forests

MDPI

Article

Exploring Fungal Communities in the Needles of Marginal Conifer Tree Populations

Jelena Lazarević [1,*] and Audrius Menkis [2,*]

[1] Biotechnical Faculty, University of Montenegro, Mihaila Lalića 1, 81000 Podgorica, Montenegro
[2] Department of Forest Mycology and Plant Pathology, Uppsala BioCenter, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden
* Correspondence: ena.lazarevic@gmail.com (J.L.); audrius.menkis@slu.se (A.M.)

Abstract: In Montenegro, coniferous forests play a key ecological role in maintaining ecosystem stability. Root-associated mycorrhizal fungi and saprotrophic fungi inhabiting forest soils are well known for their roles in nutrient cycling, organic matter decomposition, and supporting host tree health. In contrast, the fungal communities residing within conifer needles, despite potentially important ecological functions, remain largely underexplored, particularly in natural and marginal forest ecosystems such as those in the Balkans. This study aimed to investigate the diversity and community composition of needle-associated fungi in three native conifers: *Picea abies* and *Abies alba* (at the edge of their native range), and the endemic *Pinus heldreichii*, from different mountainous regions in Montenegro. High-throughput sequencing was conducted to assess fungal diversity and community composition. *Dothideomycetes* dominated fungal communities in all three tree species, followed by *Leotiomycetes* and *Tremellomycetes*. Multivariate analysis revealed distinct fungal communities in *P. heldreichii*, whereas fungal communities in *A. alba* and *P. abies* were partially overlapping. Functional classification showed a dominance of saprotrophic, pathogenic, and endophytic fungi, with *P. heldreichii* exhibiting the highest proportion of saprotrophs, while *A. alba* and *P. abies* showed a considerable proportion of pathogens. The findings highlight strong host specificity, biogeographical influences, and the ecological importance of fungal communities in coniferous forests. This study provides new insights into the diversity and functional roles of needle-associated fungi, emphasizing the need for conservation efforts to maintain microbial biodiversity in native forests of Montenegro.

Keywords: fungal community; *Picea abies*; *Abies alba*; *Pinus heldreichii*; native forests

check for updates

Academic Editor: Deividas Valiunas

Received: 26 April 2025
Revised: 26 May 2025
Accepted: 4 June 2025
Published: 7 June 2025

Citation: Lazarević, J.; Menkis, A. Exploring Fungal Communities in the Needles of Marginal Conifer Tree Populations. *Forests* **2025**, *16*, 968. https://doi.org/10.3390/f16060968

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Forests are vital ecosystems that play a key role in maintaining global biodiversity, supporting ecosystem services such as soil stabilization, including erosion control, carbon sequestration, water regulation, and nutrient cycling. Montenegro is a predominantly mountainous country in Southeastern Europe (41°52′ and 43°32′ latitude North and 18°26′ and 19°32′ longitude East), with forest covering more than 60% of the land area. Forests are predominantly natural and forest types vary across altitudes, hosting a rich biodiversity shaped by geographical and climatic diversity in the country [1].

The conifer forest zone in Montenegro is limited to mountain–subalpine regions. Norway spruce (*Picea abies* Karst) and silver fir (*Abies alba* Mill.) are keystone European conifers with important ecological and economic values [2,3]. On the Dinaric Mountains in Montenegro, both *P. abies* and *A. alba* reach the southern edge of their natural distribution. *P. abies* is a key component in the boreal forest ecosystems [4], which is well adapted to the

continental mountain climates of northern and central regions of Montenegro, where it grows in mixed stands with *A. alba*. Bosnian pine (*Pinus heldreichii* H. Christ) is a tertiary relict and subendemic to the Balkans, occurring in fragmented forest stands at high altitudes (1200–2000 m) on karst terrain in the Oro-Mediterranean zone and often under harsh environmental conditions [5,6]. The coniferous forest of *P. abies* and *A. alba*, and those of *P. heldreichii*, occupy distinct ecological niches that reflect their evolutionary adaptations to the diverse climatic and geographical conditions such as boreal and the Mediterranean, respectively (Figure 1). These mountain forests are hotspots of unique biodiversity and provide important ecosystem services. However, they represent vulnerable ecosystems, facing the effects of climate change and over-exploitation.

Figure 1. Forests of *Picea abies* and *Abies alba* (*Abieti-Piceatum abietis*) on Mt. Durmitor (**left**), photo J. Lazarević; forest of *Pinus heldreichii* (*Pinetum heldreichii mediteraneo-montanum*) on Mt. Orjen (**right**), photo Ž. Starčević.

Needle-associated fungi may have a significant effect on host tree health, ecosystem functioning, and forest resilience [7,8], particularly in regions with unique ecological conditions such as the Balkan region [9]. However, studies investigating needle-associated fungal communities in conifers from the Balkan Peninsula are scarce, leaving a significant knowledge gap regarding fungal diversity and its ecological implications.

The aim of this study was to explore the diversity and community composition of fungi associated with healthy-looking needles of *P. abies* and *A. alba* at the edge of their native range, as well as the endemic *P. heldreichii*, across different mountainous regions in Montenegro. We hypothesized that fungal communities associated with conifer needles would exhibit significant differences between tree species due to host specificity. We have implemented an extensive and wide-scale sampling with the intention to both reveal the diversity of fungal taxa with important ecological functions and to uncover the unique biodiversity of these forests by employing high-throughput sequencing techniques.

2. Materials and Methods

2.1. Study Sites and Sampling

Sampling was conducted across geographically distinct regions in Montenegro to represent the diverse climatic zones and major coniferous forest types of the studied coniferous forests in the region (Figure 2). In northwestern and central Montenegro, the Mt. Durmitor site comprises mixed coniferous forests of *P. abies* and

A. alba (Ass. *Abieti-Piceetum abietis* [10]) at 1350–1450 m alt., developed on kalkomelanosols [11] (Table 1). Associated vegetation includes species such as *Vaccinium myrtilis*, *Galium rotundifolum*, *Luzula* sp., and *Oxalis acetosella.* The Mt. Bjelasica site comprises high-altitude (1700 m) mixed *P. abies* and *A. alba* (*Abietinum albae subalpinum*) forests of low density, developed on ranker [11]. Associated vegetation includes species such as *Juniperus nana*, *Hypericum alpigenum*, *H. maculatum*, *Vaccinium sp.*, and *Rosa alpina*. Both the Mt.Durmitor and Mt.Bjelasica sites are characterized by a humid boreal mountain climate (Dfc-Dfbx'', with mean temperatures during the growing season (April–October) averaging 14.3 °C, extreme temperatures ranging from −30.4 °C to 29.6 °C, and annual precipitation of 1100 mm, of which 460 mm falls during the growing season [12].

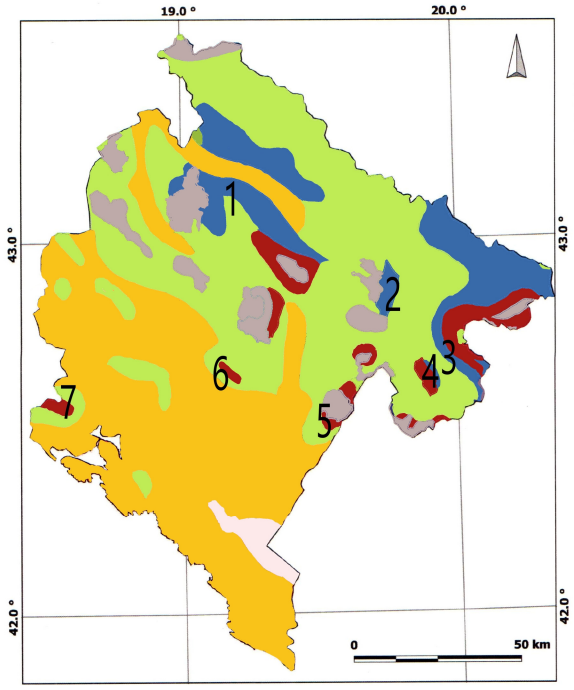

Figure 2. Map of Montenegro showing the distribution of tree vegetation. Sampling sites are numbered as follows: (1) Mt. Durmitor, (2) Mt. Bjelasica, (3) Mt. Prokletije, (4) Mt. Visitor, (5) Mt. Kuči, (6) Mt. Prekornica, and (7) Mt. Orjen. The map is adapted from [13]. Coniferous *Picea abies* and *Abies alba* forest (blue); coniferous forest of *Pinus heldreichii* (burgundy); termophillus broadleaf forest, mainly oaks (green); mesophillous broadleaf forests, mainly beech (orange); high mountain pastures and shrubs (gray); Skadar lake (pink).

Table 1. Characteristics of the study sites and tree species sampled at each location.

Forest Vegetation [10]	Site	Geographical Position	Altitude (m)	Climate [12]	Soil Type [11]	Sampled
Abieti-Piceetum abietis	Mt. Durmitor	N 43.1455 E 19.0981	1350–1450	Dfbx"	Kalkomelanosol	*P. abies*, *A. alba*
Abietinum albae subalpinum	Mt. Bjelasica	N 42.8748 E 19.6969	1700	Dfbx"	Ranker	*P. abies*, *A. alba*
Abieti-Piceetum abietis	Mt. Prokletije	N 42.5964 E 20.0306	1400–1700	Cfs"b	Dystrict cambisol	*P. abies*, *A. alba*
Abieti-Piceetum abietis	Mt. Visitor	N 42.6146 E 19.8821	1700–1900-	Cfs"b	Kakocambisol	*A. alba*
Pinetum heldreichii bertisceum	Mt. Kuči	N 44.8150 E 20.4347	1250–1700	Cfws"bx"	Leptosol/Molic leptosol	*P. heldreichi*
Pinetum heldreichii mediteraneo-montanum	Mt. Prekornica	N 42.6240 E 19.1995	1300	Cfsb/Cfsb"	Leptosol/Molic leptosol	*P. heldreichi*
Pinetum heldreichii mediteraneo-montanum	Mt. Orjen	N 42.5436 E 18.5240	1200–1300	Cfsb	Leptosol/Molic leptosol	*P. heldreichii*

In southeastern Montenegro, the Mt. Prokletije (Bogićevica) site was dominated by *P. abies* forests with admixture of *A. alba* (*Abieti-Piceetum abietis*) at 1400–1700 m alt. at

Dystrict cambisol [10,11] (Table 1). The Mt. Visitor site comprises mixed coniferous forests of *P. abies* and *A. alba* at high altitudes of 1700–1900 m, on kalkocambisol [11]. In this region, the forest understory includes many endemic and relict species such as *Wulfenia blecicci*, *Daphne blagayana*, *Pinus peuce*, and *Acer heldreichii* [10]. These forests experience a perhumid sub-Mediterranean mountain climate (Cfs″b), with a mean growing season temperature of 13.1 °C, extremes of −31 °C to 27 °C, annual precipitation of 880 mm, and growing season precipitation of 294 mm [12].

Relict pine forests dominated by *P. heldreichii* at the Mt. Kuči site are composed of *Pinetum heldreichii bertisceum* forest, across a wide zone, up to the tree line (1250–1700 m), while on the Mt. Prekornica and Mt. Orjen sites, they were shown at a lower altitude (1200–1300 m), where the bigger forest populations (Ass. *Pinetum heldreichii mediteraneo-montanum*) are present [10] (Table 1). Forests on Mt. Kuči support a diverse understory including *Daphne oleoides*, *Daphne blagayana*, *Wulfenia blecicci*, *Thymus albanicus*, *Acinos alpinus* spp. *Dinaricus*, and *Achillea abrotanoides*. Sites at Mt. Orjen and Mt. Prekornica had the following understory vegetation: *Juniperus sibirica*, *Senecio visianianus*, *Dianthus petraceus*, *Festuca heterophylla*, and *Gentiana amblyphylla*. These forests represent Mediterranean and sub-Mediterranean mountain climates (Cfsb/Cfws″bx″), with annual temperatures averaging 2–4 °C, extreme seasonal temperatures ranging from −37 °C to 40 °C, and annual precipitation between 2500 and 5000 mm, with only 8%–10% falling in the summer, and all three were developed on soils of varied development, i.e., with different depths between leptosol and molic leptosol [10–12].

At each sampling site, the forest stands were healthy-looking. Sampling took place during the autumn/winter months (November–December). Sampling was conducted by randomly selecting 1–2-year-old shoots with needles from 50 trees of each tree species per site (Table 1), growing at a minimal distance of 50 m between individual trees. Samples were placed in individual plastic bags and transported to the laboratory, where healthy-looking current-year needles were selected for DNA extraction, and stored at −20 °C before further processing. Taken together, the sampling resulted in 150 needle samples of *P. abies* from three sites, 200 needle samples of *A. alba* from four sites, and 150 of *P. heldreichii* from three forest sites.

2.2. Laboratory Work

Individual needle samples containing current-year healthy-looking needles were freeze-dried at −60 °C for 48 h (Alpha 1–4 LD, Martin Christ, Osterode Am Harz, Germany). After freeze-drying, about 200 mg of freeze-dried needle material was homogenized in a Tissue Lyser II (Qiagen, Hilden, Germany) and used for isolation of genomic DNA, using a DNeasy 96 Plant kit (Qiagen, Hilden, Germany) according to the protocol of the producer. The DNA concentration of each sample was determined using a NanoDrop^{TM} One spectrophotometer (Thermo Scientific, Rodchester, NY, USA) and adjusted to 10 ng/µL. Following DNA isolation, the fungal-specific ITS2 rDNA region was amplified by PCR using the barcoded primers gITS7 (5′-GTGARTCATCGARTCTTTG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) as described by Ihrmark et al. [14]. All DNA samples of the same tree species and site (50 samples) were amplified using primers with the same barcode, resulting in a total of 10 different barcodes. Each PCR reaction included template DNA (10 ng/µL), primers with unique barcodes, and Taq polymerase under the manufacturer's recommended conditions. PCR controls were also included. Amplifications were performed using the Applied Biosystems 2720 thermal cycler (Foster City, CA, USA). An initial denaturation step started at 95 °C for 2 min, followed by 27 amplification cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 60 s. The thermal cycling was ended by a final extension step at

72 °C for 7 min. Amplified products were verified using 1% agarose gel electrophoresis stained with Nancy-520 (Sigma-Aldrich, Stockholm, Sweden) and purified using a sodium acetate and ethanol mixture (1:15). Purified PCR products were quantified using a Qubit fluorometer 4.0 (Thermo Fisher Scientific, Waltham, MA, USA), pooled in equimolar mix, and subjected to high-throughput sequencing using the PacBio RSII platform at SciLifeLab, Uppsala, Sweden.

2.3. Bioinformatics

The bioinformatics methodology followed the principles outlined by Mischerikova et al. [15]. Sequence processing was conducted using the SCATA NGS sequencing pipeline, accessible at https://scata.mykopat.slu.se/ (accessed on 10 February 2025). The process began with quality control, which involved filtering out sequences shorter than 200 bp, low-quality reads, primer dimers, and homopolymers, which were collapsed to 3 bp prior to clustering. Only sequences containing both a barcode and primer were retained. Barcodes and primers were subsequently removed from the sequences, but metadata linking samples to sequences was preserved. Sequences were clustered into taxa using single-linkage clustering with a 98% similarity threshold. For each cluster, the most common genotype sequence was selected for taxonomic identification. In cases where clusters contained only two sequences, a consensus sequence was generated. Taxonomic identification was performed using the GenBank database and the Blastn algorithm [16]. The reliability of reference sequences was manually verified for each taxon by examining the metadata of top BLASTn hits in GenBank, prioritizing sequences from type specimens, well-annotated voucher specimens or peer-reviewed studies. Identification criteria included sequence coverage > 80%, 94%–97% similarity for genus-level identification, and ≥98% similarity for species-level identification. Sequences not meeting these criteria were assigned to higher taxonomic ranks and given unique identifiers. Assembled representative sequences of fungal non-singleton OTUs have been submitted to GenBank under accession numbers PV529837–PV530182.

2.4. Statistical Analyses

Fungal community diversity and composition were analyzed using the Shannon diversity index (to assess alpha diversity), the qualitative Sørensen similarity index (to evaluate beta diversity based on presence/absence data), and detrended correspondence analysis (DCA) (to visualize compositional differences among sites and host tree species) using Canoco 5 [17–19]. Fungal richness (total number of OTUs) among different tree species was compared using non-parametric chi-square tests. Differences in Shannon diversity index values between sites and tree species were assessed using the non-parametric Mann–Whitney U test. Fungal functional guilds were assigned using the FUNGuild online database available at https://www.funguild.org/(accessed on 15 March 2025). To assess the association between tree species and fungal OTUs, we performed an indicator species analysis using the Kruskal–Wallis test on the relative abundance of each OTU to identify those showing significant differences among host species. Only OTUs with a significance level of $p < 0.05$ were considered. These statistical analyses were conducted using Python (version 3.12.0) and the SciPy package [20]. All analyses were performed on non-transformed data.

3. Results

High-throughput sequencing and quality filtering yielded 15,269 high-quality reads. Clustering these reads at a 98% similarity threshold identified 428 OTUs. Of these, 346 OTUs, represented by 9549 reads (1364 reads per sample on average), were confirmed as fungal (Table 2) and retained for further analysis (Table S1), while 82 non-fungal OTUs

were excluded. Observed OTU richness was highest in needles of *P. abies* (229), followed by *A. alba* (218) and *P. heldreichii* (111) (Table 2). When comparing OTU richness among tree species, significant differences were observed between *A. alba* and *P. abies* ($p < 0.0001$) and between *A. alba* and *P. heldreichii* ($p < 0.0001$), while no significant difference was detected between *P. abies* and *P. heldreichii* ($p = 0.48$).

Table 2. The number of high-quality fungal reads and OTUs in needles of *Abies alba*, *Picea abies* and *Pinus heldreichii* from different sites in Montenegro.

Site	*Picea abies*		*Abies alba*		*Pinus heldreichii*		All	
	Reads	OTUs	Reads	OTUs	Reads	OTUs	Reads	OTUs
Mt. Durmitor	529	80	1341	134	-	-	1870	158
Mt. Bjelasica	737	66	61	21	-	-	798	78
Mt. Prokletije	3138	168	982	137	-	-	4120	211
Mt. Visitor	-	-	232	59	-	-	232	59
Mt. Kuči	-	-	-	-	103	24	103	24
Mt. Prekornica	-	-	-	-	1091	71	1091	71
Mt. Orjen	-	-	-	-	1341	74	1341	74
Total	4404	226	2610	218	2535	111	9549	346

The analysis of fungal OTU richness in needles among different tree species revealed that *A. alba* had 55 unique OTUs, *P. abies* had 67 unique OTUs, and *P. heldreichii* had 53 unique OTUs. A total of 113 OTUs were shared between *A. alba* and *P. abies*, 12 OTUs were shared between *A. alba* and *P. heldreichii*, and 8 OTUs were shared between *P. abies* and *P. heldreichii*. In total, 38 fungal OTUs were found to be common across all three species (*A. alba*, *P. abies*, and *P. heldreichii*) (Figure 3).

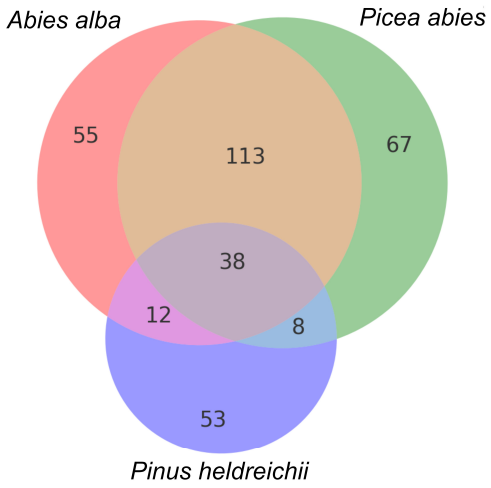

Figure 3. Venn diagram showing unique and shared OTUs among different groups of samples. Within each group, data from different sites are combined. The size of each circle represents the number of unique OTUs. *Pinus heldreichii* (blue), *Picea abies* (green), and *Abies alba* (red).

The fungal community composition varied among the three tree species, with *A. alba*, *P. abies*, and *P. heldreichii* showing distinct distributions of fungal classes (Figure 4). Dothideomycetes was the most dominant fungal class across all tree species, representing 33.1% of the community in *A. alba*, 63.7% in *P. abies*, and 54.7% in *P. heldreichii*. In *A. alba*, the second most abundant class was Leotiomycetes (23.3%), followed by Pucciniomycetes (10.8%) and Tremellomycetes (9.5%). Similarly, *P. abies* exhibited Leotiomycetes as the second most dominant class (10.5%), but with a greater relative abundance of Tremellomycetes (7.0%) and Eurotiomycetes (6.2%). For *P. heldreichii*, Leotiomycetes accounted for 21.2% of the fungal community, while Pucciniomycetes (12.0%) and Tremellomycetes (5.6%) were also common. Less abundant classes, including Pezizomycotina, Microbotryomycetes,

Exobasidiomycetes, Lecanoromycetes, Sordariomycetes, and Taphrinomycetes, contributed to the overall diversity, but their relative proportions varied among tree species.

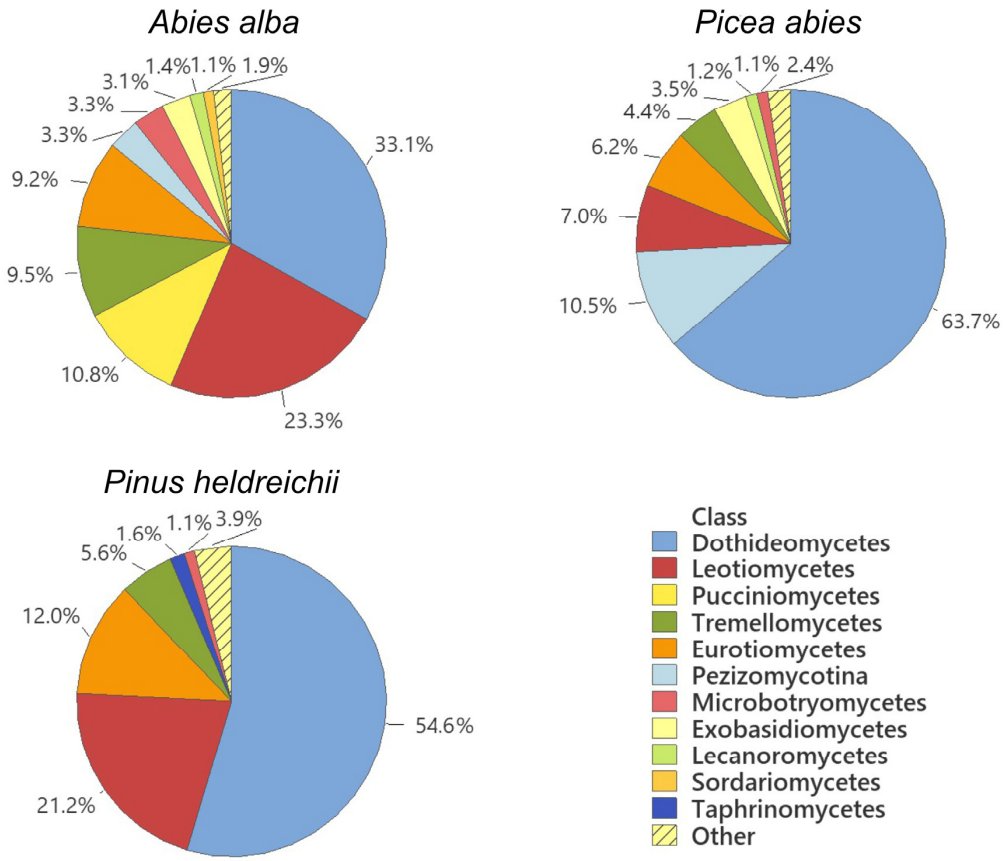

Figure 4. Relative abundance (% of sequences) of fungal classes associated with needles of *Abies alba*, *Picea abies*, and *Pinus heldreichii*. Data from different sites are combined. *Other* includes fungal classes with a relative abundance < 1%.

The 20 most abundant fungal OTUs across all tree species are shown in Table 3. Among these, the most abundant was the unidentified fungus ID 3598_3 with a total relative abundance of 10.7%. It was particularly dominant in needles of *P. heldreichii* (25.2%) but was less frequent in *P. abies* (6.4%) and *A. alba* (3.7%). Another unidentified species (ID 3598_2) was very common in *P. abies* (15.7%) but less abundant in *A. alba* (2.2%) and absent in *P. heldreichii*. Similarly, the yeast-like fungus *Vishniacozyma victoriae* showed a higher presence in *P. abies* (5.4%) but was less frequent in *A. alba* (1.3%) and absent in *P. heldreichii*. Some taxa showed a relatively balanced distribution across all species, including *Pseudeurotiaceae* sp., which was present in all three hosts with similar abundances (2.0% in *A. alba*, 4.5% in *P. abies*, and 2.2% in *P. heldreichii*). Similarly, *Cladosporium* sp. exhibited comparable abundance across all tree species (1.3%–2.7%). A few taxa demonstrated host specificity. The rust fungus *Chrysomyxa nagodhii*, known to be associated with needle infections, was exclusively detected in *P. abies* (12.6%), while unidentified fungus (ID 3598_24) was only detected in *P. heldreichii* (11.6%) (Table 3).

The DCA ordination plot shows the distribution of fungal communities associated with three tree species across different mountain sites (Figure 5). The presence of distinct clustering patterns indicated clear differences in fungal communities among the tree species. *Pinus heldreichii* was positioned to the left in the ordination, exhibiting a unique fungal community structure compared to *A. alba* and *P. abies*, which were more closely associated and showed a degree of overlap. The mountain sites demonstrated geographic variation in fungal communities. The fungal communities from Mt. Kuči (K), Mt. Orjen (O), and

Mt. Prekornica (Pr) were clustered within the *P. heldreichii* group, whereas sites from Mt. Bjelasica (B), Mt. Prokletije (P), and Mt. Visitor (V) were within the overlapping *A. alba* and *P. abies* regions. Notably, the fungal communities from Mt. Durmitor (D) and Mt. Bjelasica (B) appeared at distinct positions within the ordination, reflecting their unique communities (Figure 5).

Table 3. Relative abundance (%) of fungal taxa in needles of *Abies alba*, *Picea abies*, and *Pinus heldreichii* from Montenegro. Data from different sites were aggregated by combining all sequencing reads per tree species across all sites, without calculating averages.

OUT ID.	Taxon	Phylum *	Genbank Reference	Sequence Length, bp	Compared, bp	Similarity, %	*Abies alba*, %	*Picea abies*, %	*Pinus heldreichii*, %	All, %
3598_3	Unidentified sp.	A	KX220267	257	257/257	100%	3.8	6.4	25.2	10.7
3598_2	Unidentified sp.	A	MN902478	240	240/240	100%	2.2	15.7	-	7.8
3598_4	*Chrysomyxa nagodhii*	B	GU049432	317	312/317	98%	-	12.6	-	5.8
3598_7	*Pseudeurotiaceae* sp.	A	ON865419	240	239/240	99%	2.0	4.5	2.2	3.2
3598_24	Unidentified sp.	A	MT241934	259	259/259	100%	-	-	11.6	3.1
3598_12	*Herpotrichiellaceae* sp.	A	JF449676	258	257/258	99%	5.0	3.3	-	2.9
3598_10	*Vishniacozyma victoriae*	B	MF927673	234	234/234	100%	1.3	5.4	-	2.8
3598_20	*Cladosporium* sp.	A	LC317546	243	243/243	100%	1.3	2.7	2.7	2.3
3598_28	Unidentified sp.	A	MZ983694	242	242/242	100%	4.1	0.7	3.4	2.3
3598_38	*Mycosphaerellaceae* sp.	A	KJ406801	240	217/223	97%	0.8	0.2	7.1	2.2
3598_41	Unidentified sp.	A	MN903736	246	246/246	100%	4.3	0.6	2.4	2.1
3598_27	*Botryosphaeriales* sp.	A	PP759557	244	244/244	100%	4.7	1.5	-	2.0
3598_15	*Phaeosphaeria* sp.	A	KR909136	249	244/250	98%	7.1	0.0	-	2.0
3598_45	*Ceramothyrium* sp.	A	KC978733	252	246/253	97%	3.1	2.4	-	1.9
3598_35	*Leotiomycetes* sp.	A	OQ066890	240	240/240	100%	0.5	3.6	0.0	1.8
3598_37	*Sydowia polyspora*	A	KY659505	256	256/256	100%	1.8	1.0	2.7	1.7
3598_39	*Trichomerium dioscoreae*	A	NR_137946	265	261/265	98%	3.6	1.1	-	1.5
3598_58	*Pseudeurotiaceae* sp.	A	KR267039	243	243/243	100%	1.3	2.3	-	1.4
3598_59	*Exobasidium bisporum*	B	AB180368	281	266/268	99%	2.1	0.5	1.3	1.2
3598_49	Unidentified sp.	A	MN902540	240	235/240	98%	3.1	0.6	-	1.1
Total of the 20 taxa							62.9	56.5	56.0	59.9

* A = Ascomycota, B = Basidiomycota.

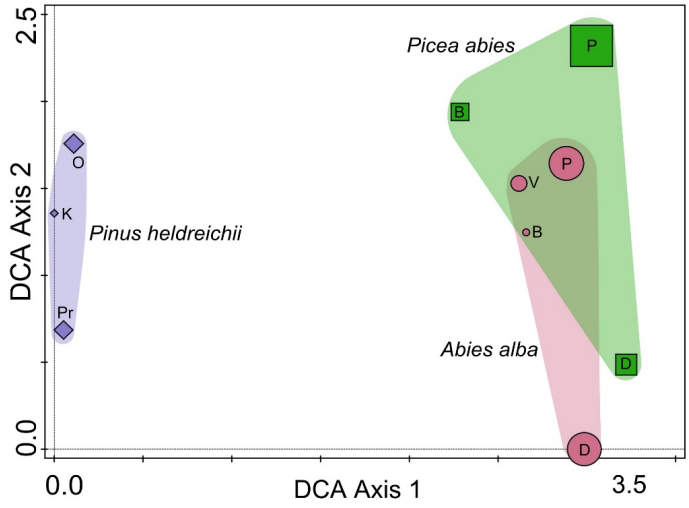

Figure 5. Ordination diagram based on detrended correspondence analysis of fungal communities in needles of *Picea abies* (points—squares; polygon—green), *Abies alba* (points—circles; polygon—red), and *Pinus heldreichii* (points—diamonds; polygon—blue). Sites of the same tree species are enclosed in color-shaded areas, illustrating the spatial distribution of fungal communities and their compositional differences and overlaps among tree species. The size of each plot (square, circle, or diamond) shows the relative richness of fungal OTUs at each site. Site names are represented as B—Mt. Bjelasica, D—Mt. Durmitor, K—Mt. Kuči, O—Mt. Orjen, Pr—Mt. Prekornica, P—Mt. Prokletije, and V—Mt. Visitor (Table 1). In the ordination, 23.9% of the variation was explained on the x-axis, and 14.8% on the y-axis.

The Shannon diversity index at different sites ranged from 2.74 to 3.97 for *A. alba*, 3.14 to 4.45 for *P. abies*, and 2.46 to 3.24 for *P. heldreichii*. The Mann–Whitney test showed

no significant differences in Shannon diversity index values among the three tree species ($p > 0.05$). The Sørensen qualitative similarity index of fungal communities was high (0.68) between *A. alba* and *P. abies*, but low (0.30) between *A. alba* and *P. heldreichii*, and low (0.27) between *P. abies* and *P. heldreichii*.

The composition of fungal trophic modes varied among *A. alba*, *P. abies*, and *P. heldreichii*, with differences in the relative abundance of unknown, saprotrophic, pathogenic, and endophytic fungi (Figure 6). For *A. alba*, fungi with an unknown trophic mode accounted for 42.5% of the total community, followed by saprotrophs (28.5%), pathogens (23.0%), and endophytes (6.0%). *P. abies* showed a higher proportion of fungi with an unknown trophic mode (59.8%), while saprotrophs represented 23.8%, pathogens 14.4%, and endophytes 2.0%. In *P. heldreichii*, unknown fungi comprised 49.2%, saprotrophs 36.2%, pathogens 8.9%, endophytes 5.4%, and mycorrhizal fungi 0.2%. The distribution patterns showed that fungi with an unknown trophic role dominated across all three tree species, particularly in *P. abies*. *P. heldreichii* exhibited the highest proportion of saprotrophs, while *A. alba* had a more even distribution of fungal functional groups (Figure 6).

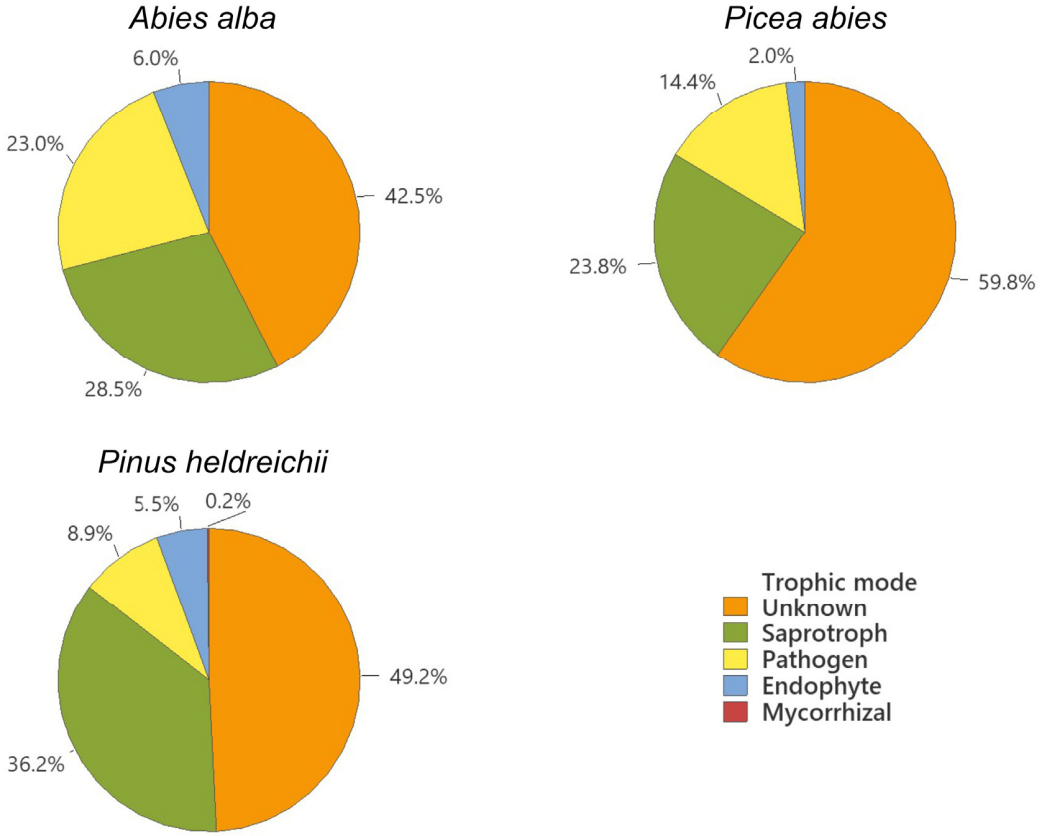

Figure 6. Relative abundance (% of sequences) of fungal trophic modes associated with needles of *Abies alba*, *Picea abies*, and *Pinus heldreichii* in Montenegro. Data from different sites are combined.

The indicator species analysis identified fungal OTUs that were significantly associated with specific tree species ($p < 0.05$). OTU 3598_24 and 3598_53 were found to be significantly associated with *P. heldreichii*, while OTUs 3598_15 and 3598_50 were linked to both *A. alba* and *P. abies*. OTU 3598_78 exhibited a broader association, being significantly linked to *A. alba*, *P. abies*, and *P. heldreichii* (Tables 1 and S1).

4. Discussion

The results of this study provided new insights into the fungal communities associated with the needles of three native conifer species (*P. abies*, *A. alba*, and *P. heldreichii*) in the

Balkan region. High-throughput sequencing revealed high fungal diversity and specificity in community composition among these tree species, hence contributing to the general knowledge on forest microbiomes by providing a detailed information of needle-associated fungal diversity in a relatively understudied region. However, a high proportion of fungal OUTs remained unidentified or were classified only at higher taxonomic levels, showing limitations in the available reference databases. The recent rapid increase in fungal barcoding studies has revealed vast fungal diversity across a broad spectrum of ecological niches. However, it is estimated that only about 15% of fungal diversity, or only about 100,000 of the 740,000 to 6,000,000 predicted fungal species, has been formally described [21]. Moreover, among the fungal species described, around 50% still lack any DNA sequence information in public databases [21]. This suggests that many of our unidentified OUTs may represent species yet to be discovered or described, emphasizing the need for future taxonomic and functional studies of phyllosphere communities in native forests [22]. In addition, many unidentified fungi also have unknown ecological functions (in terms of behavior and/or resource use), which limits the resolution of trophic mode analyses.

The diversity of fungal communities associated with tree needles was shown to vary according to host species, environmental conditions, and geographical location [15]. These fungal communities are adapted to needle-specific environments [23] and belong to diverse trophic groups, which can be functionally important and respond to changes in the environment [24].

Considering that *P. abies* represents an important commercial conifer species in Europe [2], several studies have been carried out in central and northern Europe [15,24,25]. Those studies investigated whether and how fungal communities associated with *P. abies* needles correlate with variation in tree species richness along the tree species diversity gradient [24], along the latitudinal gradient of *P. abies* distribution in Europe [15], or under different edaphic and management practices in the southern Baltics [25]. Also, other studies revealed that a plant genotype, and even different clones, affect the composition of fungal communities on assimilative plant parts [7,26]. Moreover, fungal communities evolve together with host plants [27], suggesting that outside the principal areal of distribution, such as on marginal habitats or on the edges of species distribution, *P. abies* (and *A. abies*) can be associated with a specific fungal community, what was also confirmed in the present study. In the past, fungal communities of *A. alba* needles were rarely studied, and these studies were largely based on cultivation methods [28,29]. Regarding *P. heldreichii*, our previous study [30] examined fungal communities present in older needles (2–4 years old), comparing trees growing under high environmental stress with those growing under milder conditions. In the presented study, sampling was performed in larger *P. heldreichii* populations, and under the conditions of species ecological optimum. These favorable environmental conditions may support a more stable or diverse fungal community, thereby contributing to a more comprehensive understanding of the fungal diversity associated with this relict conifer.

A key finding of this study is the host tree specificity of fungal communities as shown by the DCA. *P. heldreichii* exhibited a distinct fungal community compared to *A. alba* and *P. abies*, which shared a greater degree of overlap in fungal taxa. This pattern suggests that host species exert selective pressures on their associated fungal communities, potentially due to differences in needle chemistry, nutrient availability, and microhabitat conditions [31–33]. Furthermore, in the DCA, the separation along Axis 1 showed that the fungal communities of *P. heldreichii* were compositionally distinct, potentially due to environmental factors specific to its sites (Table 1), where this species was the dominant or sole conifer, unlike *P. abies* and *A. alba*, which occurred in mixed stands. This distinctiveness likely also reflects the relict status of this tree species and its long-term adaptation to harsh

montane environments (e.g., temperature extremes, soil composition, and precipitation patterns) in southern Europe [8,34].

The dominance of *Dothideomycetes* across all tree species aligns with global fungal diversity patterns in phyllosphere habitats, and is also consistent with the results of Nguyen et al. [24] and Mischerikova et al. [15]. The relative abundance of Leotiomycetes and Tremellomycetes suggests that these also play important roles in coniferous ecosystems.

The fungal communities were dominated by several fungal taxa (Table 3). Understanding the roles of these taxa is important for assessing their contributions to forest health, tree resilience, ecosystem stability, and nutrient cycling in ecosystems. For example, the predominant presence of *C. nagodhii*, a recently described rust pathogen [35], suggests its ecological role as a potential plant pathogen of *P. abies*. Rust fungi from the *Chrysomixa* complex (*C. ledi*) were identified as abundant species on current-year needles in Finland [24]. Rust fungi from the genus *Crysomixa* (*abietis*) are known to infect *P. abies* needles in Montenegro [36]. Similarly, the occurrence of *S. polyspora*, a well-known endophyte and opportunistic pathogen, aligns with its documented association with conifer needles under stress conditions. Only *S. polyspora* was more abundantly represented on all three hosts. In published research, it was the most common fungus in 12 *P. abies* sampling sites along a latitudinal gradient in Europe [15]; on *P. abies*, it was abundantly represented in mixed coniferous–deciduous forests in Romania and Germany, as well as in coniferous semiboreal and boreal forests in Poland and Finland [24]. Regarding *A. alba*, *S. polyspora* was abundantly detected on symptomatic and asymptomatic needles from Tatra mountains [29], and studied as a causal agent of current-season needle necrosis of *Abies* spp. [37] across Europe. On older needles of *P. heldreichii*, *S. polyspora* was among the most dominant fungi [30]. *Sydowia polyspora* has a wide geographical range [38] and is common in Europe. The high representation of this fungus on current-year needles highlights that it can be an important component in these habitats and not merely a latent pathogen of coniferous forests in this region. Its importance may increase in the future due to climate change and abiotic stress affecting trees. Yeast-like fungi, such as *V. victoriae*, are often found in extreme environments and have been tested in biocontrol and antifungal interactions [39]. It was detected in association with *P. abies* and *A. alba* needles. Their presence in conifer needles could indicate a role in microbial competition and plant defense mechanisms. Additionally, the presence of *Cladosporium* sp. across all three species suggests its role as a generalist species, commonly detected in plant tissues and known for its antifungal properties [40]. It is also abundantly detected on *P. abies* needles across different forests in Europe, including the southern Baltics [24,25].

In the present study, fungi from the genii *Ceramothyrium* and *Trichomerium dioscoreae* were detected on *P. abies* and *A. alba* needles at a relatively high abundance (Table 3). Many species of *Ceramothyrium* are found on tropical broadleaves [41,42] and *Trichomerium dioscoreae* is known to be associated with monocotyledonous flowering plants from the family Discoreaceae [43], primarily outside Europe. Interestingly, Nguyen et al. [24] have also found both *Ceramothyrium* fungi and *Trichomerium dioscoreae* [24,25] in similar abundance on *P. abies* from coniferous, hemi boreal, and temperate beech–spruce forests. The latter may suggest that some *Ceramothyrium*, as well as *Trichomerium dioscoreae*, can be specific to *P. abies* and *A. alba* as a host species. Similarly, the *Exobasidium* taxa detected on all three hosts in this study are already known from *P. abies* needles in boreal and semiboreal forests [24,25]. It is known that temperate *Ericales*, commonly found in coniferous forest understories, are preferred hosts for parasitic fungi of the genus *Exobasidium* [44].

The trophic mode analysis revealed that fungi with an unknown ecological function accounted for a significant proportion of the fungal communities in all three tree species, showing the need for continued research on the fungal communities associated with these key European conifer tree species. The presence of pathogenic, endophytic, and saprotrophic fungi

provided valuable ecological insights. The great proportion of detected potential pathogens in *A. alba* and *P. abies* may reflect increased susceptibility to fungal infections, particularly in marginal habitats, and under high environmental stresses such as drought [4]. The relatively high abundance of saprotrophic fungi in *P. heldreichii* needles may indicate latent establishment in healthy-looking tissues but also the capacity for nutrient recycling in high-altitude pine forests, where decomposition rates are generally slower [45].

These findings have important implications for forest conservation and management in Montenegro and in similar temperate and montane ecosystems. The high fungal diversity in these native conifers shows the importance of preserving natural forests, which stabilize marginal mountain environments, but also serve as reservoirs of microbial biodiversity. Future research should focus on the functional roles of dominant fungal taxa in coniferous forests, particularly in the context of climate change and forest disturbance [32].

In conclusion, this study highlights the relationships between native conifers and their associated fungi, emphasizing the ecological importance of needle-associated fungi in forest ecosystems. By advancing our understanding of fungal biodiversity and host specificity, these findings lay the foundation for future research and conservation efforts aimed at preserving the microbial diversity and ecosystem functions at the edges of tree populations in the Balkan region, particularly in Montenegrin forests.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f16060968/s1, Table S1: Relative abundance of fungal taxa associated with needles of *Abies alba*, *Picea abies* and *Pinus heldreichii* in Montenegro

Author Contributions: Conceptualization, J.L. and A.M.; methodology, J.L. and A.M.; formal analysis, J.L. and A.M.; investigation, J.L. and A.M.; resources, J.L.; data curation, J.L. and A.M.; writing—original draft preparation, J.L. and A.M.; writing—review and editing, J.L. and A.M.; funding acquisition, J.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science of Montenegro through the grants INVO HERIC No: 01-646 and 01-001 and FunGforest project No 04-082/23-2532/1. The collaboration within this project was also supported by STSM grants from COST FP 1401 and COST FP 1202.

Data Availability Statement: The original contributions presented in the study are included in the article and Supplementary Material; further inquiries can be directed to the corresponding author.

Acknowledgments: Special thanks go to Ivana Stojanović, and the Laboratory at the CNR–Institute for Biosciences and BioResources in Florence, Italy, for working on DNA extractions from all sampled material in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Čurović, M.; Jovančević, M.; Balijagić, J. Wild fruit tree species of Montenegrin forests. In *Forests of Southeast Europe Under a Changing Climate*; Šijačić-Nikolić, M., Milovanović, J., Nonić, M., Eds.; Springer: Cham, Switzerland, 2018; pp. 21–28. [CrossRef]
2. Skrøppa, T. *EUFORGEN Technical Guidelines for Genetic Conservation and Use for Norway Spruce (Picea abies)*; International Plant Genetic Resources Institute: Rome, Italy, 2003; p. 6.
3. Mauri, A.; de Rigo, D.; Caudullo, G. Abies alba in Europe: Distribution, habitat, usage and threats. In *European Atlas of Forest Tree Species*; San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A., Eds.; Publ. Off. EU: Luxembourg, 2016; p. e01493b.
4. Seppä, H.; Alenius, T.; Bradshaw, R.; Giesecke, T.; Heikkilä, M.; Muukkonen, P. Invasion of Norway spruce (*Picea abies*) and the rise of the boreal ecosystem in Fennoscandia. *J. Ecol.* **2009**, *97*, 629–640. [CrossRef]
5. Vendramin, G.G.; Fineschi, S.; Fady, B. *EUFORGEN Technical Guidelines for Genetic Conservation and Use for Bosnian Pine (Pinus heldreichii)*; Bioversity International: Rome, Italy, 2008; p. 6.
6. Lazarević, J.; Menkis, A. Fungi inhabiting fine roots of *Pinus heldreichii* in the Montenegrin montane forests. *Symbiosis* **2018**, *74*, 189–197. [CrossRef]

7. Rajala, T.; Velmala, S.M.; Vesala, R.; Smolander, A.; Pennanen, T. The community of needle endophytes reflects the current physiological state of Norway spruce. *Fungal Biol.* **2014**, *118*, 309–315. Available online: https://www.sciencedirect.com/science/article/pii/S1878614614000099 (accessed on 5 April 2025). [CrossRef]
8. Terhonen, E.; Blumenstein, K.; Kovalchuk, A.; Asiegbu, F. Forest tree microbiomes and associated fungal endophytes: Functional roles and impact on forest health. *Forests* **2019**, *10*, 42. [CrossRef]
9. Velagić-Hajrudinović, A. Regional approach to biodiversity information management and reporting in Southeast Europe. *Biodivers. Inf. Sci. Stand.* **2019**, *3*, e38190. [CrossRef]
10. Lakušić, R. Forest communities of Jugoslavia- SR Crna Gora. In *Encyclopedia of Forestry*; Potočić, Z., Ed.; Jugoslovenski Leksikografski Zavod Miroslav Krleža: Zagreb, Croatia, 1987; pp. 388–395.
11. Fuštić, B.; Đuretić, G. *Soils of Montenegro*; Biotechnical Institute, University of Montenegro: Podgorica, Montenegro, 2000.
12. Burić, M.; Micev, B.; Mitrović, L. *Atlas of Climate of Montenegro*; Montenegrin Academy of Science and Arts: Podgorica, Montenegro, 2012.
13. Lakušić, D.; Šinžar-Sekulić, J. Overview map of the potential vegetation of Montenegro. In *Botanical Lexicon of Montenegro*; Montenegrin Academy of Science and Arts: Podgorica, Montenegro, 2022; p. 679.
14. Ihrmark, K.; Bodeker, I.T.M.; Cruz-Martinez, K.; Friberg, H.; Kubartova, A.; Schenck, J.; Strid, Y.; Stenlid, J.; Brandstrom-Durling, M.; Clemmensen, K.E.; et al. New primers to amplify the fungal ITS2 region—Evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol. Ecol.* **2012**, *82*, 666–677. [CrossRef]
15. Mischerikova, V.; Marčiulynienė, D.; Marčiulynas, A.; Lynikienė, J.; Gedminas, A.; Prylutsky, O.; Menkis, A. Biogeography of fungal communities associated with *Pinus sylvestris* L. and *Picea abies* (L.) H. Karst. along the latitudinal gradient in Europe. *J. Fungi* **2023**, *9*, 829. [CrossRef]
16. Altschul, S.F.; Madden, T.L.; Schäffer, A.A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D.J. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* **1997**, *25*, 3389–3402. [CrossRef]
17. Shannon, C.E. A mathematical theory of communication. *Bell Syst. Tech. J.* **1948**, *27*, 379–423. [CrossRef]
18. Magurran, A.E. *Ecological Diversity and Its Measurement*; Princeton University Press: Princeton, NJ, USA, 1988; p. 192.
19. ter Braak, C.J.F.; Smilauer, P. *Canoco Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination, Version 4*; Microcomputer Power: Ithaca, NY, USA, 1998; p. 351.
20. Virtanen, P.; Gommers, R.; Oliphant, T.E.; Haberland, M.; Reddy, T.; Cournapeau, D.; Burovski, E.; Peterson, P.; Weckesser, W.; Bright, J.; et al. SciPy 1.0 contributors. SciPy 1.0: Fundamental algorithms for scientific computing in Python. *Nat. Methods* **2020**, *17*, 261–272. [CrossRef]
21. Xu, J. Fungal DNA barcoding. *Genome* **2016**, *59*, 913–932. [CrossRef]
22. Tedersoo, L.; Anslan, S.; Bahram, M.; Drenkhan, R.; Pritsch, K.; Buegger, F.; Padari, A. Regional scale in depth analysis of soil fungal diversity reveals strong pH and plant species effects in Northern Europe. *Front. Microb.* **2020**, *11*, 1953. [CrossRef]
23. Baldrian, P. Forest microbiome: Diversity, complexity, and dynamics. *FEMS Microbiol. Rev.* **2017**, *41*, 109–130. [CrossRef]
24. Nguyen, D.; Boberg, J.; Ihrmark, K.; Stenström, E.; Stenlid, J. Do foliar fungal communities of Norway spruce shift along a tree species diversity gradient in mature European forests? *Fungal Ecol.* **2016**, *23*, 97–108. [CrossRef]
25. Marčiulynas, A.; Marčiulynienė, D.; Mishcherikova, V.; Franić, I.; Lynikienė, J.; Gedminas, A.; Menkis, A. High Variability of Fungal Communities Associated with the Functional Tissues and Rhizosphere Soil of *Picea abies* in the Southern Baltics. *Forests* **2022**, *13*, 1103. [CrossRef]
26. Bálint, M.; Tiffin, P.; Hallström, B.; O'Hara, R.B.; Olson, M.S.; Fankhauser, J.D.; Piepenbring, M.; Schmitt, I. Host Genotype Shapes the Foliar Fungal Microbiome of Balsam Poplar (*Populus balsamifera*). *PLoS ONE* **2013**, *8*, e53987. [CrossRef]
27. Rodriguez, R.J.; Redman, R.S. More than 400 million years of evolution and some plants still can't make it on their own: Plant stress tolerance via fungal symbiosis. *J. Exp. Bot.* **2008**, *59*, 1109–1114. [CrossRef]
28. Siber-Canavesi, F.; Sieber, T.N. Successional patterns of fungal communities in needles of European silver fir (*Abies alba* Mill.). *New Phytol.* **1993**, *25*, 149–161. [CrossRef]
29. Pusz, W.; Baturo-Ciesniewska, A.; Kaczmarek-Pienczewska, A.; Zwijacz-Kozica, T.; Patejuk, K. The mycobiota of needles and shoots of silver fir (*Abies alba* Mill.) with symptoms of *Herpotrichia* needle browning in the Tatra Mts. (Poland). *Ann. For. Res.* **2020**, *63*, 45–56. [CrossRef]
30. Lazarević, J.; Menkis, A. Fungal biodiversity in the phyllosphere of *Pinus heldreichii* H. Christ-an endemic and high-altitude pine of Mediterranean region. *Diversity* **2020**, *12*, 172. [CrossRef]
31. Peay, K.G.; Kennedy, P.G.; Talbot, J.M. Dimensions of biodiversity in the Earth's mycobiome. *Nat. Rev. Microbiol.* **2016**, *14*, 434–447. [CrossRef]
32. Tedersoo, L.; Bahram, M.; Põlme, S. Global diversity and geography of soil fungi. *Science* **2014**, *346*, 1256688. [CrossRef]
33. Baldrian, P.; López-Mondéjar, R.; Kohout, P. Forest microbiome and global change. *Nat. Rev. Microbiol.* **2023**, *21*, 487–501. [CrossRef]

34. Barbi, F.; Martinović, T.; Odriozola, I.; Machac, A.; Moravcová, A.; Algora, C.; Ballian, D.; Barthold, S.; Brabcová, V.; Awokunle Hollá, S.; et al. Disentangling drivers behind fungal diversity gradients along altitude and latitude. *New Phytol.* **2025**, *247*, 295–308. [CrossRef]
35. Crane, P.E. Morphology, taxonomy, and nomenclature of the *Chrysomyxa ledi* complex and related rust fungi on spruce and Ericaceae in North America and Europe. *Can. J. Bot.* **2001**, *79*, 57–982. [CrossRef]
36. Karadić, D. *Forest Phytopathology*; University of Belgrade, Faculty of Forestry: Belgrade, Serbia and University of Banja Luka, Faculty of Forestry: Banja Luka, Bosnia and Herzegovina, 2010; pp. 330–331.
37. Talgo, V.; Chastanger, G.; Thomsen, I.M.; Cech, T.; Riley, K.; Lang, K.; Klemsdal, S.S. *Sydowia polyspora* associated with current season needle necrosis (CSNN) on true fir (*Abies* spp.). *Fungal Biol.* **2010**, *114*, 545. [CrossRef]
38. Botella, L.; Diez, J.J. Phylogenetic diversity of fungal endophytes in Spanish stands of *Pinus halepensis*. *Fungal Divers.* **2011**, *47*, 9–18. [CrossRef]
39. Buzzini, P.; Turchetti, B.; Yurkov, A. Extremophilic yeasts: The toughest yeasts around? *Yeast* **2018**, *35*, 487–497. [CrossRef]
40. Bensch, K.; Braun, U.; Groenewald, J.Z.; Crous, P.W. The genus *Cladosporium*. *Stud. Mycol.* **2012**, *72*, 1–401. [CrossRef]
41. Tennakoon, D.S.; Thambugala, K.M.; Jeewon, R.; Hongsanan, S.; Kuo, C.-H.; Hyde, K.D. Additions to *Chaetothyriaceae* (Chaetothyriales): *Longihyalospora* gen. nov. and *Ceramothyrium longivolcaniforme*, a new host record from decaying leaves of *Ficus ampelas*. *MycoKeys* **2019**, *61*, 91–109, ISSN 1314–4057. Available online: https://www.sciencedirect.com/science/article/pii/S1314405719000892 (accessed on 5 April 2025). [CrossRef]
42. Wijesinghe, S.N.; Dayarathne, M.C.; Maharachchikumbura, S.S.N.; Wanasinghe, D.N.; Hyde, K.D. Ceramothyrium chiangraiense, a novel species of Chaetothyriales (*Eurotiomycetes*) from Ficus sp. in Thailand. *Asian J. Mycol.* **2019**, *2*, 269–280. [CrossRef]
43. Trichomerium Dioscoreae. Available online: https://ontosight.ai/glossary/term/trichomerium-dioscoreae-fungus-species-overview--67a29eaec445bf945af37842 (accessed on 10 April 2025).
44. Callan, B.E.; Carris, L.M. 7-Fungi on Living Plant Substrata, Including Fruits. In *Biodiversity of Fungi*; Mueller, M.D., Bills, G.F., Foster, M.S., Eds.; Academic Press: Cambridge, MA, USA, 2004; pp. 105–126. Available online: https://www.sciencedirect.com/science/article/pii/B9780125095518500106 (accessed on 5 April 2025). [CrossRef]
45. Ottosson, E.; Norden, J.; Dahlberg, A.; Edman, M.; Jönsson, M.; Larsson, K.H.; Lindahl, B.D. Species associations during the succession of wood-inhabiting fungal communities. *Fungal Ecol.* **2015**, *14*, 27–38. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.