



Fungal diversity in Atlantic oak root systems: a pioneering study in the Cantabrian Mountains

Norma Alas-Gutiérrez¹ · Elena Fernández-Miranda Cagigal² · José Carlos Pérez-Girón^{3,4} · Marcos Viejo⁵ · Candela Cuesta¹ · Ricardo Javier Ordás¹ · Pedro Álvarez-Álvarez¹

Received: 10 November 2025 / Revised: 19 December 2025 / Accepted: 28 December 2025
© The Author(s) 2026

Abstract

Oak forests are native ecosystems that support diverse life forms and provide essential services. This study focused on elucidating the taxonomic characterization and diversity of fungal species in Atlantic oak woodlands dominated by *Quercus petraea* at its southwestern distribution limit in the Iberian Peninsula. Sampling was conducted in three protected natural areas to assess fungal root diversity by macroscopic and microscopic methods, including histological analysis of root sections. Additionally, metabarcoding techniques were used to sequence the internal transcribed spacer (ITS) region, targeting fungal diversity. The analysis identified 1,043 Amplicon Sequence Variants (ASVs), corresponding to 297 species. Although fungal richness was consistent across locations, community composition was geographically specific. Tree social classes also influenced fungal diversity, which was higher in the intermediate trees in some areas. Functional guilds varied markedly, with symbiotic fungi (notably ectomycorrhizal fungi and dark septate endophytes) most abundant based on sequencing reads, while saprotrophs were the most diverse genus. These findings highlight the structural and ecological complexity of oak-associated fungal communities and their potential importance in forest conservation and ecosystem functioning.

Keywords DNA sequencing · Metabarcoding · Mycorrhizal fungi · *Quercus petraea* · Root systems · Systematic sampling · Taxonomic characterization

Introduction

Plants interact with the diverse communities of organisms living in the soil around their roots, a biodiversity-rich zone known as the rhizosphere (Igiehon and Babalola 2018). Within this zone, arbuscular mycorrhizal associations

Section editor: Marco Thines

✉ Pedro Álvarez-Álvarez
alvarezpedro@uniovi.es

Norma Alas-Gutiérrez
normaalasgutierrez@gmail.com

Elena Fernández-Miranda Cagigal
elena@ixcelium.com

José Carlos Pérez-Girón
jcperezgiron@gmail.com

Marcos Viejo
marcos.viejo@usc.es

Candela Cuesta
cuestacandela@uniovi.es

Ricardo Javier Ordás
rordas@uniovi.es

¹ Department of Organisms and Systems Biology, Polytechnic School of Mieres, University of Oviedo, 33600 Mieres, Asturias, Spain

² ixCelium, 17 King Edwards Road, Ruislip, London HA4 7AE, UK

³ Department of Botany, Faculty of Sciences, University of Granada, Av. Fuente Nueva S/N, 18071 Granada, Spain

⁴ Inter-University Institute for Research On the Earth System in Andalucía (IISTA), University of Granada, Av. Mediterráneo S/N, Ronda 18006, Granada, Spain

⁵ Department of Functional Biology, University of Santiago de Compostela, Santiago de Compostela, 15782 A Coruña, Spain

engage in complex and symbiotic relationships with approximately 85% of plant species (Strullu-Derrien et al. 2018), enhancing nutrient absorption and contributing to the productivity, health, and overall functioning of forest ecosystems (Luo et al. 2023). These associations also increase plant resilience to environmental stresses, such as drought, and make an indispensable contribution to biodiversity conservation (Cosme 2023). Consequently, a comprehensive understanding of fungal diversity and dynamics in tree root systems is basic for unravelling the complexity of below-ground biodiversity patterns and ecosystem processes.

Several techniques have enabled scientists to unveil the diverse range of fungal species that play an essential function in the symbiotic relationships of forest ecosystems (Tedersoo et al. 2022; Adnan et al. 2022). However, recent studies have highlighted the value of metabarcoding in providing a holistic perspective of fungal communities (Adamo et al. 2020), revealing the intricate species webs and their adaptive responses to environmental changes (Gorzela et al. 2015; Tedersoo et al. 2020). Given their ecological relevance, such methodological approaches are particularly valuable for studying tree-dominated ecosystems where below-ground interactions play a central role. This technology is indispensable for generating the holistic data required to inform conservation and management strategies in vulnerable ecosystems.

Oak forests are renowned for maintaining ecological equilibrium and providing a suite of ecosystem services, including carbon sequestration, soil stabilization, water regulation, and cultural benefits, which are invaluable to environmental health and human well-being (Stavi et al. 2022). They support a rich tapestry of life, including a wide variety of flora and fauna, as well as a high diversity of root fungal associations (Toju et al. 2013a, b; García-Guzmán et al. 2017). However, despite their ecological importance, there is a notable gap in our understanding of the identification of whole fungal diversity supported by Atlantic oak-dominated forests, which include pedunculate oak (*Quercus robur* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.).

This knowledge gap is particularly evident at the southwestern limit of the distribution of these forests, a critical ecotone where populations are under severe pressure due to projected climate-driven shifts that reduce suitable habitat and force upward niche displacement (Pérez-Girón et al. 2025). In this region, oak stands are increasingly shrinking or shifting northward in response to changing environmental conditions (Benito Garzón et al. 2008; del Río et al. 2021). This geographic boundary represents a natural laboratory for studying ecological vulnerability. As these ecosystems change, the associated fungal communities, which underpin host health, are also predicted to shift, fundamentally altering ecosystem function (Che et al. 2019). Studying the complex fungal interactions and diversity within Atlantic

oak forests, particularly in the most threatened species, *Quercus petraea*, is important to determine the biodiversity and functional roles of these communities within the ecosystems (Toju et al. 2018).

The main fungal groups, *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Glomeromycota* and *Zygomycota*, are cosmopolitan, being present in multiple ecosystems worldwide, with similar fungal communities depending on environmental conditions (Tedersoo et al. 2014). However, Tedersoo et al. (2014) also argued that fungal communities vary with the type of vegetation, and that even within the same species they are conditioned by the state of development of the individuals.

Accordingly, the main objectives of this study were: (i) to characterize the fungal communities in Atlantic oak forests in northern Spain, focusing on *Q. petraea*, through systematic identification and classification of fungal taxa involved; and (ii) to determine how the relationships between site and tree social class influence fungal diversity and composition.

Methods

Site description

Three locations within Natura 2000 protected areas in the Cantabrian Mountains (northern Spain) were selected: Redes Natural Park (RDS), Fuentes del Narcea, Degaña e Ibias Natural Park (FDI), and Ancares Special Area of Conservation (ANC). All sites share a temperate, humid Atlantic climate, with annual precipitation of 1000–1300 mm and mean annual temperatures of 14–17 °C (Ninyerola et al. 2005). Forests are dominated by mature oak (*Quercus* spp.) and beech (*Fagus sylvatica* L.) stands (Allué Andrade 1990). Fungal diversity analysis was conducted within an inventory plot in each protected area (Fig. 1). The abiotic and biotic characteristics of each plot, including soil pH, organic matter content, humus type, texture, slope, and canopy composition, were recorded by the authors and are summarized below. All plots were selected as representative of their respective protected areas and provide a suitable environmental gradient for investigating the factors shaping fungal diversity.

Río del Alba plot (RDS) was NE-facing, with a 54% slope at 676 m a.s.l. (coordinates 298686 E, 4782038 N -ETRS89, UTM Zone 30-). Soil was strongly acidic (pH 4.57), with high organic matter content (MO 27.76%), elevated nitrogen levels (1.24%) and Moder humus type (C/N 15.59). The soil texture was sandy loam. Monasterio de Hermo plot (FDI) faced SE, had a 33% slope at 1200 m a.s.l. (coordinates 699868 E, 4761828 N -ETRS89, UTM Zone 29-), with strongly acidic soil (pH 3.79), very high organic matter (MO 25.63%) and nitrogen content (0.90%). The humus was

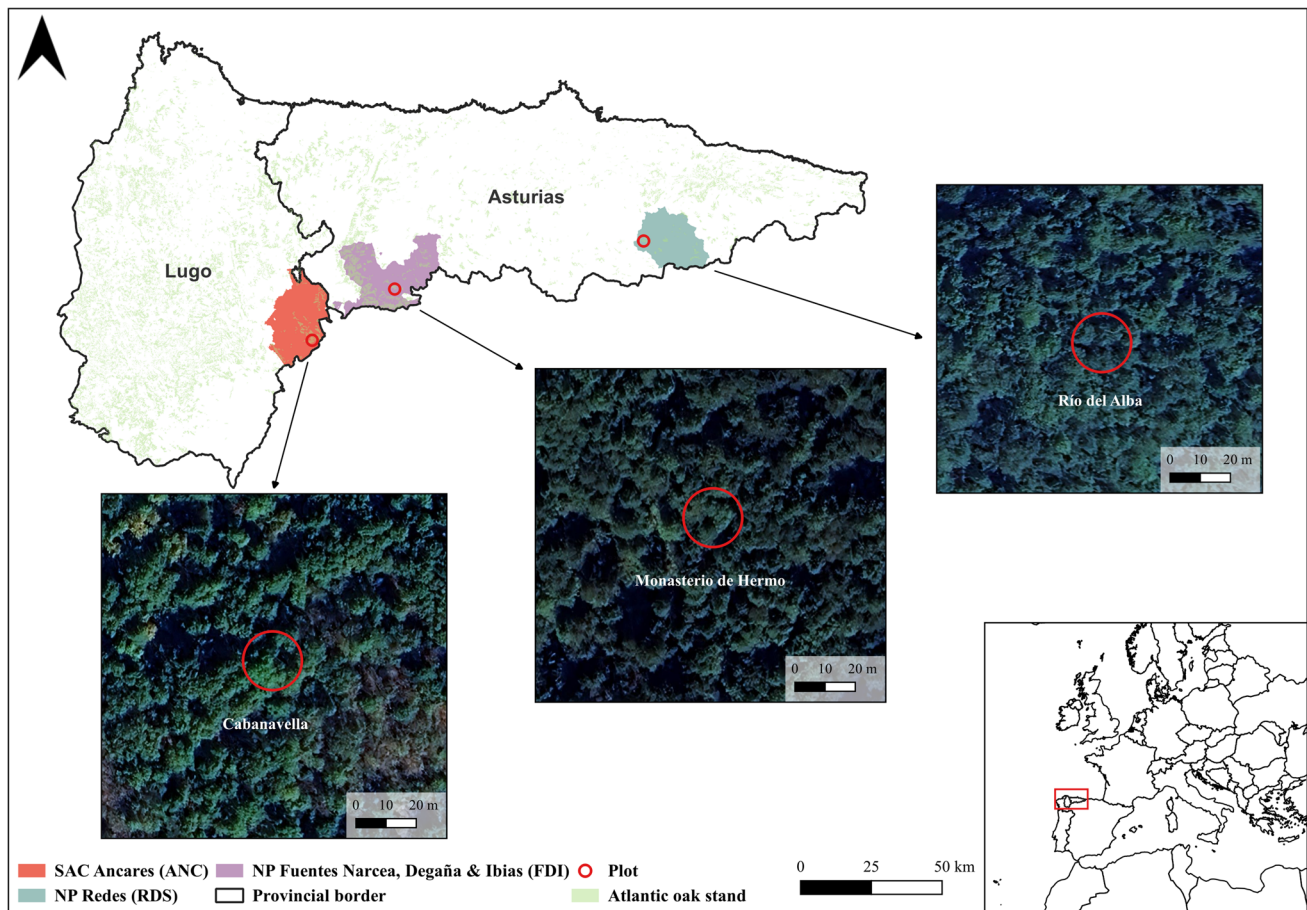


Fig. 1 Location of the three inventory plots within the Redes Natural Park (RDS), Fuentes del Narcea, Degaña e Ibias (FDI) Natural Park and the Ancares Special Area of Conservation (ANC). Atlantic oak

stands represent forest stands dominated by *Q. petraea* according to the Forest Map of Spain

Moder type (C/N 15.56) and the texture was sandy loam. Cabanavella plot (ANC) was SW-facing, with 59% slope at 1191 m a.s.l. (coordinates 672082 E, 4741679 N -ETRS89, UTM Zone 29-). Soil was moderately acidic (pH 5.51), with high organic matter (9.13%) and moderate nitrogen content (0.35%). As in the previous inventory plots, the humus was Moder (C/N 15.63) and the texture was sandy loam. All plots were dominated by mature oak stands (*Quercus petraea*). Pure stands in Río del Alba and Cabanvella, with occasional representation of *Q. pyrenaica* Willd and *Fagus sylvatica* at Monasterio de Hermo.

Sample collection and root processing

Four individual oak trees were chosen at random in each inventory plot to include one tree in each social class (submerged, intermediate, codominant and dominant). This choice is based on social class reflecting the structural position of the tree within the canopy and, therefore, adequately summarizes its level of competence, access to resources

(especially light), and its role within the vertical structure of the forest. Dominant trees extend above the general canopy and receive full vertical and substantial lateral light, while co-dominant trees form the main canopy layer and obtain full light from above but only limited light from the sides due to neighbouring crowns of similar height. Intermediate trees are positioned within the lower part of the canopy and receive reduced vertical light and minimal lateral illumination, reflecting a moderate to high level of competition. Submerged trees remain entirely below the canopy, receiving only diffuse light and experiencing the highest competitive pressure (Bechtold 2003).

A total of 12 individual trees were analysed (Table 1). The roots of each selected tree were carefully dug up to ensure that they belonged to the target individual. The main root was traced to the location of the fine roots, where samples were collected. Sections of the root were cut in different cardinal directions, obtaining a sample per tree of ≈ 2.4 m long. These samples were placed in sterile Ziplock bags and stored at 4 °C until further analysis. Samples were

Table 1 Dasometric characteristics, sociological classes and sample characteristics of the 12 selected trees

Plot	Species	Tree identification code	Diameter (cm)	Height (m)	Sociological class
ANC	<i>Q. petraea</i>	A1	42.3	18.4	Dominant
		A2	33.8	16.8	Codominant
		A3	18.8	13.5	Intermediate
		A4	11.5	6.3	Submerged
FDI	<i>Q. petraea</i>	F1	26.1	17.5	Dominant
		F2	26.5	14.6	Codominant
		F3	32.5	12.9	Intermediate
		F4	17.8	12	Submerged
RDS	<i>Q. petraea</i>	R1	41.4	24.9	Dominant
		R2	26.7	16.7	Codominant
		R3	23.9	16.6	Intermediate
		R4	11.9	11.2	Submerged

obtained by digging to a depth of 20 cm, covering the entire topsoil, where biological activity is greatest. All the samples were collected during a period of less than 30 days between March and April of 2023, trying to capture the maximum fungal diversity. This timing coincided with the production of fine roots, where most of the associations occur, and an increase of root exudates, which stimulate the formation of fungal associations.

The roots were washed with tap water to remove adhering soil particles and then divided into 10 cm subsamples. Two different types of analysis were then conducted: *i*) analysis of individual ectomycorrhizal morphotypes located at the root tips and clarification of roots to confirm the presence of arbuscular mycorrhizal and endophytes by histological analyses, and *ii*) DNA extraction to determine the endogenous and exogenous fungal communities present in the roots by metabarcoding analyses.

Histological analysis

In each subsample, the ectomycorrhizal morphotypes were macroscopically characterized, using a Leica EZ 410 magnifying glass, based on the methods proposed by Agerer (1987; 1995) and Goodman et al. (1996). Based on the methods indicated, an identification sheet was developed in which, for each morphotype analysed, the branching type, shape, texture, colour and lustre were recorded. Additionally, the rate of colonization of each morphotype was calculated based on the total number of ectomycorrhizal root tips per cm of root.

To validate the presence of the Hartig net in the morphotype, 20 subsamples of each sample were collected into 1.5 mL tubes containing FAA fixative and were stored at 4 °C for later microscopic examination. Slices of thickness between 10 and 30 µm were prepared in a cryostat (Leica,

model CM1510-1). The slices were examined with a Leica DM 2000 microscope and a Leica DFC295 camera. Histological characterization was performed using the methods described by Goodman et al. (1996) and Ingleby et al. (1990), consisting of the determination of the type of parenchyma or pseudoparenchyma that forms the mantle, recording the mantle thickness and, also, the presence or absence of Hartig net and its extent when present.

To determine the presence of arbuscular mycorrhizas and/or endophytes, several segments of root (2 cm), sampled every 10 cm along the root of all sampled trees were collected and placed in 1.5 mL tubes containing FAA fixative for later microscopic examination and stored at 4 °C. The clarification and staining method described (Phillips and Hayman 1970) was used to histologically validate the presence of arbuscular mycorrhizas and/or endophytes. Root samples were submerged in 10% KOH (w/v potassium hydroxide in water) at 100 °C for 10–15 min, then bleached with fresh alkaline 10% H₂O₂ (v/v in water) at room temperature for 10 min and finally acidified with 1 N HCl for 10 min. The roots were stained with Trypan blue at 0.05% (w/v) at 100°C for 10 min using lactoglycerol (1:1:1 lactic acid, glycerol and water) to remove excess dye. A Leica DM 2000 microscope, along with a Leica DFC295 camera, was used for verification.

DNA extraction

DNA was extracted for metabarcoding analysis to characterize the fungal communities associated with the roots. In total, DNA was extracted from 45 samples: 11 from individual ectomycorrhizal morphotypes and 34 from root fragments of the 12 selected trees. Two types of samples were processed: **(a)** individual ectomycorrhizal morphotypes to determine the main fungal components associated with each type (20 ectomycorrhizal root tips of each morphotype were

collected and frozen at -80°C for DNA analysis), and (b) selected root fragments from the 12 selected trees to capture the overall fungal community along the root system. For these, 3–4 fragments each of 2–4 cm were selected from each subsample and frozen at -80°C until extraction, resulting in a total of 34 DNA extractions. DNA was extracted following the manufacturer's protocol, with the GeneMATRIX Plant & Fungi DNA Purification Kit (EURx). Samples of 20–30 μL (DNA concentration $> 10\text{ ng}/\mu\text{L}$ and A260/A280 ratio > 1.8 –2) were used. DNA was quantified using a Qubit High Sensitivity DNA Assay (Thermo Fisher Scientific).

Metabarcoding

The ITS1 spacer region of the rRNA locus, consisting of approximately 280 base pairs, was amplified with primer pairs ITS1F (Gardes and Bruns 1993) and ITS2 (White et al. 1990). The primers included the Illumina sequencing platform with 5' end attachments. A blocking primer (*Quercus*_ITS2BP: ATGCGAGAGCCTAGATATCCGTTGCC) based on the sequences of *Q. petraea* and *Q. robur* was designed, with Geneious 11.1.5, to prevent non-specific amplification of *Quercus* spp, in the region where the reverse primer (ITS2) hybridizes. A C3 CPG spacer (a non-nucleotidic modification) was also added to the 3' end of the blocking primer to prevent elongation. This enables the primer to bind unwanted DNA targets without being amplified, therefore selectively suppressing *Quercus* spp. DNA amplification during PCR.

PCRs were conducted with a reaction mixture containing 1.25 μL of template DNA, 0.5 μM of the primers, 10 μM of the blocking primers, 3.13 μL of Supreme NZYTaq (NZYTech) and 2 $\times$ Green Master Mix (NZYTech), made up to a volume of 12.5 μL with ultrapure water. The reaction mixture was incubated as follows: initial denaturation at 95°C for 5 min; followed by 35 cycles of 95°C for 30 s, 65°C for 45 s, 52.9°C for 45 s, and 72°C for 45 s; and final extension at 72°C for 7 min.

The oligonucleotide indices required for multiplexing different libraries in the same sequencing pool were attached in a second amplification step under identical conditions but with only five cycles and an annealing temperature of 60°C . According to the manufacturer's instructions, the library was purified using Mag-Bind RXNPure Plus magnetic beads (Omega Bio-tek). The finished libraries were pooled in equimolar amounts according to a QubitdsDNA HS Assay (Thermo Fisher Scientific) and sequenced using NovaSeq technology (Illumina). 250 bp paired-end reads were used and 4 Gb of data (19,471,306 reads) were generated (accessible in <https://www.ncbi.nlm.nih.gov/>, BioProject PRJNA1372172).

Bioinformatics

Cutadapt v3.5 software was used to remove non-biological DNA sequences (primers, indices, and sequencing adapters). Amplicons were processed using the DADA2 tool (Callahan et al. 2016) and implemented with QIIME 2 (Bolyen et al. 2019). The resulting output of the DADA2 pipeline consisted of a table including the number of occurrences of each Amplicon Sequence Variant (ASV) observed in each sample and a table listing all ASVs and the corresponding representative sequences. To reduce the potential impact of low-abundance reads, ASVs occurring at a frequency below 0.01% in each sample were excluded from further analyses. This filtering step was applied to reduce the potential impact of low-abundance reads, which can result from sequencing errors or technical artefacts.

Taxonomic analysis

The taxonomic assignment was conducted in two steps. We used the UNITE fungal database v8.2 (Abarenkov et al. 2020) and BLASTn v2.13.0 + algorithm (Camacho et al. 2009) for the initial analysis. We then cross-referenced each sequence using the Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). We implemented this dual verification process because the data available on the BLAST website is more recent and up-to-date. This approach ensures the validity and rigour of the data obtained through molecular analysis. Taxonomic assignment was based on the following criteria: *i*) query coverage $> 80\%$, indicating the proportion of the query sequence aligning with the reference; *ii*) E-value closest to 0, representing the probability of random matches; and *iii*) percentage of identity $> 80\%$, reflecting the proportion of identical bases between sequences. Identification levels were determined based on query coverage and percentage of identity, and ASVs below the 80% threshold were excluded.

Additionally, ASVs with identical values across multiple individuals of the same taxonomic category (provided the data were homogeneous) were assigned to the next higher taxonomic level whenever feasible, and otherwise they were excluded. After thorough analysis and verification of the data, 4.8% of the ASVs were discarded. At the genus level, fungal functional guilds were assigned using the FungalTraits database (Pölme et al. 2020) and FUNGuild database (Nguyen et al. 2016), in a complementary manner. This approach maximizes the coverage and reliability of functional assignments and reduces ambiguities. When a taxon was present in both databases, trait assignments were verified for consistency.

Diversity metrics

Diversity metrics and community composition analyses were conducted using the *vegan* package (Oksanen et al. 2022) in R Software (R Core Team 2024). Species richness, Shannon equitability (Pielou's evenness), and Gini-Simpson indices were calculated to quantify alpha diversity. Non-parametric pairwise comparisons were conducted using the Wilcoxon rank-sum test for two group comparisons and the Kruskal–Wallis test for those involving multiple groups.

Beta diversity was assessed using the Sørensen (Sørensen 1948), Jaccard (Jaccard 1912) and Whittaker (Whittaker 1960) dissimilarity matrices, using a distance-to-group centroid approach based on presence/absence data, providing a robust measure of community composition differences. This enables the variance in species composition across areas to be gauged by inter-site comparisons.

For each dissimilarity matrix, we assessed homogeneity of multivariate dispersions using PERMDISP (Anderson 2006) based on the distance of each site to its group centroid using 999 permutations to test whether beta diversity differed among sampling sites due to differences in within-group dispersion. To visualize multivariate patterns, we performed Principal Coordinates Analysis (PCoA) on each dissimilarity matrix. PCoA ordinations were used to inspect the spatial configuration of sites and to qualitatively assess group separation in fungal community composition. Differences among sampling sites in centroid location (i.e., community composition) were formally tested using PERMANOVA (Anderson 2008) with 999 permutations for each of the three dissimilarity matrices. This allowed us to determine whether fungal communities differed significantly among sampling sites after accounting for within-group dispersion.

Additionally, we also computed the indicator value (IndVal) with the *indicspecies* package (De Cáceres and Legendre 2009) in R Software (R Core Team 2024) using the *multipatt* function for seeking fungal species associated with individual or combined site groups (De Cáceres et al. 2010).

Results

Fungal community richness and composition

Root histology observations

The results of root analysis showed that all samples have internal fungal structures corresponding to dark septate endophytes, sclerotium, endophytes and vesicles, which may indicate the presence of arbuscular mycorrhizal fungi. Arbuscules were not found in any case (see Supplementary

Information Fig. S2). These observations are purely descriptive.

ASV richness and composition

1,043 ASVs were obtained from DNA extractions (1.748.225 reads in FDI, 1.535.035 reads in ANC and 1.118.325 reads in RDS); the highest number was found at the ANC site and the lowest at the RDS site. Overall, 7.4% of ASVs were shared among the sites (Fig. 2). The richness of the fungal community did not differ significantly across the sampling areas (ANC, FDI and RDS). Similarly, comparison of the richness among the tree social classes within each area did not reveal any statistically significant differences. Additionally, no significant differences in diversity were found in the comparison of tree social classes across the various sampling areas (Supplementary Information Table S1).

Cladophialophora emerged as the most diverse genus through the sites, with 43 ASVs in ANC, 28 ASVs in RDS and 25 ASVs in FDI. The next most diverse genera were *Mortierella*, with 19 ASVs in RDS, *Hyaloscypha* with 18 ASVs in FDI, and *Oidiodendron*, which had 17 ASVs in ANC (Supplementary Information Table S2). At the species level (Supplementary Information Table S2), *Cladophialophora chaetospora* was the most diverse (21 ASVs in ANC, 18 ASVs in FDI and 14 ASVs in RDS). This was followed by *Rhizodermea veluwensis* (15 ASVs in ANC), *Lactifluus vellereus* and *Oidiodendron majus* (7 ASVs in FDI) and *Lachnum virgineum* (6 ASVs in RDS).

Analysis of the social class of the trees (Table 2) showed that *Cladophialophora* was the dominant genus, especially in the protected areas of the ANC and FDI. By contrast, in the RDS site, *Cladophialophora* was less abundant, and other genera such as *Mortierella* and *Oidiodendron* were important.

Based on the taxonomic assignment and the number of reads for each ASV (view full taxonomic assignment in Supplementary Data), the genera with the highest relative abundance were *Rhizodermea* in ANC and RDS and *Lachnum* in FDI. The species with the highest relative abundance were *Rhizodermea veluwensis*, in ANC and RDS, and *Lachnum pygmaeum* (Fig. 3a and 3b). This pattern was followed by the individual trees analysed at each site. In ANC, *Rhizodermea* was the main genus in the dominant, codominant and intermediate classes, whereas *Lactarius* was predominant in the submerged class. In FDI, *Lachnum* was the most abundant genus in the dominant, codominant and submerged classes, while *Rhizoscyphus* was the most abundant in the intermediate class. Finally, in RDS, the main genera were *Lachnum* in the dominant class, *Chaetosphaeria* in the codominant class, *Phacidium* in the submerged class and *Rhizodermea* in the intermediate class (Supplementary Information Table S3). *Rhizodermea veluwensis* was the main species in

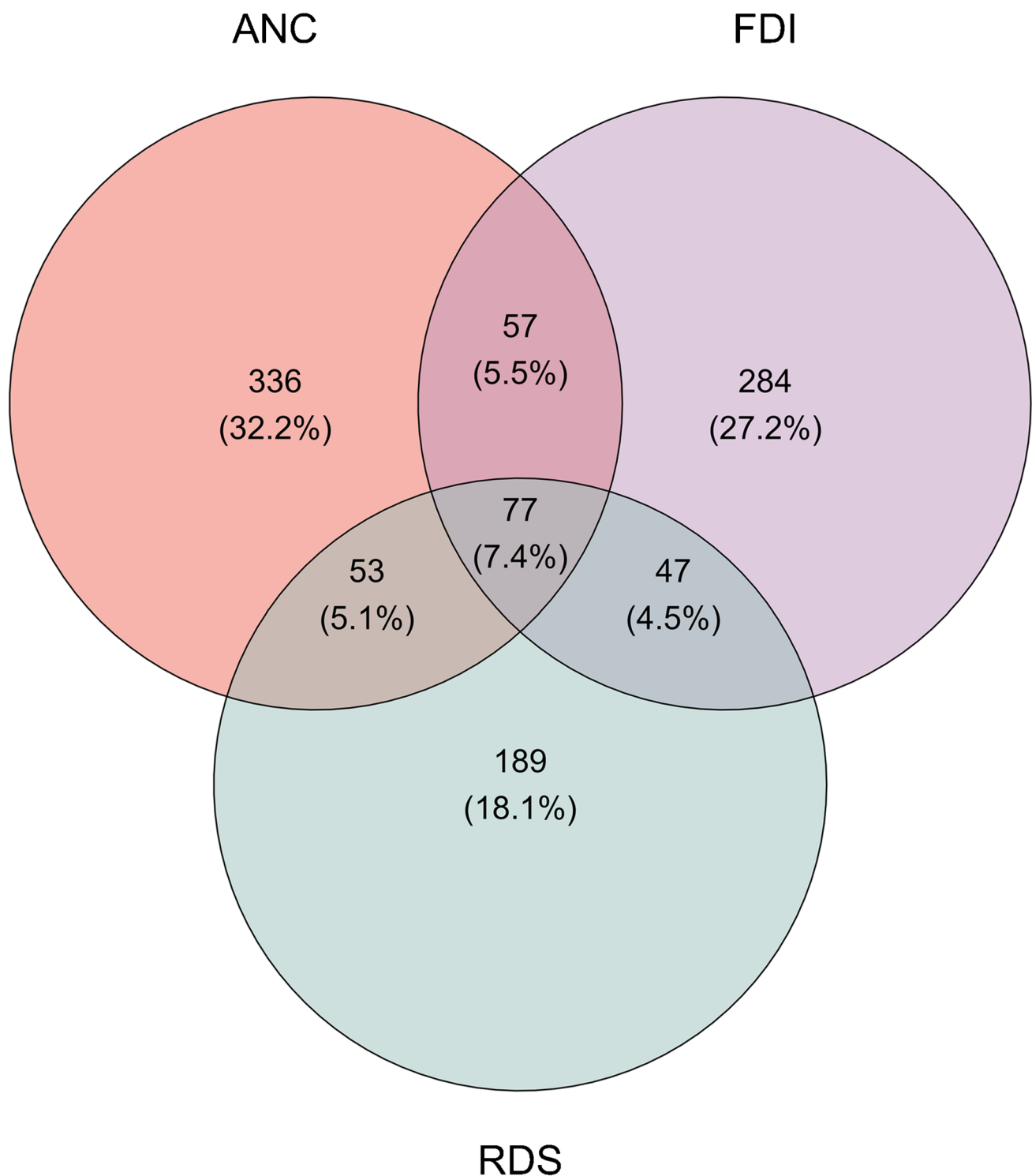


Fig. 2 Venn diagram of ASVs in different sites and overlap in composition among sites

the dominant, codominant and intermediate classes in the ANC site, in the codominant class in FDI and in the dominant and intermediate classes in RDS. In addition, the following were the main species: *Chaetosphaeria vermicularioides* in the codominant class in RDS; *Lachnum pygmaeum*

in the dominant class in FDI, *Lactarius camphoratus* in the submerged class in ANC, *Phacidium mollerianum* in the submerged class in RDS and *Rhizoscyphus monotropae* in the intermediate class in FDI (Supplementary Information Table S3).

Table 2 Most common ASVs (genus and species) depending on tree social class and site. Values within parentheses indicate the number of different ASVs

Protected area	Social class	Genus	Species
ANC	Dominant	<i>Cladophialophora</i> (16)	<i>Rhizodermea veluwensis</i> (12)
	Codominant	<i>Cladophialophora</i> (19)	<i>Cladophialophora chaetospora</i> (9)
	Intermediate	<i>Cladophialophora</i> (29)	<i>Cladophialophora chaetospora</i> (19)
	Submerged	<i>Cladophialophora</i> (8)	<i>Cladophialophora chaetospora</i> (6)
FDI	Dominant	<i>Cladophialophora</i> (12)	<i>Cladophialophora chaetospora</i> (7)
	Codominant	<i>Cladophialophora</i> (7)	<i>Lactifluus vellereus</i> (7)
	Intermediate	<i>Cladophialophora</i> (13)	<i>Cladophialophora chaetospora</i> (11)
	Submerged	<i>Cladophialophora</i> (10)	<i>Cladophialophora chaetospora</i> (8)
RDS	Dominant	<i>Mortierella</i> (13)	<i>Oidiodendron majus</i> (5)
	Codominant	<i>Cladophialophora</i> (23)	<i>Cladophialophora chaetospora</i> (11)
	Intermediate	<i>Mortierella</i> (9)	<i>Mortierella macrocystis</i> (3)
	Submerged	<i>Cladophialophora</i> (8)	<i>Cladophialophora chaetospora</i> & <i>Oidiodendron majus</i> (5)

The findings show that the fungal community appeared to be less diverse in RDS than in ANC and FDI. The observed differences were based on tree social classes, suggesting that the fungal composition is influenced by the position of the trees in the forest.

Fungal taxonomic richness as a function of sampling site and tree social class is summarised in Table 3. The highest number of taxa in the intermediate class and the highest proportion of unique taxa across all taxonomic levels were found in ANC and FDI. By contrast, the overall diversity and taxonomic exclusivity were generally lower in the dominant trees. This pattern was not observed in RDS, where intermediate trees exhibited lower uniqueness than dominant trees. Considering the observed data, future studies involving fungal populations associated with temperate forests should perhaps focus on intermediate trees.

The indicator value analysis revealed that 39 species were associated with a single group, whereas five species were associated with the two groups (Table 4). The species *Lactarius camphoratus* and *Lecidea nylanderii* were identified as strong indicators for the ANC group, with IndVal statistics of 0.920 and 0.874, respectively, with a significant p-value of 0.001. Similarly, for the FDI group, *Rhizoscyphus monotropae* and *Tyrannosorus hystrionides* were identified as significant indicators, with IndVal values of 0.925 and 0.909, respectively.

Alpha and beta diversity

Examination of the alpha diversity yielded valuable insights into the ecological richness within each area. Shannon and Gini-Simpson indices showed a decrease at the species level relative to the genus or ASVs levels. Overall, the RDS exhibits a less complex community with lower diversity values than ANC and FDI. Interestingly, Pielou's evenness index reflected a different trend, with higher values at the species

level in RDS, suggesting that the community is not dominated by a few individuals (Fig. 4).

Based on tree type, some differences were observed. In ANC, the Shannon and Gini-Simpson diversity indices generally showed greater diversity in dominant and intermediate trees than in codominant and submerged trees. In FDI, the diversity values were highest for intermediate and submerged trees, and in RDS, they were highest for the dominant and submerged trees. Regarding evenness, the values indicate that, despite some diversity, the distribution of abundance is not completely uniform. In ANC and RDS, the lowest evenness values corresponded to the dominant trees, whereas in FDI they corresponded to the intermediate trees (Supplementary Information Table S4).

At the genus level, both the Jaccard and Sørensen indices indicated higher multivariate dispersion (i.e., higher beta diversity) in FDI, followed by RDS and ANC, as shown by the distances to group centroids (Fig. 5). This pattern suggests greater heterogeneity in genus composition among FDI sites. In contrast, the Whittaker index showed the opposite trend, with ANC exhibiting the largest dispersion and the clearest separation in PCoA space, followed by RDS and FDI, indicating stronger turnover in ANC. These visual patterns were consistent across PERMDISP and PCoA. PERMANOVA (Supplementary Information Table S6) further supported significant differences in community composition among sampling sites for all three indices, particularly for Whittaker, which showed the strongest separation in multivariate space. Results at the species level showed a similar pattern (see Fig. S1).

Fungal traits and guilds

After completion of the taxonomic assignment, functional assignment was conducted. The genus was assigned to one of five categories: no data (nd, i.e. not included in the databases

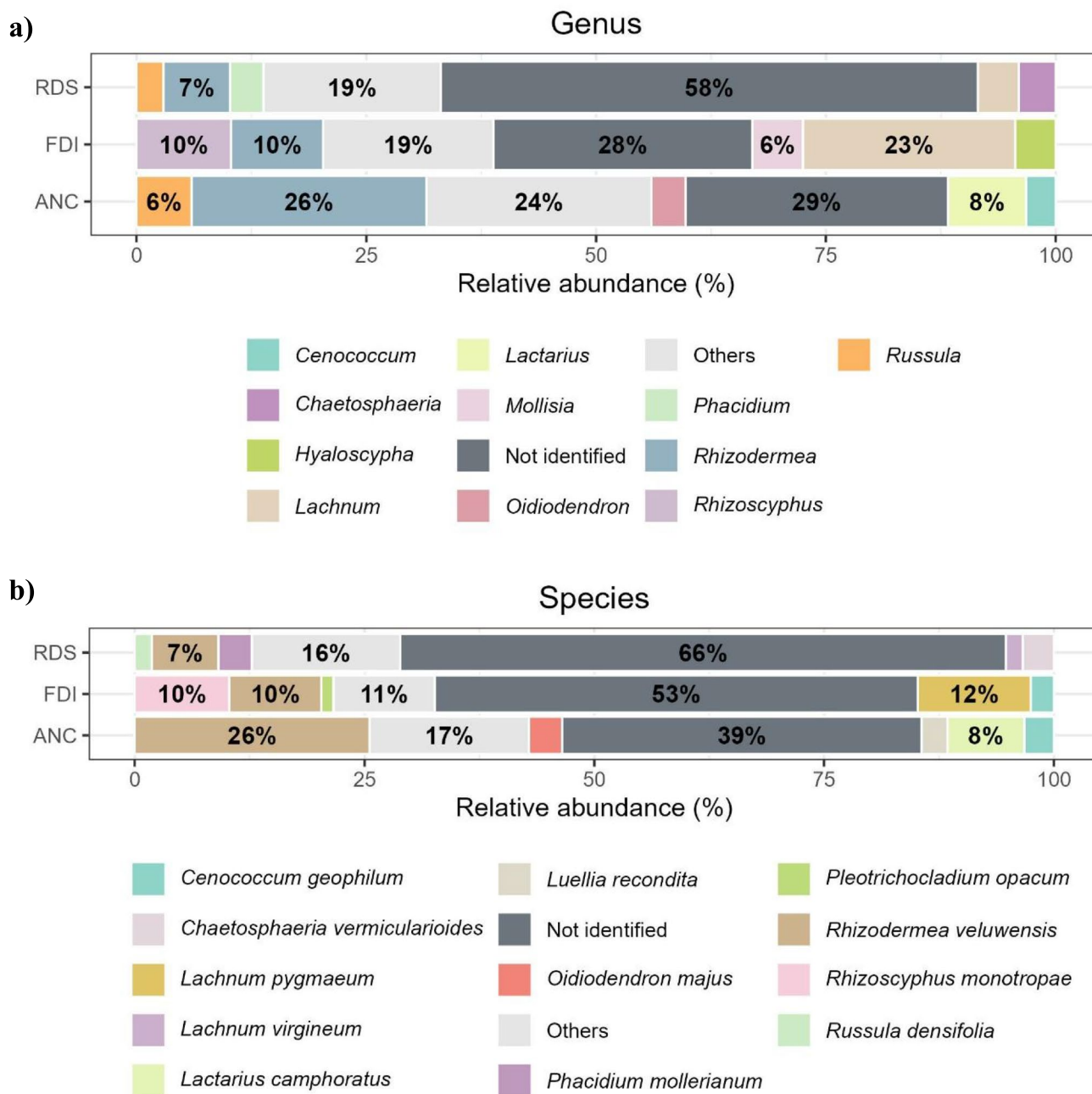


Fig. 3 Relative abundance of the five most important **a** genera and **b** species within the sampling areas. The relative abundance (percentage) of genera and species representing less than 5% of the total is

not shown in the graph. The “Others” category includes the remaining orders or families not included in the five most important groups

analysed), saprobes, symbionts, pathogens and unspecified (i.e. not belonging to any of the other categories). At all the sampling sites, saprophytes were the predominant trophic type in terms of genus richness, followed by symbionts and pathogens. However, analysis of the distribution of reads (and not considered) showed that symbionts were the most abundant, followed by saprobes and pathogens (Fig. 6). Considering the sociological class, the patterns of richness and

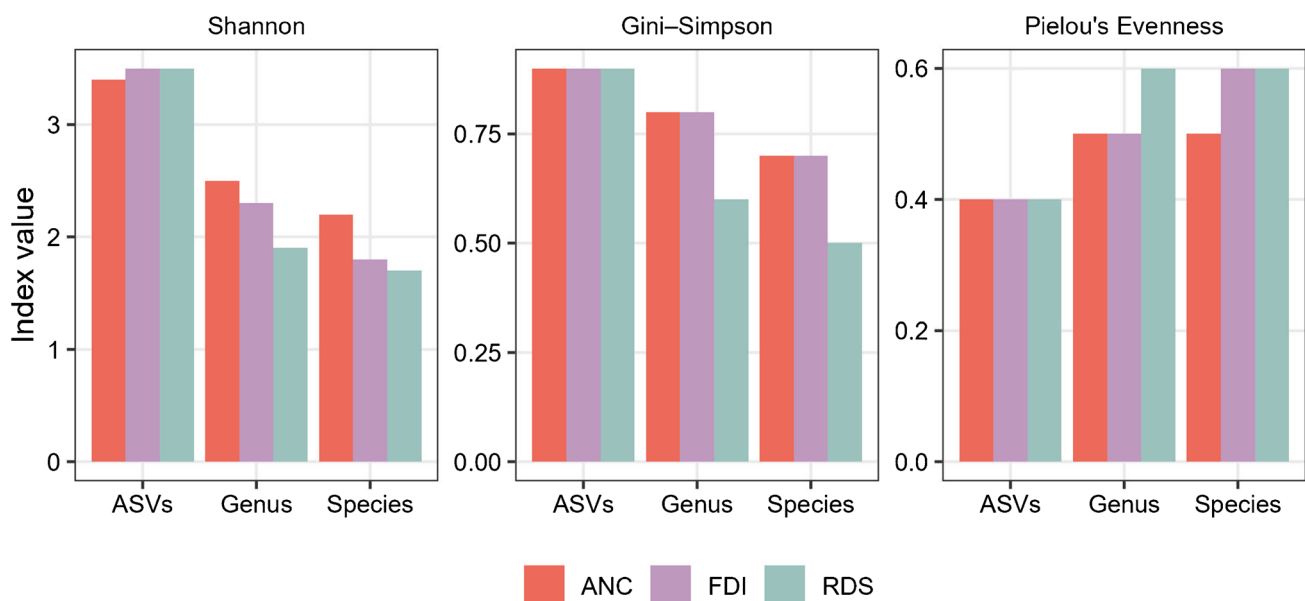
abundance followed the previous trend. In terms of abundance (number of reads), symbiotrophs were the dominant group, except in the codominant tree at RDS, indicating strong mutualistic activity in the communities. Saprotrophs, although generally less abundant, showed the highest richness in most combinations, indicating the presence of a high diversity of decomposers, albeit with lower relative abundance. Pathotrophs presented both low richness and

Table 3 Taxonomic assignment by sampling site and individual tree. Values in parentheses indicate the number of unique Phyla, Classes, Orders, Families, Genera and Species observed within each tree relative to the other trees in the protected area

Protected area	Social class	Phylum	Class	Order	Family	Genus	Species
ANC	Intermediate	5 (2)	12 (2)	40 (12)	66 (19)	84 (32)	92 (43)
	Codominant	4 (1)	12 (1)	30 (3)	54 (12)	73 (25)	64 (25)
	Dominant	3 (0)	11 (0)	28 (5)	50 (9)	54 (14)	56 (19)
	Submerged	3 (0)	11 (2)	28 (3)	50 (10)	63 (18)	56 (21)
FDI	Intermediate	5 (1)	13 (1)	36 (7)	59 (19)	76 (24)	78 (33)
	Submerged	4 (0)	13 (1)	33 (2)	44 (7)	68 (21)	61 (20)
	Codominant	4 (0)	10 (0)	27 (2)	40 (7)	55 (14)	55 (18)
	Dominant	3 (0)	9 (0)	28 (3)	38 (5)	51 (7)	46 (12)
RDS	Codominant	3 (0)	14 (2)	34 (6)	49 (11)	63 (24)	64 (31)
	Dominant	4 (0)	12 (0)	32 (3)	46 (7)	64 (19)	63 (26)
	Submerged	4 (0)	11 (0)	31 (3)	49 (11)	64 (21)	48 (16)
	Intermediate	3 (0)	9 (0)	16 (0)	20 (2)	22 (3)	20 (5)

Table 4 Multilevel Pattern Analysis Results. N° sp. indicates the number of species associated with each group, and Species, the name of the corresponding species. Level of statistical significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Protected area	N° sp.	Species
ANC	7	<i>Lactarius camphoratus</i> ***, <i>Lecidea nylanderii</i> ***, <i>Cladophialophora minutissima</i> **, <i>Tomentella papuae</i> **, <i>Ramularia endophylla</i> *, <i>Cortinarius parvoacetosus</i> *, <i>Neocurbitaria cava</i> *
FDI	16	<i>Rhizoscyphus monotropae</i> **, <i>Tyrannosorus hystrioides</i> ***, <i>Hyaloscypha finlandica</i> **, <i>Lachnum pygmaeum</i> **, <i>Metapochonia suchlasporia</i> ***, <i>Xerocomellus poederi</i> ***, <i>Chaetosphaeria myriocarpa</i> *, <i>Tomentella coerulea</i> *, <i>Lactifluus vellereus</i> *, <i>Chaetosphaeria ciliata</i> *, <i>Exophiala equina</i> *, <i>Inocybe assimilata</i> *, <i>Russula parazurea</i> *, <i>Solicoccozyma terreia</i> *, <i>Phialocephala fortinii</i> *, <i>Tricholoma saponaceum</i> *
RDS	16	<i>Chaetosphaeria vermicularioides</i> *, <i>Epicoccum nigrum</i> ***, <i>Linnemannia longigemmata</i> *, <i>Tausonia pullulans</i> **, <i>Trichoderma asperellum</i> *, <i>Nadsonia starkeyi-henricii</i> **, <i>Phaeostalagmus cycloporus</i> **, <i>Polyozellus tristis</i> **, <i>Cladosporium delicatulum</i> *, <i>Trichoderma longipilis</i> *, <i>Glutinomyces brunneus</i> *, <i>Chaetomium amesii</i> *, <i>Helicoon myosuroides</i> *, <i>Polycephalomycetes tomentosus</i> *, <i>Ramgea ozimecii</i> *, <i>Scytalidium album</i> *
ANC + RDS	1	<i>Oidiodendron pilicola</i> *
FDI + RDS	4	<i>Solicoccozyma terricola</i> **, <i>Mortierella macrocystis</i> **, <i>Psychrophila antarctica</i> *, <i>Leptodontidium trabinellum</i> *

**Fig. 4** Alpha diversity for the different sampling sites (ASVs: Amplicon Sequence Variants)

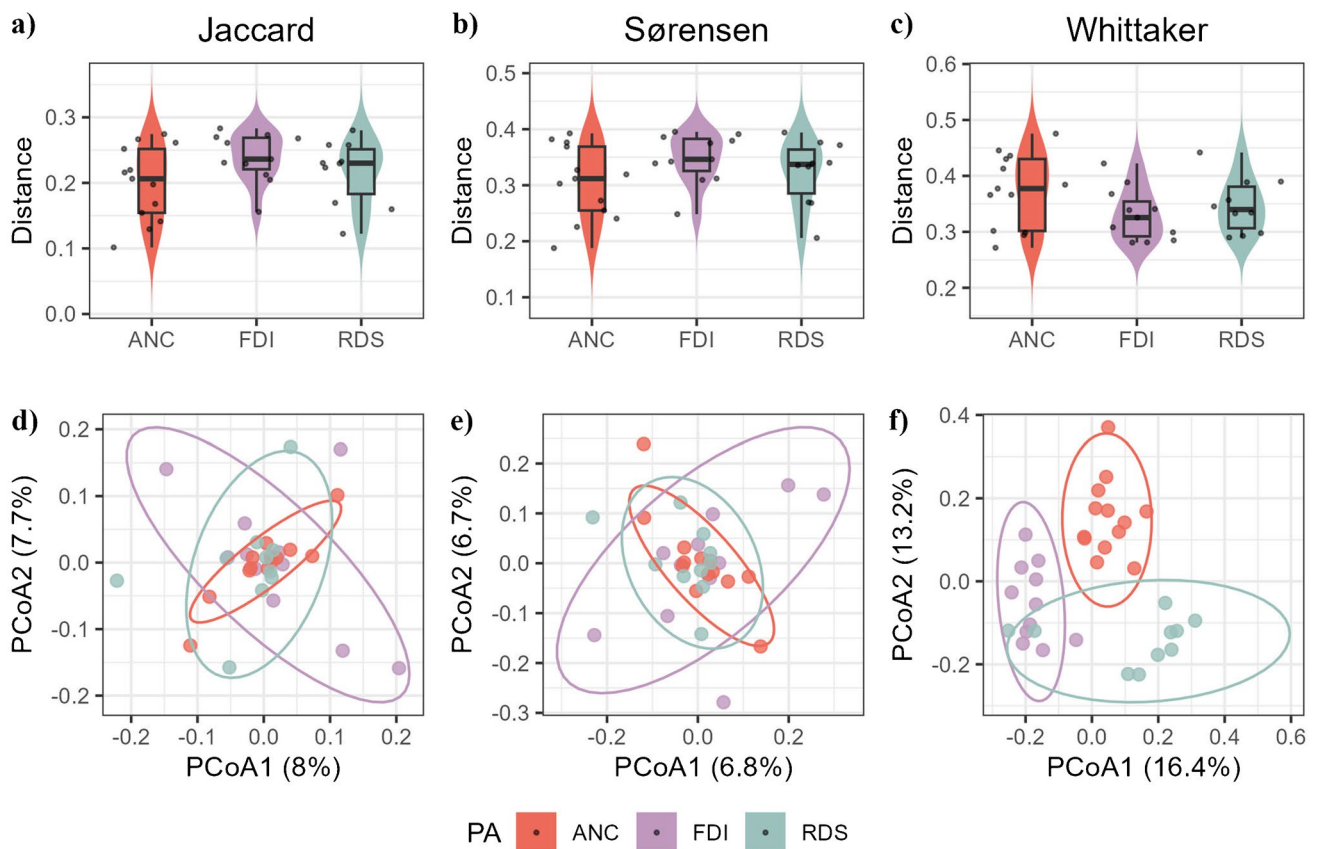


Fig. 5 Beta diversity patterns at the genus taxonomic level across sampling sites (ANC, FDI, RDS) based on three dissimilarity indices. Panels (a–c) show pairwise community distances using Jaccard, Sørensen, and Whittaker indices, respectively. Panels (d–f) present

the corresponding PCoA ordinations, with ellipses representing 95% confidence regions for sampling site. See Figure S1 in the Supplementary Material for equivalent analyses at the species level

abundance (Supplementary Information Table S5). These findings suggest that the richness of a specific trophic mode does not necessarily correspond to its relative abundance in the fungal community.

Ectomycorrhizal analysis

A total of 11 morphotypes were identified within the ectomycorrhizal community (Fig. 7). Each morphotype was found to have a Harting net. The histology of the 11 morphotypes is described in detail in the Supplementary Information (see histological description section, Fig S3–S4).

The levels of colonization of each morphotype per tree and location are indicated in Table 5. The lowest level of colonization and the lowest variability of systems were observed in the specimens in the RDS plot. Overall, the highest values -in terms of colonization- correspond to the FDI plot. Only morphotypes 1, 2 and 3 were observed in all root systems studied. Considering the individual trees analysed, the fungal diversity and colonization rate were highest

in the intermediate, dominant and submerged classes at the ANC, FDI and RDS sites.

To complement the anatomical and colonization analyses of the ectomycorrhizal morphotypes, we performed metabarcoding on the 11 DNA extractions yielded 332 ASVs. These represent a subset of the total 1,043 ASVs obtained from all roots, and some of these ASVs were also detected in the other root samples. The ASVs included 163 assigned to the phylum *Ascomycota*, 143 to *Basidiomycota*, 11 to *Mucoromycota*, and 11 were classified at the kingdom level (i.e. the ASVs could not be assigned them to a particular phylum), and 4 were assigned to *Cryptomycota*. In all morphotypes, the fungus responsible for the ectomycorrhizal structure was predominantly identified as belonging to the phylum *Basidiomycota* (Table 6), except one morphotype, where *Cenococcum geophilum* was identified as the primary fungus (Table 6). In all morphotypes analysed, a diverse group of fungi from the same or another phylum was accompanied by the main ectomycorrhizal fungus (see histological description in Supplementary Information).

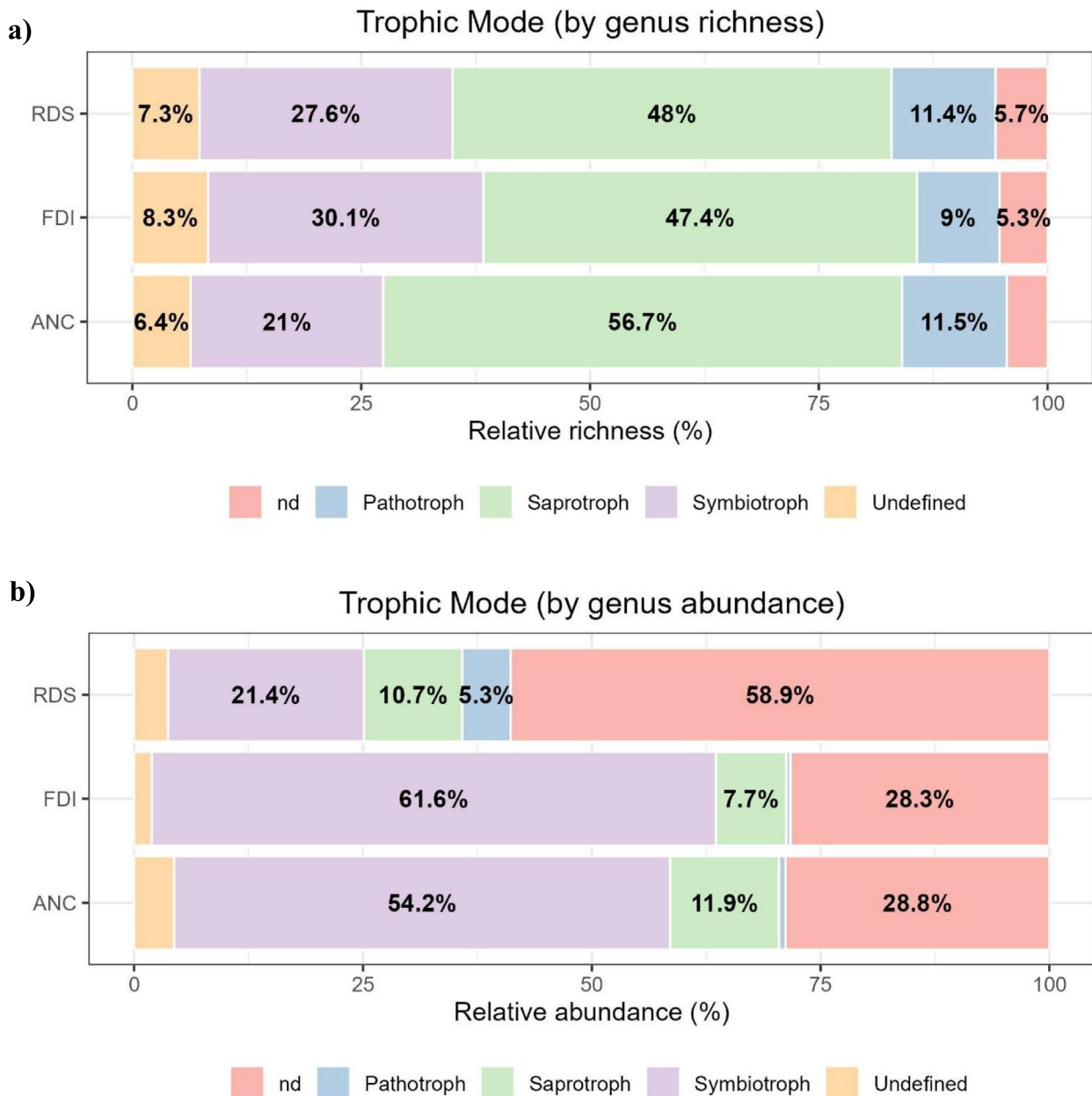


Fig. 6 Trophic modes of the different sampling sites: **a** richness, **b** relative abundance

Discussion

Fungal communities play a fundamental role in the dynamics and functioning of ecosystems. Therefore, it is important to understand their composition and how they affect the environment. Our taxonomic study of the rhizospheres of *Quercus petraea*, at the southern limit of its European distribution, revealed highly diverse fungal communities, whose composition and structure depend on local environmental conditions and host-specific factors.

Effects of sampling sites in fungal communities

Our results indicate that local environmental heterogeneity factors exert a strong effect on community composition and diversity. While fungal richness (alpha diversity) was surprisingly similar across sites, the community composition differed significantly. Only 7.4% of ASVs were shared, indicating a strong compositional turnover, suggesting that species richness alone is insufficient to understand community similarity. Different environments select for different sets of taxa even if the number of taxa is similar. Similar patterns

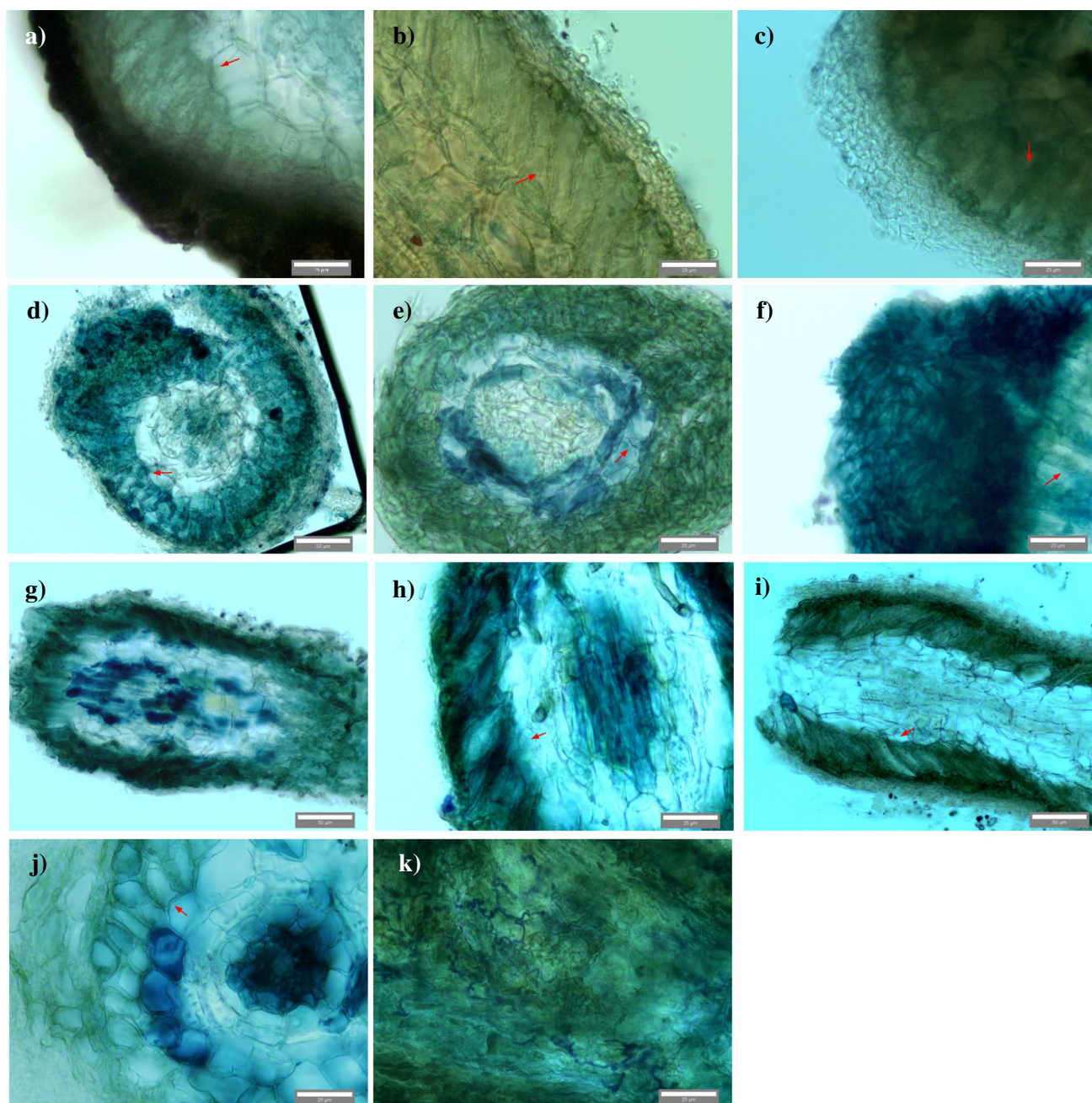


Fig. 7 Histological section stained with cotton blue of each morphotype (MF): **a** MF1, **b** MF2, **c** MF3, **d** MF4, **e** MF5, **f** MF6, **g** MF7, **h** MF8, **i** MF9, **j** MF10, and **k** MF11. The scale bar corresponds to 25 μm in **a–c**, **e**, **f**, **h**, **j**, **k**, and 50 μm in **d**, **g**, **i**. The arrow indicates the Hartig Net

have been observed in other ecosystems. For example, Wang et al. (2015), found that soil pH determined alpha diversity but did not explain beta diversity.

The signals from the different multivariate approaches used to evaluate dispersion and community structure demonstrate that fungal communities diverge significantly among environments, reinforcing the idea that local soil and climatic conditions strongly structure community composition (certain taxa are favoured or excluded depending on local

soil and microclimatic conditions). The most notable environmental differences detected among sampling sites were the soil nitrogen content, pH, and solar exposure, suggesting that these factors would play a key role in the development of the fungal community.

High nitrogen availability in the soil for plants decreases ectomycorrhizal colonization (Pellitier and Zak 2021), as observed in the colonization rates of root tips of RDS. The supply of this element would imply less carbon investment

Table 5 Colonization and variability of ectomycorrhizal systems in the studied root systems. CO: Colonisation rate, calculated as the mean number of ectomycorrhizas root tips per cm of root sampled; MF: morphotype; ×: Present; –: Absent

Protected area	Social class	CO	MF1	MF2	MF3	MF4	MF5	MF6	MF7	MF8	MF9	MF 10	MF 11
ANC	Intermediate	5.8	×	×	×	×	×	×	×	×	×	–	–
	Submerged	1.4	×	×	×	×	–	×	–	–	–	–	–
	Codominant	2.4	×	×	×	–	–	×	–	×	×	–	–
	Dominant	3.6	×	×	×	×	–	×	–	×	×	–	–
FDI	Intermediate	4.5	×	×	×	×	×	×	–	–	×	×	–
	Codominant	3.2	×	×	×	–	×	–	–	–	–	–	–
	Dominant	5.4	×	×	×	×	×	×	–	×	×	×	×
	Submerged	4.3	×	×	×	×	×	×	–	–	×	–	×
RDS	Submerged	2.6	×	×	×	×	×	–	–	–	–	–	–
	Dominant	0.4	×	×	×	–	×	–	–	–	–	–	–
	Intermediate	0.3	×	×	×	×	×	–	–	–	–	–	–
	Codominant	0.6	×	×	×	–	×	–	–	–	–	–	–

Table 6 Main members of each phylum identified in each morphotype. Asterisks indicate the representatives with the highest number of readings. – indicates absence in the morphotype

Morphotype	Ascomycota	Basidiomycota	Mucoromycota
M1	<i>Cenococcum geophilum</i> *	Trechisporales	–
M2	<i>Genea hispidula</i>	<i>Lactarius</i> spp.*	–
M3	<i>Helotiales</i>	<i>Tomentella lilacinogrisacea</i> *	–
M4	<i>Helotiales</i>	Agaricales*	<i>Mortierellales</i>
M5	<i>Helotiales</i>	<i>Russula lepida</i> *	<i>Mortierellales</i>
M6	<i>Helotiales</i>	<i>Tomentella coerulea</i> *	<i>Mortierellales</i>
M7	<i>Helotiales</i>	<i>Melanogaster ambiguous</i> *	<i>Mortierellales</i>
M8	<i>Helotiales</i>	<i>Trechisporales</i> *	<i>Gryganskiella cystojenkinii</i>
M9	<i>Helotiales</i>	<i>Cortinarius</i> spp.*	<i>Mortierellales</i>
M10	<i>Helotiales</i> *	<i>Russulales</i>	<i>Mortierellales</i>
M12	<i>Helotiales</i>	<i>Luellia recondita</i> *	–

by plants in this type of symbiotic network, thereby altering the fungal composition of the rhizosphere. Soil pH exerts strong selective pressure on fungal taxa, as different pH regimes favour different functional groups, as reported by Tedersoo et al. (2014), and verified, among others, by Maghnia et al. (2017) and Shen et al. (2022) in their studies on oak communities. Solar exposure modulates soil temperature and moisture, creating microhabitats that further filter fungal colonizers. Guo et al. (2022) indicate that soil moisture, among other parameters, is highly correlated with the distribution of fungal communities.

Consequently, contrary to our initial expectations that the fungal composition of the target trees would remain constant across different locations, our findings showed, also, several differences. In terms of ASVs richness, the genus *Cladophialophora* was dominant in all analysed specimens in ANC and FDI plots, while *Mortierella* was also present in RDS (in dominant and intermediate states). This suggests that, despite the existence of host-derived micro conditions, fungal organisms depend on edaphic and climatic

conditions. Differences were also observed in terms of relative abundance.

Ecological roles of dominant fungal taxa

We observed differences between the most abundant genera and/or species and the number of ASVs, indicating that functional diversity is not reflected in relative abundance, as some of the genera or species displaying high genetic diversity were not the most abundant in terms of reads.

The dominant ASV–rich genus in our samples was *Cladophialophora*, which is considered a dark septate endophyte (DSE), a broad root-fungal colonizing group characterized by melanized cell walls and intracellular colonization of healthy plants (Jumpponen and Trappe 1998), whose effects in plants are still unclear (Mandyam and Jumpponen 2014). This dominance is due to the high diversity, reflected in the high number of distinct ASVs, suggesting a high capacity for adaptation to different environmental conditions, perhaps contributing to host stress tolerance.

In terms of relative abundance, the most important genus was *Rhizoderma* in ANC and RDS sites and *Lachnum* in the FDI site. Both are members of the order *Helotiales*, which play an essential part in plant nutrition, particularly in the absence of arbuscular mycorrhizal symbiosis (Bruyant et al. 2024). Given the low presence of *Glomeromycota* in our oaks, these *Helotiales* may provide alternative nutrient uptake pathways.

The Río del Alba (RDS) site presented a fungal composition different from the rest, characterized by lower diversity and the presence of the genus *Mortierella* as the second most abundant in ASVs richness. While they are considered saprophytes (Bonfante and Venice 2020), several studies show that species of the genus *Mortierella* are isolated as endophytes of various plant species, where they promote their growth (Ozimek and Hanaka 2021). Species of the genus *Mortierella* protect plants against pathogens through several complementary mechanisms that strengthen soil health and plant resistance (Ozimek and Hanaka 2021). The dominance of this genus in the plot where pathogenic organisms are more prevalent suggests that they are acting as a natural control agent contributing to ecosystem stability.

The role of tree sociological classes in fungal communities

Although richness did not vary significantly among sociological classes, our outcomes indicate the important function of tree classes in shaping fungal diversity. We expected that dominant trees would harbour the most diverse communities, but it only happened in RDS. Instead, we found that trees of intermediate social class exhibited the greatest fungal richness and unique taxa at ANC and FDI sites. There is evidence that understory trees can survive under restrictive conditions thanks to shared mycorrhizal networks, which allow them to benefit from resources captured by canopy trees (Simard et al. 1997). Intermediate trees may rely more on diverse fungal associations to maximize nutrient uptake from the soil, thus compensating for their competitive disadvantage on the surface. Our results suggest that intermediate trees could be critical reservoirs of biodiversity within the forest. This fact suggests that canopy structure and micro-environmental heterogeneity influence fungal associations. Further studies of this type are needed, considering the position of the analysed tree within the canopy, to determine the influence of microenvironmental conditions on the establishment of root fungal communities.

Morphological vs. molecular ectomycorrhizal characterization

This work highlights the importance of combining molecular metabarcoding with morphological characterization

to obtain a holistic view of the entire fungal community coexisting in one single root tip. Visual inspection of root tips confirmed that the classical ectomycorrhizal structures (mantle and Hartig net) were formed primarily by *Basidiomycota* genera, such as *Tomentella*, *Russula*, and *Lactarius*, which dominate the external structures – essential for nutrient exchange-, and a wide range of *Ascomycota* genera, mainly DSE members, within the root cortex, whose role is still unclear. The detected ectomycorrhizal fungi cluster was consistent with those of studies with *Q. robur* and *Q. petraea*, such as those carried out by Courty et al. (2008), Suz et al. (2014), and Milović et al. (2023). It is worth mentioning that our results regarding the composition of the fungal communities present in the mycorrhizal tips are consistent with those of Tedersoo et al. (2009) and Pellitier and Zak (2021).

Functional diversity and forest resilience implications

The use of *IndVal* analysis reinforced the ecological specificity and prospective conservation value of these fungal communities. It revealed taxa strongly associated with sites, underscoring the ecological specificity of the fungal communities. These taxa may be used as ecological indicators of environmental change and as a reliable tool for conservation. The effectiveness of *IndVal* has been demonstrated in numerous studies, e.g., Casazza et al. (2008), Leote et al. (2019), and Morelli et al. (2021).

Functionally, the presence of various groups of fungi coexisting has been determined, suggesting the formation of several fungal networks simultaneously (symbionts, endophytes, saprophytes, and pathogens). The combined action of these organisms would be greater than the sum of their individual contributions (Rilling et al. 2025). Such multifaceted interactions highlight the ecological significance of fungal diversity and underscore the need for further research into the mechanisms by which these communities bolster forest vitality, especially under changing environmental conditions. These networks could make a relevant, yet frequently underestimated, contribution to stress tolerance and nutrient cycling for *Quercus petraea* populations under marginal environmental conditions. They are considered significant components of overall ecosystem stability.

Symbionts were the most important group (in terms of genus reads), highlighting the importance that this type of interaction has in the exchange of nutrients (Brundrett and Tedersoo 2018). It is worth mentioning the high prevalence of fungal organisms characterized as DSE (endophytes). Ruotsalainen et al. (2022) suggest that they have saprophytic and colonizing capacity and could benefit the plant without additional costs. The genetic diversity observed could be an indicator of the adaptive strategies these fungi employ to

thrive under varying conditions, helping their host to cope with stress linked to marginal habitats.

Symbionts dominated in terms of the number of genus reads, but saprophytes were the most abundant in terms of genus richness. The saprophytic community identified (specializing in leaf litter, wood, dung, and soil, among others) appears to provide an important contribution to soil maintenance, improving fertility and structure, and supporting plant development. While some saprophytes are symbiotically associated with orchids (Martos et al. 2009), this rarely occurs with tree species (Smith et al. 2017). This group of fungi could influence plant growth, protect against pathogens, and increase nutrient uptake (Oh et al. 2018). Despite the number of fungal phytopathogens described, it is not possible to determine the influence of these on the health of the trees.

Limitations and future perspectives

To contextualize these findings, it is necessary to acknowledge certain limitations. First, regarding the sample size, while this may seem limited for a community ecology analysis (it was similar to that used in other studies, such as Adamo et al. 2022), it was offset by intensive per-individual sampling. Second, sampling was restricted to a single season (spring), which may prioritize taxa active during the onset of vegetative growth and underrepresent species that reach their peak development in autumn. However, given the current lack of data at this southern distribution margin, these results provide a valuable basis for long-term monitoring of ecosystem dynamics in Atlantic oak forests.

Conclusions

In this study, we investigated the taxonomic characterization and ecological interactions between fungal communities associated with the roots of *Quercus petraea* (sessile oak) in the Cantabrian Mountains of northern Spain, providing valuable insights into the taxonomic composition and ecological interactions between fungal communities and these oak forests. Examination of the fungal communities revealed a diverse range of fungi thriving in these oak-dominated environments. Exploration of the novel diversity within the arboreal root systems of sessile oak forests has uncovered unique ecological roles and adaptations of fungi, expanding our understanding of fungal diversity in these unique ecosystems. Moreover, the results reveal variations in fungal community composition across different protected areas and tree sociological classes, reflecting the influence of environmental and ecological factors on fungal diversity. Finally, this research contributes to a greater understanding of the complex interactions shaping forest biodiversity.

Further studies of fungal communities in forest ecosystems are necessary to enhance our knowledge of their ecological importance and to inform sustainable management practices for these valuable natural resources.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11557-025-02119-7>.

Author contribution NA-G: was involved in conceptualization; data curation; formal analysis; supervision; validation; investigation; visualization; methodology; and writing – original draft, review and editing. EF-MC: was involved in conceptualization; data curation; formal analysis; supervision; validation; investigation; visualization; methodology; and writing – original draft, review and editing. JCP-G: was involved in data curation, formal analysis, supervision, validation, investigation, methodology, and writing—review and editing. MV: was involved in supervision, validation, investigation, visualization, methodology, and writing—review and editing. CC: was involved in supervision, validation, investigation, methodology, software and writing—review and editing. RJO: was involved in supervision, validation, investigation, methodology, and writing—review and editing. PÁ-Á: was involved in conceptualization; data curation; formal analysis; supervision; validation; investigation; visualization; methodology; project administration; and writing—original draft, review and editing. All authors read and approved the manuscript.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. This study was carried out as part of the SOE4/P1/E1012 project “Integrated and intelligent information management of complex forests and mixed-species plantations in Southwest Europe (COMFOR-SUDOE),” funded by the Interreg Sudoe Programme and coordinated by The National Museum of Natural Sciences (CSIC).

Data availability The taxonomic assignment data that support the findings of this study are available in this open access database: <https://www.ncbi.nlm.nih.gov/BioProject/PRJNA1372172>.

Declarations

Ethical approval Not applicable.

Consent to participate All authors confirm that they have provided their consent to participate in this study.

Consent for publication All authors consent to the publication of this manuscript.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abarenkov K, Zirk A, Piirmann T, Pöhönen R, Ivanov F, Nilsson RH, Kõljalg U (2020) UNITE QIIME release for Fungi. <https://doi.org/10.15156/BIO/786385>
- Adamo M, Voyron S, Chialva M, Marmesse R, Girlanda M (2020) Metabarcoding on both environmental DNA and RNA highlights differences between fungal communities sampled in different habitats. *PLoS One* 15:e0244682. <https://doi.org/10.1371/journal.pone.0244682>
- Adamo I, Dashevskaya S, Alday JG (2022) Fungal perspective of pine and oak colonization in Mediterranean degraded ecosystems. *Forests* 13(1):88. <https://doi.org/10.3390/f13010088>
- Adnam M, Islam W, Gang L, Chen HYH (2022) Advanced research tools for fungal diversity and its impact on forest ecosystem. *Environ Sci Pollut Res* 29:45044–45062. <https://doi.org/10.1007/s11356-022-20317-8>
- Agerer R (1987–1995) Colour atlas of ectomycorrhizae. Einhorn, Schwäbisch Gmünd; Munich
- Allue Andrade JL (1990) Atlas Fitoclimático de España. Taxonomías. Ministerio de Agricultura, Pesca y Alimentación, Instituto Nacional de Investigaciones Agrarias, Departamento de Sistemas Forestales, Madrid
- Anderson MJ (2006) Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 62:245–253. <https://doi.org/10.1111/j.1541-0420.2005.00440.x>
- Anderson MJ (2008) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 26:32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.x>
- Bechtold WA (2003) Crown position and light exposure classification—an alternative to field-assigned crown class. *North J Appl For* 20:154–160
- Benito Garzón M, Sánchez de Dios R, Sainz Ollero H (2008) Effects of climate change on the distribution of Iberian tree species. *Appl Veg Sci* 11:169–178. <https://doi.org/10.3170/2008-7-18348>
- Bolyen E, Rideout JR, Dillon MR et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 37:852–857. <https://doi.org/10.1038/s41587-019-0209-9>
- Bonfante P, Venice F (2020) *Mucoromycota*: going to the roots of plant-interacting fungi. *Fungal Biol Rev* 34:100–113. <https://doi.org/10.1016/j.fbr.2019.12.003>
- Brundrett MC, Tedersoo L (2018) Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol* 220:1108–1115. <https://doi.org/10.1111/nph.14976>
- Bruyart P, Moënné-Loccoz Y, Almario J (2024) Root-associated *Helotiales* fungi: overlooked players in plant nutrition. *Soil Biol Biochem* 109363. <https://doi.org/10.1016/j.soilbio.2024.109363>
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 13:581. <https://doi.org/10.1038/nmeth.3869>
- Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: architecture and applications. *BMC Bioinformatics* 10:421. <https://doi.org/10.1186/1471-2105-10-421>
- Casazza G, Zappa E, Mariotti MG, Médail F, Minuto L (2008) Ecological and historical factors affecting distribution pattern and richness of endemic plant species: the case of the Maritime and Ligurian Alps hotspot. *Divers Distrib* 14:47–58. <https://doi.org/10.1111/j.1472-4642.2007.00412.x>
- Che R, Wang S, Wang Y, Xu Z, Wang W, Rui Y, Wang F, Hu J, Tao J, Cui X (2019) Total and active soil fungal community profiles were significantly altered by six years of warming but not by grazing. *Soil Biol Biochem* 139:107611. <https://doi.org/10.1016/j.soilbio.2019.107611>
- Cosme M (2023) Mycorrhizas drive the evolution of plant adaptation to drought. *Commun Biol* 6:346. <https://doi.org/10.1038/s42003-023-04722-4>
- Courty PE, Franc A, Pierrat JC, Garbaye J (2008) Temporal changes in the ectomycorrhizal community in two soil horizons of a temperate oak forest. *Appl Environ Microbiol* 74:5792–5801. <https://doi.org/10.1128/AEM.01592-08>
- De Cáceres M, Legendre P, Moretti M (2010) Improving indicator species analysis by combining groups of sites. *Oikos* 119:1674–1684. <https://doi.org/10.1111/j.1600-0706.2010.18334.x>
- del Río S, Canas R, Cano E, Cano-Ortiz A, Musarella C, Pinto-Gomes C, Penas A (2021) Modelling the impacts of climate change on habitat suitability and vulnerability in deciduous forests in Spain. *Ecol Indic* 131:108202. <https://doi.org/10.1016/j.ecolind.2021.108202>
- García-Guzmán OM, Garibay-Orijel R, Hernández E et al (2017) Worldwide meta-analysis of *Quercus* forests ectomycorrhizal fungal diversity reveals southwestern Mexico as a hotspot. *Mycorrhiza* 27:811–822. <https://doi.org/10.1007/s00572-017-0793-9>
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol Ecol* 2:113–118
- Goodman DM, Durall DM, Trofymow JA, Berch SM (1996) A manual of concise descriptions of North American ectomycorrhizae. Mycologue Publications and Canada–BC Forest Resource Development Agreement, Pacific Forestry Centre, Victoria, BC
- Gorzela MA, Asay AK, Pickles BJ, Simard SW (2015) Inter-plant communication through mycorrhizal networks mediates complex adaptive behavior in plant communities. *AoB Plants* 7:plv050. <https://doi.org/10.1093/aobpla/plv050>
- Guo P, Xing PJ, Song J, Wu LL, Li BQ, et al. (2022) Fungal community in roots and the root zone of *Quercus mongolica* and the correlations with the environmental factors. *J For Res*. <https://doi.org/10.13341/j.jfr.2022.1489>
- Ingleby K, Manson PA, Last FT, Fleming LV (1990) Identification of ectomycorrhizas. Institute of Terrestrial Ecology Research Publication No. 5, Natural Environment Research Council, London.
- Igiehon NO, Babalola OO (2018) Rhizosphere microbiome modulators: contributions of nitrogen-fixing bacteria towards sustainable agriculture. *Int J Environ Res Public Health* 15:574. <https://doi.org/10.3390/ijerph15040574>
- Jaccard P (1912) The distribution of the flora in the Alpine zone. *New Phytol* 11:37–50. <https://doi.org/10.1111/j.1469-8137.1912.tb05611.x>
- Jumpponen A, Trappe JM (1998) Dark-septate root endophytes: a review with special reference to facultative biotrophic symbiosis. *New Phytol* 140:295–310. <https://doi.org/10.1046/j.1469-8137.1998.00265.x>
- Leote P, Cajaiba RL, Cabral JA, Brescovit AD, Santos M (2019) Are data-mining techniques useful for selecting ecological indicators in biodiverse regions? Bridges between market basket analysis and indicator value analysis from a case study in the neotropics. *Ecol Indic* 107:105833. <https://doi.org/10.1016/j.ecolind.2019.105833>
- Luo S, Phillips RP, Jo I et al (2023) Higher productivity in forests with mixed mycorrhizal strategies. *Nat Commun* 14:1377. <https://doi.org/10.1038/s41467-023-36888-0>
- Maghnia FZ, Abbas Y, Mahé F, Kerdouh B, Tournier E, Ouadji M et al (2017) Habitat- and soil-related drivers of the root-associated fungal community of *Quercus suber* in the Northern Moroccan forest. *PLoS One* 12:e0187758. <https://doi.org/10.1371/journal.pone.0187758>

- Mandyam KG, Jumpponen A (2014) Mutualism–parasitism paradigm synthesized from results of root-endophyte models. *Front Microbiol* 5:776. <https://doi.org/10.3389/fmicb.2014.00776>
- Martos F, Dulormne M, Pailler T et al (2009) Independent recruitment of saprotrophic fungi as mycorrhizal partners by tropical achlorophyllous orchids. *New Phytol* 184:668–681. <https://doi.org/10.1111/j.1469-8137.2009.02987.x>
- Milović M, Kovačević B, Drekić M et al (2023) Ectomycorrhizal diversity in a mature pedunculate oak stand near Morović. *Serbia Iforest* 16:345. <https://doi.org/10.3832/ifor4362-016>
- Morelli F, Reif J, Díaz M, Tryjanowski P et al (2021) Top ten birds indicators of high environmental quality in European cities. *Ecol Indic* 133:108397. <https://doi.org/10.1016/j.ecolind.2021.108397>
- Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) Funguild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol* 20:241–248. <https://doi.org/10.1016/j.funeco.2015.06.006>
- Ninyerola M, Pons X, Roure JM (2005) Atlas Climático Digital de la Península Ibérica. Universidad Autónoma de Barcelona, Bellaterra, Metodología y aplicaciones en bioclimatología y geobotánica
- Oh SY, Cho HJ, Eimes JA, Han SK, Kim CS, Lim YW (2018) Guild patterns of basidiomycetes community associated with *Quercus mongolica* in Mt. Jeombong, Republic of Korea. *Mycobiology* 46:13–23. <https://doi.org/10.1080/12298093.2018.1454009>
- Oksanen J, Simpson G, Blanchet F et al (2022) Vegan: community ecology package. R package version 2.6–4. <https://CRAN.R-project.org/package=vegan>
- Ozimek E, Hanaka A (2021) *Mortierella* species as the plant growth-promoting fungi present in the agricultural soils. *Agriculture* 11:7. <https://doi.org/10.3390/agriculture11010007>
- Pellitier PT, Zak DR (2021) Ectomycorrhizal root tips harbor distinctive fungal associates along a soil nitrogen gradient. *Fungal Ecol* 54:101111. <https://doi.org/10.1016/j.funeco.2021.101111>
- Pérez-Girón JC, López-Bao JV, Díaz-Varela E, Álvarez-Álvarez P (2025) Predicting climate-related compositional shifts in nut-producing species that are important for bears during hyperphagia. *Front Glob Change* 8:1624612. <https://doi.org/10.3389/fgc.2025.1624612>
- Phillips JM, Hayman DS (1970) Improved procedures for clearing root and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc* 55:158–181. [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3)
- Pölme S, Abarenkov K, Henrik Nilsson R et al (2020) FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Divers* 105:1–16. <https://doi.org/10.1007/s13225-020-00466-2>
- R Core Team (2024) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Ruotsalainen AL, Kauppinen M, Wäli PR, Saikkonen K, Helander M, Tuomi J (2022) Dark septate endophytes: mutualism from by-products? *Trends Plant Sci* 27:247–254. <https://doi.org/10.1016/j.tplants.2021.10.001>
- Shen H, Yan B, Wang H, Sun W, Jiao K, Qin G (2022) Changes in soil ectomycorrhizal fungi community in oak forests along the urban–rural gradient. *Forests* 13:675. <https://doi.org/10.3390/f13050675>
- Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R (1997) Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388:579–582
- Smith GR, Finlay RD, Stenlid J, Vasaitis R, Menkis A (2017) Growing evidence for facultative biotrophy in saprotrophic fungi: data from microcosm tests with 201 species of wood-decay basidiomycetes. *New Phytol* 215:747–755. <https://doi.org/10.1111/nph.14551>
- Sørensen T (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. *Biol Skr* 5:1–34. Munksgaard, Copenhagen.
- Stavi I, Thevs N, Welp M et al (2022) Provisioning ecosystem services related with oak (*Quercus*) systems: a review of challenges and opportunities. *Agroforest Syst* 96:293–313. <https://doi.org/10.1007/s10457-021-00718-3>
- Strullu-Derrien C, Selosse MA, Kenrick P, Martin FM (2018) The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. *New Phytol* 220:1012–1030. <https://doi.org/10.1111/nph.15076>
- Suz LM, Barsoum N, Benham S et al (2014) Environmental drivers of ectomycorrhizal communities in Europe’s temperate oak forests. *Mol Ecol* 23:5628–5644. <https://doi.org/10.1111/mec.12947>
- Tedersoo L, Pärtel K, Jairus T, Gates G, Pöldmaa K, Tamm H (2009) Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the *Helotiales*. *Environ Microbiol* 11:3166–3178. <https://doi.org/10.1111/j.1462-2920.2009.02020.x>
- Tedersoo L, Bahram M, Pölme S et al (2014) Global diversity and geography of soil fungi. *Science* 346:1256688. <https://doi.org/10.1126/science.1256688>
- Tedersoo L, Bahram M, Zobel M (2020) How mycorrhizal associations drive plant population and community biology. *Science* 367:eaba1223. <https://doi.org/10.1126/science.aba1223>
- Tedersoo L, Bahram M, Zinger L, Nilsson RH, Kennedy PG, Yang T, Anslan S, Mikryukov V (2022) Best practices in metabarcoding of fungi: from experimental design to results. *Mol Ecol* 31:2769–2795. <https://doi.org/10.1111/mec.16460>
- Toju H, Sato H, Yamamoto S et al (2013a) How are plant and fungal communities linked to each other in belowground ecosystems? A massively parallel pyrosequencing analysis of the association specificity of root-associated fungi and their host plants. *Ecol Evol* 3:3112–3124. <https://doi.org/10.1002/ece3.706>
- Toju H, Yamamoto S, Sato H, Tanabe AS, Gilbert GS, Kadowaki K (2013b) Community composition of root-associated fungi in a *Quercus*-dominated temperate forest: “codominance” of mycorrhizal and root-endophytic fungi. *Ecol Evol* 3:1281–1293. <https://doi.org/10.1002/ece3.546>
- Toju H, Tanabe AS, Sato H (2018) Network hubs in root-associated fungal metacommunities. *Microbiome* 6:116. <https://doi.org/10.1186/s40168-018-0497-1>
- Wang JT, Zheng YM, Hu HW et al (2015) Soil pH determines the alpha diversity but not beta diversity of soil fungal community along altitude in a typical Tibetan forest ecosystem. *J Soils Sediments* 15:1224–1232. <https://doi.org/10.1007/s11368-015-1070-1>
- White TJ, Bruns T, Lee SJ, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: a Guide to Methods and Applications* 18(1):315–322
- Whittaker RH (1960) Vegetation of the Siskiyou Mountains, Oregon and California. *Ecol Monogr* 30:279–338. <https://doi.org/10.2307/1943563>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.