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Fungal Diversity in Fire-Affected Pine Forest Soils at the Upper Tree Line

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Abstract: Forest fires represent a significant ecological disturbance in ecosystems that increasingly affects *Pinus heldreichii* H. Christ forests at the upper tree line in Montenegro, due to climate change and anthropogenic factors. Soil samples were collected from five high-altitude sites in the Kuči Mountains, including three post-fire sites (2-, 4-, and 6-years post-fire) and two unburned control sites. High-throughput sequencing and soil chemical analyses were conducted to assess fungal diversity, community composition, and soil nutrient properties. The results showed that fungal diversity was significantly higher in unburned soils compared to post-fire soils, with the most prominent changes in ectomycorrhizal fungi, which are crucial for pine regeneration. The fungal community composition differed markedly between the post-fire and unburned sites, with specific taxa such as *Hygrocybe conica* (Schaeff.) P. Kumm. and *Solicoccozyma aeria* (Saito) Yurkov dominating the post-fire environments. Despite this, the fungal richness did not significantly change over time (2-, 4-, or 6-years post-fire), suggesting the slow recovery of fungal communities in high-altitude environments. In addition to shifts in fungal biodiversity, the post-fire soils exhibited higher levels of available phosphorus, likely due to the conversion of organic phosphorus into soluble forms during combustion. However, the organic matter content remained unchanged. This study provided important insights into the long-term ecological impacts of forest fires on high-altitude *P. heldreichii* forests and underlined the importance of preserving unburned forest areas to maintain fungal biodiversity and support natural regeneration, as well as the potential need for active restoration strategies in fire-affected regions.

Keywords: *Pinus heldreichii*; disturbance recovery; fungal community; forest fire; high-altitude pine forests; ectomycorrhiza; soil nutrient dynamics; phosphorus availability

1. Introduction

Forest fires represent a significant ecological disturbance in forest ecosystems, significantly affecting the landscape, biodiversity, soil properties, and biogeochemical cycles. The Mediterranean basin, characterized by its hot, dry summers and wet winters, is particularly prone to wildfires. In Europe, the Mediterranean region and the Balkan Peninsula have the highest incidences of forest fires and burned areas. However, in recent years, forest fires have also become a significant issue in Central Europe [1]. The total burned areas in Europe annually amounts to more than half a million hectares, as in 2023, but can also reach up to 1,000,000 ha, as in 2017 [2]. According to Alexandrian et al. [3], the average annual number of forest fires throughout the Mediterranean basin was close to 50,000 and the annual cumulated burn area in Mediterranean countries is estimated to be approximately 600,000 ha. In the Mediterranean region, *Pinus heldreichii* H. Christ is a high-altitude conifer tree species with a discontinuous and restricted distribution. As a tertiary relict, *P. heldreichii* forests are of key importance for nature and landscape conservation, protection against gravitational natural hazards, and recreation [4–6]. It is expected to adapt well to climate change and could be a promising tree species for forestry and nature conservation in high-altitude mountain areas [7].

However, in Montenegro, *P. heldreichii* has been experiencing an increased incidence of forest fires over the last 20 years, making it a pertinent case study for investigating fire impacts on forest ecosystems in this specific environment at the upper tree line. These fires have been frequent and intense, causing the destruction of the aboveground forest ecosystem and leading to poor or lacking natural regeneration of pine forests in the burned areas (Figure 1).

Figure 1. Fire-destroyed *Pinus heldreichii* forest stands at the upper tree line at the Hum Orahovski site in Montenegro.

Natural regeneration refers to the process by which forests regenerate without human intervention. In healthy ecosystems, this process is crucial for maintaining biodiversity, forest structure, and ecosystem services [8,9]. However, in the case of *P. heldreichii* forests impacted by frequent fires, the ability to naturally regenerate has been severely hampered [10,11]. The heat from these fires often destroys seed banks and damages the soil, creating unfavorable conditions for seed germination and sapling growth [12,13]. In addition, fires also play a crucial role in altering soil nutrient dynamics [14–16], which can have lasting effects on ecosystem recovery. For example, alternations can be expected in the dynamic of nitrogen and phosphorus [17], which are key nutrients for plant growth, as well as in organic matter. These changes in nutrient availability can influence plant regeneration and microbial activity [18–20], particularly in marginal habitats. Under high-altitude conditions, post-fire environments may also experience intensive soil erosion and loss of organic matter [21].

Soil fungal communities play a critical role in ecosystem functioning. They are involved in nutrient cycling, decomposition, and the formation of mycorrhizal associations, which are vital for plant health and soil structure [22]. However, the resilience and response of these communities to forest fires remain poorly understood, particularly under harsh environmental conditions such as high-altitude Mediterranean environments and in the context of rapid environmental changes. The disturbance caused by fires can lead to significant shifts in fungal diversity and community composition, affecting their functional capabilities and, consequently, the recovery of soil health and ecosystem services after a forest fire [14,23]. Following changes in community composition, such as the reduction in ectomycorrhizal (ECM) fungi, the regeneration of high-altitude pine forests can also be affected. ECM fungi can be particularly important for natural regeneration, as they form symbiotic relationships with pine roots. These fungi enhance nutrient and water uptake, improve soil structure, and help pines to establish and grow in challenging environments. Following a fire, the disruption of ECM fungal networks can severely hinder pine regeneration, as the reduced fungal diversity and abundance limit the availability of essential nutrients to seedlings.

In this study, we aimed to show the impact of forest fires on the composition and diversity of fungal communities in forest soils at the upper forest line. By comparing fungal diversity and abundance in soils collected from burned and unburned *P. heldreichii* sites, we aimed to understand the dynamics of soil–fungal community responses to forest

fires. Additionally, to estimate the fungal communities present on tree roots, which are relevant for pine forest regeneration, we collected rootlets from naturally regenerating pines in unburned forest sites that were lost during the forest fire. This information can be essential for understanding the mechanisms of ecosystem recovery and resilience following fire disturbances. We hypothesized that after a forest fire, the diversity of fungal species in the soil would be drastically reduced, and their recovery would be slow due to harsh environmental conditions at burned high altitude sites. Although relict pine forests support high and specific biodiversity, this diversity can be lost during forest fires, making the recovery of ECM fungi crucial for the successful re-establishment of pine forests afterward.

Our research is positioned within the broader context of high-altitude Mediterranean fire ecology, contributing to the growing body of knowledge on how wildfires shape ecosystem dynamics and biodiversity. As climate change continues to alter fire regimes worldwide, understanding the response of belowground microbial communities to fire is of key importance for predicting and mitigating the long-term impacts of fires on ecosystem health and resilience [15,24]. Furthermore, this study may also contribute to the development of more effective management and restoration strategies for high-altitude Mediterranean forest ecosystems affected by fires. The absent or delayed natural regeneration in *P. heldreichii* forests after fire poses significant challenges for their long-term survival and stability. Without sufficient natural regeneration, these ecosystems may fail to recover fully, leading to a loss in forest cover, reduction in biodiversity, and the potential conversion of forested areas into grasslands or bare rock. Active restoration efforts, such as reforestation, may become necessary to ensure the persistence of *P. heldreichii* in fire-affected regions.

2. Materials and Methods

2.1. Experimental Setup and Study Sites

This study was carried out in the Kuči Mountains in southeastern Montenegro. Samples were taken from five sites situated in a high-mountain karst region with pronounced terrain, representing the typical habitats of *P. heldreichii* forests. *P. heldreichii* grows at altitudes of 1200–2000 m, primarily on soils derived from limestone, in high mountain regions influenced by the Mediterranean climate, both in the Balkans and southern Italy [2–4].

Among the selected sites, three were post-fire, and two were unburned control sites. Climate, soil conditions, and the type of vegetation (*Pinetum heldreichii* s.l.) were similar throughout the area. Forest fires were frequent in this region over the last 20 years (i.e., between 2000 and 2020). The fires at these sites were of high intensity and long duration, i.e., lasting more than two weeks. While the tree crowns were burned, the dead trunks mostly remained standing. The needle litter layer was largely burned and left as soil cover on sites with lower inclination, though the fires may have had varying local effects on the organic soil and litter layer depending on site conditions.

Sampling was performed at the beginning of June 2016. The exact dates (year and month) of fire events were established using forestry records. The three post-fire sites differed in age since the fire (two, four, and six years after the fire) and represented a time series, allowing for the study of spatio-temporal changes in the composition and structure of fungal communities after site disturbance. As no data on the state of fungal diversity before the forest fires was available, two control sites were selected to represent the soil–fungal diversity of native *P. heldreichii* forests. In parallel with soil sampling, rootlets from natural regeneration were collected from control sites. On post-fire sites, natural regeneration had been burned. The distance between the two control sites was about 20 km, with the post-fire sites situated between them. The study sites were at an altitude of 1320–1730 m, and the site inclinations ranged from 5° to 40° (Table 1).

Table 1. Characteristics of the study sites.

Site	Geographical Position	Altitude (m)	Inclination	Soil Type	Site Condition
Sovrh (S)	N42°35′ E19°37′	1720	>30%	Molic leptosol	Unburned
Kučka Korita (KK)	N 42°29′ E19°30′	1350	>5%	Leptosol	Unburned
Hum Orahovski (HO)	N 42°30′ E 19°31′	1800	>40%	Molic leptosol	2 y after fire
Kastrat (K)	N 42°35′ E 19°29′	1500	5–10%	Leptosol	4 y after fire
Treskavac (T)	N 45°35′ E 19°30′	1550	5–10%	Molic leptosol	6 y after fire

2.2. Climatic and Edaphic Conditions

The climate at the study sites was characterized as a humid warm temperate climate type (Cf), which was represented by subtypes Cfws"bx" [25]. The summers are short, dry, and chilly, and the winters are cold and windy. The mean annual air temperature is approximately 0 °C; the mean daily summer maximum is 6 °C; and the mean daily winter minimum is −2 °C. The absolute minimum temperature is in winter at approximately −30 °C, and the maximum in summer is approximately 30 °C. The mean annual precipitation is approximately 2200–2600 mm. The rainfall reaches its maximum in late autumn and early winter, while the minimum is during the summer months, which are often followed by 40–70 day-long periods of drought. The mean summer precipitation is approximately 220–260 mm, making up approximately 10% of the total annual precipitation [25].

The bedrock at the study sites is solid chalk limestone, which contains a small proportion of insoluble residues. In general, the soils are poorly developed, very water-porous, skeletal leptosols (rendzina), having only an A horizon, characterized by the accumulation of humus (A-R profile). According to the classification of texture, the soil is a sandy loam with a powdery structure. The soil is rich in humus content (10–25%), poor in calcium–carbonate, and has a weak acid reaction (pH approximately 6) [26,27].

The vegetation at the unburned *P. heldreichii* forests usually included *Juniperus sibirica* Burgsd., *Festuca heterofilla* Lam., *Luzula sylvatica* Gaudich., *Veronica officinalis* L., *Aremonia agrymonioides* (L.) DC., *Stachys scardica* (Griseb.) Hayek, *Helianthemum canum* (L.) Hornem, *Dianthus petraeus* Waldst. & Kit., *Senecio visianianus* Viss., *Gentiana amblyphylla* Borbas, and *Cerastium grandiflorum* Waldst. & Kit. [28].

The unburned control sites included two *P. heldreichii* stands in Kučka Korita and Sovrh. The Kučka Korita (KK) site was represented by trees of different ages, with moderate growth conditions for *P. heldreichii* due to relatively low site inclination, moderate elevation, and the presence of a leptosol. The Sovrh (S) site was characterized by middle-aged and young *P. heldreichii* trees (a developing forest stand) growing under harsh environmental conditions due to high site inclination, high elevation, and the early development of soil–molic leptosol [27].

The post-fire sites included Hum Orahovski (HO), which was two years post-fire. HO is a prominent mountain peak in the southeastern part of the study area, in close proximity to the KK control site. Large old-growth forest stands were present there but burned in consecutive years. The sampling site was situated on a steep mountain slope (ca. 40% inclination) with a southern exposure. The vegetation at the HO site consisted of dense grasses, including *Luzula sylvatica*, *Festuca heterophylla*, and *Epilobium angustifolium* L.

The Kastrat (K) site, four years post-fire, and the Treskavac (T) site, six years post-fire, were located approximately 1.5 km apart. In this area, forest stands covering more than 300 ha burned in consecutive fires over less than a decade. The K site was situated on a mountain plateau, and the sampling site was located on slopes of slight inclination. The vegetation at the K site included species such as *Verbascum*, *Senecio*, *Festuca*, and *Hypericum*.

The T site was a prominent mountain peak, but the sampling location was on a moderately inclined slope, on a flattened shelf. At the T site (six years after the fire), vegetation was dominated by *Epilobium angustifolium*, with some pioneer tree species such as *Salix caprea* L. appearing.

2.3. Sampling of Soil

The sampling sites ranged from 100×150 m to 100×300 m, depending on the terrain topography. Post-fire sampling was based on known fire distribution, ensuring the sites were distinct from the neighboring areas potentially affected by other fires. At each site, five (5) individual soil samples were taken. In the control sites, soil samples were collected in the vicinity of mature living *P. heldreichii* trees and on post-fire sites; sampling of the soil was performed in the vicinity of trees that had died due to forest fires. The position of trees was chosen based on the topography of the terrain (the presence of soil cover). The trees were situated at a minimal distance of 25 m between each other. The soil samples were taken using a spade, down to 20 cm depth, over the entire depth of the soil profile to the bedrock. The samples were individually packed into plastic bags, transported to the laboratory, and kept at 4 °C for no more than one week before being processed. In the laboratory, individual samples of the fine soil fraction, sieved through a 2 mm × 2 mm mesh, were placed in plastic bags and stored at −20 °C until they were used for DNA extraction.

2.4. Sampling of Rootlets

Rootlets were randomly collected at the unburned control sites, under 3- to 8-year-old *P. heldreichii* trees, growing in the vicinity of mature living *P. heldreichii* trees which were at least 25 m apart, in parallel with soil sampling. The rootlets were taken using a spade and included lateral roots with attached fine roots (up to 20 cm depth). The samples were individually packed into plastic bags, transported to the laboratory, and kept at 4 °C for a maximum of one week before processing. For each sample, rootlets were separated from the soil, soaked in cold water for 12 h, and gently washed with tap water to remove any remaining soil. The rootlets were then separated from the lateral roots, placed in 1.5 mL centrifuge tubes, and stored at −20 °C until DNA extraction. Taken together, the sampling resulted in 25 soil samples from 5 sites (3 post fire and 2 control unburned sites) and in 10 root samples from 2 unburned sites. The sampling approach is shown in a Supplementary Figure S1.

2.5. Soil Analyses

At least 100 g of soil from each of the 25 samples was dried at room temperature (ca. 21 °C) for 24 h, before analysis. The soil chemical properties were determined by the methods described in the Practicum for Agrochemistry [29]. The soil pH (active and potential) was determined using a combined glass–electrode method in a water as well as 1 M KCl of soil suspension 1:2.5 (*w/v*). The content of the total carbonates was determined by the volumetric method; organic C via wet oxidation with 0.02 M $KMnO_4$; total nitrogen via the Kjeldahl method; the available phosphorus and potassium (extraction with an ammonium–acetate–lactate solution at pH = 3.7) according to the Egner–Riehm–Domingo method using spectrophotometry and flame photometry, respectively. The exchangeable Ca and Mg (extraction with 1 M CH_3COONH_4) and available Fe, Mn, Zn, and Cu (extraction with 0.005 M DTPA) were determined using flame atomic absorption spectrometry (AA-6800, Shimadzu, Tokyo, Japan). The particle size distribution was analyzed using the sieve and the pipette method [30].

2.6. Molecular Analysis

DNA extractions were performed from 25 individual soil samples and 10 samples containing rootlets, which were previously freeze-dried at −60 °C for 48 h (Alpha 1-4 LD, Martin Christ, Germany). The samples were placed in separate 2 mL tubes with a screw cap and homogenized in a Fastprep machine (Precellys, Montigny-le-Bretonneux,

France). About 200 mg of freeze-dried soil per sample was used for DNA extraction, which was performed using the NucleoSpin soil kit (Marcherey-Nagel, Düren, Germany). For the isolation of genomic DNA from the rootlets, 200 mg of material was taken from each respective sample and extraction was completed using the CTAB protocol [31]. The DNA isolated from the rootlets was directly used in PCR reactions, while the DNA isolated from the soil was additionally purified using the JetQuick DNA purification kit (Genomed GmbH, Leinfelden, Germany). The concentration of DNA in each sample was determined using a NanoDrop™ One spectrophotometer (Thermo Scientific, Rochester, NY, USA) and adjusted to 10 ng/μL. Amplification via the PCR of the ITS2 rDNA region was performed using a barcoded fungal-specific primer gITS7 (5′-GTGARTCATCGARTCTTTG-3′) [32] and a barcoded universal primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [33]. All the samples from the same site were amplified using primers with the same barcode, resulting in 5 different barcodes for soil samples and 2 for rootlet samples. 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 extending at 72 °C for 60 s. The thermal cycling was ended by a final extension step at 72 °C for 7 min [34]. The PCR products were analyzed using gel electrophoresis on 1% agarose gels stained with Nancy-520 (Sigma-Aldrich, Stockholm, Sweden). The PCR products were purified using the sodium acetate protocol [35]. The purified PCR products were quantified using a Qubit fluorometer 4.0 (Thermo Fisher Scientific, Waltham, MA, USA), and an equimolar mix of all the PCR products was used as part of a larger sample for high-throughput sequencing using a Pacific Biosciences RS II platform (Menlo Park, CA, USA) and one SMRT cell at the SciLifeLab (Uppsala, Sweden).

2.7. Bioinformatics

The principles of bioinformatics followed Lynikienė et al. [36]. The sequences obtained were subjected to quality control and clustering in the SCATA NGS sequencing pipeline available at https://scata.mykopat.slu.se/, accessed on 1 June 2024. The initial procedure started with the quality filtering of the sequences that included the removal of sequences shorter than 200 bps, sequences with low read quality, primer dimers, and homopolymers, which were collapsed to 3 bps before clustering. Only the sequences containing a barcode and primer were retained. Then, the primer and sample barcodes were removed from the sequence, but information on the sample and sequence association was stored as meta-data. A single-linkage clustering based on 98.5% similarity was used to cluster sequences into different taxa. For each cluster, the sequence of the most common genotype was used for taxonomic identification. For clusters containing only two sequences, a consensus sequence was produced. The taxa were taxonomically identified using the GenBank database and the BLASTn algorithm [37]. The reliability of each reference sequence was manually checked for each taxon. If available, the sequence of a type species was used as a reference. The following criteria were used for identification: sequence coverage >80%; 94–97% similarity to the genus level, and $\geq$ 98% similarity to the species level. Sequences deviating from these criteria were identified only to a high taxonomic rank and were given unique names [34,36]. The representative sequences of fungal non-singletons are available from GenBank under accession numbers PQ364141-PQ364830.

2.8. Statistical Analyses

The soil data were processed using IBM SPSS version 23 (Armonk, New York, NY, USA). Significant differences between the means were determined using an independent *t*-test with a 95% confidence interval. A cluster analysis (squared Euclidean distance and Ward's method) was applied to the standardized data (Z-scores by soil variable). The differences in richness of fungal OTUs in different study sites were compared by nonparametric chi-square testing [38]. As each of the datasets was subjected to multiple comparisons, confidence limits for the *p*-values of the chi-square test were reduced to the

corresponding number of times as required by the Bonferroni correction. The Shannon diversity index, the qualitative Sørensen similarity index, and the detrended correspondence analysis (DCA) in Canoco 5 were used to characterize the diversity and composition of the fungal communities [39–41]. Hierarchical clustering was conducted using Ward's method, which minimized the total within-cluster variance [42]. The Euclidean distance metric was employed to measure the dissimilarity between the OTU profiles. The results were visualized using a cluster map generated with the Seaborn package in Python [43], with a heatmap displaying the abundance of each OTU and dendrograms illustrating the hierarchical relationships among the OTUs and samples.

3. Results

3.1. Soil Properties

Soil analyses indicated that *Pinus heldreichii* at unburned sites on solid chalk limestone were growing on leptosols with a neutral to slightly acidic reaction, rich in organic matter and nitrogen (Table 2). These soils had a very low content of available phosphorus (P) but an optimal level of available potassium (K). No previous data exists on the content of secondary elements and micronutrients in the leptosols of *P. heldreichii* high-altitude forests. The exchangeable calcium (Ca) and magnesium (Mg) were at optimal to high levels, while the content of the available iron (Fe) was considered very high, and the content of the zinc (Zn), manganese (Mn), and copper (Cu) levels were optimal. The values of electrolytic conductivity (EC) were within the range typically found in moderately fertile soils. Based on these parameters, soils from fire-affected sites could be similarly categorized as those from unburned sites.

Table 2. Soil chemical characteristics and texture (minimum, maximum, mean, and standard deviation) of *Pinus heldreichii* stands at unburned (Sovrh—S and Kučka Korita—KK) and post fire (Hum Orahovski—HO; Kastrat—K; and Treskavac—T) forest sites.

Soil Parameter	Min	Max	Unburned	Post Fire
pH (H$_2$O)	6.6 (KK)	7.7 (HO)	7.1 ± 0.7	7.2 ± 0.4
pH (KCl)	5.8 (KK)	7.1 (S)	6.5 ± 0.9	6.4 ± 0.5
CaCO$_3$ (%)	0 (T, K, KK, S)	2.4 (HO)	0.0 ± 0.0	0.8 ± 1.4
Organic C (%)	9.1 (S)	13.9 (T)	10.1 ± 1.4	12.7 ± 1.0
Total N (%)	0.52 (S)	1.01 (K)	0.65 ± 0.18	0.85 ± 0.18
P$_2$O$_5$ (mg/100 g) *	0.3 (S)	6.2 (HO)	0.5 ± 0.3	5.0 ± 1.6
K$_2$O (mg/100 g)	13.6 (K)	21.4 (T)	15.6 ± 1.0	17.3 ± 3.9
Ca (mg/100 g)	606 (KK)	1321 (S)	964 ± 506	914 ± 83
Mg (mg/100 g)	34.4 (T)	156 (HO)	91.9 ± 74.9	76.8 ± 68.7
Fe (mg/kg)	46.2 (S)	77.1 (KK)	61.7 ± 21.8	59.6 ± 9.5
Mn (mg/kg)	5.6 (T)	13.4 (KK)	10.4 ± 4.3	7.2 ± 2.5
Zn (mg/kg)	0.65 (T)	3.81 (HO)	1.7 ± 0.5	2.3 ± 1.6
Cu (mg/kg)	0.48 (T)	1.52 (KK)	1.1 ± 0.6	0.8 ± 0.3
EC (μS/cm)	96.5 (KK)	229 (K)	140 ± 61.7	182 ± 60
Coarse sand (%)	1.60 (KK)	6.84 (T)	3.04 ± 2.04	4.86 ± 1.77
Fine sand (%)	45.98 (S)	55.99 (T)	50.42 ± 6.27	51.50 ± 4.37
Silt (%)	29.30 (T)	40.80 (HO)	35.95 ± 2.86	35.72 ± 5.87
Clay (%)	7.63 (HO)	11.57 (S)	10.59 ± 1.38	7.92 ± 0.31
Total sand (%)	50.46 (S)	62.82 (T)	53.46 ± 4.24	56.36 ± 5.81
Total clay (%)	37.18 (T)	49.54 (S)	46.55 ± 4.24	43.64 ± 5.81

* The means are significantly different at $p < 0.05$.

To assess the effect of fire on soil properties, an independent t-test was applied. Although it was anticipated that organic matter would change significantly after the fire, no significant difference was found (Table 2). The content of available phosphorus was significantly higher in soil samples from fire-affected sites compared to unburned control sites. The soil texture across the different study sites was consistent, characterized as sandy

loam with a powdery structure. The soil showed slight differentiation with depth, was highly porous to water, and maintained similar physical properties throughout the sites.

The similarity between the physical and chemical soil properties among localities is given as a hierarchical clustering dendrogram (Figure 2). Two cluster groups were formed based on all the analyzed soil parameters: (1) Kastrat and Hum Orahovski combined with Sovrh; and (2) Treskavac and Kučka korita.
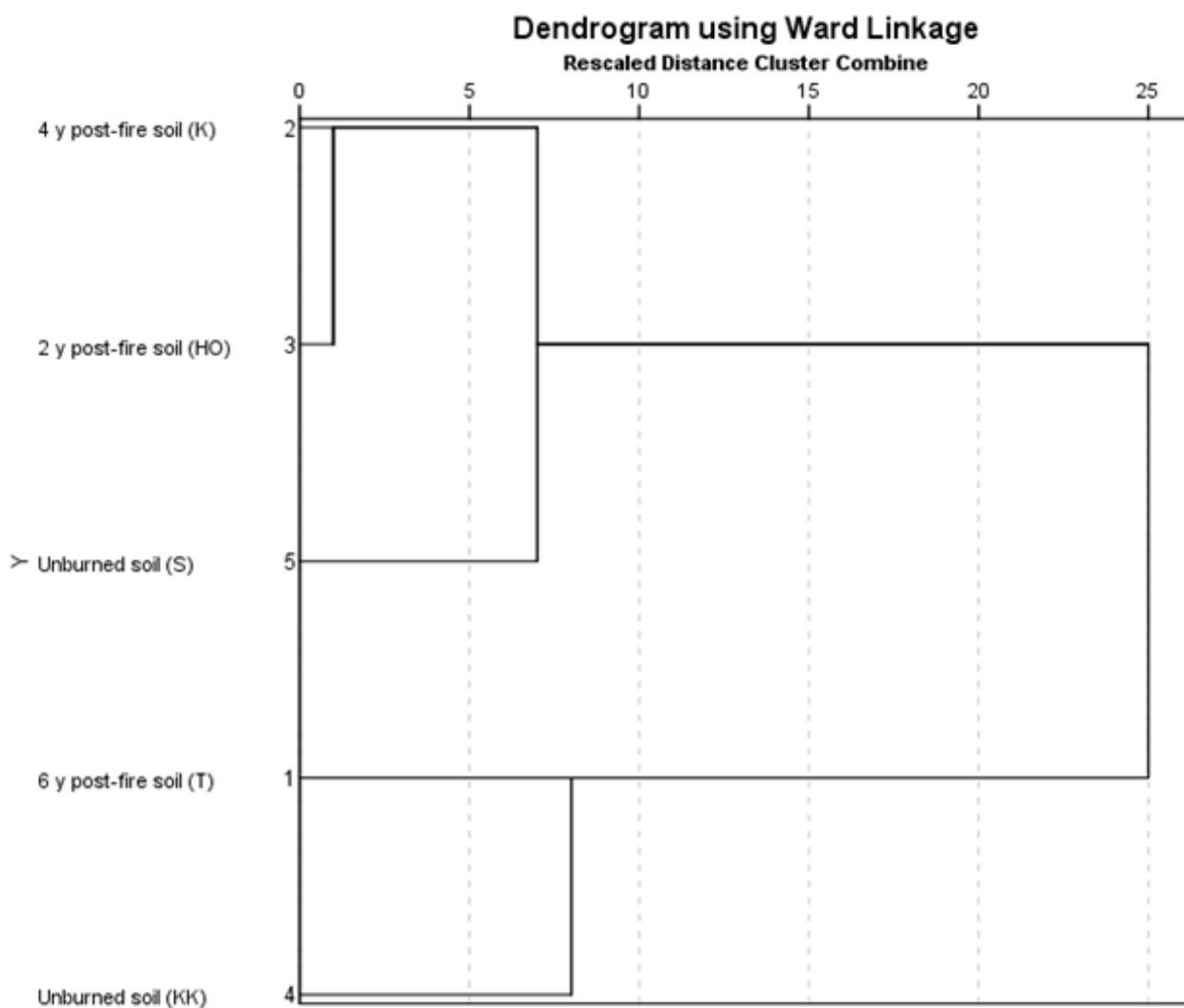

Figure 2. Hierarchical clustering dendrogram on physical and chemical soil properties in post-fire and unburned forest sites. Site names are shown in parentheses: T—Treskavac; K—Kastrat; HO—Hum Orahovski; KK—Kučka Korita; and S—Sovrh.

3.2. Fungal Communities

High-throughput sequencing and quality filtering showed the presence of 10,006 high-quality reads. The clustering of these reads at 98.5% similarity revealed the presence of 747 OTUs. A BLASTn analysis showed that 690 OTUs (represented by 9258 reads) were fungal and were retained (Table 2), while 57 non-fungal OTUs were excluded from further analyses. The absolute OTU richness was highest in the unburned soil, then in the post-fire soil, and the least was in rootlets (Table 3, Table S1).

The comparison among the same type of samples, i.e., either post-fire forest soil, unburned forest soil, or the rootlets of *P. heldreichii*, showed that the number of OTUs among these was similar ($p > 0.05$). Consequently, the time past after the forest fire (2, 4 or 6 years) did not have a significant effect on the richness of the fungal OTUs (Table 3). By

contract, the number of OTUs among the different types of samples differed significantly ($p < 0.05$) (Table 3).

Table 3. Generated number of high-quality fungal sequences and detected diversity of fungal OTUs. Number of OTUs from different sites and substrates followed by the same letter do not differ significantly at $p > 0.05$. Abbreviated names of each site are in parentheses.

Site	Site Condition (Year of Forest Fire)	Substrate	No. of Reads	No. of OTUs	Shannon Diversity Index
Treskavac (T)	6 years post-fire	Soil	507	118 a	4.06
Kastrat (K)	4 years post-fire	Soil	583	155 a	4.46
Hum Orahovski (HO)	2 years post-fire	Soil	669	165 a	4.39
Kučka Korita (KK)	Unburned	Soil	2665	340 b	4.78
Sovrh (S)	Unburned	Soil	2456	344 b	4.72
Kučka Korita (KK)	Unburned	Rootlets	722	70 c	2.98
Sovrh (S)	Unburned	Rootlets	1656	120 c	3.48
Total			9258	690	

The Venn diagram shows the unique and shared number of OTUs across the three types of samples. In a combined dataset, the number of unique OTUs was largest in the unburned forest soils, then in the post-fire forest soils, and the least was in rootlets of *P. heldreichii* (Figure 3). The number of shared OTUs was highest between the unburned soil and the post-fire soil, then between unburned soil and rootlets, and the lowest between post-fire soil and rootlets (Figure 3). The Shannon diversity index of the fungal communities ranged 4.06–4.46 in the post-fire forest soils, 4.72–4.78 in the unburned forest soils, and 2.98–3.48 in the rootlets (Table 3). The Sørensen qualitative similarity index was moderate between the unburned soil and the post-fire soil (0.44), moderate between the unburned soil and the rootlets (0.33), and low between the post-fire soil and the rootlets (0.23).
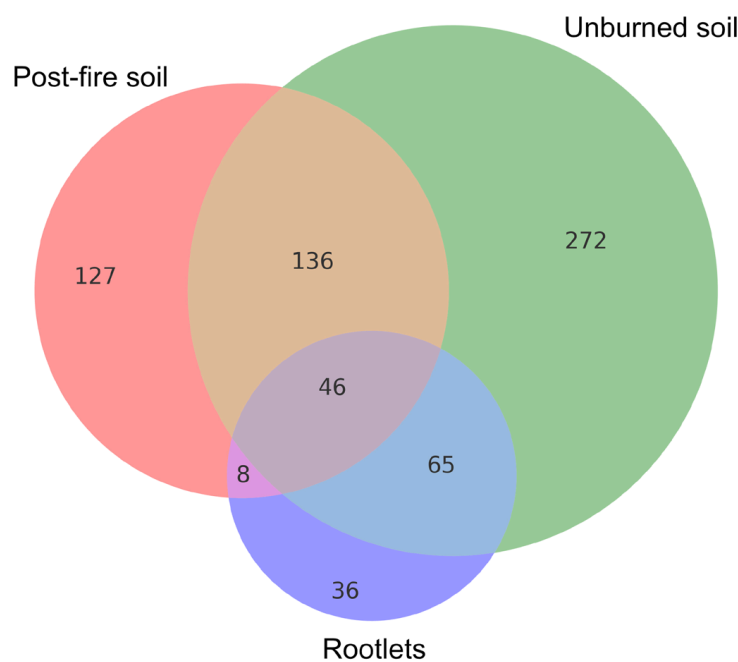

Figure 3. Venn diagram showing unique and shared OTUs among different groups of samples. Within each group, data from different sites is combined. The size of each circle represents the number of unique OTUs. Roolets (blue), Unburned soil (green), post-fire soil (red).

The overall fungal community was dominated by Ascomycota (61.2%), followed by Basidiomycota (29.7%), Mucoromycota (4.3%), Chytridiomycota (3.4%), and Glomeromycota (1.4%). The most abundant class was *Agaricomycetes*, comprising 27.4% of the total fungal community (Figure 4). *Sordariomycetes* and *Leotiomcetes* each followed with a relative abundance of 10.5%. Other more abundant classes included *Dothideomycetes* (9.8%), *Eurotiomycetes* (7.5%), and *Archaeorhizomycetes* (7.3%) (Figure 4).
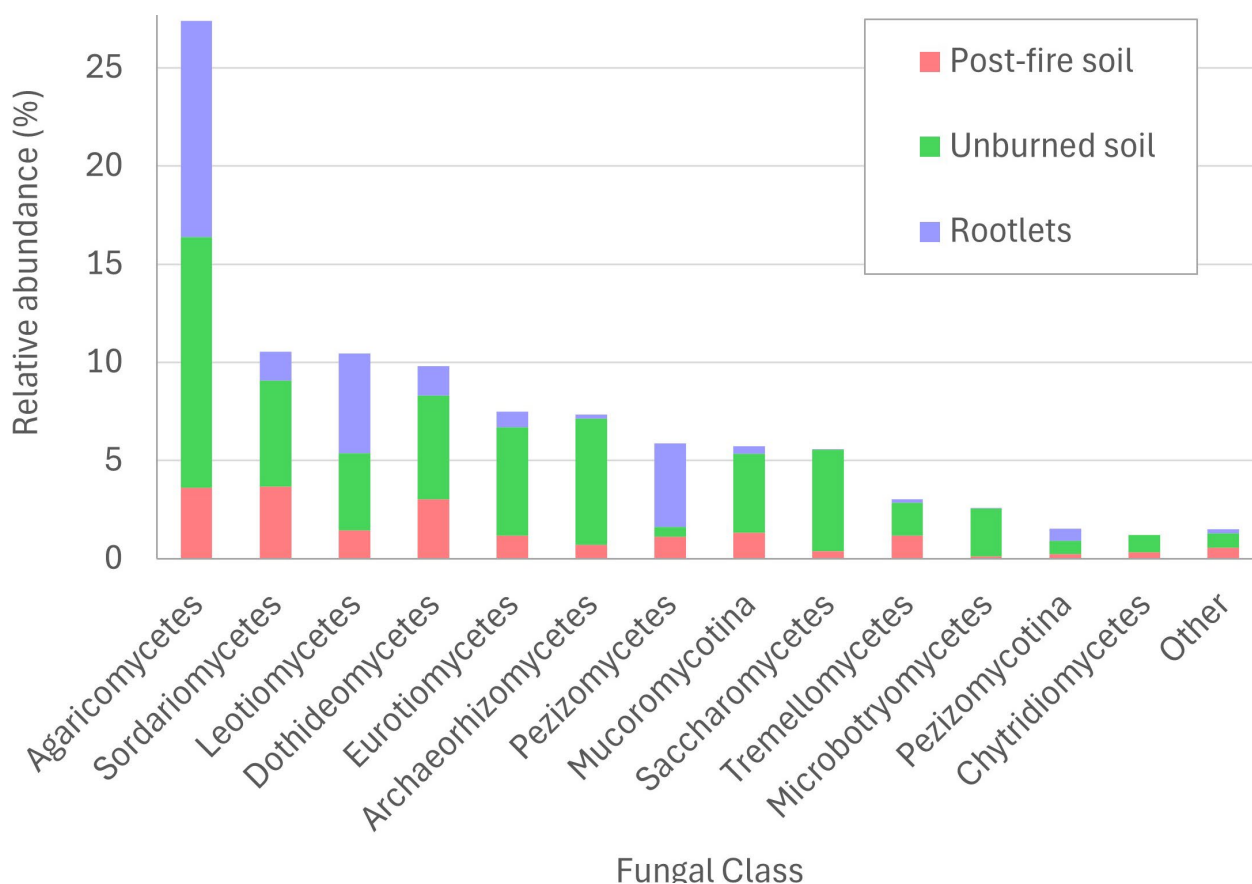

Figure 4. Bar chart showing the relative abundance of different fungal classes. Classes that accounted for less than 1% of the total fungal community were combined into an Other category.

The most common fungi of the study (different sites and substrates combined) were *Tomentella* sp. (4.1%), *Geminibasidium* sp., (3.1%) and *Wilcoxina rehmii* Chin S. Yang & Korf (3.1%) (Table 4). The most common fungi in the post-fire forest soils (different sites combined) were *Hygrocybe conica* (Schaeff.) P. Kumm. (5.6%), *Solicoccozyma aeria* (Saito) Yurkov (5.5%), and *Pseudogymnoascus roseus* Raillo (3.5%), and in the unburned forest soils *Geminibasidium* sp. (5.1%), unidentified sp. strain 3598_36 (4.3%), and unidentified sp. strain 3598_11 (4.0%) were the most common; in the rootlets, *Wilcoxina rehmii* (11.4%), *Tomentella* sp. (10.6%), and unidentified sp. strain 3598_43 (8.1%) were the most common fungi (Table 4). The results showed that several dominant fungal taxa, including *Tomentella* sp., *Amphinema* sp., unidentified sp. strain 3598_43, and *Agaricomycetes* sp., which were abundant in the unburned forest soils and/or rootlets, were absent in the post-fire soils. In contrast, the relative abundance of *H. conica* and *S. aeria* significantly increased in response to fire disturbance (Table 4). Other dominant fungal taxa persisted across all the substrates (unburned soils, rootlets, and post-fire soils) though their relative abundance was either comparable or reduced in the post-fire soils (Table 4).

Table 4. Relative abundance (%) of the 20 most common fungal OTUs detected at post-fire soils (PFS), unburned soils (US), and rootlets (R). Sites are shown in parentheses: T—Treskavac; K—Kastrat; HO—Hum Orahovski; KK—Kučka Korita; and S—Sovrh.

Strain	Taxon	Phylum *	Genbank No.	Reference No.	Identities	6-y PFS (T)	4-y PFS (K)	2-y PFS (HO)	US (KK)	US (S)	R (KK)	R (S)	All
3598_6	*Tomentella* sp.	B	PQ364141	LR873297	314/314(100%)	-	-	-	4.7	0.0	32.4	1.1	4.1
3598_23	*Geminibasidium* sp.	B	PQ364142	LS447497	319/321(99%)	0.4	-	3.1	3.6	6.7	-	-	3.1
3598_22	*Wilcoxina rehmii*	A	PQ364143	MF926519	254/254(100%)	1.0	-	-	-	0.2	-	16.4	3.1
3598_36	Unidentified sp.	A	PQ364144	LR876984	215/215(100%)	0.4	1.5	-	0.3	8.8	-	0.5	2.6
3598_33	*Penicillium* sp.	A	PQ364145	MT557492	257/257(100%)	1.8	3.3	1.6	2.8	4.6	0.1	-	2.5
3598_11	Unidentified sp.	B	PQ364146	KC818337	303/303(100%)	0.8	-	0.1	5.9	2.0	-	-	2.3
3598_14	*Amphinema* sp.	B	PQ364147	JN943914	263/280(94%)	-	-	-	0.6	0.8	0.7	10.1	2.3
3598_25	*Saccharomycetales* sp.	A	PQ364148	MK627007	301/301(100%)	0.4	-	0.1	5.8	1.8	-	-	2.2
3598_43	Unidentified sp.	B	PQ364149	KY322596	250/250(100%)	-	-	-	-	0.2	-	11.7	2.1
3598_9	*Solicoccozyma aeria*	B	PQ364150	OM743906	325/325(100%)	4.5	2.9	8.4	1.0	2.5	-	0.2	2.0
3598_26	Unidenfied sp.	A	PQ364151	MG207370	241/241(100%)	4.3	0.2	0.4	3.4	2.0	0.1	0.1	1.8
3598_21	*Hygrocybe conica*	B	PQ364152	MN992415	333/335(99%)	8.5	-	8.2	-	2.0	-	-	1.6
3598_29	*Leptodophora echinata*	A	PQ364153	NR_170730	241/242(99%)	1.0	1.2	0.7	0.2	1.3	8.0	1.8	1.5
3598_47	*Cadophora fastigiata*	A	PQ364154	MF182432	242/242(100%)	0.6	-	0.3	0.2	0.1	2.4	6.2	1.4
3598_77	*Tetracladium maxilliforme*	A	PQ364155	KU519119	238/239(99%)	1.0	0.5	1.8	0.3	0.7	5.3	2.4	1.3
3598_86	*Archaeorhizomyces* sp.	A	PQ364156	OW846612	210/214(98%)	-	1.5	-	0.5	3.8	-	0.1	1.3
3598_74	*Mortierella clonocystis*	M	PQ364157	LC515184	333/333(100%)	2.0	-	0.6	2.4	0.4	2.5	0.1	1.2
3598_54	*Sagenomella verticillata*	A	PQ364158	MH860215	263/263(100%)	3.7	0.5	-	0.6	2.5	-	-	1.1
3598_40	*Agaricomycetes* sp.	B	PQ364159	KT182914	284/284(100%)	-	-	-	0.7	-	4.8	2.6	1.0
3598_88	*Pseudeurotium hygrophilum*	A	PQ364160	MF375774	236/241(98%)	-	3.6	1.9	0.6	0.8	0.6	1.0	1.0
	Total of 20 taxa					30.4	15.3	27.5	28.9	41.0	24.5	53.2	35.3

* A = Ascomycota; B = Basidiomycota; M = Mucoromycota.

A detrended correspondence analysis showed that the fungal community composition differed among the different types of samples (post-fire forest soils, unburned forest soils, and rootlets) (Figure 5). Furthermore, fungal communities in three post-fire sites were positioned closely on the *x*-axis but separated from each other on the *y*-axis (Figure 5). The samples of both the unburned forest soils and the rootlets were each grouped together (Figure 5).
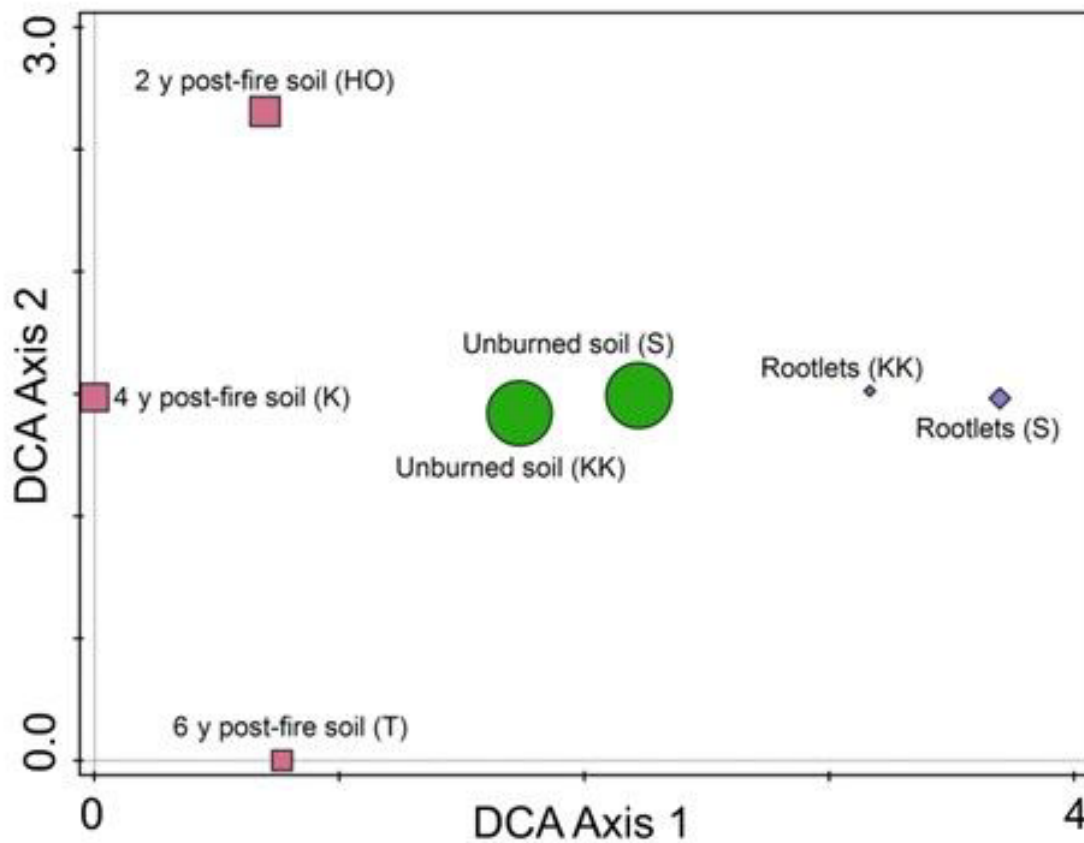

Figure 5. Ordination diagram based on detrended correspondence analysis of fungal communities from post-fire forest soil (square), unburned forest soil (circle), and rootlets of *Pinus heldreichii* from unburned forest stands (diamonds). Size of each plot shows relative richness of fungal OTUs. Site names are shown in parentheses: T—Treskavac; K—Kastrat; HO—Hum Orahovski; KK—Kučka Korita; and S—Sovrh. In ordination, 21.6% of variation was explained on *x*-axis, and 9.7% on *y*-axis.

A hierarchical clustering analysis was performed on the 10 most common fungal OTUs (Figure 6). The cluster map revealed distinct groups of OTUs based on their co-occurrence and abundance patterns across the samples. For example, certain OTUs such as *Wilcoxina rehmii*, *Amphinema* sp., and unidentified sp. formed a tight cluster, showing that these OTUs share similar abundance patterns. The samples were also clustered into distinct groups, reflecting the similarity in their fungal community composition. The unburned soil samples and fine root samples from each S and KK site clustered together (Figure 6), showing that each of these sites supported similar fungal communities. The post-fire soil samples from different sites also showed clustering, showing that fire disturbance leads to a different but consistent fungal community structure. The heatmap showed the abundance of each OTU, highlighting key OTUs that were highly abundant across the multiple samples. For example, *Geminibasidium* sp. and *Penicillium* sp. had high abundances in several samples, showing their dominance or adaptability to a range of soil conditions. By contrast, certain OTUs such as *Wilcoxina rehmii* and *Amphinema* sp., showed high abundance in specific samples (Figure 6).
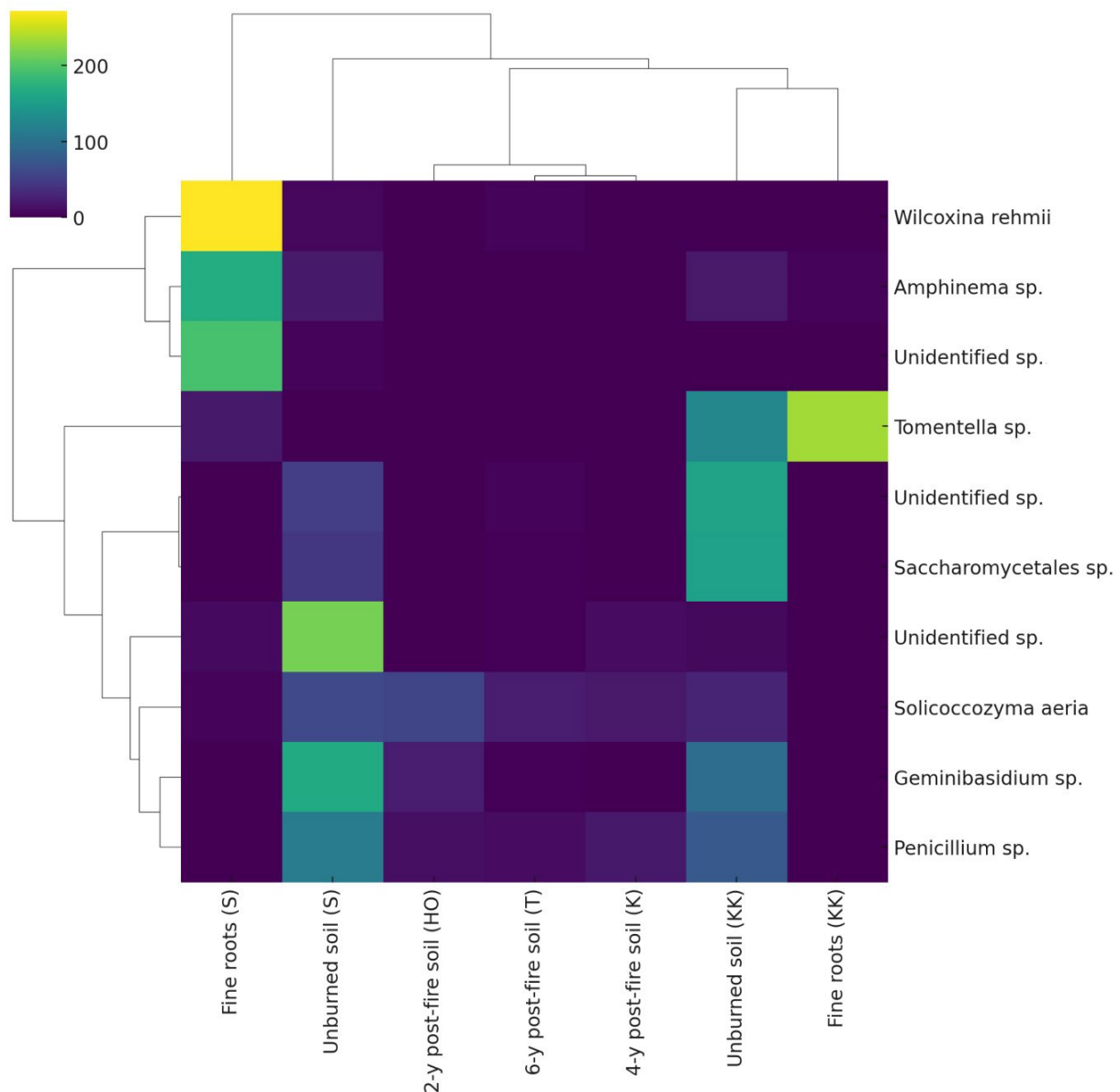
Figure 6. A cluster map of the 10 most common fungal OTUs across various soil and root samples. The dendrograms illustrate the hierarchical relationships among the OTUs and the samples based on their abundance profiles.

4. Discussion

Forest fires can have profound effects on soil biodiversity and ecosystem services. Understanding the dynamics of soil–fungal communities in response to fires is essential for predicting their long-term impacts on forest ecosystems, especially in high-altitude environments. Such knowledge is vital for developing effective forest management and restoration strategies [15].

However, current information about soil–fungal communities in response to fires is limited and based on a few studies, primarily focused on forest ecosystems in Mediterranean, boreal, and temperate climates, with other land uses and climatic conditions often underrepresented [18].

This study provided new insights into the impact of forest fires on soil–fungal communities in relic high-altitude *P. heldreichii* forests. By integrating field sampling with molecular techniques, such as DNA metabarcoding, we revealed how fire disturbances influence fungal diversity and community composition. Additionally, the results expanded

our understanding of these forests' soils, particularly in relation to their fungal communities, which had previously not been studied, along with their key chemical and physical soil properties.

Our results showed that fungal diversity was significantly higher in the unburned forest soils compared to the post-fire soils, aligning with our hypothesis that fire reduces fungal diversity. This observation is consistent with other studies in fire-affected ecosystems, such as those by Pérez-Valera et al. [19] and Bastias et al. [20], who reported that fire led to a reduction in fungal richness and a shift in community composition. These findings suggest that fire creates a more selective environment, where only certain fungal species, particularly those adapted to post-fire conditions, can persist.

The chemical analysis of soil samples from unburned and post-fire sites revealed significant differences in available phosphorus levels, with post-fire soils showing elevated phosphorus content. This can be explained by the conversion of organic phosphorus into soluble forms during the fire, which increases the available fraction in the soil. Studies, such as those by Deng et al. [17] and Coan et al. [16], have reported similar findings, noting that fires lead to the mineralization of organic phosphorus and its accumulation in the topsoil layer, where it remains due to its low mobility. This increase in phosphorus availability can benefit pioneer tree species, although the absence of ECM fungi in post-fire soils could hinder nutrient uptake by regenerating trees. Organic matter was expected to change significantly after a fire, but no notable differences were found between the unburned and the post-fire soils. This suggests that the variability in soil organic matter content at the sites was already high before the fire. The soil texture was consistent across the study sites, characterized as sandy loam with high water porosity.

Interestingly, the time elapsed since the fire (2, 4, or 6 years) did not significantly affect the fungal richness, indicating that fungal community recovery might be a slow process, not easily observable within a few years post-disturbance [23]. This suggests that the recovery of fungal communities, especially in high-altitude environments, may take longer than expected. This study also demonstrated that fungal community composition differed markedly between post-fire and unburned sites. Post-fire soils were characterized by the dominance of different fungal OTUs compared to unburned soils. This indicates that fire creates a selective environment that favors specific fungal taxa, likely those more adapted to nutrient-poor and harsh conditions following a fire [44]. For example, the resilience of certain ECM and saprotrophic fungi was evident in post-fire environments, highlighting their potential roles in ecosystem recovery.

ECM fungi play a key role in the natural regeneration of pine forests, particularly in harsh environments like those found at high altitudes. In this study, we found that ECM fungi such as *Tomentella*, *Wilcoxina*, and *Amphinema* were key components of the fungal communities associated with *P. heldreichii*. *Tomentella* species, widely distributed in coniferous forests worldwide, were among the dominant ECM fungi in our study. Previous research has shown that *Tomentella* can dominate root mycorrhizal communities in mature forests and post-disturbance environments, including post-fire and clear-cut sites [12,45,46]. Their ability to survive disturbances through spore deposits in the soil makes them resilient to environmental changes [47]. Our results support previous studies on the importance of *Tomentella* for pine forests, as this genus was dominant in both rootlets and soil in unburned and post-fire sites. Similarly, *Wilcoxina* and *Amphinema*, both known for their resilience in disturbed environments, were also significant components of the ECM fungal community. *Wilcoxina rehmii*, a fire-adapted species, played a key role in the early stages of post-fire succession by initiating new mycorrhizal associations with regenerating pine seedlings [13]. Although *Amphinema* was commonly found in post-disturbance coniferous forests [48], it was notably absent from our post-fire sites. This may be due to the fire, which likely eliminated potential ECM hosts, such as tree roots or surviving woody vegetation, which are critical for the survival of ECM fungi in a fire [12].

In addition to ECM fungi, root endophytes have been shown to increase host tolerance to abiotic factors such as drought and heat stress, thereby increasing plant health [49],

and potentially shaping plant community diversity and distribution [50]. Species from *Cadophora* have been shown to promote pine seedlings growth [51,52], and similar effects could be assumed for the recently described genus *Leptodophora,* which was proposed to accommodate selected root-associated members of the genus *Cadophora* [53]. *Tetracladium* spp. may also exist as root endophytes in terrestrial environments [49]. Importantly, all three taxa appear in both the rootlets and soil, and remain consistent even after fire.

Saprotrophic fungi, which dominated high altitude *P. heldreichii* forests, such as *Sacharomycetales, Geminibasidium, Penicillium, Solicoccozyma aerea, Mortierella,* and *Pseudeurotium hygrophilum* (Sogonov, W. Gams, Summerb. & Schroers) Minnis & D.L. Lindner, played an essential role in the post-fire soil environment, thereby representing the core fungal community in the high altitude pine forest soils.

Yeasts (*Saccharomycetales*) are cosmopolitan and widespread, but with mainly saprotrophic growth. They are often found in specialized habitats, which can be nutrient poor, but rich in organic carbon [54,55]. Fungi from the genus *Penicillium* are common in the rhizosphere soil in various terrestrial environments [56], acting not only as saprotrophs, but also as producers of solubilized phosphorus, siderophores, and phytohormones [57,58], which are important for plant health. Similarly, filamentous fungi *Mortierella* (Mucoromycota) are commonly found in environments such as the bulk soil, the rhizosphere, and plants tissues. Species of *Mortierella* are capable of surviving under very unfavorable environmental conditions, while utilizing carbon sources contained in polymers such as cellulose, hemicellulose, and chitin. In extremely hostile environments, *Morierellas* are responsible for improving access to the bioavailable forms of P (phosphorous) and Fe (iron) in soils, for the synthesis of phytohormones, and for the protection of herbaceous plants from pathogens [59]. Although usually reported as humus-associated fungi, there is a certain specification of species within the genera *Penicillium* and *Mortriella* in relation to the type of humus and soil in which they occur [60]. *Solicoccosima aerea* belongs to the basidiomicete yeasts detected from temperate [61] and Mediterranean [62] soils, which was also recently shown as a promoter of plant growth [63]. Although *S. aeria* was abundantly represented in the undisturbed *P. heldreichii* forest soil, it was sustained as one of the most common fungi in post-fire forest soils together with a saprotrophic fungus *Hygrocybe conica*. Species of the genus *Hygrocybe* are known to be common in montane grasslands in Montenegro; they are often associated with the habitats of *P. heldreichii* [27,64,65], as well as with the high-altitude forests of *Pinus peuce* Griseb. [66]. Further, fungi from the genus *Geminibasidium* were showing particular resilience, owing to their presence in both unburned and post-fire soils. These fungi are known for their roles in organic matter decomposition and nutrient cycling, which are vital processes for soil recovery after a fire [67]. *Geminibasidium*, a heat-resistant and xerotolerant basidiomycete, has been reported as a dominant post-fire fungal species in the coniferous forests of North America [13]. The presence of the species, such as *H. conic*a, *S. aeria* and *Geminibasidium*, in both unburned and post-fire soils, suggests that these may be important bioindicators of the soil recovery stages in fire-affected ecosystems [68]. Being present in undisturbed soils, but also in post-fire soils, *Pseudeurotium* species was well-represented. *Pseudeurotium hygrophila* was relatively common within the north temperate montane habitat and is thought to be a common fungus in similar habitats worldwide [69]. It has not shown any specific plant–host association or other relationships that would limit its distribution in montane soils. Although the species of *Sagenomella* are usually reported as animal pathogens, *Sagenomella verticillata* W. Gams & B.E. Söderstr. was the most common species recorded in soils on pine forest clear-cuts in Sweden [70] and in the clear-cut boreal forests of *Picea mariana* (Mill.) Britton, Sterns & Poggenb. in Canada [60], where similar to our sites, the presence of *S. verticillata* was observed in combination with the *Mortierella* species. Additionally, it is worth mentioning that *Archaeorhizomyces*, often combined with the taxa of *Geminibasidium* and *Penicillium*, predominated in the post-fire soils of *Pinus ponderosa* Douglas ex C.Lawson following the severe fires [13]. Ubiquitous soil fungi from the genus *Archaeorhizomyces* [71] have also been detected in undisturbed high-altitude pine forest habitats in Montenegro [27,66]. Although more research is needed

for definitive conclusions, repeated detections of these fungal taxa in similar environmental and ecological conditions suggest their specific ecological niche and indicate that these taxa may represent an important part of the fungal community in high-altitude pine forest soils.

The clustering analysis provided valuable insights into the ecological dynamics of fungal communities in different soil environments. The patterns among the unburned samples suggested more stable fungal communities, while the distinct clustering of post-fire soils indicated the impact of fire disturbance on fungal community composition. The presence of highly abundant OTUs across the different environments highlighted their potential as bioindicators for soil health and ecological changes. These findings can inform conservation strategies aimed at protecting and restoring soil health in fire-affected regions. Given the slow recovery of ECM fungi in post-fire environments, forest management practices should consider strategies that promote the recolonization of these fungi, such as the introduction of ECM inoculants in reforestation efforts [60]. Moreover, the presence of pioneer ECM species like *Tomentella* and *Wilcoxina* in post-fire environments suggests their potential use in promoting pine regeneration in disturbed areas. These fungi may help establish new pine seedlings in areas where natural regeneration is hindered by the absence of ECM hosts [45].

5. Conclusions

In conclusion, this study shed light on the significant shifts in soil–fungal communities following forest fires in high-altitude *P. heldreichii* forests. This study recognized a core fungal community that persisted both before and after the fire disturbances, underscoring the resilience of specific fungal taxa in post-fire environments. The results also emphasized the importance of ECM fungi in forest regeneration in high-altitude habitats. These findings underline the need for further research to fully understand the long-term dynamics of soil–fungal communities and their implications for ecosystem health and management. Maintaining unburned forest areas and utilizing fungal bioindicators to monitor soil recovery will be crucial for preserving biodiversity and ensuring the resilience of fire-affected ecosystems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f15112012/s1, Figure S1: sampling of soil and rootlets at unburned (a) and burned (b) forest sites; Table S1: relative abundance (%) of fungal OTUs detected in post-fire soils, unburned soils, and rootlets. Sites are shown in parentheses: T—Treskavac; K—Kastrat; HO—Hum Orahovski; KK—Kučka Korita; and S—Sovrh.

References

1. Milanović, S.; Kaczmarowski, J.; Ciesielski, M.; Trailović, Z.; Mielcarek, M.; Szczygieł, R.; Kwiatkowski, M.; Bałazy, R.; Zasada, M.; Milanović, S.D. Modeling and Mapping of Forest Fire Occurrence in the Lower Silesian Voivodeship of Poland Based on Machine Learning Methods. *Forests* **2023**, *14*, 46. [CrossRef]
2. San-Miguel-Ayanz, J.; Durrant, T.; Boca, R.; Maianti, P.; Liberta', G.; Oom, D.; Branco, A.; De Rigo, D.; Suarez Moreno, M.; Ferrari, D.; et al. *Advance Report on Forest Fires in Europe, Middle East and North Africa 2023*; Publications Office of the European Union: Luxembourg, 2024. [CrossRef]
3. Alexandrian, D.; Esnault, F.; Calabri, G. Forest Fire in Medierranean Area, FAO Report 1998. Available online: https://www.fao.org/4/x1880e/x1880e07.htm (accessed on 1 July 2024).
4. Jovanović, B. *Dendrology*, 6th ed.; Faculty of Forestry, University of Belgrade: Belgrade, Serbia, 2007; p. 536. (In Serbian)
5. Lazarević, J.; Menkis, A. Fungal diversity in the phyllosphere of *Pinus heldreichii* H. Christ—An endemic and high-altitude pine of the Mediterranean region. *Diversity* **2020**, *12*, 172. [CrossRef]
6. 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; ISBN 978-92-9043-789-5.
7. The Second National Communication on Climate Change of Montenegro to the United Nations Framework Convention on Climate Change (UNFCCC), Montenegro Ministry of Sustainable Development and Tourism and the Centre for Sustainable Development, Office of the United Nations Development Programme (UNDP), Jablan, N., Ed. 2015. Available online: https://www.undp.org/montenegro/publications/second-national-communication-climate-change (accessed on 1 July 2024).
8. Borja, M.E.L. Climate Change and Forest Natural Regeneration in Mediterranean Mountain Areas. *Forest Res.* **2014**, *3*, e108. [CrossRef]
9. Ambs, D.; Schmied, G.; Zlatanov, T.; Kienlein, S.; Pretzsch, H.; Simeonova Nikolova, P. Regeneration dynamics in mixed mountain forests at their natural geographical distribution range in the Western Rhodopes. *For. Ecol. Manag.* **2024**, *552*, 121550. [CrossRef]
10. Lazarević, J. Forests and biodiversity of Kuči mountains. In *The Katuns of the Kuči Mountains*; Laković, I., Ed.; University of Montenegro: Podgorica, Montenegro, 2017; pp. 62–78.
11. Stevanović, V.; Vasić, V. Overview of anthropogenic factors that threaten the biodiversity of Yugoslavia. In *Biodiversity of Yugoslavia with List of Species of Special Importance*; Radović, I., Angelus, J., Eds.; Ecolibri: Beograd, Serbia, 1995; pp. 19–36.
12. Baar, J.; Horton, T.R.; Kretzer, A.M.; Bruns, T.D. Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytol.* **1999**, *143*, 409–418. [CrossRef]
13. Pulido-Chavez, M.F.; Alvarado, E.C.; DeLuca, T.H.; Edmonds, R.L.; Glassman, S.I. High-severity wildfire reduces richness and alters composition of ectomycorrhizal fungi in low-severity adapted ponderosa pine forests. *For. Ecol. Manag.* **2021**, *485*, 118923. [CrossRef]
14. Certini, G. Effects of fire on properties of forest soils: A review. *Oecologia* **2005**, *143*, 1–10. [CrossRef]
15. Bowman, D.M.J.S.; Balch, J.K.; Artaxo, P.; Bond, W.J.; Carlson, J.M.; Cochrane, M.A.; D'Antonio, C.M.; DeFries, R.S.; Doyle, J.C.; Harrison, S.P.; et al. Fire in the Earth System. *Science* **2009**, *324*, 481–484. [CrossRef]
16. Caon, L.; Vallejo, V.R.; Ritsema, C.J.; Geissen, V. Effects of wildfire on soil nutrients in Mediterranean ecosystems. *Earth-Sci. Rev.* **2014**, *139*, 47–58. [CrossRef]
17. Deng, Y.; Zhang, Y.; Zheng, X.; Cui, X. Effect of severe wildfire on soil phosphorus fractions and adsorption in a cold temperate coniferous forest after 5 years. *Eur. J. Soil Sci.* **2024**, *75*, e13464. [CrossRef]
18. Barreiro, A.; Díaz-Raviña, M. Fire impacts on soil microorganisms: Mass, activity, and diversity. *Curr. Opin. Environ. Sci. Health* **2021**, *22*, 100264. [CrossRef]
19. Pérez-Valera, E.; Goberna, M.; Verdú, M. Fire modulates ecosystem functioning through the phylogenetic structure of soil bacterial communities. *Soil Biol. Biochem.* **2018**, *120*, 80–88. [CrossRef]
20. Bastias, B.A.; Huang, Z.Q.; Cairney, J.W.G. Influence of long-term repeated prescribed burning on mycelial communities of ectomycorrhizal fungi. *New Phytol.* **2006**, *172*, 149–158. [CrossRef] [PubMed]
21. Noske, P.J.; Nyman, P.; Lane, P.N.J.; Rengers, F.K.; Sheridan, G.J. Changes in soil erosion caused by wildfire: A conceptual biogeographic model. *Geomorphology* **2024**, *459*, 109272. [CrossRef]
22. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*, 3rd ed.; Academic Press: London, UK, 2008; 800p.
23. Holden, S.R.; Treseder, K.K. A meta-analysis of soil microbial biomass responses to forest disturbances. *Front. Microbiol.* **2013**, *4*, 163. [CrossRef]
24. Treseder, K.K.; Maltz, M.; Hawkins, B.A.; Fierer, N.; Stajich, J.E.; McGuire, K.L. Evolutionary histories of soil fungi are reflected in their large-scale biogeography. *Ecol. Lett.* **2014**, *17*, 1086–1093. [CrossRef]
25. Burić, D.; Ducić, V.; Mihajlović, J. The climate of Montenegro: Modificators and types-part two. *Bull. Serbian Geogr. Soc.* **2014**, *44*, 73–90. [CrossRef]
26. Fuštić, B.; Đuretić, G. *Soils of Montenegro, Biotechnical Institute*; University of Montenegro: Podgorica, Montenegro, 2000; pp. 156–172. (In Serbian)
27. Lazarević, J.; Menkis, A. Fungi inhabiting fine roots of *Pinus heldreichii* in the Montenegrin montane forests. *Symbiosis* **2018**, *74*, 189–197. [CrossRef]
28. Blečić, V.; Lakušić, R. Forests of *Pinius heldreichii* Christ at Štitovo and Bjelasica in Montenegro. *Bull. Repub. Inst. Prot. Nat. Mus. Nat. Hist. Titogr.* **1969**, *4*, 5–10.

29. Topalović, A. *Practicum in Agrochemistry—Methods of Chemical Analysis and Data Processing*; University of Montenegro: Podgorica, Montenegro, 2022; 220p.

30. Topalović, A.; Knežević, M.; Trifunović, S.; Novaković, M.; Pešić, M.; Đurović, D. Effects of Soil Properties and Fertilization on Quality and Biological Activity of Swiss Chard. *Eur. J. Hortic. Sci.* **2018**, *83*, 374–381. [CrossRef]

31. Menkis, A.; Ihrmark, K.; Stenlid, J.; Vasaitis, R. Root-associated fungi of *Rosa rugosa* grown on the frontal dunes of the Baltic Sea coast in Lithuania. *Microb. Ecol.* **2014**, *67*, 769–774. [CrossRef] [PubMed]

32. 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] [PubMed]

33. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press, Inc.: San Diego, CA, USA, 1990; pp. 315–322.

34. Lazarević, J.; Menkis, A. *Cytospora friesii* and *Sydowia polyspora* are associated with the sudden dieback of *Abies concolor* in Southern Europe. *Plant Protect. Sci.* **2022**, *58*, 258–263. [CrossRef]

35. Menkis, A.; Marčiulynas, A.; Gedminas, A.; Lynikienė, J.; Povilaitienė, A. High-throughput sequencing reveals drastic changes in fungal communities in the phyllosphere of Norway spruce (*Picea abies*) following invasion of the spruce bud scale (*Physokermes piceae*). *Microb. Ecol.* **2015**, *70*, 904–911. [CrossRef]

36. Lynikienė, J.; Marčiulynienė, D.; Marčiulynas, A.; Gedminas, A.; Vaičiukynė, M.; Menkis, A. Managed and unmanaged *Pinus sylvestris* forest stands harbour similar diversity and composition of the phyllosphere and soil fungi. *Microorganisms* **2020**, *8*, 259. [CrossRef]

37. 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]

38. Magurran, A.E. *Ecological Diversity and Its Measurement*; Princeton University Press: Princeton, NJ, USA, 1988; p. 192.

39. Sokal, R.R.; Rohlf, F.J. *Biometry: The Principles and Practice of Statistics in Biological Research*, 4th ed.; H. Freeman & Company: New York, NY, USA, 2005; 896p.

40. Shannon, C.E. A mathematical theory of communicastion. *Bell Syst. Tech. J.* **1948**, *27*, 379–423. [CrossRef]

41. 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.

42. Murtagh, F.; Legendre, P. Ward's hierarchical agglomerative clustering method: Which algorithms implement Ward's criterion? *J. Classif.* **2014**, *31*, 274–295. [CrossRef]

43. Waskom, M. Seaborn: Statistical data visualization. *J. Open Source Softw.* **2021**, *6*, 3021. [CrossRef]

44. Hawkes, C.; Kivlin, S.; Rocca, J.; Huguet, V.; Thomsen, M.; Suttle, K. Fungal community responses to precipitation. *Glob. Change Biol.* **2010**, *17*, 1637–1645. [CrossRef]

45. Lilleskov, E.A.; Bruns, T.D. Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. *Mycologia* **2005**, *97*, 762–769. [CrossRef] [PubMed]

46. Zhu, Y.-Q.; Li, X.-L.; Zhao, D.-X.; Wei, Y.-L.; Yuan, H.-S. Four New Species of *Tomentella* (Thelephorales, Basidiomycota) from Subtropical Forests in Southwestern China. *J. Fungi* **2024**, *10*, 440. [CrossRef] [PubMed]

47. Kjoller, R. Disproportionate abundance between ectomycorrhizal root tips and their associated mycelia. *FEMS Microbiol. Ecol.* **2006**, *58*, 214–224. [CrossRef] [PubMed]

48. Hagerman, S.; Jones, M.; Bradfield, G.; Gillespie, M.; Durall, D. Effects of clear-cut logging on the diversity and persistence of ectomycorrhizae at a subalpine forest. *Can. J. Forest Res.* **1999**, *29*, 124–134. [CrossRef]

49. Lazar, A.; Mushinski, R.M.; Bending, G.D. Landscape scale ecology of *Tetracladium* spp. fungal root endophytes. *Environ. Microbiome* **2022**, *17*, 40. [CrossRef]

50. Abrego, N.; Roslin, T.; Huotari, T.; Tack, A.J.M.; Lindahl, B.D.; Tikhonov, G.; Somervuo, P.; Schmidt, N.M.; Ovaskainen, O. Accounting for environmental variation in co-occurrence modelling reveals the importance of positive interactions in root-associated fungal communities. *Mol. Ecol.* **2020**, *29*, 2736–2746. [CrossRef]

51. Alberton, O.; Kuyper, T.W.; Summerbell, R.C. Dark septate root endophytic fungi increase growth of Scots pine seedlings under elevated CO_2 through enhanced nitrogen use efficiency. *Plant Soil* **2010**, *328*, 459–470. [CrossRef]

52. Poteri, M.; Kasanen, R.; Asiegbu, F.O. Mycobiome of forest tree nurseries. In *Tree Microbiome: Phyllosphere, Endosphere and Rhizosphere, Forest Microbiology*; Asiegbu, F.O., Kovalchuk, A., Eds.; Academic Press: New York, NY, USA, 2021; Chapter 17; pp. 305–325. [CrossRef]

53. Koukol, O.; Macia-Vicente, J. *Leptodophora* gen. nov. (*Helotiales*, *Leotiomycetes*) proposed to accommodate selected root-associated members of the genus *Cadophora*. *Czech Mycol.* **2022**, *74*, 57–66. [CrossRef]

54. Moore, D.; Robson, G.D.; Trinci, A.P.J. *21st Century Guidebook to Fungi*; Cambridge University Press: Cambridge, UK, USA, 2011; pp. 200–202.

55. Eriksson, O.E.; Winka, K. Supraordinal taxa of *Ascomycota*. *Myconet* **2017**, *1*, 1–16.

56. Park, M.S.; Lee, J.W.; Kim, S.H.; Park, J.H.; You, Y.H.; Lim, Y.W. *Penicillium* from Rhizosphere Soil in Terrestrial and Coastal Environments in South Korea. *Mycobiology* **2020**, *48*, 431–442. [CrossRef]

57. Altaf, M.M.; Imran, M.; Abulreesh, H.; Khan, M.; Ahmad, I. Diversity and applications of *Penicillium* spp. in plant-growth promotion. In *New and Future Developments in Microbial Biotechnology and Bioengineering: Penicillum System Properties and Applications*; Gupta, V.K., Rodriguez-Couto, S., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 261–276.

58. Shi, L.; Dossa, G.G.O.; Paudel, E.; Zang, H.; Xu, J.; Harrison, R.D. Changes in Fungal Communities across a Forest Disturbance Gradient. *Appl. Environ. Microbiol.* **2019**, *85*, e00080-19. [CrossRef] [PubMed]

59. Ozimek, E.; Hanaka, A. *Mortierella* Species as the Plant Growth-Promoting Fungi Present in the Agricultural Soils. *Agriculture* **2021**, *11*, 7. [CrossRef]

60. Summerbell, E.C. Root endophyte and mycorrhizosphere fungi of black spruce, *Picea mariana*, in a boreal forest habitat: Influence of site factors on fungal distributions. *Stud. Mycol.* **2005**, *53*, 121–145. [CrossRef]

61. Mašínová, T.; Doreen Bahnmann, B.; Větrovský, T.; Tomšovský, M.; Merunková, K.; Baldrian, P. Drivers of yeast community composition in the litter and soil of a temperate forest. *FEMS Microbiol. Ecol.* **2017**, *93*, fiw223. [CrossRef]

62. Diez-Hermano, S.; Poveda, J.; Benito, Á.; Peix, Á.; Martín-Pinto, P.; Diez, J.J. Soil mycobiome and forest endophytic fungi: Is there a relationship between them? *For. Ecol. Manag.* **2024**, *562*, 121924. [CrossRef]

63. Carvajal, M.; Godoy, L.; Gebauer, M.; Catrileo, D.; Albornoz, F. Screening for indole-3-acetic acid synthesis and 1-aminocyclopropane-carboxylate deaminase activity in soil yeasts from Chile uncovers *Solicoccozyma aeria* as an effective plant growth promoter. *Plant Soil* **2023**, *496*, 83–93. [CrossRef]

64. Perić, B.; Perić, O. Diversity of macromycetes in Montenegro. *MASA* **1997**, *11*, 45–142.

65. Perić, B.; Perić, O. Preliminary red list of Macromycetes of Montenegro 2. *Mycol. Monten.* **2004**, *7*, 7–33.

66. Lazarević, J.; Topalović, A.; Menkis, A. Patterns of fungal diversity in needles, rootlets and soil of endemic *Pinus peuce*. *Balt. For.* **2022**, *28*, 58. [CrossRef]

67. Nguyen, H.D.T.; Nickerson, N.L.; Seifert, K.A. *Basidioascus* and *Geminibasidium*: A new lineage of heat-resistant and xerotolerant basidiomycetes. *Mycologia* **2013**, *105*, 1231–1250. [CrossRef]

68. Nguyen, N.H.; Song, Z.; Bates, S.T.; Branco, S.; Tedersoo, L.; Menke, J.; Schilling, J.S.; Kennedy, P.G. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **2016**, *20*, 241–248. [CrossRef]

69. Sogonov, M.; Schroers, H.-J.; Dijksterhuis, J.; Summerbell, R. The hyphomycete *Teberdinia hygrophila* gen. nov., sp. nov. and related anamorphs of *Pseudeurotium* species. *Mycologia* **2005**, *97*, 695–709. [CrossRef] [PubMed]

70. Bååth, E. Microfungi in a clearcut pine forest soil in central Sweden. *Can. J. Bot.* **1981**, *59*, 1331–1337. [CrossRef]

71. Rosling, A.; Cox, F.; Cruz-Martinez, K.; Ihrmark, K.; Grelet, G.A.; Lindahl, B.D.; Menkis, A.; James, T.Y. Archaeorhizomycetes: Unearthing an ancient class of ubiquitous soil fungi. *Science* **2011**, *333*, 876–879. [CrossRef]