

Article

# Diversity and Composition of Belowground Fungal Communities Associated with *Picea abies* L. (H.) Karst. and *Larix* sp. Mill.: A Comparative Study

Jūratė Lynikienė <sup>1,\*</sup>, Adas Marčiulynas <sup>1</sup>, Diana Marčiulynienė <sup>1</sup>, Artūras Gedminas <sup>1</sup>, Valeriia Mishcherikova <sup>1</sup> and Audrius Menkis <sup>2</sup>

<sup>1</sup> Institute of Forestry, Lithuanian Research Centre for Agriculture and Forestry, Liepų str. 1, Kaunas District, LT-53101 Girionys, Lithuania; adas.marciulynas@lammc.lt (A.M.); diana.marciulyniene@lammc.lt (D.M.); arturas.gedminas@lammc.lt (A.G.); valeriia.mishcherikova@lammc.lt (V.M.)

<sup>2</sup> Department of Forest Mycology and Plant Pathology, Uppsala BioCenter, Swedish University of Agricultural Sciences, P.O. Box 7026, SE-75007 Uppsala, Sweden; audrius.menkis@slu.se

\* Correspondence: jurate.lynikiene@lammc.lt

**Abstract:** The aim of the presented study was to compare the diversity and composition of fungal communities associated with the roots and the rhizosphere soil of *P. abies* and *Larix* sp. in mid-age and mature managed forest stands in Lithuania. We also aimed to assess the presence of fungi–host-specific associations, i.e., whether *Larix* sp. stands could provide habitats for soil fungi currently associated with *P. abies*. The study sites were 10 *Larix* sp. and 10 *P. abies* forest stands in Lithuania. For the study, 100 root samples and 10 organic and 10 mineral soil samples were collected in *P. abies* stands as well as the same number in *Larix* sp. stands, and DNA was isolated, amplified using ITS2 rDNA as a marker and subjected to high-throughput sequencing. The results showed that the Shannon diversity index of fungal communities was similar between the two tree species when compared either between root ( $H = 4.26$  *P. abies* and  $H = 3.82$  *Larix* sp.), organic soil ( $H = 5.12$  *P. abies* and  $H = 5.13$  *Larix* sp.) or mineral soil ( $H = 4.71$  *P. abies* and  $H = 4.29$  *Larix* sp.) samples. Multivariate analysis showed that the fungal community composition in the organic and mineral soil samples of both *P. abies* and *Larix* sp. were similar, and thus, overlapping. The analysis also showed that the distribution of fungal species was denser in the roots and organic soil but more scattered in mineral soil. However, several fungi in the roots of either *P. abies* or *Larix* sp. showed a certain host specificity.

**Keywords:** fungal diversity; fungal communities; *Picea abies*; *Larix* sp.; roots; rhizosphere soil

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## 1. Introduction

Terrestrial plants including forest trees are generally known to live in close association with microbial organisms [1]. Fungal communities together with bacteria, archaea and protists colonizing plant tissues and inhabiting outer plant surfaces, collectively comprise plant microbiota. Different plant tissues host distinct microbial communities, which are commonly divided into the rhizosphere (communities associated with root surface and adjacent soil layer), phyllosphere (microbial communities of outer surfaces of aerial plant parts) and endosphere (microbial communities residing within plant tissues) [2].

Globally, the biomass and relative proportions of microbial groups, including fungi, co-vary depending on the concentration of growth-limiting nutrients in soils and plant tissues including roots [3]. As an important component of soil microorganisms, soil fungi participate in the recycling of soil nutrients [3] through the decomposition of plant litter and the mineralization of soil carbon and nitrogen [4,5]. Moreover, microorganisms in the

rhizosphere may increase the supply of water to plants. Directly, this takes place through the uptake and transportation of water, while indirectly this occurs when fungal hyphae increase the availability of water by exploring a larger volume of soil by penetrating between small particles [6]. Soil fungi are also among the most abundant and species-rich taxonomic groups of organisms that can secrete a variety of extracellular enzymes [4,7]. Their activity may have beneficial, neutral or detrimental effects on plants, thereby affecting the overall functioning of forest ecosystems [1,3]. For instance, soil saprophytic fungi, which obtain nutrients by degrading dead organic matter, were shown to increase the turnover of soil nutrients [3,8,9]. Symbiotic mycorrhizal fungi, including arbuscular mycorrhizal and ectomycorrhizal (ECM) fungi, are known to improve the nutrition of plants. ECM fungi form beneficial symbioses with tree roots and can be essential for tree growth, particularly under harsh environmental conditions. Mycorrhizal fungi may also modify interactions between plants and other soil organisms such as pathogens, root-inhabiting nematodes or nitrogen-fixing bacteria [10]. By contrast, pathogenic fungi may cause tree diseases, resulting in reduced growth or even mortality. They often attack trees, which are affected by other biotic or/and abiotic factors [11]. Taken together, soil microbes play vital roles in forest ecosystems due to their participation in nutrient, carbon and water cycling [3], while the rhizosphere is a biologically active zone where tree roots and soil microbes interact [12].

Many fungi, which are associated with plants including forest trees, show a certain level of host specificity. The structural and functional diversity of rhizosphere fungal communities in forest ecosystems are usually affected by tree species as their litter often possess specific chemical qualities [13]. Although the effects of host phylogeny on fungal communities are widely documented, the magnitude of any host phylogenetic effects could differ among fungal guilds owing to factors such as co-evolutionary history, ecological specificity or interactions between ecological and evolutionary processes [14,15]. Generally, a stronger host phylogenetic constraint is expected on the community composition of parasitic organisms than of mutualists, owing to the need to avoid host defenses [16]. Several studies showed that certain endophytes belonging to Heliales have preferences for conifer trees [17], whereas a number of ECM fungi show specificity for a single host species [18]. In addition, woody tissues of trees often host fungal species, which are absent from herbaceous species including members of the orders Polyporales and Russulales [19]. However, empirical evidence for fungi–host-specific associations is lacking [20].

One of the most ubiquitous and economically, ecologically and geographically significant coniferous tree species in Eurasia's forest ecosystems is *Picea abies* [21–23]. Its native range is very wide, extending from central Europe to Fennoscandia and western Siberia [24]. *P. abies* was widely planted both inside and beyond its natural range of distribution due to its easy management and valuable timber. As a result, the area of *P. abies* stands has increased significantly during the past century [25]. Nonetheless, previous studies showed that one of the main reasons for *P. abies*' growth disruptions across its distribution range is climate change [26]. This tree species is more vulnerable to wind damage [27] and drought stress [28] due to its relatively shallow root system. Additionally, over the past ten years, *Ips typographus* L. has caused extensive damage to *P. abies* over a wide area in Europe including the Baltic countries. Further increases in these disturbances is anticipated, especially along the edge of *P. abies*' distribution, where the consequences of climate change are likely to be most pronounced [29]. From the ecological point of view, *Larix* spp. (including *L. decidua*) are characterized by a high adaptability to warmer climatic conditions, especially in comparison to *P. abies*, and has thus received increasing attention in recent years [30].

Furthermore, *Larix* spp. constitute an essential part of subalpine and boreal forests, growing natively in various parts of the northern hemisphere. *Larix* spp. grow more rapidly, produce wood that is more durable, and are better adapted to various environmental conditions than *P. abies*. This partly depends on their deeper root system, which also helps the trees to better survive windstorms and droughts [31]. In the future, *Larix* spp. trees

may be considered as an alternative to *P. abies* as they thrive in environments that are currently occupied by *P. abies* [32]. Nonetheless, native tree species frequently coexist with a wide range of other organisms, including fungi [33]. The biodiversity of these organisms may be disturbed by changes in the natural forest's structure and composition, thereby affecting the functioning of forest ecosystems.

*P. abies* is known to be associated with diverse fungal communities [11] both above- [11,34–36] and belowground [36–38]. These fungal communities were extensively studied in mid-age and mature *P. abies* forest stands. Differently to *P. abies*, in Europe, similar studies on fungal communities associated with *Larix* spp. are scarce. Moreover, most studies on fungal communities associated with *Larix* spp. were limited to young trees, including either naturally regenerated *Larix* spp. seedlings [39], seedlings growing in forest nurseries [40] or second-rotation young forest plantations [41]. Remarkably, it was previously demonstrated that a significant number of lichen and insect species associated with stems are shared among *P. abies* and *Larix* spp. [42]. As *P. abies* is predicted to suffer greatly from climate change in this region, it was suggested that *Larix* spp. could support indigenous insect and lichen species, which are currently associated with *P. abies* [42].

The development of high-throughput sequencing methods has revolutionized fungal community studies. Despite some limitations, high-throughput sequencing has become a predominant method to characterize the alpha and beta diversity in fungal communities. These methods, which are widely used to study natural habitats in terrestrial ecosystems worldwide, generated more than 200 publications until 2019 and over 250 million ITS sequences [43]. High-throughput sequencing can also complement fungal diversity predictions, which were previously based on traditional mycology methods, and increase our understanding of fungal biodiversity and community composition overall [43].

The aim of the presented study was to compare the diversity and composition of fungal communities associated with the roots and the rhizosphere soil of *P. abies* and *Larix* sp. in mid-age and mature managed forest stands in Lithuania. We also aimed to assess the presence of fungi–host-specific associations, i.e., whether *Larix* sp. stands could provide habitats for soil fungi currently associated with *P. abies*.

## 2. Materials and Methods

### 2.1. Study Site and Sampling

Ten *P. abies* and ten *Larix* sp. study sites were selected in different locations in Lithuania. The geographical position of each study site is available in Lynikiene et al., 2022 [42]. Due to frequent hybridization, the identification of larch trees to species level is problematic [44,45], and thus, in this study, they are referred to as *Larix* sp. At each site, there was one *P. abies* stand and one *Larix* sp. stand, which were within a radius of 200 m, i.e., within the same geographical area and with similar climatic conditions. The topography was also similar in these stands. The study sites were selected based on forest inventory information from the State Forest Cadastre database. The following indicators were taken into consideration when selecting each study site: (a) tree species of *P. abies* or *Larix* sp. were the most common in each site; (b) similar soil type [46]; and (c) similar type of ground vegetation [47]. The majority of the study sites were of normal humidity, moderate fertility soils and *oxalidos*a vegetation type. The characteristics of the selected study sites were as follows: the mean tree age ranged from 47 to 80 years; the mean tree height was between 21.7 and 35.9 m; the mean tree diameter was between 23.5 and 43.5 cm; and the stocking level was between 0.6 and 1.3 [42].

To study fungal communities, tree roots and the rhizosphere soil were sampled in 2019. At each *P. abies* and *Larix* sp. study site, five soil samples without litter were taken from the rhizosphere of randomly selected *P. abies* or *Larix* sp. trees using a 2 cm diameter soil core. Soil samples were taken down to 20 cm depth, which included ca. 50 g of organic soil layer and ca. 50 g of mineral soil layer. Organic and mineral soil layers were separated, and individual replicates of each layer were mixed together resulting in 10 organic and 10

mineral soil samples from all *P. abies* study sites and the same number from all *Larix* sp. study sites. A total of 40 soil samples that consisted of 250 g of soil from each layer were collected. For the sampling of roots, five *P. abies* and five *Larix* sp. trees were randomly selected in each study site. Within a distance of 0.5 m from each tree stem, fine lateral roots with root tips were excavated using a spade, which was carefully cleaned between individual samples. Collected roots were separated from the soil and other particles. Each sample consisted of root material up to 10 g in weight, and included up to 7 roots, which were up to 15 cm long. A total of 100 root samples were taken from *P. abies* stands and the same number from *Larix* sp. stands. All equipment was thoroughly cleaned between individual soil and root samples. After the collection, individual root and soil samples were placed in sterile plastic bags, labeled, transported the same day to the laboratory and stored at  $-20\text{ }^{\circ}\text{C}$  until further processing. Collected root and soil samples were used for DNA extraction.

## 2.2. DNA Procedure

Root samples were carefully washed in sterile water removing any of the remaining soil and sectioned into 5 mm long segments, prior to freeze-drying and DNA extraction. Immediately after the preparation, all root and soil samples were freeze-dried for 48 h using Labconco FreeZone Benchtop Freeze Dryer (Cole-Parmer, Vernon Hills, IL, USA). Then, ca. 0.5 g of freeze-dried material from each sample was powdered using a Fast prep machine (Montigny-le-Bretonneux, France). For DNA extraction, 30 mg of this powder per sample was used. The total DNA was extracted using the CTAB method as presented by Marčiulygienė et al. (2022) [48]. The concentration of genomic DNA was determined using a NanoDrop™ One spectrophotometer (Thermo Scientific, Rochester, NY, USA) and diluted to 10 ng/ $\mu\text{L}$ . Amplification by PCR of the ITS2 rRNA region was completed using barcoded primers gITS7 [49] and barcoded primers ITS4 [50], following the protocol by Clemmensen et al. (2016) [51]. Within the same tree species and site, replicate samples of the same substrate (roots or the soil) were amplified using primers with the same barcode. This resulted in these samples being pooled together following PCR. Individual PCRs for each replicate sample were used to increase the representativeness of each site. PCR amplification was carried out in 50  $\mu\text{L}$  reactions using an Applied Biosystems 2720 thermal cycler (Foster City, CA, USA). The PCR started with an initial denaturation at  $94\text{ }^{\circ}\text{C}$  for 5 min, then followed by 30 cycles of  $94\text{ }^{\circ}\text{C}$  for 30 s, and annealing at  $56\text{ }^{\circ}\text{C}$  for 30 s and  $72\text{ }^{\circ}\text{C}$  for 30 s, and ended by a final extension at  $72\text{ }^{\circ}\text{C}$  for 7 min. The PCR products were assessed on 1% agarose gels stained with GelRed (Biotium, Fremont, CA, USA). The PCR products were purified using 3 M sodium acetate (pH 5.2) (Applichem GmbH, Darmstadt, Germany) and 96% ethanol mixture (1:25). Cleaned PCR products were quantified using a Qubit fluorometer 4.0 (Life Technologies, Stockholm, Sweden), and then pooled in an equimolar mix. Sequencing was carried out on the PacBio RSII platform using two SMRT cells at the SciLifeLab in Uppsala, Sweden.

## 2.3. Bioinformatics

The SCATA NGS sequencing pipeline (<http://scata.mykopat.slu.se>, accessed on 14 April 2021) was used for sequence quality control and clustering. The procedures included the removal of primer dimers, short sequences (<200 bp), low-quality sequences ( $Q < 20$ ) and homopolymers, which were collapsed to 3 base pairs (bp) before clustering. Sequences lacking a tag or primer were removed, but sample information was stored as metadata. Single-linkage clustering based on 98.5% similarity was used to cluster different OTUs. OTUs were assigned taxonomic names using the GenBank (NCBI) database and Blastn algorithm. The criteria used were sequence coverage >80%, similarity to genus level 94–97% and similarity to species level 98–100%. Representative sequences of fungal nonsingletons as part of the Targeted Locus Study project have been deposited in GenBank under accession number (KIEZ00000000). Taxonomical information was also associated with each cluster using the SH mapping feature using the UNITE database

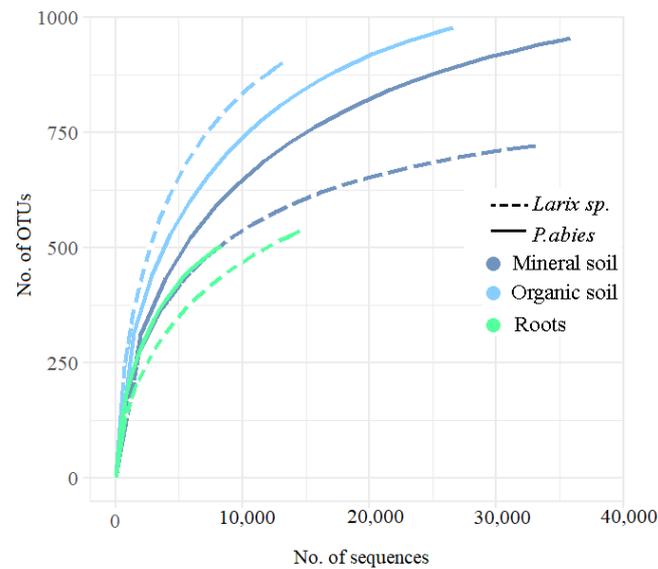
(<https://unite.ut.ee/analysis.php>, accessed on 26 May 2023). The FUNGuild database (<https://github.com/UMNFuN/FUNGuild>, accessed on 26 May 2023) was used to assign fungal functional groups according to Nguyen et al. (2016) [52] and Tedersoo et al. (2014) [53].

#### 2.4. Statistical Analysis

To estimate the relationship between the cumulative number of fungal OTUs and the number of ITS2 rRNA sequences, species accumulation curves were generated using Analytical Rarefaction v.1.3 available at <http://www.uga.edu/strata/software/index.html> (accessed on 10 June 2023). The Shannon diversity index and qualitative Sørensen similarity index [54,55] were used to characterize fungal communities associated with roots and the soil of *P. abies* and *Larix* sp. The nonparametric Mann–Whitney test in Minitab was used to test statistical differences of the Shannon diversity index among different sites and samples. The number of unique and shared fungal species among different substrates and tree species was visualized using a Venn diagram available at (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) (accessed on 25 May 2023). The differences in the relative abundance of fungal OTUs between study sites were tested using the Kruskal–Wallis test using XLSTAT (Addinsoft, NY, USA). The composition of fungal communities was examined using nonmetric multidimensional scaling (NMDS) based on the Bray–Curtis similarity index using open access software R version 4.0.5 (R Core team, Austria (<http://www.R-project.org/>)) (accessed on 10 June 2023). Permanova was performed to assess the significance in the distribution of fungal communities between different samples (roots and the soil) and between both tree species. Multilevel Pattern Analysis (MPA) was performed to assess the indicator fungal species associated with *P. abies* and *Larix* sp., using function `multipatt` with 999 numbers of permutation from the library `Indicspecies`, (<https://cran.r-project.org/web/packages/indicspecies/index.html>) (accessed on 5 June 2023) A heatmap was built based on a  $\log + 1$  transformed matrix using the 30 most common fungal OTUs using the `heatmap` function from the `heatmap` package in R.

### 3. Results

High-throughput sequencing of pooled root and soil samples resulted in 329,109 reads. Filtering showed the presence of 173,705 (52.8%) high-quality reads, while 155,404 (47.2%) low-quality reads were excluded. Clustering of high-quality reads showed the presence of 2273 nonsingleton contigs at 98.5% similarity level representing different OTUs. Among these, 2142 (94.3%) were fungal (Table S1), which were retained, while 131 (5.7%) nonfungal OTUs were excluded. Rarefaction analysis showed that species accumulation curves did not reach the asymptote, showing that a higher species richness could be detected with deeper sequencing (Figure 1).



**Figure 1.** Species accumulation curves showing the relationship between the cumulative number of fungal OTUs and the number of fungal sequences from different substrates (roots, organic and mineral soil) of *Picea abies* and *Larix sp.*

When all study sites were taken together, there were 519 fungal OTUs among 7034 (33.3%) reads in the roots of *P. abies* and 530 fungal OTUs among 14,066 (66.7%) reads in the roots of *Larix sp.* (the difference significant at  $p < 0.05$ ) (Table 1). In a similar comparison, there were 974 fungal OTUs among 27,028 (71.3%) reads in the organic soil of *P. abies* and 828 fungal OTUs among 10,881 (28.7%) reads in the organic soil of *Larix sp.* ( $p < 0.05$ ). Furthermore, there were 952 fungal OTUs among 38,128 (48.4%) reads in the mineral soil of *P. abies* and 814 fungal OTUs among 40,690 (51.6%) reads in the mineral soil of *Larix sp.* ( $p > 0.05$ ). The Shannon diversity index did not differ significantly between the tree species: in the roots ( $H = 4.26$  in *P. abies* and  $H = 3.82$  in *Larix sp.*), in the organic soil ( $H = 5.12$  in *P. abies* and  $H = 5.13$  in *Larix sp.*) and in the mineral soil ( $H = 4.71$  in *P. abies* and  $H = 4.29$  in *Larix sp.*) ( $p > 0.05$ ) (Table 1). The Sørensen similarity index of fungal communities was low to moderate between the corresponding datasets (roots, organic and mineral soil) of both tree species (Table 1).

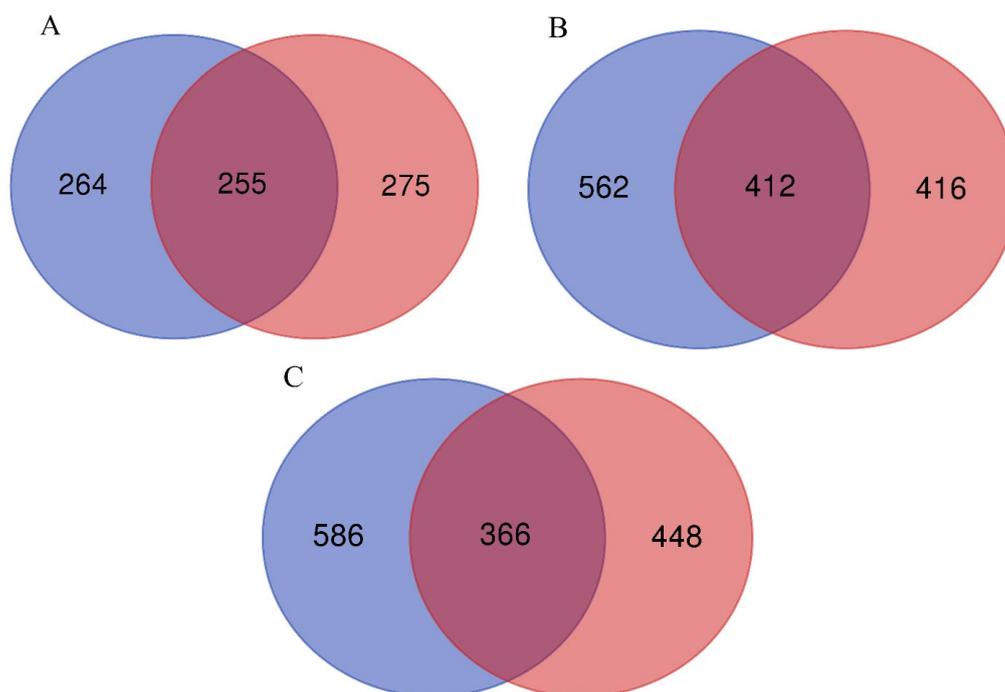
The comparison of fungal species richness showed that in the roots, there were 264 unique OTUs associated with *P. abies*, 275 unique OTUs associated with *Larix sp.* and 255 OTUs shared between *P. abies* and *Larix sp.* (Figure 2A). In the organic soil, there were 562 unique OTUs associated with *P. abies*, 416 unique OTUs associated with *Larix sp.* and 412 OTUs shared between both tree species (Figure 2B). In the mineral soil, there were 586 unique OTUs associated with *P. abies*, 448 unique OTUs associated with *Larix sp.* and 366 OTUs shared between *P. abies* and *Larix sp.* (Figure 2C).

**Table 1.** Fungal diversity parameters in the roots and the rhizosphere soil (organic and mineral layers) at *Picea abies* and *Larix* sp. study sites.

Site	Tree species	Relative Abundance (%)	Richness (%)	Shannon H	Sørensen Cs	Relative Abundance (%)	Richness (%)	Shannon H	Sørensen Cs	Relative Abundance (%)	Richness (%)	Shannon H	Sørensen Cs	Relative Abundance (%)	Richness (%)	Shannon H	Sørensen Cs
		Roots					Soil										
							Organic Layer			Mineral Layer			TOTAL SOIL				
S1/L1	<i>Picea</i>	2.2	11.9	3.37	0.31	7.2	13.4	3.90	0.31	1.0	6.4	3.29	0.33	3.0	11.2	3.90	0.35
	<i>Larix</i>	5.3	15.2	2.34		6.3	13.5	3.90		3.1	13.3	4.12		4.1	15.4	4.31	
S2/L2	<i>Picea</i>	4.5	13.8	3.06	0.34	0.1	1.2	2.71	0.10	2.6	9.7	3.50	0.25	1.8	7.3	3.53	0.32
	<i>Larix</i>	11.6	16.2	3.05		1.4	8.7	4.12		0.7	4.5	2.26		0.9	8.3	3.70	
S3/L3	<i>Picea</i>	2.4	12.9	3.71	0.21	3.2	11.4	3.84	0.22	1.0	5.9	3.14	-	1.7	10.3	3.81	0.27
	<i>Larix</i>	7.2	10.2	1.71		5.5	22.1	4.55		-	-	-		1.8	15.5	4.55	
S4/L4	<i>Picea</i>	2.6	12.8	3.55	0.36	7.6	15.8	4.07	0.24	0.3	8.1	4.26	0.17	2.7	15.1	4.31	0.29
	<i>Larix</i>	7.0	16.6	2.90		0.6	6.1	3.99		1.1	8.0	3.74		1.0	8.7	4.14	
S5/L5	<i>Picea</i>	1.8	13.2	3.80	0.10	5.3	14.7	4.13	0.23	4.3	10.6	3.76	0.23	4.6	14.1	4.18	0.25
	<i>Larix</i>	0.3	4.0	3.04		1.7	10.0	4.11		3.2	12.1	3.78		2.7	12.2	4.04	
S6/L6	<i>Picea</i>	1.1	7.2	3.12	0.18	17.4	17.9	3.50	-	3.9	15.3	4.14	0.23	8.3	18.3	4.08	0.28
	<i>Larix</i>	4.6	11.2	2.47		-	-	-		12.5	22.6	3.75		8.4	15.9	3.75	
S7/L7	<i>Picea</i>	5.8	16.2	3.29	0.27	25.1	20.3	4.15	0.23	26.9	29.8	4.12	0.29	26.3	27.6	4.33	0.32
	<i>Larix</i>	4.8	12.6	2.92		2.4	12.3	3.92		25.9	15.4	3.31		18.3	16.4	3.41	
S8/L8	<i>Picea</i>	3.6	10.9	1.99	0.32	2.3	5.5	3.19	0.21	3.0	5.5	0.80	0.20	2.8	6.7	1.89	0.34
	<i>Larix</i>	12.4	14.2	2.65		3.4	9.9	3.78		0.2	4.3	3.36		1.2	9.0	4.04	
S9/L9	<i>Picea</i>	4.1	13.7	3.29	0.31	1.2	9.1	4.16	0.28	0.3	3.6	2.71	0.16	0.6	7.8	4.11	0.27
	<i>Larix</i>	7.8	12.6	2.96		1.4	7.7	3.60		5.0	10.0	3.72		3.8	10.7	3.92	
S10/L10	<i>Picea</i>	5.2	15.3	3.06	0.25	2.1	10.3	3.75	0.21	5.1	15.3	3.12	-	4.1	16.1	3.57	0.24
	<i>Larix</i>	5.6	12.4	2.75		5.9	7.9	3.41		-	-	-		1.9	5.5	3.41	
All sites *	<i>Picea</i>	33.3 (7034)	66.4 (519)	4.26	0.49	71.3 (27,028)	70.1 (974)	5.12	0.47	48.4 (38,128)	68.0 (952)	4.71	0.44	55.8 (65,156)	72.1 (1429)	5.13	0.53
	<i>Larix</i>	66.7	67.8	3.82		28.7	59.6	5.13		51.6	58.1	4.29		44.2	63.9	4.67	

	(14,066)	(530)		(10,881)	(828)		(40,690)	(814)		(51,571)	(1267)	
Total	100.0	100.0	4.20	100.0	100.0	5.43	100.0	100.0	4.79	100.0	100.0	5.22
	(21,100)	(782)		(37,909)	(1389)		(78,818)	(1401)		(116,727)	(1983)	

\* The number of fungal OTUs and the number of fungal sequences are shown in the brackets.



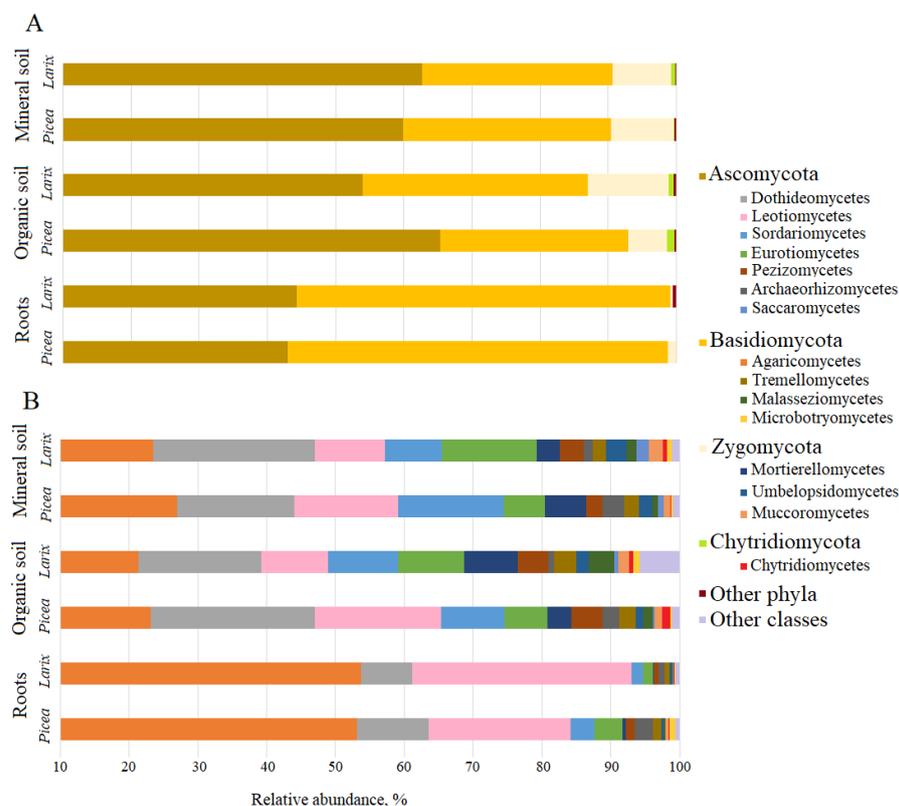
**Figure 2.** Venn diagram showing the number of unique and shared fungal OTUs detected in different samples of *Picea abies* and *Larix sp.*: (A) roots; (B) organic soil; (C) mineral soil. Blue: *P. abies*, pink: *Larix sp.* Within each tree species, different study sites are combined.

Ascomycota and Basidiomycota dominated the fungal communities in different substrates: 43.0% and 55.7% in the roots of *P. abies*, and 44.3% and 55.1% in the roots of *Larix sp.*; 65.3% and 27.6% in the organic soil of *P. abies*, and 54.0% and 33.0% in the organic soil of *Larix sp.*; and 59.9% and 30.5% in the mineral soil of *P. abies*, and 62.7% and 27.9% in the mineral soil of *Larix sp.*, all respectively (Figure 3A).

For both *P. abies* and *Larix sp.*, in the roots, Basidiomycota fungi showed a significantly higher relative abundance than Ascomycota ( $p < 0.01$ ), while it was vice versa in the soil (Figure 3A). Zygomycota fungi showed a low relative abundance in the roots of both *P. abies* (1.2%) and *Larix sp.* (0.4%), but they were significantly ( $p < 0.05$ ) higher in the organic (5.7% and 9.1%) and mineral soils (11.9% and 8.5%) of *P. abies* than those of *Larix sp.*, respectively. Chytridiomycota were mainly detected in soil samples of both tree species (ca. 1.0%), and the difference was not significant ( $p > 0.05$ ) between *P. abies* and *Larix sp.* Taken together, the relative abundance of fungal phyla differed significantly ( $p < 0.01$ ) among the roots and soil samples of both tree species.

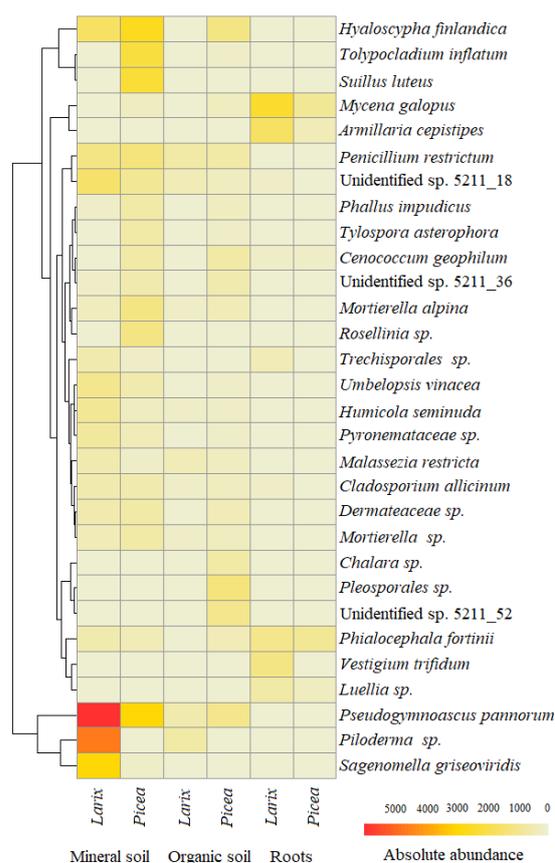
The most common classes within Ascomycota were Leotiomyces, Dothideomyces, Sordariomyces and Eurotiomyces, which were detected at variable abundances in all substrates of both *P. abies* and *Larix sp.* (Figure 3B). Among these, Leotiomyces showed 20.6% relative abundance in the roots of *P. abies* and 31.9% in the roots of *Larix sp.*, in the organic soil, it was 18.9% for *P. abies* and 9.6% for *Larix sp.*, and in the mineral soil, it was 15.2% for *P. abies* and 10.2% for *Larix sp.* (Figure 3B). The difference was significant ( $p < 0.05$ ) between *P. abies* and *Larix sp.*, and between root and soil samples. Differently from Leotiomyces, Dothideomyces, Sordariomyces and Eurotiomyces were more common in organic soil (*P. abies*: 23.8%, 9.2%, 6.2% and *Larix sp.*: 17.9%, 10.2%, 9.7%, respectively) and mineral soil (*P. abies*: 17.0%, 15.3%, 5.9% and *Larix sp.*: 23.5%, 8.2%, 13.8%, respectively) than in roots (*P. abies*: 10.4%, 3.4%, 4.1% and *Larix sp.*: 7.5%, 1.6%, 1.4%, respectively). The difference was significant ( $p < 0.05$ ) between the root and soil samples but not between *P. abies* and *Larix sp.* Other fungal classes within Ascomycota were relatively rare (Figure 3B). Within Basidiomycota, Agaricomycetes dominated the fungal communities, while Tremellomyces, Malasseziomyces and Microbotryomyces were

less abundant, but all of these were detected at variable abundances in all substrates of both *P. abies* and *Larix* sp. (Figure 3B). Agaricomycetes showed a significantly higher relative abundance in the roots of *P. abies* (53.2%) and *Larix* sp. (53.7%) ( $p < 0.01$ ) than in the organic (23.2% and 21.3%) or mineral soils (27.0% and 23.5%) of *P. abies* and *Larix* sp., respectively. Within each substrate, the relative abundance of Agaricomycetes did not differ significantly between the two tree species ( $p > 0.05$ ).



**Figure 3.** Relative abundance of fungal phyla (A) and classes (B) in roots and the rhizosphere soil (organic and mineral) of *P. abies* and *Larix* sp. Within each tree species and substrate, different study sites are combined. *Other phyla* and *Other classes* include phyla and classes with a relative abundance of <1%.

Hierarchical clustering of the 30 most abundant fungal OTUs showed a specific association of fungal OTUs with each tree species that was more pronounced for fungi from soil samples than those from root samples of both *P. abies* and *Larix* sp. (Figure 4). In mineral soil, *Pseudogymnoascus pannorum*, *Piloderma* sp., *Sagenomella griseoviridis* and *Hyaloscypha finlandica* showed the highest association with *Larix* sp., while *P. pannorum*, *H. finlandica*, *Tylopocladium inflatum* and *Suillus luteus* showed the highest association with *P. abies* (Figure 4). Similar patterns were also observed for organic soil. In roots, *Mycena galopus* and *Phialocephala fortinii* showed the highest association with *P. abies*, while *M. galopus*, *Armillaria cepistipes*, *P. fortinii*, *Vestigium trifidum* and *Luellia* sp. showed the highest association with *Larix* sp. (Figure 4).



**Figure 4.** A heatmap with a dendrogram showing hierarchical clustering of the 30 most abundant fungal OTUs from different samples of each tree species (columns) and the level of species co-occurrence (rows). Within each tree species, different study sites are combined.

The 30 most common fungal OTUs comprising 52.2% of all fungal sequences are shown in Table 2. The most common fungi in the roots of *P. abies* were *M. galopus* (13.0%), *P. fortinii* (12.9%), *A. cepistipes* (6.8%) and *Luellia* sp., while those in the roots of *Larix* sp. were *M. galopus* (16.4%), *A. cepistipes* (11.9%), *V. trifidum* (7.9%) and *P. fortinii* (6.8%) (Table 2). The most common fungi in the organic soil of *P. abies* were *Pleosporales* sp. (4.8%), *Hyaloscypha finlandica* (4.3%), *Pseudogymnoascus pannorum* (3.8%) and Unidentified sp. 5211\_52 (3.7%), while those in the organic soil of *Larix* sp. were *Piloderma* sp. (5.6%), *Penicillium restrictum* (5.6%), *P. pannorum* (4.7%) and Unidentified sp. 5211\_18 (3.9%) (Table 2). The most common fungi in the mineral soil of *P. abies* were *P. pannorum* (7.5%), *H. finlandica* (6.7%), *Suillus luteus* (5.9%) and *Tolyptocladium infratum* (5.6%), while those in the mineral soil of *Larix* sp. were *P. pannorum* (14.6%), *Piloderma* sp. (11.2%), *Sagenomella griseoviridis* (7.0%) and Unidentified sp. 5211\_18 (3.7%) (Table 2).

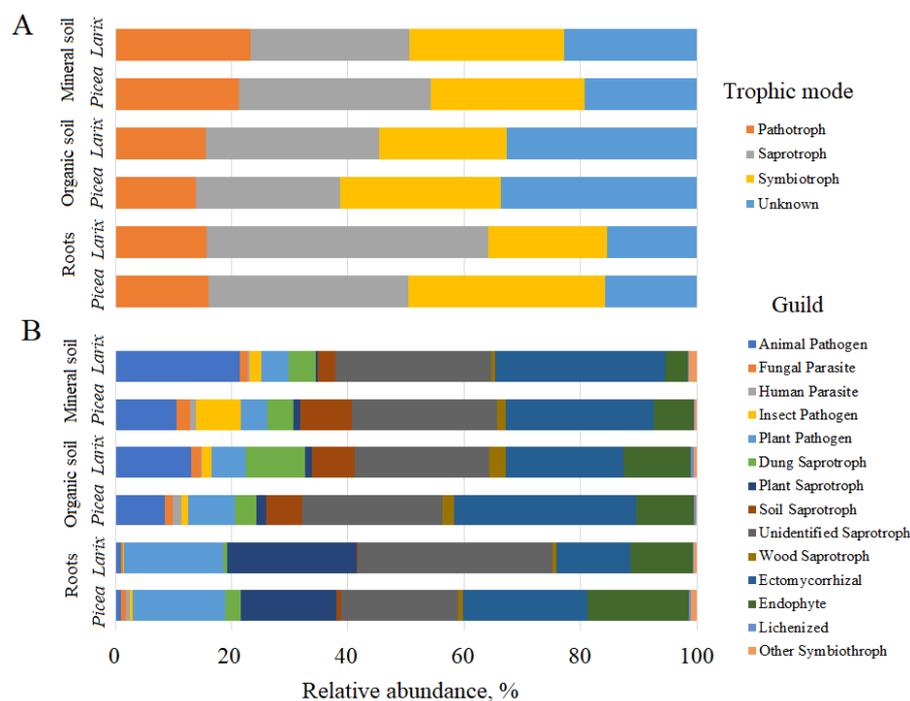
**Table 2.** Relative abundance (%) of the 30 most common fungal OTUs detected in roots, organic and mineral soil of *Picea abies* and *Larix* sp. study sites. Within each tree species, different study sites are combined.

Phyla *	Fungal OTU	Genbank Reference	Similarity, bp (%)	Roots			Organic Soil			Mineral Soil			All
				<i>P. abies</i>	<i>Larix</i> sp.	Both	<i>P. abies</i>	<i>Larix</i> sp.	Both	<i>P. abies</i>	<i>Larix</i> sp.	Both	
A	<i>Pseudogymnoascus pannorum</i>	MN879385	241/241 (100)	0.4	0.5	0.4	3.8	4.7	4.1	7.5	14.6	11.2	7.6
A	<i>Hyaloscypha finlandica</i>	MK965770	232/236 (98)	1.4	0.4	0.7	4.3	0.7	3.2	6.7	4.0	5.3	4.0
B	<i>Piloderma</i> sp.	MK838260	289/289 (100)	-	0.6	0.4	0.01	5.6	1.6	-	11.2	5.8	3.8
B	<i>Mycena galopus</i>	MK795846	305/305 (100)	13.0	16.5	15.3	1.0	0.1	0.8	0.8	0.1	0.5	2.8
A	<i>Penicillium restrictum</i>	MT090009	257/257 (100)	1.6	0.5	0.9	2.3	5.6	3.2	3.2	2.9	3.0	2.8
A	Unidentified sp. 5211_18	MZ442004	234/235 (99)	0.7	1.3	1.1	1.2	3.9	2.0	2.4	3.7	3.1	2.5
A	<i>Phialocephala fortinii</i>	MT028045	238/238 (100)	12.9	6.8	8.9	1.5	0.5	1.2	1.2	1.2	1.2	2.4
A	<i>Sagenomella griseoviridis</i>	LC177648	249/249 (100)	0.0	0.0	0.0	0.2	0.3	0.2	0.6	7.0	3.9	2.3
A	<i>Tolyposcladium inflatum</i>	MT294423	246/246 (100)	0.0	-	0.0	0.5	0.2	0.4	5.6	0.1	2.8	1.7
B	<i>Suillus luteus</i>	KU059580	331/331 (100)	0.03	0.02	0.02	0.3	0.01	0.2	5.9	-	2.8	1.7
B	<i>Armillaria cepistipes</i>	OK324330	477/477 (100)	6.8	11.9	10.2	-	-	-	-	0.04	0.02	1.6
A	<i>Cladosporium allicinum</i>	OP965390	243/243 (100)	1.1	1.3	1.2	1.1	1.3	1.2	1.3	1.3	1.3	1.2
Z	<i>Mortierella alpina</i>	MT529891	345/345 (100)	0.1	0.0	0.0	1.5	1.6	1.6	2.8	0.8	1.8	1.4
Z	<i>Umbelopsis vinacea</i>	KU727816	294/294 (100)	0.1	0.1	0.1	0.8	1.0	0.9	1.3	2.6	2.0	1.4
A	<i>Dermateaceae</i> sp.	MK965746	237/237 (100)	0.2	0.2	0.2	1.4	0.7	1.2	1.7	1.4	1.5	1.2
A	<i>Cenococcum geophilum</i>	MN450580	241/241 (100)	1.9	1.0	1.3	2.2	0.6	1.8	1.6	0.3	0.9	1.2
A	<i>Humicola seminuda</i>	LT993594	249/249 (100)	0.0	-	0.0	0.8	1.8	1.1	0.8	2.2	1.5	1.2
Z	<i>Mortierella</i> sp.	KP311419	344/344 (100)	0.1	0.1	0.1	1.3	1.6	1.4	1.6	1.0	1.3	1.1
A	<i>Pyronemataceae</i> sp.	LR603942	256/256 (100)	0.5	0.1	0.2	0.8	0.7	0.8	1.2	1.8	1.5	1.1
B	<i>Malassezia restricta</i>	LT854697	369/369 (100)	0.2	0.2	0.2	1.0	3.5	1.8	0.6	1.3	1.0	1.1
A	<i>Pleosporales</i> sp.	KT269193	238/245 (97)	0.9	0.0	0.3	4.8	0.0	3.4	0.0	-	0.0	1.0
A	Unidentified sp.	MN902437	216/216(100)	1.3	0.0	0.5	1.9	0.1	1.4	1.5	0.3	0.9	0.9
B	<i>Trechisporales</i> sp.	JF519283	297/297 (100)	1.2	3.4	2.6	0.0	0.1	0.1	0.4	1.3	0.8	0.9
A	<i>Vestigium trifidum</i>	KP783486	236/239 (99)	0.1	7.9	5.3	0.0	0.5	0.2	-	0.0	0.0	0.9
B	<i>Phallus impudicus</i>	OP603024	290/290 (100)	0.0	-	0.0	1.0	0.8	0.9	1.7	0.4	1.0	0.8
A	<i>Rosellinia</i> sp.	KT264658	257/258 (99)	0.0	-	0.0	-	-	-	2.9	0.0	1.4	0.8
B	<i>Tylospora asterophora</i>	HM190017	288/288 (100)	0.6	0.1	0.3	0.7	0.5	0.7	1.8	0.0	0.9	0.7

A	Unidentified sp. 5211_52	KT196588	201/226 (89)	0.0	-	0.0	3.7	-	2.6	0.0	-	0.0	0.7
B	<i>Luellia</i> sp.	LS447499	319/320 (99)	4.3	4.4	4.3	0.0	0.2	0.1	0.0	0.0	0.0	0.7
A	<i>Chalara</i> sp.	MK965775	238/238 (100)	0.3	0.3	0.3	2.4	0.7	1.9	0.2	0.0	0.1	0.7
<b>Total of 30 fungal OTUs</b>				49.7	57.6	55.0	40.8	37.5	39.8	55.2	59.6	57.5	52.2

\* Phyla: A (Ascomycota), B (Basidiomycota), Z (Zygomycota).

FUNGuild classification and statistical analysis showed that the relative abundance of fungal trophic modes did not differ significantly between corresponding substrates of the two tree species ( $p > 0.05$ ). In a similar comparison, the difference was significant ( $p = 0.0002$ ) between root and soil samples. Saprotrophs were most abundant and composed 34.4% and 48.4% of fungal communities in the roots, 24.9% and 29.8% in the organic soil, and 33.0% and 27.2% in the mineral soil of *P. abies* and *Larix* sp., all respectively (Figure 5A).



**Figure 5.** FUNGuild classification of fungal trophic modes (A) and fungal guilds (B) from roots, organic and mineral soil of *Picea abies* and *Larix* sp. Within each substrate and tree species, different study sites are combined.

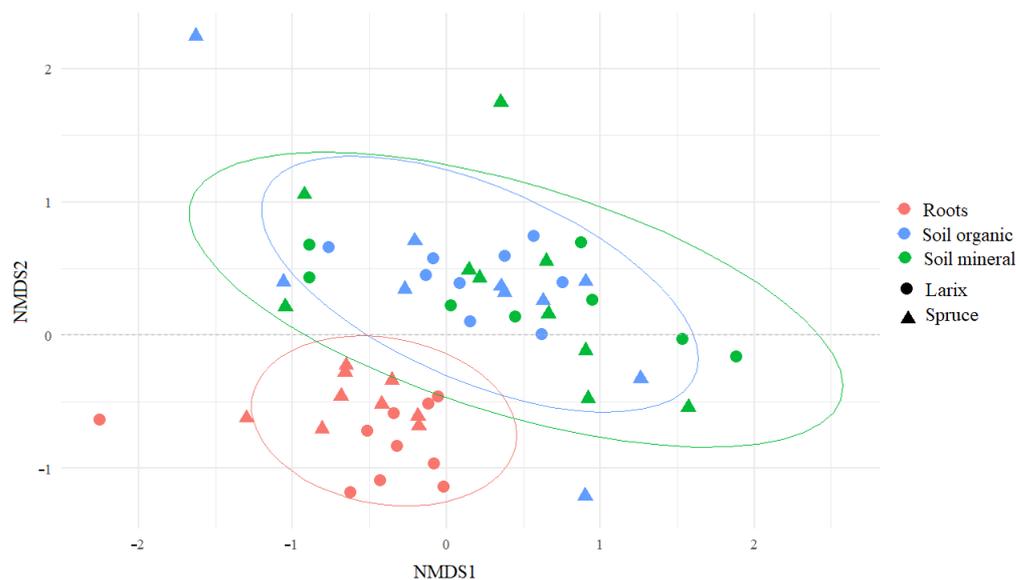
Symbiotrophs were the second most abundant trophic mode representing 33.8% and 20.5% of fungal communities in the roots, 27.5% and 21.9% in the organic soil, and 26.5% and 26.6% in the mineral soil of *P. abies* and *Larix* sp., all respectively. Pathotrophs composed between 15.0% and 20.0% of fungal communities in different substrates of each tree species. Unclassified fungi composed between 15.8% and 15.4% of fungal communities in the roots, 33.8% and 32.8% in the organic soil, and 19.2% and 22.9% in the mineral soil of *P. abies* and *Larix* sp., all respectively (Figure 5A). Among saprotrophs, the most common fungal guild was unidentified saprotrophs, which composed 16.8% and 28.2% of fungal communities in the roots, 15.8% and 15.5% in the organic soil, and 20.1% and 20.5% in the mineral soil of *P. abies* and *Larix* sp., all respectively (Figure 5B). Among all symbiotrophs, the most common guild was ectomycorrhizal (ECM) fungi, which composed 18.0% and 10.8% of fungal communities in the roots, 20.7% and 13.5% in the organic soil, and 20.6% and 22.5% in the mineral soil of *P. abies* and *Larix* sp., all respectively. For both unidentified saprotrophs and ECM fungi, the differences between the corresponding substrates of each tree species were insignificant ( $p > 0.05$ ). Plant pathogens were more common in the roots of *P. abies* (13.3%) and *Larix* sp. (14.5%) but were relatively rare in the soil (Figure 5B). The relative abundance of plant pathogens was not significant ( $p > 0.05$ ) between *P. abies* and *Larix* sp. samples. Other fungal guilds were generally less common, and their relative abundance varied among different substrates and tree species (Figure 5B).

Among common ECM fungi, *Amphinema byssoides* (5.8% and 3.5%) and *Cenococcum geophilum* (2.4% and 2.2%) were the most common in the roots of *P. abies* and *Larix* sp.,

respectively. *C. geophilum* (2.2%) and *Inocybe* sp. (2.1%) were the most common in the organic soil of *P. abies*, while *Piloderma* sp. (5.6%) and *C. geophilum* (0.5%) were the most common in the organic soil of *Larix* sp. *Suillus luteus* and *Tylospora asterophora* were the most common fungi in the mineral soil of *P. abies*, while *Piloderma* sp. (11.2%) and *Inocybe geophylla* (0.9%) were the most common in the mineral soil of *Larix* sp. (Table S3). Interestingly, *Piloderma* sp. was either not detected in the roots and mineral soil of *P. abies* or showed very low relative abundance (0.01%) in the organic soil. *S. luteus* was not detected in the mineral soil of *Larix* sp. but was common in the mineral soil of *P. abies* (Table S3). The other most common ECM fungi showed a generally low relative abundance in the different substrates and tree species (Table S3).

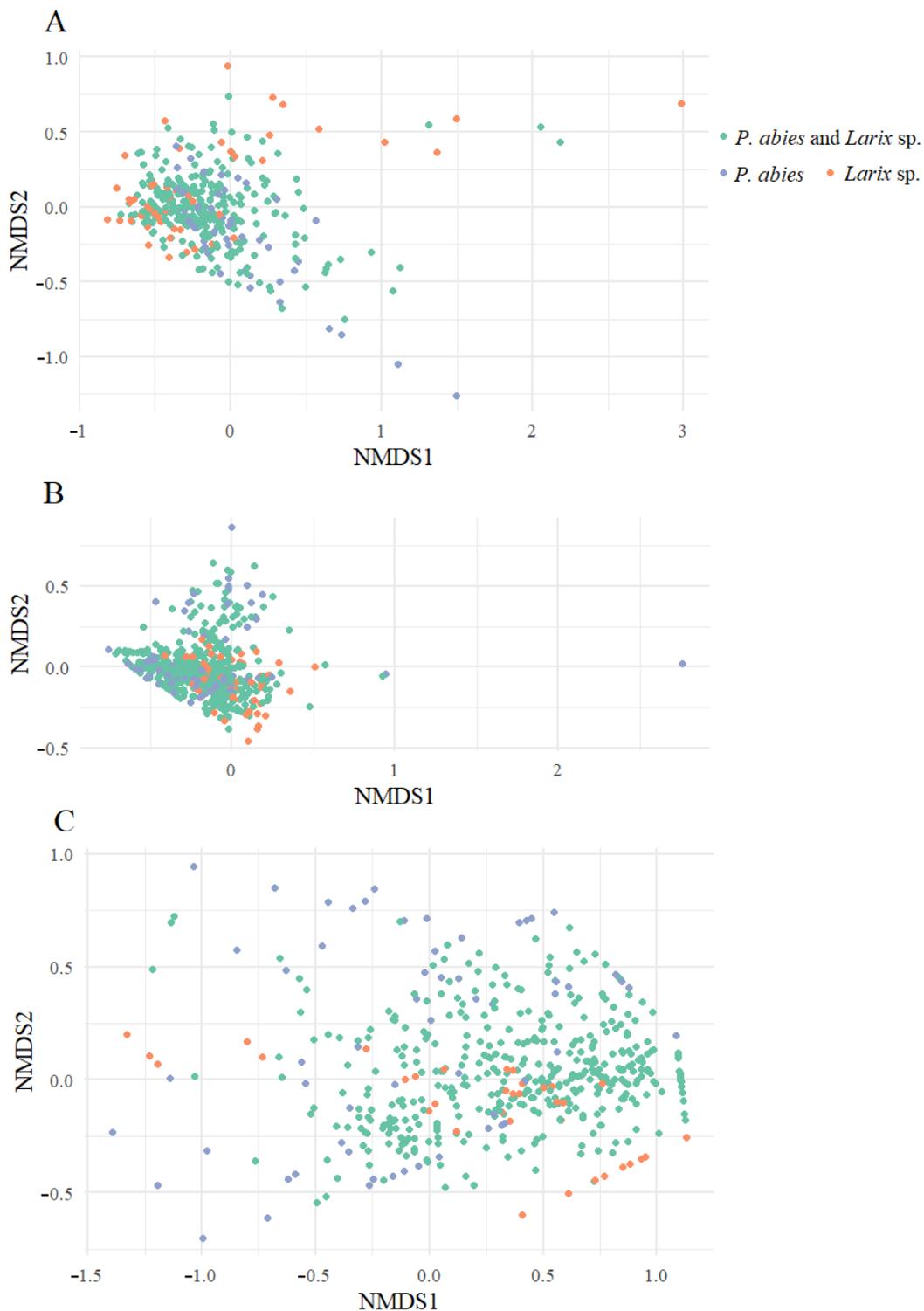
Among common plant pathogenic fungi, *Armillaria cepistipes* was the most common in the roots of *P. abies* (6.8%) and *Larix* sp. (11.9%), but it was absent in the organic soil and seldomly found in the mineral soil of the *Larix* sp. (0.04%) study sites (Table S3). *Heterobasidion annosum* was the second most abundant plant pathogen in the roots of *P. abies* (2.5%), but it was not detected in the roots of *Larix* sp. Interestingly, this species was detected at low abundances in the organic soil of *Larix* sp. and in the mineral soil of *P. abies* but not in other soils of these tree species. (Table S3). Plant pathogens *Fusarium saloni* and *Fusarium equiseti* were detected exclusively in soil samples of both tree species. The other most common plant pathogens such as *Sydowia polyspora*, *Neonectria candida* and *Ganoderma applanatum* were detected at low abundances in different substrates and tree species (Table S3).

NMDS analysis showed that within each substrate (roots, organic and mineral soil), the fungal communities between *P. abies* and *Larix* sp. were similar and overlapping (Figure 6). Permanova showed that the difference was not significant ( $p > 0.05$ ) between the fungal communities in the mineral and organic soils of these two tree species.



**Figure 6.** NMDS ordination diagram based on nonmetric multidimensional scaling of fungal communities in roots, organic and mineral soil of *Picea abies* and *Larix* sp.

The majority of fungal OTUs were common to both tree species in the roots and organic and mineral soil (Figure 7). Therefore, the distribution of fungal communities between the two tree species was not significant in the roots ( $p = 0.23$ ), or in the organic ( $p = 0.14$ ) and mineral ( $p = 0.72$ ) soils (Figure 7B,C). Generally, fungal communities were distributed more densely in the roots and organic soil but were more scattered in mineral soil (Figure 7A–C).



**Figure 7.** NMDS ordination diagram based on nonmetric multidimensional scaling of fungal communities showing graphical representation of fungi common to both tree species, specific to *P. abies* or specific to *Larix* sp. (A) in root; (B) in organic soil; (C) in mineral soil.

Multilevel Pattern Analysis (MPA) showed that eight and seven different fungal OTUs were indicator species significantly associated with the roots of *P. abies* and *Larix* sp., respectively. Among these, *Pyronemataceae* sp. (0.47,  $p = 0.013$ ) and *Russula vinosa* (0.53,  $p = 0.002$ ) showed a strong association with *P. abies* roots, while *Mycena galopus* (0.62,  $p = 0.0002$ ), *Vestigium trifidum* (0.53,  $p = 0.002$ ) and *Helotiaceae* sp. 5211\_61 (0.50,  $p = 0.007$ )

showed a strong association with *Larix* sp. roots (Table S2). Interestingly, *M. galopus* was among the most abundant OTUs associated with both *Larix* sp. and *P. abies* roots (Table 2). Differently from *M. galopus*, *Mycena cinerella* showed a strong association with the roots of *Larix* sp. but not with the roots of *P. abies*. Unidentified sp. 5211\_606 showed a strong association exclusively with *P. abies* roots. Fourteen and twelve fungal OTUs showed a significant association with the organic soil of *P. abies* and *Larix* sp., respectively (Table S2). Among these, Unidentified sp. 5211\_827 (0.52,  $p = 0.004$ ), Unidentified sp. 5211\_130 (0.47,  $p = 0.02$ ) and *Thysanophora penicillioides* (0.46,  $p = 0.01$ ) showed a strong association with the organic soil of *P. abies*, while Unidentified sp. 5211\_1651 (0.44,  $p = 0.04$ ), Unidentified sp. 5211\_1895 (0.44,  $p = 0.04$ ) and *Trechispora bryssinella* (0.42,  $p = 0.04$ ) showed a strong association with the organic soil of *Larix* sp. There were 45 and 4 fungal OTUs, significantly associated with the mineral soil of *Larix* sp. and *P. abies*, respectively. Among these, *Malassezia globosa* (0.53,  $p = 0.003$ ), *Wallemia sebi* (0.51,  $p = 0.002$ ), *Alternaria alternata* (0.50,  $p = 0.002$ ) and *Wilcoxina* sp. (0.50,  $p = 0.007$ ) showed a strong association with the mineral soil of *Larix* sp., while *Cytospora sophoricola* (0.53,  $p = 0.003$ ), *Parmeliaceae* sp. (0.51,  $p = 0.02$ ), *Paraconiothyrium* sp. (0.40,  $p = 0.02$ ) and *Mortierella* sp. (0.40,  $p = 0.04$ ) showed a strong association with the mineral soil of *P. abies*. The remaining fungal OTUs, which showed a significant association with a certain substrate of either tree species, are shown in Table S2.

#### 4. Discussion

The results showed that the fungal species richness associated with the roots and organic and mineral soils of *P. abies* and *Larix* sp. was similar between corresponding substrates, showing that both tree species provide habitats for and support a similar biodiversity of belowground fungal communities (Table 1). Interestingly, the species richness, the Shannon diversity index and the number of unique fungal OTUs was higher in the organic and mineral soils than in the roots of each tree species (Table 1, Figure 2), showing different capacities of each of these habitats to support fungal biodiversity. In agreement with this, fungal species richness in the soil was shown to be particularly high compared with terrestrial plant tissues including roots [56,57]. In general, soil fungi can occupy different ecological niches depending on the available resources [58]. Organic and mineral nutrients present in the soil create favorable conditions for fungal activities such as decomposition and nutrient assimilation [59,60]. With a high diversity and complexity of fungal communities in the soil, the rate of decomposition and the release of nutrients increases [61], which also stimulates the uptake of nutrients by plants [62,63]. These factors promote tree viability and growth, at the same time making them more tolerant to abiotic and biotic stress factors [11].

The results also showed that in different substrates, the fungal community composition largely overlapped between *P. abies* and *Larix* sp. (Figure 6) even though PerMANOVA showed that the fungal communities in the roots differed between the two tree species. This may suggest that *Larix* sp. can provide habitats to a great number of rhizosphere fungi associated with *P. abies*. Therefore, a more extensive planting of *Larix* sp. and a partial replacement of *P. abies* stands can be expected to support indigenous soil fungal communities, which are adapted to local environmental conditions and provide important ecosystem services. The difference in the composition of fungal communities in the roots of *P. abies* and *Larix* sp. was likely due to the fact that certain fungal OTUs were host-specific (Figure 7A). It is known that the structural and functional diversity of rhizosphere fungal communities can be affected by the tree species [13] even though the rhizosphere is a biologically active zone where roots and soil fungal communities interact [12]. Several studies involving multiple plant hosts have indicated that fungal specificity patterns display a host-associated phylogenetic signal [64–67]. Alternatively, Molina et al. (1992) [68] showed that host–fungal compatibility may be influenced by environmental factors, a phenomenon referred to as ecological specificity. In a broad sense, specificity simply refers to nonrandom host–symbiont associations between compatible partners, which is more

commonly termed as host preference when specificity is nonexclusive. In a strict sense, host specificity is defined as exclusive host–symbiont associations, which are probably governed by co-evolutionary events [69]. The observed patterns could also be influenced by specific characteristics of roots as the amount of root biomass, root architecture and root exudates vary among different tree species [70]. Thus, variations in root fungal communities may have also been determined by differences in the root systems of *P. abies* and *Larix* sp. trees explored by Da Ronch et al. (2016) [31].

This study also revealed a number of strong associations between certain fungal OTUs and specific substrates of *P. abies* and/or *Larix* sp. For example, several *Mycena* fungi showed significant associations with the roots of both *P. abies* and *Larix* sp. (Table S2). *Mycena* is considered as one of the largest genera in Agaricales (over 500 species), which is widespread across habitats and climate zones, possessing a saprobic and/or endophytic lifestyle [71]. However, Thoen et al. (2020) [72] showed that multiple species of *Mycena* can colonize tree roots, e.g., of *Betula pendula* seedlings in vitro, and form a gradient of interactions from harmful to neutral or beneficial, with some species or strains being able to transfer nutrients to the plant host. Prior to this, representatives of *Mycena* were known primarily as quantitatively important litter and wood debris decomposers [9,73,74]. Harder et al. (2023) [71] showed that *M. galopus*, *M. leptoccephala*, *M. epipterygia*, *M. sanguinolenta* and *M. metata* were fungi that frequently invaded plant roots. In the present study, *M. galopus* was among the most common fungi detected in the roots of *P. abies* and *Larix* sp. (Table 2, Figure 4), but *M. galopus*, *M. cinerella* and *M. sanguinolenta* showed a significant association with *Larix* sp. roots, while *M. rebaudengoi* showed a significant association with *P. abies* roots (Table S2). These results support previous observations that different *Mycena* species possess the ability to invade living plant roots [75] and generally lack a host specificity, which was also reported in other studies [71,76].

Saprotrophs and symbiotrophs were found to be the most common trophic modes, while ECM fungi represented one of most abundant fungal guilds in the different substrates of both *P. abies* and *Larix* sp. (Figure 5). Most European forest trees live in symbiosis with ECM fungi, which facilitate both nutrient and water uptake, increase resistance to certain root diseases and enhance the stress tolerance of these trees. As studies on ECM fungi in mid-age or mature larch stands are scarce [77], the present study provided new information on this group of fungi. For example, ECM fungi *Wilcoxina mikolae* and *Wilcoxina* sp. were significantly associated with the mineral soil of *Larix* sp. (Table S2). Leski et al. (2008) [77] demonstrated that *W. mikolae* was the most common ECM symbiont colonizing roots of one-year-old seedlings of *Larix decidua* in bare-root forest nurseries. *Wilcoxina* spp. are known to be common colonizers of nursery-grown conifer seedlings including *P. abies* and *Larix* sp. [77–81]. Several other ECM fungi were also detected including fungi from genera *Amphinema*, *Cenococcum*, *Piloderma*, *Russula* and *Suillus* (Table S3). *Amphinema byssoides* was reported as an important ECM symbiont of *P. abies* seedlings [82], but the composition and abundance of ECM fungi may change with tree age [40]. *Piloderma* sp. and *C. geophilum* were reported as ECM fungi that are distributed through numerous habitats, environments and geographic regions and associated with a large variety of host species including gymnosperms and angiosperms [83]. In the present study, *Piloderma* sp. showed a high relative abundance (Tables 2 and S3) and was significantly associated with the mineral soil of *Larix* sp. (Table S2). It appears that specific species of *Piloderma* can be associated with different host trees, having different roles in different forest ecosystems. Differently from *Piloderma* and *Cenococcum*, members of the genus *Suillus* form ECM associations almost exclusively with members of the Pinaceae family (*Pinus*, *Picea*, *Larix*, *Pseudotsuga* and *Abies*) and display a high degree of host specificity ([67,84]. Magrit et al. (2010) [40] reported that *Suillus* spp. were among mycobionts especially important for the development of larch seedlings. *Russula* spp. were shown to be able to form ECM symbiosis with many plant species in a broad range of plant families, including Leguminosae, Fagaceae, Dipterocarpaceae and Pinaceae [85]. In the present study, *Russula adusta* was significantly associated with *Larix* sp. roots, while *Russula vinosa* was significantly

associated with *P. abies* roots (Table S2), supporting findings by Wang et al. (2015) [85] that the reproductive biology of *Russula* and other ECM fungi likely vary among different species of the same genus and among ecological niches. Other more common ECM fungi were detected at a variable abundance in different substrates of both tree species, showing a low host specificity. Taken together, the results showed a high relative abundance and varying specificity of different ECM fungi, thereby showing their adaptation and potential importance for tree growth and nutrition in the investigated mid-age plantations of *P. abies* and *Larix* sp.

This study also revealed the presence of several tree pathogens (Table S3). For example, *Armillaria cepistipes* was detected at a similar relative abundance in the roots of *P. abies* and *Larix* sp. (Table S3). *Armillaria* root rot is a major disease of woody plants, which may significantly affect the structure and function of forest ecosystems. *A. cepistipes* is among the most common *Armillaria* species, which also include *A. borealis*, *A. ostoyae*, *A. mellea*, *A. gallica* and *A. tabescens*, infecting living trees in Europe. Due to the lack of direct evidence, *A. cepistipes* is generally considered to behave similarly to *A. gallica*, a species that is categorized, according to field observations and inoculation experiments, as a weak pathogen [86,87]. *A. cepistipes* appears to have a low host specificity and to be a weak pathogen likely to have a low impact on stands of both *P. abies* and *Larix* sp. By contrast, the root rot pathogen *Heterobasidion annosum* showed a preference for the roots of *P. abies* (Table S3), which was also reported in other studies, e.g., [88]. Other more commonly detected pathogens included species such as *Sydowia polyspora*, *Ganoderma applanatum*, *Gemmamyces piceae*, *Fusarium saloni* and *Rhizosphaera kalkhoffii*, which can cause diseases to conifers including *P. abies* and *Larix* sp. However, these pathogens generally showed a low host specificity and were also found at low relative abundances in the different substrates of *P. abies* and *Larix* sp. (Table S3), indicating that the investigated forest stands were relatively healthy.

## 5. Conclusions

The richness of fungal OTUs associated with the roots and organic and mineral soils was similar between *P. abies* and *Larix* sp., showing that both tree species provide habitats for and support a similar biodiversity of belowground fungal communities. The fungal community composition in the organic and mineral soils of *P. abies* and *Larix* sp. was similar, while in the roots, several fungal OTUs were specific to either *P. abies* or *Larix* sp., indicating the potential importance of fungi associated with the roots of each tree species. Nevertheless, the composition of fungal communities was strongly dependent on the substrate, showing the importance of their different ecological functions in forest ecosystems. The results may, therefore, suggest that *Larix* sp. can provide habitats to a large number of rhizosphere fungi associated with *P. abies*, and that a more extensive planting of *Larix* sp. and a partial replacement of *P. abies* stands should not restrict the development of indigenous root and soil fungal communities.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d16030160/s1>, Table S1: Relative abundance (%) of fungal OTUs detected in roots, organic and mineral soil of *Picea abies* and *Larix* sp. study sites. Within each tree species, different study sites are combined.; Table S2: Association among fungal taxa and different group samples (roots, organic and mineral soil) of *Picea abies* and *Larix* sp. trees according to Multilevel Pattern Analysis (MPA of indicator species), significance level (alpha): 0.05.; Table S3: Relative abundance (%) of the 15 most common ectomycorrhizal and the 15 most common plant pathogenic fungal OTUs in roots and organic and mineral soil of *Picea abies* and *Larix* sp. Within each tree species, different study sites are combined.

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