to the north: Drivers of disease emergence in Northern Europe. Front. Plant Sci. 9, 1818. https://doi.org/10.3389/fpls.2018.01818.

Brodde, L., Stein Åslund, M., Elfstrand, M., Oliva, J., Wågström, K., Stenlid, J., 2023. *Diplodia sapinea* as a contributing factor in the crown dieback of Scots pine (*Pinus sylvestris*) after a severe drought. For. Ecol. Manag. 546, 121436. https://doi.org/10.1016/j.foreco.2023.121436.

Bugzacki, S., 1972. Zalerion arboricola, a new helicosporous hyphomycete from conifer stems. Trans. Br. Mycol. Soc. 59 (1), 159–IN122.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E.V.L., Schulze-Lefert, P., 2013. Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant Biol. 64, 807–838. https://doi.org/10.1146/annurev-arplant-050312-120106.

Bullington, L.S., Lekberg, Y., Sniezko, R., Larkin, B., 2018. The influence of genetics, defensive chemistry and the fungal microbiome on disease outcome in whitebark pine trees. Mol. Plant Pathol. 19 (8), 1847–1858. https://doi.org/10.1111/mpp.12663.

Bußkamp, J., Langer, G.J., Langer, E.J., 2020. *Sphaeropsis sapinea* and fungal endophyte diversity in twigs of Scots pine (*Pinus sylvestris*) in Germany. Mycol. Prog. 19, 985–999. https://doi.org/10.1007/s11557-020-01617-0.

Clemmensen, K.E., Ihrmark, K., Durling, M.B., Lindahl, B.D., 2016. Sample preparation for fungal community analysis by high-throughput sequencing of barcode amplicons. Methods Mol. Biol. (Clifton, N. J.) 1399, *61–88*. https://doi.org/10.1007/978-1-4939-3369-3_4.

De Cáceres, M., Jansen, F., & Dell, N. (2020). Package 'indicspecies': Relationship between Species and Groups of sites version 1.7. 6. In.

Eusemann, P., Schnittler, M., Nilsson, R.H., Jumpponen, A., Dahl, M.B., Würth, D.G., Buras, A., Wilmking, M., Unterseher, M., 2016. Habitat conditions and phenological tree traits overrule the influence of tree genotype in the needle mycobiome–*Picea glauca* system at an arctic treeline ecotone. N. Phytol. 211 (4), 1380–1392. https://doi.org/10.1111/nph.13988.

Flowers, J., Hartman, J., Vaillancourt, L., 2003. Detection of Latent *Sphaeropsis sapinea* Infections in Austrian Pine Tissues Using Nested-Polymerase Chain Reaction. Phytopathology 93, 1471–1477. https://doi.org/10.1094/PHYTO.2003.93.12.1471.

Hassani, M.A., Durán, P., Hacquard, S., 2018. Microbial interactions within the plant holobiont. Microbiome 6, 1–17. https://doi.org/10.1186/s40168-018-0445-0.

Huson, D.H., Auch, A.F., Qi, J., Schuster, S.C., 2007. MEGAN analysis of metagenomic data. Genome Res. 17 (3), 377–386 doi:http://www.genome.org/cgi/doi/10.1101/gr.5969107.

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82, 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x.

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.-H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22 (21), 5271–5277. https://doi.org/10.1111/mec.12481.

Koskella, B., Meaden, S., Crowther, W.J., Leimu, R., Metcalf, C.J.E., 2017. A signature of tree health? Shifts in the microbiome and the ecological drivers of horse chestnut bleeding canker disease. N. Phytol. 215 (2), 737–746. https://doi.org/10.1111/nph.14560.

Larsson, R., Menkis, A., Olson, Å., 2024. Fungal communities associated with Picea abies, Pinus sylvestris and Larix sp. seeds of different geographic origin: Implications for disease management. For. Pathol. 54 (4), e12880. https://doi.org/10.1111/efp.12880.

Leopold, D.R., Busby, P.E., 2020. Host genotype and colonist arrival order jointly govern plant microbiome composition and function. e3265 Curr. Biol. 30 (16), 3260–3266. https://doi.org/10.1016/j.cub.2020.06.011.

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., Kõljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J., Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers: A user's guide. N. Phytol. 199 (1), 288–299. https://doi.org/10.1111/nph.12243.

Marčiulynas, A., Marčiulynienė, D., Lynikienė, J., Bakys, R., Menkis, A., 2022. Fungal Communities in Leaves and Roots of Healthy-Looking and Diseased *Ulmus glabra*. Microorganisms 10 (11), 2228. https://doi.org/10.3390/microorganisms10112228.

Maresi, G., Luchi, N., Pinzani, P., Pazzagli, M., Capretti, P., 2007. Detection of *Diplodia pinea* in asymptomatic pine shoots and its relation to the normalized Insolation index. For. Pathol. https://doi.org/10.1111/j.1439-0329.2007.00506.x.

McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8 (4), e61217. https://doi.org/10.1371/journal.pone.0061217.

Millberg, H., Boberg, J., Stenlid, J., 2015. Changes in fungal community of Scots pine (*Pinus sylvestris*) needles along a latitudinal gradient in Sweden. Fungal Ecol. 17, 126–139. https://doi.org/10.1016/j.funeco.2015.05.012.

Muñoz-Adalia, E.J., Sanz-Ros, A.V., Flores-Pacheco, J.A., Hantula, J., Diez, J.J., Vainio, E.J., Fernández, M., 2017. Sydowia polyspora dominates fungal communities carried by two tomicus species in pine plantations threatened by Fusarium circinatum. Forests 8 (4), 127. ⟨https://www.mdpi.com/1999-4907/8/4/127⟩.

Oliva, J., Ridley, M., Redondo, M.A., Caballol, M., 2021. Competitive exclusion amongst endophytes determines shoot blight severity on pine. Funct. Ecol. 1–17. https://doi.org/10.1111/1365-2435.13692.

Osono, T., Mori, A., 2004. Distribution of phyllosphere fungi within the canopy of giant dogwood. Mycoscience 45 (3), 161–168. https://doi.org/10.1007/S10267-003-0167-3.

Porras-Alfaro, A., Bayman, P., 2011. Hidden fungi, emergent properties: endophytes and microbiomes. Annu. Rev. Phytopathol. 49, 291–315. https://doi.org/10.1146/annurev-phyto-080508-081831.

Rajala, T., Velmala, S.M., Vesala, R., Smolander, A., Pennanen, T., 2014. The community of needle endophytes reflects the current physiological state of Norway spruce. Fungal Biol. 118 (3), 309–315. https://doi.org/10.1016/j.funbio.2014.01.002.

Redondo, M.A., Berlin, A., Boberg, J., Oliva, J., 2020. Vegetation type determines spore deposition within a forest–agricultural mosaic landscape. FEMS Microbiol. Ecol. 96 (6), fiaa082. https://doi.org/10.1093/femsec/fiaa082.

Redondo, M.A., Oliva, J., Elfstrand, M., Boberg, J., Capador-Barreto, H.D., Karlsson, B., Berlin, A., 2022. Host genotype interacts with aerial spore communities and influences the needle mycobiome of Norway spruce. Environ. Microbiol. 24 (8), 3640–3654. https://doi.org/10.1111/1462-2920.15974.

Ridout, M., Newcombe, G., 2018. Sydowia polyspora is both a foliar endophyte and a preemergent seed pathogen in pinus ponderosa. Plant Dis. 102 (3), 640–644. https://doi.org/10.1094/pdis-07-17-1074-re.

Rodriguez, R.J., White, J.F., Arnold, A.E., Redman, R.S., 2009. Fungal endophytes: diversity and functional roles. N. Phytol. 182, 314–330. https://doi.org/10.1111/j.1469-8137.2009.02773.x.

Roy, G., Bussieres, G., Laflamme, G., Dessureault, M., 2001. *In vitro* inhibition of *Heterobasidion annosum* by *Phaeotheca dimorphospora*. For. Pathol. 31 (6), 395–404. https://doi.org/10.1046/j.1439-0329.2001.00259.x.

Schulz, B., Boyle, C., 2005. The endophytic continuum. Mycol. Res. 109, 661–686. https://doi.org/10.1017/S095375620500273X.

Schulz, B., Haas, S., Junker, C., Andrée, N., Schobert, M., 2015. Fungal endophytes are involved in multiple balanced antagonisms. Curr. Sci. 109, 39–45 doi:https://www.jstor.org/stable/24905689.

Sherwood, P., Villari, C., Capretti, P., Bonello, P., 2015. Mechanisms of induced susceptibility to Diplodia tip blight in drought-stressed Austrian pine. Tree Physiol. 35, 549–562. https://doi.org/10.1093/treephys/tpv026.

Sieber, T.N., 2007. Endophytic fungi in forest trees: are they mutualists? Fungal Biol. Rev. 21 (2-3), 75–89. https://doi.org/10.1016/j.fbr.2007.05.004.

Smith, H., Wingfied, M.J., Coutinho, T.A., 2002. The role of latent *Sphaeropsis sapinea* infections in post-hail associated die-back of *Pinus patula*. For. Ecol. Manag. 164, 177–184. https://doi.org/10.1016/S0378-1127(01)00610-7.

Solheim, H., Torp, T.B., Hietala, A.M., 2013. Characterization of the ascomycetes *Therrya fuckelii* and *T. pini* fruiting on Scots pine branches in Nordic countries. Mycol. Prog. 12, 37–44. https://doi.org/10.1007/s11557-012-0813-2.

Somervuo, P., Koskela, S., Pennanen, J., Henrik Nilsson, R., Ovaskainen, O., 2016. Unbiased probabilistic taxonomic classification for DNA barcoding. Bioinformatics 32 (19), 2920–2927. https://doi.org/10.1093/bioinformatics/btw346.

Stein Åslund, M., 2020. Forest health and resilience in a changing climate: a case study of Scots pine decline on Gotland. Master Thesis, Swed. Univ. Agric. Sci.

Stein Åslund, M., Reichelt, M., Zhang, K., Castaño, C., Stenlid, J., Gershenzon, J., Elfstrand, M., 2024. Scots pines with tolerance to *Melampsora pinitorqua* and *Diplodia sapinea* show distinct metabolic profiles. Plant, Cell Environ. https://doi.org/10.1111/pce.15218.

Stone, J.K., Bacon, C.W., White Jr, J.F., 2000. An overview of endophytic microbes: endophytism defined. In: Bacon, C.W., White Jr, J.F. (Eds.), Microbial endophytes. Marcel Dekker, pp. 3–29.

Swart, W.J., Wingfield, M.J., Knox-Davies, P.S., 1987. Factors associated with *Sphaeropsis sapinea* infection of pine trees in South Africa. Phytophylactica 19, 505–510.

Terhonen, E., Blumenstein, K., Kovalchuk, A., Asiegbu, F.O., 2019. Forest tree microbiomes and associated fungal endophytes: functional roles and impact on forest health. Forests 10, 1–32. https://doi.org/10.3390/f10010042.

Terhonen, E.-L., Babalola, J., Kasanen, R., Jalkanen, R., Blumenstein, K., 2021. Sphaeropsis sapinea found as symptomless endophyte in Finland. article id 10420. Silva Fenn. 55 (1). https://doi.org/10.14214/sf.10420.

Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., Singh, B.K., 2020. Plant–microbiome interactions: from community assembly to plant health. Nat. Rev. Microbiol. 18 (11), 607–621. https://doi.org/10.1038/s41579-020-0412-1.

Unterseher, M., Tal, O., 2006. Influence of small-scale conditions on the diversity of wood decay fungi in a temperate, mixed deciduous forest canopy. Mycol. Res. 110 (2), 169–178. https://doi.org/10.1016/j.mycres.2005.08.002.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., Dufresne, A., 2015. The importance of the microbiome of the plant holobiont. N. Phytol. 206 (4), 1196–1206. https://doi.org/10.1111/nph.13312.

Vilanova, L., Caballol, M., Zhang, K., Olson, Å., Barnes, I., Wingfield, M.J., Oliva, J., 2024. New simple sequence repeat markers reveal undetected diversity in Spanish and Californian Diplodia sapinea populations. Fungal Genet. Biol. 174, 103937. https://doi.org/10.1016/j.fgb.2024.103937.

Wagner, M.R., Lundberg, D.S., Del Rio, T.G., Tringe, S.G., Dangl, J.L., Mitchell-Olds, T., 2016. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat. Commun. 7 (1), 12151. https://doi.org/10.1038/ncomms12151.

Yang, D., Plante, F., Bernier, L., Piché, Y., Dessureault, M., Laflamme, G., Ouellette, G., 1993. Evaluation of a fungal antagonist, *Phaeotheca dimorphospora*, for biological control of tree diseases. Can. J. Bot. 71 (3), 426–433. https://doi.org/10.1094/PD-78-0821.

Yang, D., Bernier, L., Dessureault, M., 1994. Biological control of Septoria leaf spot of poplar by *Phaeotheca dimorphospora*. Plant Dis. 78 (8), 821–825. https://doi.org/10.1094/PD-78-0821.

Yang, D., Laflamme, G., Bernier, L., Dessureault, M., 1995. *Phaeotheca dimorphospora* as a potential biocontrol agent for shoot blight caused by *Gremmeniella abietina*. Can. J. Plant Pathol. 17 (1), 7–12. https://doi.org/10.1080/07060669509500713.