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DNA-Metabarcoding of Belowground Fungal Communities in Bare-Root Forest Nurseries: Focus on Different Tree Species

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Abstract: The production of tree seedlings in forest nurseries and their use in the replanting of clear-cut forest sites is a common practice in the temperate and boreal forests of Europe. Although conifers dominate on replanted sites, in recent years, deciduous tree species have received more attention due to their often-higher resilience to abiotic and biotic stress factors. The aim of the present study was to assess the belowground fungal communities of bare-root cultivated seedlings of *Alnus glutinosa*, *Betula pendula*, *Pinus sylvestris*, *Picea abies* and *Quercus robur* in order to gain a better understanding of the associated fungi and oomycetes, and their potential effects on the seedling performance in forest nurseries and after outplanting. The study sites were at the seven largest bare-root forest nurseries in Lithuania. The sampling included the roots and adjacent soil of 2–3 year old healthy-looking seedlings. Following the isolation of the DNA from the individual root and soil samples, these were amplified using ITS rRNA as a marker, and subjected to high-throughput PacBio sequencing. The results showed the presence of 161,302 high-quality sequences, representing 2003 fungal and oomycete taxa. The most common fungi were *Malassezia restricta* (6.7% of all of the high-quality sequences), *Wilcoxina mikolae* (5.0%), *Pustularia* sp. 3993_4 (4.6%), and *Fusarium oxysporum* (3.5%). The most common oomycetes were *Pythium ultimum* var. *ultimum* (0.6%), *Pythium heterothallicum* (0.3%), *Pythium spiculum* (0.3%), and *Pythium sylvaticum* (0.2%). The coniferous tree species (*P. abies* and *P. sylvestris*) generally showed a higher richness of fungal taxa and a rather distinct fungal community composition compared to the deciduous tree species (*A. glutinosa*, *B. pendula,* and *Q. robur*). The results demonstrated that the seedling roots and the rhizosphere soil in forest nurseries support a high richness of fungal taxa. The seedling roots were primarily inhabited by saprotrophic and mycorrhizal fungi, while fungal pathogens and oomycetes were less abundant, showing that the cultivation practices used in forest nurseries secured both the production of high-quality planting stock and disease control.

Keywords: fungal diversity; community composition; ectomycorrhiza; pathogens; oomycetes; tree seedlings

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1. Introduction

In Europe, forest tree planting has increased considerably in recent decades [1,2], thereby increasing the demand for planting stock. The increased planting is primarily due to the commitment in many countries to increase the forest area and/or to rehabilitate degraded forest ecosystems, and thus to reclaim disturbed sites for forestry. This also allows us to maintain and increase biodiversity, and to mitigate the negative effects of global climate change [2,3]. In European forests, tree planting is a principal reforestation practice that also contributes to the sustainability and productivity of forests. Nowadays, most of the planting stock, which is used in forestry, is produced in forest nurseries [4]. The quality of the seedlings produced is one of the critical factors that contributes to successful

forest restoration programs [5]. Although the seedling quality may depend on different factors, the associated microbial communities can be of key importance, and may determine the success of the seedlings' survival, establishment and growth after outplanting [6–8].

In nature, healthy and asymptomatic plants cohabit with diverse microbes that form complex microbial consortia and impact plant growth and productivity [9,10]. Several studies have reported a wide range of beneficial effects of microbiota members on plant health, including disease suppression [11,12], the priming of the plants' immune systems [13], the induction of systemic resistance [14], increased nutrient acquisition [15], increased tolerance to abiotic stresses [16], better adaptation to different environmental conditions [17], and the promotion of root mycorrhization [18]. Among the beneficial microbes, ectomycorrhizal (ECM) fungi are known to provide nutritional benefits to host trees, and may also mitigate negative effects of different abiotic and biotic factors [19]. For example, Zak [20] has postulated several mechanisms by which ECM fungi may provide disease protection to the feeder roots of plants. However, the conditions that often prevail in forest nurseries—namely confined space, high soil fertility, the use of fungicides, and abundant watering—may discourage the ECM colonization of seedling roots [21–23]. Such nursery conditions may select for opportunistic ECM fungal species, while limiting root colonisation by ECM fungi present in forest ecosystems [23]. In contrast to ECM fungi, pathogenic fungi or bacteria and plant nematodes may have a negative effect on seedling health, thereby causing abnormal growth or even mortality [24]. Infestations caused by oomycetes, including *Phytophthora* species, can be another threat to seedling production in forest nurseries. Jung et al. [25] demonstrated that nursery seedlings across Europe are commonly infested with a large array of *Phytophthora* spp. Nursery diseases may also have a negative effect on the field performance of outplanted seedlings. Besides this, nursery diseases may also be a threat to local forests when infected seedlings are outplanted, especially in areas where the disease was not present [26]. For example, nursery stock has been shown to be the most common means for the introduction of new *Phytophthora* species into landscapes and habitats worldwide [27,28]. Although many previous studies have linked *Phytophthora* diseases to ornamental plants [25,29–31], recent studies suggest that these are also found on native plants produced in forest nurseries [32–34].

Interactions between plants and soil microbes are highly dynamic in nature [35–38], and rhizosphere microbial communities may differ between different plant species [39–41], different genotypes within the species [42,43], and between the different developmental stages of a given plant [44,45]. For example, the assessment of fungal communities in the roots of healthy-looking *P. sylvestris* and *P. abies* seedlings in Swedish forest nurseries has showed the dominance of ECM and/or endophytic fungi, but has also revealed some differences in the fungal community composition between the two tree species [46]. Bzdyk et al. [47] demonstrated that the roots of nursery-grown *Fagus sylvatica* and *Q. robur* were inhabited by rather different communities of fungi. By contrast, Beyer-Ericson et al. [48] studied the root-associated fungi of diseased seedlings in Swedish forest nurseries, showing that the commonly-detected fungi were fungal pathogens, including species from the genera *Cylindrocarpon*, *Fusarium*, *Pythium*, *Botrytis*, *Alternaria* and *Ulocladium*. Similarly, in Norwegian forest nurseries, the roots of diseased seedlings were associated with pathogens from the genera *Pythium* and *Rhizoctonia* [49–51], while in Finnish forest nurseries, there were pathogens from the genera *Fusarium*, *Rhizoctonia*, *Pythium* and *Phytophthora* [52,53]. The above studies demonstrate that the root-associated fungal communities in forest nurseries may depend not only on the health status of the tree seedlings, but also on the tree species. Although fungal communities in the healthy and decaying roots of nursery-grown tree seedlings are relatively well understood, studies comparing the belowground fungal communities associated with different tree species are still scarce.

Incidences of locally-occurring, and especially of invasive, forest pathogens have increased exponentially in the last two centuries, causing extensive economic and ecological damage [54]. As nursery seedlings may also serve as a probable source for forest infestation [55–57], a better understanding of the fungal and oomycete communities as-

sociated with different tree species could be of considerable practical importance. The aim of the present study was to assess the belowground fungal communities of five principle bare-root cultivated tree species in the seven largest forest nurseries in Lithuania using DNA-metabarcoding in order to gain a better understanding of the beneficial and pathogenic fungi and oomycetes, and their potential effects on seedling performance in forest nurseries and after outplanting.

2. Materials and Methods

2.1. Study Site and Sampling

The study sites were at the seven largest bare-root forest nurseries in Lithuania, namely: Alytus (N 54°24′21.91″, E 24°2′33.27″), Anykščiai (N 55°34′15.07″, E 25°7′3.73″), Dubrava (N 54°50′11.42″, E 24°1′59.23″), Kaišiadorys (54°48′41.97″, 24°33′15.98″), Kretinga (N 56°1′29.82″, E 21°14′2.53″), Panevėžys (N 55°45′32.72″, E 24°30′28.33″) and Trakai (N 54°30′13.05″, E 24°49′46.97″). All of these forest nurseries were situated within a radius of 300 km. Information on each forest nursery, the climate within the area and the soil type is in Table 1. The sampling was conducted between April and May 2018, i.e., at the end of dormancy, but before the time of the seedlings' outplanting in the forest, by sampling the roots and adjacent soil of 2–3 year old healthy-looking seedlings of *A. glutinosa, B. pendula, P. sylvestris*, *P. abies* and *Q. robur*. The latter seedlings were selected as they represent the principal tree species in the given geographical area. The approximate seedling height was 20 cm for *P. sylvestris* and *P. abies*, 30 cm for *Q. robur,* and 50 cm for *B. pendula* and *A. glutinosa*. During the seedling cultivation in the forest nurseries, fungicides are not used, but fertilizers are applied according to the established routines.

Table 1. Characteristics of the seven largest bare-root forest nurseries in Lithuania that were sampled in the present study.

Forest Nursery	Area of the Nursery, ha	Total No. of Seedlings Produced, Millions	No. of Seedlings Produced by Tree Species, Millions					Mean Monthly Temperature, °C *	Mean Monthly Precipitation, mm *	Soil Type
			Alnus glutinosa	*Betula pendula*	*Picea abies*	*Pinus sylvestris*	*Quercus robur*			
Alytus	23.1	4.7	0.2	0.4	2.6	1.1	0.3	10.5	49.6	Nc
Anykščiai	34.0	5.6	0.3	0.3	4.6	0.2	0.2	10.1	38.2	Nc
Dubrava	56.3	7.1	0.3	0.2	5.2	1.2	0.2	10.2	64.8	Nc
Kaišiadorys	27.0	4.6	0.2	0.2	3.6	0.2	0.3	9.9	52.1	Nc
Kretinga	66.0	5.5	0.1	0.1	4.4	0.4	0.2	8.8	61.6	Nc
Panevėžys	66.0	8.8	0.4	0.3	7.4	0.3	0.5	9.8	46.0	Lc
Trakai	31.4	5.1	0.1	0.2	3.2	1.3	0.1	10.3	41.0	Nb

* The climate data is for the period of sampling, i.e., April–May 2018. the soil type: Nb—oligotrophic mineral soils of normal humidity; Nc—mesotrophic mineral soils of normal humidity; Lc—eutrophic gleyic sandy loam [58].

The sampling in each forest nursery included 10 seedling root systems of each tree species, and 10 soil samples that were collected in the vicinity of each collected root system. In total, 350 root samples (7 nurseries × 5 tree species × 10 root samples) and 350 soil samples (7 nurseries × 5 tree species × 10 soil samples) were collected. For the collection of the roots, seedlings were randomly selected, their roots were excavated, they were gently shaken to remove the larger particles of soil, and they were cut off from the shoots. The soil samples (ca. 100 g) were taken using a 2 cm diameter soil core down to a 20 cm depth. The soil core was carefully cleaned between taking different samples. The collected root and soil samples were individually placed into plastic bags, labelled, transported to the laboratory and stored at −20 °C before being further processed.

In the laboratory, the roots were carefully washed in tap water in order to remove any of the remaining soil, and the fine roots with root tips were separated from the coarse roots

(which were discarded). The fine roots were cut into ca. 1 cm-long segments, and each forest nursery and tree species was pooled together, resulting in a total of 35 root samples (7 nurseries × 5 tree species). The soil samples were sieved (mesh size 2 × 2 mm) in order to remove larger particles and roots, and each forest nursery and tree species was pooled together, resulting in a total of 35 samples (7 nurseries × 5 tree species).

2.2. DNA Isolation, Amplification and Sequencing

The principles of the DNA work followed the study by Marčiulynas et al. [59]. No surface sterilization of the roots was carried out. Prior to the isolation of the DNA, each sample (soil or roots) was freeze-dried (Labconco FreeZone Benchtop Freeze Dryer, Cole-Parmer, Vernon Hills, IL, USA) at −60 °C for two days. After the freeze-drying, ca. 0.03 g dry weight of each root sample was placed into a 2-mL screw-cap centrifugation tube together with glass beads. All of the samples were homogenized using a Fast prep shaker (Montigny-le-Bretonneux, France). The DNA from the roots was isolated using CTAB extraction buffer (0.5 M EDTA pH 8.0, 1 M Tris-HCL pH 8.0, 5 M NaCl, 3% CTAB) followed by incubation at 65 °C for 1 h. After the centrifugation, the supernatant was transferred to a new 1.5-mL Eppendorf tube, mixed with an equal volume of chloroform, centrifuged at 13,000 rpm for 8 min, and the upper phase was transferred to new 1.5-mL Eppendorf tubes. Then, an equal volume of 2-propanol was used to precipitate the DNA into a pellet by centrifugation at 13,000 rpm for 20 min. The pellet was washed in 500 µL 70% ethanol, dried, and dissolved in 50 µL sterile milli-Q water. Unlike the roots, ca. 1 g of freeze-dried soil was used for the isolation of the DNA from each sample using a NucleoSpin®Soil kit (Macherey-Nagel GmbH & Co. Duren, Germany) according to the manufacturer's recommendations. Following the isolation of the DNA, the DNA concentration in the individual samples was determined using a NanoDrop™ One spectrophotometer (Thermo Scientific, Rodchester, NY, USA). The amplification of the ITS rRNA region was achieved using a forward primer ITS6 [60] and barcoded universal primer ITS4 [61]. This primer pair was shown to amplify both fungi and oomycetes [60]. The polymerase chain reaction (PCR) was performed in 50 µL reactions, and consisted of the following final concentrations: 0.02 ng/µL template DNA, 200 µM dNTPs, 750 µM $MgCl_2$, 0.025 µM DreamTaq Green polymerase (5 U/µL) (Thermo Scientific, Waltham, MA, USA), and 200 nM of each primer; sterile milli-Q water was added in order to make up the final volume (50.0 µL) of the reaction. The amplifications were performed using a Applied Biosystems 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR program started with an initial denaturation step at 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, and annealing at 55 °C for 30 s and 72 °C for 1 min, followed by a final extension step at 72 °C for 7 min. The PCR products were assessed using gel electrophoresis 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). After the quantification of all of the PCR products using a Qubit fluorometer 4.0 (Life Technologies, Stockholm, Sweden), they were pooled in an equimolar mix and sequenced using a PacBio platform and one Sequel SMRT cell at a SciLifeLab facility in Uppsala, Sweden.

2.3. Bioinformatics

The sequences were filtered and clustered using the Sequence Clustering and Analysis of Tagged Amplicons (SCATA) next-generation sequencing (NGS) pipeline (http://scata.mykopat.slu.se). The sequences were filtered for quality, removing short sequences (<200 bp), sequences with low mean read quality (Q < 20), primer dimers, and homopolymers, which were collapsed to three base pairs (bp) before clustering. The sequences were screened for primers and sample-identifying barcodes, and those sequences that were missing a barcode or primer were removed. The sequences were clustered into different taxa by single linkage clustering, with a 2.0% maximum distance allowed for the sequences to enter the clusters. The fungal taxa were taxonomically classified using a Ribo-

somal Database Project (RDP) pipeline classifier (https://pyro.cme.msu.edu/index.jsp), and sequences showing <80% similarity to the phylum level were considered to be of non-fungal or oomycete origin, and were excluded from further analyses. All of the fungal and oomycete sequences were taxonomically identified using the GenBank (NCBI) database and the Blastn algorithm (Table S1). The following criteria were used for the identification: sequence coverage > 80%, similarity to taxon level 98–100%, and similarity to genus level 94–97%. Sequences not matching these criteria were considered unidentified, and were given unique names. Representative sequences of the fungal and oomycete non-singletons are available from GenBank under accession numbers MW214720–MW216333.

2.4. Statistical Analysis

The rarefaction analysis was performed using Analytical Rarefaction v.1.3, which is available at http://www.uga.edu/strata/software/index.html. The differences in the richness of the fungal and oomycete operational taxonomic units (OTUs) in the roots and soil among the different tree species were compared using the nonparametric chi-square test [62]. As each of the datasets was subjected to multiple comparisons, the confidence limits for the *p*-values of the chi-square tests were reduced the corresponding number of times required by the Bonferroni correction [63]. The Shannon diversity index, the qualitative Sørensen similarity index, and non-metric multidimensional scaling (NMDS) in Canoco v.5.02 (Microcomputer Power, Ithaca, NY, USA) [62,64] were used in order to characterise the diversity and composition of the fungal communities. A MANOVA in Minitab v. 18.1 (Pennsylvania State University, State College, Pennsylvania, PA, USA) was used in order to evaluate the degree of separation (along NMDS axes 1 and 2) between the fungal communities in the different types of samples (roots and soil) and among the different tree species. For each type of sample (roots or soil), the nonparametric Mann-Whitney test in Minitab was used to test whether the Shannon diversity index differed among the different tree species. The assignment of ecological roles was based on FUNGuild [65].

3. Results

A total of 305,139 sequences was generated by the PacBio sequencing. The quality filtering showed the presence of 161,302 (52.9%) high-quality sequences, while the remaining 143,837 (47.1%) low quality sequences were excluded from further analyses. The clustering of the high-quality sequences showed the presence of 3564 non-singleton contigs representing different OTUs. Singletons were excluded. The taxonomic classification showed that 2003 (56.2%) of the OTUs represented fungi (Table S1 and Table 2), while 1561 (43.8%) non-fungal OTUs were excluded. Among all of the fungal OTUs, 390 (19.5%) were identified to the species level, 345 (17.2%) were identified to the genus level, and 1268 (63.3%) were identified only to a higher taxonomic level.

Of the 2003 fungal OTUs (all of which identified at least to the phylum level, Table S1) across all of the soil and root samples, Ascomycota was the most dominant phylum, which accounted for 50.4% of all of the fungal OTUs, followed by Basidiomycota (31.4%), Mucoromycota (6.2%), Chytridiomycota (6.0%), Oomycota (3.4%) and Zoopagomycota (2.0%). Entorrhizomycota, Blastocladiomycota, Cryptomycota, Zygomycota, Olpidiomycota and Blastoclamidiomycota were also detected, but in a very low proportions (each < 0.2%). Among all of the fungal OTUs (different tree species and nurseries combined), 266 (13.3%) were exclusively found in roots, 589 (29.4%) were exclusively found in the soil, and 1148 (57.3%) were shared between both types of samples (Figure 1).

Table 2. Generated high-quality fungal sequences and the detected diversity of the fungal taxa in the roots and soil of the five different tree species from the seven forest nurseries in Lithuania. The data from the different forest nurseries are combined.

Samples	Tree Species	No. of Fungal Sequences/Taxa	Shannon Diversity Index H [a]
Roots	*Alnus glutinosa*	6979/691	2.16–4.33
	Betula pendula	2620/256	1.93–4.09
	Picea abies	12,532/591	1.75–4.28
	Pinus sylvestris	11,910/718	2.62–4.32
	Quercus robur	5270/453	2.14–4.23
Total Roots		39,311/1414	
Soil	*Alnus glutinosa*	4292/664	3.28–5.03
	Betula pendula	7525/836	2.57–5.20
	Picea abies	7961/873	3.28–4.89
	Pinus sylvestris	8745/881	3.56–4.69
	Quercus robur	3862/646	3.83–5.17
Total Soil		32,385/1737	
All		71,696/2003	

[a] The Shannon diversity index H column shows a variation among the different forest nurseries.

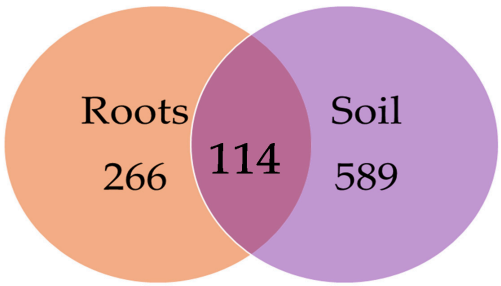

Figure 1. Venn diagram showing the diversity of the fungal OTUs found in the seedling roots and soil, and the number of fungal OTUs shared between both types of samples. The samples from different tree species and forest nurseries are combined.

Information on the 30 most common fungal OTUs—representing 62.03% of all of the high-quality fungal sequences in the roots and 38.95% of all of the high-quality fungal sequences in the soil of the different tree species—is in Table 3. The most common fungi (all samples combined) were *Malassezia restricta* (6.7% of all of the high-quality sequences), *Wilcoxina mikolae* (5.0%), *Pustularia* sp. 3993_4 (4.6%), *Fusarium oxysporum* (3.5%), *Tomentella* sp. 3993_7 (2.6%) and *Suillus luteus* (1.3%) (Table 3). Among the 30 most common fungal OTUs, five OTUs represented plant pathogens, including *F. oxysporum*, *Dactylonectria macrodidyma* (1.1%), *Fusarium solani* (0.9%), *Pestalotiopsis* sp. 3993_40 (0.8%), and *Fusarium* sp. 3993_41 (0.8%) (Table 3). The phylum Oomycota was represented by 68 OTUs, which comprised 1.5% of all of the high-quality sequences in the roots, and 3.2% of all of the high-quality sequences in the soil. Among these, there were 35 (51.5%) OTUs of *Pythium* spp., 4 (5.9%) of *Phytophthora* spp., 3 (4.4%) of *Phytopythium* spp., 2 (2.9%) of *Hyaloperonospora* spp., 2 (2.9%) of *Peronospora* spp., and 1 (1.5%) of *Pythiogeton* sp., while 21 (30.9%) could not be identified to the species or genus level (Table S1). Information on the 30 most common oomycete OTUs is in Table 4. The most common oomycetes were *Pythium ultimum* var. *ultimum* (0.6% of all of the high-quality sequences), *Pythium heterothallicum* (0.3%), *Pythium spiculum* (0.3%), *Pythium sylvaticum* (0.2%), *Pythium irregulare* (0.2%) and *Peronospora* sp. 3993_148 (0.1%) (Table 4).

Table 3. Occurrence and relative abundance of the 30 most common fungal OTUs (show as a proportion of all of the high-quality fungal sequences) in the roots and soil of the five tree species that were bare-root cultivated in the forest nurseries. The data from the different forest nurseries are combined.

OTU	Phylum	Reference	Similarity,% [a]	*Alnus glutinosa*		*Betula pendula*		*Picea abies*		*Pinus sylvestris*		*Quercus robur*		All	All	Total%
				Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	
Malassezia restricta	Basidiomycota	CP030254	727/728 (99)	5.79	3.59	8.02	4.96	6.57	2.85	10.43	6.00	20.75	0.85	9.01	4.05	6.70
Wilcoxina mikolae	Ascomycota	KU061020	592/593 (99)	0.11	0.09	0.38	0.07	22.28	0.95	3.82	1.46	0.88	0.08	8.82	0.67	5.03
Pustularia sp. 3993_4	Ascomycota	MF352743	513/518 (99)	-	0.26	0.15	4.52	7.50	0.04	15.53	0.03	0.82	-	7.56	1.10	4.56
Fusarium oxysporum	Ascomycota	GU136492	547/547 (100)	2.41	5.24	0.73	4.44	3.28	4.81	2.99	4.63	1.91	2.54	2.72	4.46	3.53
Tomentella sp. 3993_7	Basidiomycota	KX095160	648/663 (98)	0.01	0.05	-	0.07	14.20	0.15	0.07	0.01	0.15	0.00	4.80	0.06	2.60
Suillus luteus	Basidiomycota	KU721223	688/688 (100)	-	0.02	0.04	0.00	0.06	0.01	6.90	0.77	0.12	0.08	2.23	0.22	1.30
Mycoarthris corallina	Ascomycota	AH009124	467/473 (99)	6.53	0.44	0.57	9.13	0.02	0.82	0.06	0.11	0.09	0.16	1.30	2.43	1.82
Paraphaeosphaeria sporulosa	Ascomycota	KY977581	594/594 (100)	1.83	1.49	0.53	1.62	1.29	2.42	1.22	3.90	0.52	1.11	1.25	2.36	1.76
Chaetomium cochliodes	Ascomycota	KT895345	570/570 (100)	0.42	2.10	0.27	1.73	1.51	3.13	1.20	3.56	0.40	1.32	1.02	2.57	1.74
Pleotrichocladium opacum	Ascomycota	NR155696	545/549 (99)	0.44	0.40	28.66	0.54	0.80	0.78	0.42	0.29	0.79	0.54	2.57	0.51	1.61
Halenospora varia	Ascomycota	AJ608987	538/538 (100)	1.09	0.91	4.77	0.43	1.39	1.88	1.39	1.44	3.71	1.58	1.78	1.26	1.54
Tomentella sublilacina	Basidiomycota	HM189981	648/662 (98)	12.94	1.28	0.04	0.00	0.06	-	0.03	0.01	0.18	0.05	2.47	0.18	1.41
Umbelopsis vinacea	Mucoromycota	KC489498	628/633 (99)	0.46	0.91	0.42	0.33	1.39	3.49	1.11	2.53	0.27	0.13	0.96	1.75	1.33

Table 3. *Cont.*

OTU	Phylum	Reference	Similarity,% [a]	*Alnus glutinosa*		*Betula pendula*		*Picea abies*		*Pinus sylvestris*		*Quercus robur*		All	All	Total%
				Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	
Cladosporium cladosporioides	Ascomycota	MG664765	547/547 (100)	4.28	0.70	1.22	4.16	0.22	0.14	0.49	0.27	1.12	0.54	1.22	1.23	1.22
Suillus granulatus	Basidiomycota	AJ272409	677/678 (99)	-	-	-	-	0.01	-	6.83	0.01	0.03	-	2.18	0.00	1.17
Solicoccozyma terricola	Basidiomycota	KY558367	641/641 (100)	0.50	2.89	0.15	1.10	0.26	3.52	0.29	1.53	0.12	1.84	0.29	2.14	1.15
Phialocephala fortinii	Ascomycota	AY078131	560/561 (99)	0.96	0.19	0.27	0.09	1.61	1.42	2.34	0.35	1.22	0.05	1.59	0.50	1.08
Tuber sp. 3993_24	Ascomycota	KT215193	646/646 (100)	0.03	0.02	0.04	0.00	2.35	0.06	2.64	0.00	5.20	0.03	2.10	0.02	1.13
Dactylonectria macrodidyma	Ascomycota	JN859422	541/541 (100)	0.63	2.10	0.38	0.69	0.46	0.90	2.17	0.89	1.22	1.04	1.10	1.03	1.06
Mortierella sp. 3993_26	Mucoromycota	KP311420	641/641 (100)	0.99	1.10	0.31	0.98	0.78	1.53	0.97	1.02	0.30	1.97	0.80	1.26	1.02
Saitozyma podzolica	Basidiomycota	KY320605	511/511 (100)	1.00	1.56	0.11	1.48	0.34	1.75	0.26	1.97	0.21	1.14	0.41	1.65	0.99
Unidentified sp. 3993_29	Ascomycota	FN393100	546/550 (99)	0.34	0.12	0.34	0.56	1.33	0.40	1.90	1.01	0.91	0.41	1.22	0.57	0.92
Pseudogymnoascus sp. 3993_33	Ascomycota	KY977601	562/562 (100)	0.19	3.84	3.21	1.01	0.33	1.39	0.22	0.59	0.46	0.67	0.48	1.33	0.87
Penicillium sp. 3993_47	Ascomycota	MK226541	579/579 (100)	0.29	2.54	0.04	1.05	0.29	1.04	0.43	2.24	0.12	0.80	0.30	1.54	0.87
Mortierella sp. 3993_38	Mucoromycota	HG935763	640/640 (100)	1.43	1.77	-	0.74	0.34	1.42	0.39	1.36	0.15	1.29	0.52	1.28	0.87
Trichoderma crassum	Ascomycota	NR134370	610/610 (100)	0.97	0.98	0.15	0.74	0.51	0.88	0.79	1.22	1.64	0.70	0.76	0.93	0.84

Table 3. *Cont.*

OTU	Phylum	Reference	Similarity,% [a]	*Alnus glutinosa*		*Betula pendula*		*Picea abies*		*Pinus sylvestris*		*Quercus robur*		All	All	Total%
				Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	Roots%	Soil%	
Fusarium solani	Ascomycota	MF782768	562/562 (100)	0.14	3.87	0.08	1.09	0.31	1.65	0.31	0.82	0.12	1.45	0.25	1.57	0.86
Pestalotiopsis sp. 3993_40	Ascomycota	KT963804	588/588 (100)	1.65	0.70	0.08	0.52	0.26	0.92	0.26	1.77	0.49	1.68	0.53	1.12	0.80
Fusarium sp. 3993_41	Ascomycota	MH550484	559/559 (100)	1.03	0.70	1.11	1.24	0.41	1.19	0.29	1.18	0.18	0.44	0.51	1.04	0.76
Sebacina sp. 3993_35	Basidiomycota	JX844771	641/643 (99)	0.03	0.00	0.27	0.03	3.67	0.00	0.06	0.48	0.06	-	1.28	0.14	0.75
Total of 30 OTUs				46.51	39.84	52.33	43.31	73.83	39.56	65.78	41.48	44.15	22.50	62.03	38.95	51.3

[a] Sequence similarity column shows base pairs compared between the query sequence and the reference sequence in the NCBI databases, with the percentage of the sequence similarity in parentheses.

Table 4. Occurrence and relative abundance of the 30 most common oomycete OTUs (shown as a proportion of all of the high-quality fungal sequences) in the roots and soil of the five tree species that were bare-root cultivated in the forest nurseries. The data from the different forest nurseries are combined.

OTU	Reference	Similarity % [a]	*Alnus glutinosa*		*Betula pendula*		*Picea abies*		*Pinus sylvestris*		*Quercus robur*		All	All	Total %
			Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	
Pythium ultimum var. *ultimum*	AY598657	917/917 (100)	0.258	0.559	0.076	1.435	0.048	0.829	0.067	1.349	0.030	1.554	0.094	1.161	0.59
Pythium heterothallicum	AY598654	882/889 (99)	0.143	0.210	0.076	0.545	-	0.251	0.008	0.812	0.030	0.829	0.038	0.534	0.27
Pythium spiculum	HQ643790	972/978 (99)	0.072	0.047	0.038	0.040	0.032	0.088	1.243	0.114	-	-	0.423	0.068	0.26
Pythium sylvaticum	AY598645	997/999 (99)	0.172	0.326	0.496	0.385	0.088	0.138	0.243	0.057	0.030	0.466	0.177	0.238	0.21
Pythium irregulare	AY598702	1026/1029 (99)	0.043	0.280	0.076	0.133	0.128	0.301	0.151	0.114	0.091	0.207	0.113	0.198	0.15

Table 4. *Cont.*

OTU	Reference	Similarity % [a]	*Alnus glutinosa*		*Betula pendula*		*Picea abies*		*Pinus sylvestris*		*Quercus robur*		All	All	Total %
			Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	
Peronospora sp. 3993_148	MF372507	803/852 (94)	-	-	-	-	-	-	-	-	2.765	-	0.244	-	0.13
Pythium intermedium	KU211482	957/959 (99)	-	0.489	0.038	0.133	-	0.038	0.059	0.057	-	0.026	0.021	0.124	0.07
Pythium amasculinum	AY598671	856/857 (99)	0.029	-	-	0.159	0.008	0.025	0.008	0.023	-	0.363	0.011	0.093	0.05
Pythium sp. 3993_349	KU211471	894/907 (99)	0.014	0.047	-	0.053	0.024	-	0.008	0.057	-	0.363	0.013	0.077	0.04
Unidentified sp. 3993_508	MF570293	101/119 (85)	0.029	0.047	0.038	0.040	0.008	0.050	0.025	0.069	0.030	0.104	0.021	0.059	0.04
Pythium acanthicum	AY598617	858/859 (99)	0.014	-	-	0.013	0.040	0.050	0.017	0.057	0.030	0.104	0.024	0.043	0.03
Pythium apiculatum	HQ643443	948/954 (99)	0.072	0.023	-	0.120	-	0.063	-	0.011	0.061	-	0.019	0.049	0.03
Pythium rostratifingens	KU211363	1053/1064 (99)	0.115	0.023	0.038	0.066	-	-	-	0.034	0.030	0.104	0.027	0.040	0.03
Phytopythium citrinum	HM061322	852/857 (99)	-	-	0.496	-	-	-	0.008	-	0.030	-	0.040	-	0.02
Unidentified sp. 3993_709	KJ716873	724/865 (84)	-	-	-	-	0.016	-	0.109	0.011	-	-	0.040	0.003	0.02
Pythium pleroticum	AY598642	958/959 (99)	-	0.093	-	-	-	0.063	-	-	-	0.078	-	0.037	0.02
Pythium sp. 3993_1159	AY598639	940/966 (97)	0.086	-	-	-	-	0.075	-	-	-	-	0.016	0.019	0.02
Unidentified sp. 3993_729	HQ643756	226/240 (94)	-	-	-	-	0.064	0.013	0.008	0.023	-	-	0.024	0.009	0.02
Pythium rostratifingens	KU209835	962/969 (99)	-	0.047	-	0.053	-	0.050	-	-	-	-	-	0.031	0.01

Table 4. *Cont.*

OTU	Reference	Similarity % [a]	*Alnus glutinosa*		*Betula pendula*		*Picea abies*		*Pinus sylvestris*		*Quercus robur*		All	All	Total %
			Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	Roots %	Soil %	
Pythium sp. 3993_1163	AY598696	993/1036 (96)	0.043	-	-	-	-	0.013	-	0.034	-	0.078	0.008	0.022	0.01
Unidentified sp. 3993_943	MH671329	870/883 (99)	-	-	-	-	0.048	0.025	-	0.011	-	0.026	0.016	0.012	0.01
Unidentified sp. 3993_1191	KF318041	606/754 (80)	-	0.023	-	-	-	-	-	0.046	-	0.104	-	0.028	0.01
Phytophthora fragariae	KJ755093	896/905 (99)	0.029	0.116	0.038	-	-	-	-	-	-	-	0.008	0.015	0.01
Phytophthora pseudosy-ringae	EU074793	838/848 (99)	-	-	-	-	-	-	-	0.011	-	0.181	-	0.025	0.01
Pythium violae	AY598717	1001/1006 (99)	0.043	0.023	-	0.013	-	0.025	-	0.011	-	-	0.008	0.015	0.01
Unidentified sp. 3993_1954	LC176476	143/157 (91)	0.057	-	-	0.053	-	-	-	-	-	-	0.011	0.012	0.01
Unidentified sp. 3993_981	KF318041	602/754 (80)	-	0.023	-	0.040	-	-	-	-	-	0.104	-	0.025	0.01
Pythium sp. 3993_1117	JF431913	804/842 (95)	-	-	-	0.013	-	0.050	-	-	-	0.052	-	0.022	0.01
Unidentified sp. 3993_1171	KJ716873	797/854 (93)	0.014	-	-	0.040	-	-	-	-	-	0.078	0.003	0.019	0.01
Hyaloperonospora brassicae	MG757782	940/943 (99)	-	-	-	-	-	-	0.050	-	-	-	0.016	-	0.01
Total of 30 OTUs			1.232	2.377	1.412	3.336	0.503	2.148	2.007	2.905	3.130	4.816	1.414	2.977	2.14

[a] Sequence similarity column shows base pairs compared between the query sequence and the reference sequence in the NCBI databases, with the percentage of the sequence similarity in parentheses.

The rarefaction analysis showed that the number of fungal and oomycete OTUs did not reach species saturation (Figure 2). When the same number of fungal and oomycete sequences had been taken, the richness of the fungal and oomycete OTUs was significantly lower in the roots than in the soil (tree species and nurseries combined) ($p < 0.0001$) (Figure 2A). A similar comparison among the different tree species (root and soil data combined) showed that the deciduous tree species (*A. glutinosa*, *B. pendula* and *Q. robur*) had a significantly lower richness of fungal and oomycete OTUs taxa compared to the coniferous tree species (*P. abies* and *P. sylvestris*) ($p < 0.0001$) (Figure 2B). A higher variation in the richness of the fungal and oomycete OTUs was observed when the root and soil samples were analysed separately (nurseries combined) (Figure 2C,D). For example, in the roots, the richness of the fungal and oomycete OTUs was significantly lower compared *B. pendula* vs. other tree species ($p < 0.0001$), *Q. robur* vs. *A. glutinosa*, *P. abies* or *P. sylvestris* ($p < 0.0001$), and *P. abies* vs. *A. glutinosa* or *P. sylvestris* ($p < 0.02$) (Figure 2C). In a similar comparison, *P. sylvestris* and *A. glutinosa* did not differ significantly from each other ($p > 0.05$) (Figure 2C). In the soil, the richness of the fungal and oomycete OTUs was significantly lower compared to *A. glutinosa* vs. other tree species ($p < 0.0001$) (except *A. glutinosa* vs. *Q. robur*, $p > 0.05$) and *Q. robur* vs. *B. pendula*, *P. abies* or *P. sylvestris* ($p < 0.0001$), while *B. pendula*, *P. abies* and *P. sylvestris* did not differ significantly from each other ($p > 0.05$) (Figure 2D).

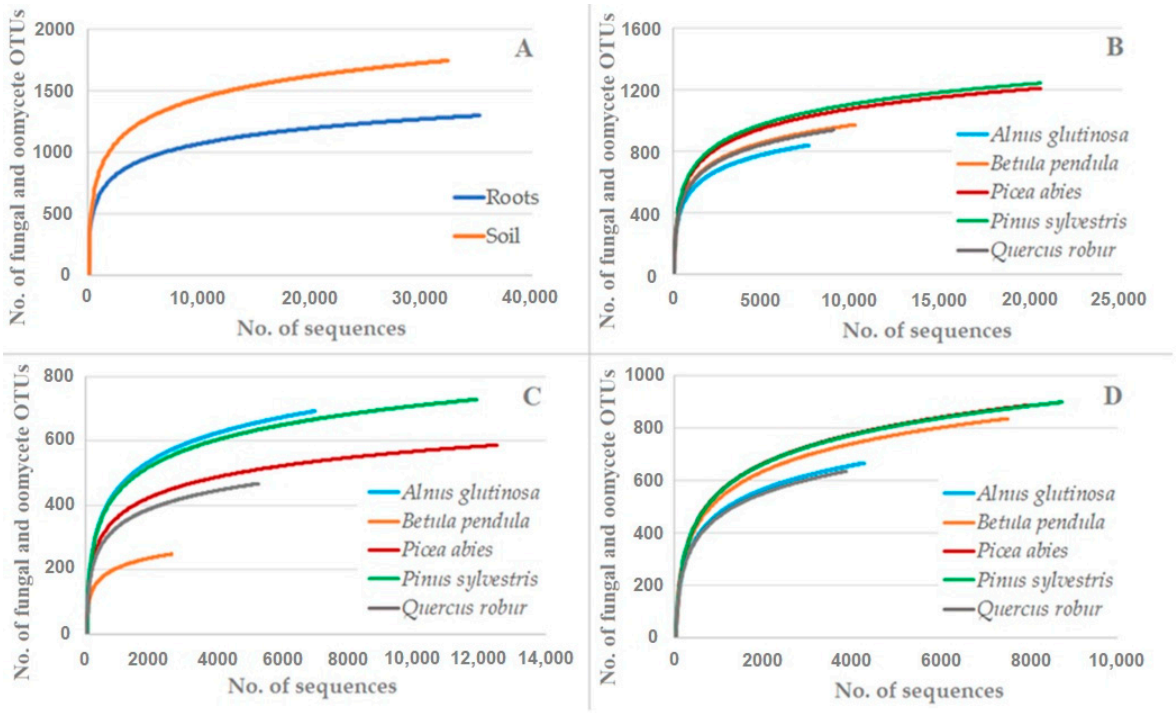

Figure 2. Rarefaction curves showing the relationship between the cumulative number of fungal and oomycete OTUs and the number of ITS rRNA sequences compared: (**A**) roots vs. soil; (**B**) the different tree species (roots and soil samples combined); (**C**) root samples of the different tree species; and (**D**) soil samples of the different tree species.

The non-metric multidimensional scaling of the fungal and oomycete communities showed a partial separation of the root and soil samples (tree species and nurseries combined) (Figure 3). The MANOVA showed that this separation was statistically significant ($p < 0.0001$) (Figure 3). For both the root and soil samples, a higher degree of separation of the fungal communities was between the coniferous tree species (*P. abies* and *P. sylvestris*) and the deciduous tree species (*A. glutinosa*, *B. pendula* and *Q. robur*) (Figure 4A,B). The MANOVA showed that the fungal and oomycete communities in the roots of *P. abies* and *P. sylvestris* differed significantly from those in *A. glutinosa* and *B. pendula* ($p < 0.0001$), while the fungal community in the roots of *Q. robur* were similar to all of the other tree species

(Figure 4A). A similar comparison of fungal communities between *P. abies* and *P. sylvestris*, and between *A. glutinosa* and *B. pendula* showed that these did not differ significantly from each other ($p > 0.05$), respectively. In the soil, the fungal communities did not differ significantly among the different tree species ($p > 0.05$) (Figure 4B).

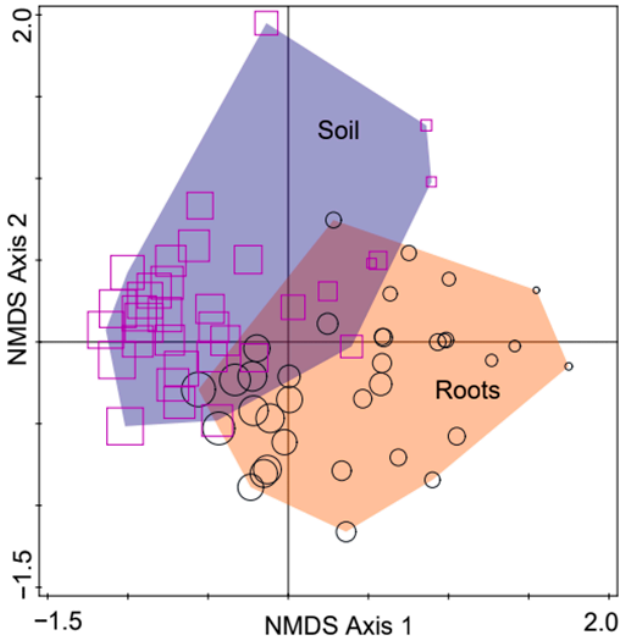

Figure 3. Nonmetric multidimensional scaling (NMDS) of the fungal and oomycete communities in the roots and soil of the five tree species grown in the forest nurseries. Each point (circles for the roots and squares for the soil) represents a separate sample of different tree species, and the size of each point reflects the relative richness of the fungal and oomycete OTUs. The NMDS of the fungal and oomycete communities explained 52.8% of the variation on Axis 1 and 26.8% of the variation on Axis 2.

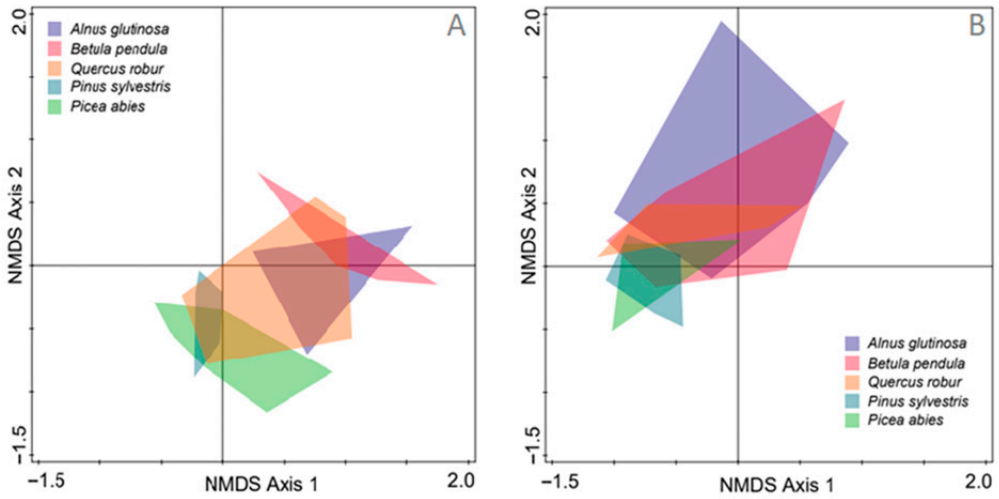

Figure 4. NMDS of the fungal and oomycete communities in the roots (**A**) and soil (**B**) of the five tree species grown in the forest nurseries. The data from the different forest nurseries are combined.

A total of 31 fungal classes was detected. A comparison among the different tree species showed that the relative abundance of fungal classes was more uniform among the soil samples than among the root samples (Figure 5). Nevertheless, in both the root and soil samples, *Sordariomycetes*, *Leotiomycetes*, *Dothideomycetes* and *Agaricomycetes* were the

most common (Figure 5). An exception was the class *Pezizomycetes*, which showed a high relative abundance in the roots of *P. abies* and *P. sylvestris*, while *Ustilaginomycotina_Incertae sedis* showed a high relative abundance in the roots of *Q. robur* (Figure 5).

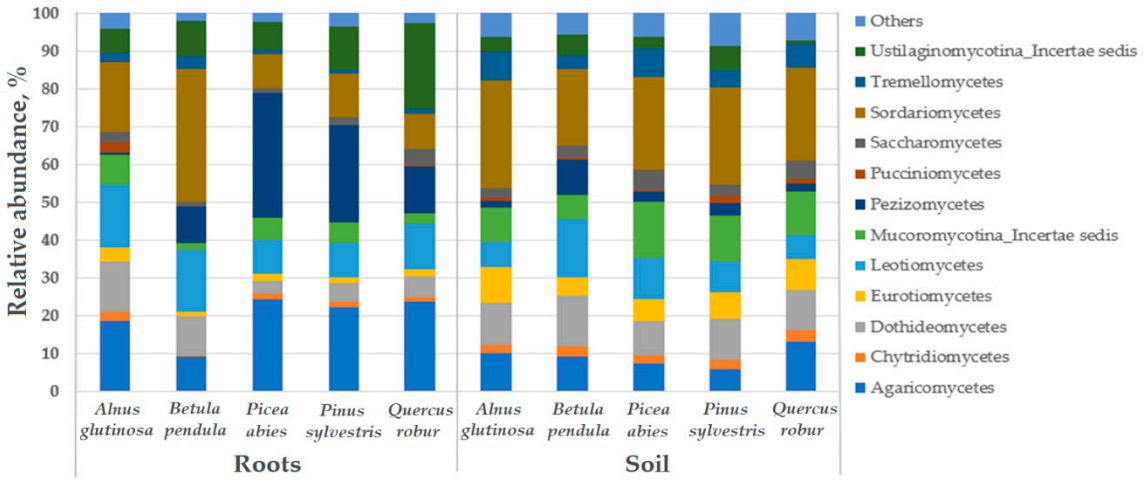

Figure 5. Relative abundance of the fungal classes in the roots and soil of the different tree species, and the other fungal classes that presented with a relative abundance of <1%. The data from the different forest nurseries are combined.

In the different tree species, the Sørensen similarity index of the fungal and oomycete communities was moderate, and ranged between 0.35 and 0.55 among the root samples, and between 0.43 and 0.56 among the soil samples. The Shannon diversity (H) index of the fungal and oomycete communities was high in both the root and soil samples (Table 2). The Mann-Whitney test showed that the Shannon diversity index did not differ significantly among the root or soil samples of the different tree species ($p > 0.05$). The assignment of fungal and oomycete ecological roles (nurseries combined) revealed a higher variation in the relative abundance among the root samples than among the soil samples of the different tree species (Figure 6). In the roots, the most common fungal and oomycete OTUs were of unknown ecological roles (23.9–46.1%, a range represents different tree species), followed by saprotrophs (19.6–37.7%), mycorrhizal fungi (5.7–46.6%), and pathogens (7.5–16.3%), and the least common were endophytes (1.9–4.5%) (Figure 6). Similarly, in the soil, the most common fungal and oomycete OTUs were unknown (34.7–41.2%), followed by saprotrophs (31.2–40.7%), pathogens (17.6–24.2%), and endophytes (1.8–3.6%), and the least common were mycorrhizal fungi (1.2–3.8%) (Figure 6).

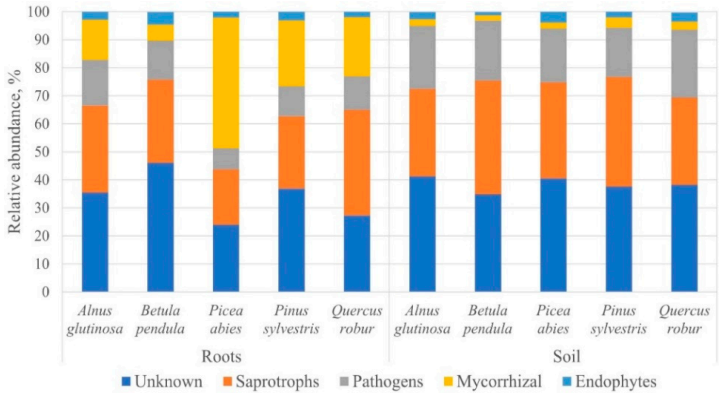

Figure 6. Ecological roles (shown as a proportion of the high-quality sequences) of the fungal and oomycete OTUs detected in the roots and soil of the different tree species. The data from the different forest nurseries are combined.

4. Discussion

In the present study, a comparison of the five economically-important tree species cultivated under similar conditions in bare-root forest nurseries provided valuable insights into the specificity of the associated fungal and oomycete OTUs. Firstly, the results demonstrated that the seedling roots and the rhizosphere soil were inhabited by a high diversity of fungal and oomycete OTUs (Figures 1 and 2, Table 2), thereby corroborating previous observations that the belowground habitat in forest nurseries supports species-rich communities of fungi [66]. Interestingly, the detected richness of the fungal OTUs can be comparable to those present in the forest stands of the same geographical area [59], even though the rarefaction analysis showed that the observed richness of fungal OTUs can potentially be higher with increased sequencing effort (Figure 2). Secondly, our results revealed that the diversity and composition of the fungal and oomycete communities were partly dependant on the substrate (roots or soil) and/or on the host tree species (Figures 1–5). As a result, there was generally a higher richness of fungal and oomycete OTUs in the soil than in the seedling roots, which was probably due to a higher heterogeneity of the soil environment compared to the roots [67], even though the intensive soil preparations (e.g. plowing and harrowing) in bare-root forest nurseries may have a homogenising effect on the soil's fungal communities (Figures 4–6). The segregation of the fungal and oomycete communities between the root and soil samples (Figure 3) was likely influenced by the host tree species, owing to a higher degree of modification of the associated fungal and oomycete communities in the roots than in the soil. In agreement with this, several studies have shown that plants may modify the structure of the associated microbial communities in their roots [68,69]. By contrast, the fungal communities in the soil can only be indirectly controlled by plants through the release of organic compounds that may contribute to the unique rhizosphere nutrient pool which is accessible to the soil microorganisms [70–72].

The coniferous tree species (*P. abies* and *P. sylvestris*) generally showed a higher richness of fungal and oomycete OTUs, and a rather distinct community composition compared to the deciduous tree species (*A. glutinosa*, *B. pendula* and partially *Q. robur*) (Figures 2 and 4). These distinctions were more apparent among the fungal and oomycete communities in the roots, but were expressed less in the soil (Figures 4–6). As certain root-associated fungi can be host-dependent, and some can even be host-specific [73–77], this demonstrates the relative importance of the host [78]. For example, Ishida et al. [79] showed that taxonomically-close host species harbour similar communities of mycorrhizal fungi. In agreement with this, it appears that mycorrhizal fungi play a key role in shaping the fungal communities in the roots of different tree species, as the abundance of fungi of other ecological roles was rather similar among the different tree species and substrates (roots or soil) (Figure 6). The latter may suggest that fungi of unknown ecological roles, saprotrophs, pathogens and endophytes generally possess a lower host or substrate specificity than mycorrhizal fungi; thus, the former were likely often represented by fungal generalists. Furthermore, in agreement with the previous studies on fungal communities in forest nurseries [7,46,80], the results have shown the dominance of fungal OTUs belonging to Ascomycota (50.4%) and Basidiomycota (31.4%). A higher relative abundance of ascomycetes in the soil and roots from all of the forest nurseries may reflect their better adaptation to a highly-transformed forest nursery environment compared to basidiomycetes. Indeed, members of Ascomycota have been shown to dominate on sites following site disturbances [81].

Among the dominant fungi, *Malassezia restricta* was shown to be exceedingly widespread and ecologically diverse in the environmental samples [82]. It was found in deep-sea sediments [83], hydrothermal vents [84], stony corals [85], Antarctic soils [86,87], on the exoskeleton of soil nematodes [88], and on various plant roots [89]. A recent study has also indicated that *M. restricta* is one of the most frequently-occurring species in the irrigation water of forest nurseries [59]. Despite many investigations, remarkably little is known about the impact of *M. restricta* on plant health. *Wilcoxina mikolae*, which was the second most commonly-detected fungus, was found in different forest nurseries, tree species

and substrates (roots and soil) (Table 3), thereby showing a broad ecological niche. In agreement with this, this mycorrhizal symbiont was commonly reported in association with the roots of forest nursery seedlings [90,91]. Besides this, it was shown that *Wilcoxina* fungi can reduce the negative effect of salt stress on the plants [92] and support tree growth in high-altitude marginal habitats [93]. Its common occurrence in forest nurseries and on different hosts raises the question of its potential effect on seedling performance in forest nurseries, but such information is scarce. For example, Smaill and Walbert [94] showed that, on the roots of *Pinus radiata* seedlings, the abundance of *Wilcoxina* increases with increased applications of fertilizers and fungicides. Jones et al. [95] found that seedlings colonised by *Wilcoxina* showed an increased accumulation of ^{15}N. *Suillus luteus* was another mycorrhizal fungus commonly detected in forest nurseries (Table 3). *Suillus* spp. are known as pioneer fungi, occurring in association with *Pinus* spp. in forest nurseries and in newly established forest plantations [6,96]. *Pinus sylvestris* seedlings inoculated with *S. luteus* were shown to have significantly better survival and growth rates after outplanting compared to controls [97], thereby demonstrating that this fungus can benefit the host trees.

Although the fungal communities were dominated by saprotrophs (Figure 6, Table S1), pathogens were also detected, indicating their potential threat to the plants. *Fusarium oxysporum* was the most commonly detected pathogen (Table 3), and it is known as one of the most destructive soil-borne pathogens, causing seedling diseases in forest nurseries worldwide [98]. *Fusarium solani* was also detected, but at lower proportions (Table 3). It is often found on dead organic matter, but under certain conditions it can cause disease in various hosts [99]. *Dactylonectria macrodidyma* (previously *Neonectria macrodidyma*) was also commonly recorded both in the root and soil samples of different tree seedlings (Table 3). *Dactylonectria macrodidyma* was shown to be an economically-important pathogen in forest nurseries [48,52,100–102]. Interestingly, the above-mentioned fungal pathogens showed generally low host or habitat specificity, but their relative abundance was often higher in the soil than in the roots of the different tree species (Table 3).

Oomycetes represent one of the most problematic groups of disease-causing microorganisms in different growing environments, including forest nurseries [25]. They can also cause diseases in different hosts, including trees, ornamental plants, and crops [103]. In the present study, oomycetes were often more abundant in the soil than in the seedlings' roots (Table 4), suggesting that, in healthy roots, their development was largely restricted. The oomycetes that were most common in this study (Table 4) are also known to cause seedling diseases in forest nurseries, including taxa such as *P. ultimum* var. *ultimum*, *P. heterothallicum* [104,105] and *P. spiculum* [106–108]. *Pythium spiculum* was previously detected in the feeder roots and in the rhizosphere soil of declining oaks [109], but information on its pathogenicity to oaks is limited [106]. Among other oomycetes, *P. irregulare* is known to be associated with more than 200 host plants, and can cause root rot in deciduous and coniferous trees [110]. Interestingly, *Peronospora* sp. 3993_148 was exclusively detected in oak roots (Table 4), which may be due to the fact that representatives of this genus are known to cause downy mildew disease [111]. Among the 30 most common oomycetes, only two *Phytophthora* species were detected, i.e., *Phytophthora pseudosyringae* and *Phytophthora fragariae* (Table 4). *Phytophthora pseudosyringae* is known to cause root and collar rot in deciduous trees [112]. *Phytophthora fragariae* is a root pathogen that causes red stele disease in strawberries [113,114], but in the present study, it was associated with the soil and roots of *A. glutinosa,* and with the roots of *B. pendula* (Table 4).

5. Conclusions

The results demonstrated that the seedling roots and the rhizosphere soil in bare-root forest nurseries support a high richness of fungal taxa, which can be comparable to those present in forest stands.

Although the fungal communities in the roots were generally different between coniferous and deciduous tree species, the corresponding fungal communities in the soil were similar, thereby showing the relative importance of fungal generalists. The

seedling roots were primarily inhabited by saprotrophic and mycorrhizal fungi, while fungal pathogens and oomycetes were less abundant, showing that the cultivation practices used in forest nurseries secured both the production of high-quality planting stock and disease control.

Supplementary Materials: The following are available online at https://www.mdpi.com/2076-2607/9/1/150/s1. Table S1: Relative abundance of fungal and oomycete OTUs sequenced from the root and soil samples of the five tree species bare-root cultivated in forest nurseries in Lithuania.

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References

1. Karjalainen, T.; Liski, J.; Pussinen, A.; Lapvetela Èinen, T. *Sinks in the Kyoto Protocol and Considerations for Ongoing Work in the UNFCCC Work*; The Nordic Ministry Council; 2000; in press.
2. APAT—Auditory Processing Abilities Test. *Nursery Production and Stand Establishment of Broad-Leaves to Promote Sustainable Forest Management*; APAT—Auditory Processing Abilities Test: Roma, Italy, 2003.
3. Bastin, J.F.; Finegold, Y.; Garcia, C.; Mollicone, D.; Rezende, M.; Routh, D.; Zohner, C.M.; Crowther, T.W. The global tree restoration potential. *Science* **2019**, *365*, 76–79. [CrossRef]
4. Krasowski, M.J. *Forests and Forest Plants—Volume III: Producing Planting Stocks in Forest Nurseries*; Eolss Publishers Co.: Oxford, UK, 2012.
5. Grossnickle, S.C.; MacDonald, J.E. Seedling Quality: History, Application and Plant Attributes. *Forests* **2018**, *9*, 283. [CrossRef]
6. Menkis, A.; Vasiliauskas, R.; Taylor, A.F.S.; Stenlid, J.; Finlay, R. Fungal communities in mycorrhizal roots of conifer seedlings in forest nurseries under different cultivation systems, assessed by morphotyping, direct sequencing and mycelial isolation. *Mycorrhiza* **2005**, *16*, 33–41. [CrossRef]
7. Menkis, A.; Vasiliauskas, R.; Taylor, A.F.S.; Stenström, E.; Stenlid, J.; Finlay, R. Fungi in decayed roots of conifer seedlings in forest nurseries, afforested clear-cuts and abandoned farmland. *Plant. Pathol.* **2006**, *55*, 117–129. [CrossRef]
8. Menkis, A.; Vasiliauskas, R.; Taylor, A.F.S.; Stenlid, J.; Finlay, R. Afforestation of abandoned farmland with conifer seedlings inoculated with three ectomycorrhizal fungi—Impact on plant performance and ectomycorrhizal community. *Mycorrhiza* **2007**, *17*, 337–348. [CrossRef]
9. Berendsen, R.L.; Pieterse, C.M.J.; Bakker, P.A.H.M. The rhizosphere microbiome and plant health. *Trends Plant. Sci.* **2012**, *17*, 478–486. [CrossRef]
10. Vorholt, J.A. Microbial life in the phyllosphere. *Nat. Rev. Microbiol.* **2012**, *10*, 828–840. [CrossRef]
11. Mendes, R.; Kruijt, M.; de Bruijn, I.; Dekkers, E.; van der Voort, M.; Schneider, J.H.M.; Piceno, Y.M.; DeSantis, T.Z.; Andersen, G.L.; Bakker, P.A.; et al. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* **2011**, *332*, 1097–1100. [CrossRef]
12. Ritpitakphong, U.; Falquet, L.; Vimoltust, A.; Berger, A.; Metraux, J.P.; L'Haridon, F. The microbiome of the leaf surface of *Arabidopsis* protects against a fungal pathogen. *New Phytol.* **2016**, *210*, 1033–1043. [CrossRef]
13. Van der Ent, S.; Van Hulten, M.; Pozo, M.J.; Czechowski, T.; Udvardi, M.K.; Pieterse, C.M.J.; Ton, J. Priming of plant innate immunity by rhizobacteria and β-aminobutyric acid: Differences and similarities in regulation. *New Phytol.* **2009**, *183*, 419–431. [CrossRef]
14. Zamioudis, C.; Korteland, J.; Van Pelt, J.A.; van Hamersveld, M.; Dombrowski, N.; Bai, Y.; Hanson, J.; Van Verk, M.C.; Ling, H.Q.; Schulze-Lefert, P.; et al. Rhizobacterial volatiles and photosynthesis-related signals coordinate MYB72 expression in *Arabidopsis* roots during onset of induced systemic resistance and iron-deficiency responses. *Plant. J.* **2015**, *84*, 309–322. [CrossRef] [PubMed]

15. Van der Heijden, M.; Bruin, S.; Luckerhoff, L.; van Logtestijn, R.S.; Schlaeppi, K. A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *ISME J.* **2016**, *10*, 389–399. [CrossRef] [PubMed]
16. Rolli, E.; Marasco, R.; Vigani, G.; Ettoumi, B.; Mapelli, F.; Deangelis, M.L.; Gandolfi, C.; Casati, E.; Previtali, F.; Gerbino, R.; et al. Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environ. Microbiol.* **2015**, *17*, 316–331. [CrossRef] [PubMed]
17. Haney, C.H.; Samuel, B.S.; Bush, J.; Ausubel, F.M. Associations with rhizosphere bacteria can confer an adaptive advantage to plants. *Nat. Plants.* **2015**, *1*, 15051. [CrossRef]
18. Garbaye, J. Helper bacteria—A new dimension to the mycorrhizal symbiosis. *New Phytol.* **1994**, *128*, 197–210. [CrossRef]
19. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*, 2nd ed.; Academic Press: London, UK, 1997; p. 605.
20. Zak, B. The role of mycorrhizae in root disease. *Ann. Rev. Phytopathol.* **1964**, *2*, 377–392. [CrossRef]
21. Vaario, L.M.; Tervonen, A.; Haukioja, K.; Haukioja, M.; Pennanen, T.; Timonen, S. The effect of nursery substrate and fertilization on the growth and ectomycorrhizal status of containerized and out planted seedlings of *Picea abies*. *Can. J. For. Res.* **2009**, *39*, 64–75. [CrossRef]
22. Kazantseva, O.; Bingham, M.; Simard, S.W.; Berch, S.M. Effects of growth medium, nutrients, water, and aeration on mycorrhization and biomass allocation of greenhouse-grown interior Douglas-fir seedlings. *Mycorrhiza* **2009**, *20*, 51–66. [CrossRef]
23. Henry, C.; Raivoarisoa, J.; Razafimamonjy, A.; Ramanankierana, H.; Andrianaivomahefa, P.; Ducousso, M.; Selosse, M.A. Transfer to forest nurseries significantly affects mycorrhizal community composition of *Asteropeia mcphersonii* wildings. *Mycorrhiza* **2017**, *27*, 321–330. [CrossRef]
24. Raj, A.J.; Lal, S.B. *Forestry Principles and Applications*; Scientific Publishers: Rajasthan, India, 2013.
25. Jung, T.; Orlikowski, L.; Henricot, B.; Abad-Campos, P.; Aday, A.G.; Aguín Casal, O.; Bakonyi, J.; Cacciola, S.O.; Cech, T.; Chavarriaga, D.; et al. Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of *Phytophthora* diseases. *For. Path.* **2016**, *46*, 134–163. [CrossRef]
26. Peterson, G.W.; Smith, R.S., Jr. *Forest Nursery Diseases in the United States*; Agriculture Hand-Book No 470. Forest Service 1975. Library of Congress Catalog No. 74-600103; U.S. Department of Agriculture: Washington, DC, USA, 1975; p. 125.
27. Brasier, C.M. The biosecurity threat to the UK and global environment from international trade in plants. *Plant. Pathol.* **2008**, *57*, 792–808. [CrossRef]
28. Liebhold, A.M.; Brockerhoff, E.G.; Garrett, L.J.; Parke, J.L.; Britton, K.O. Live plant imports: The major pathway for forest insect and pathogen invasions of the US. *Front. Ecol. Environ.* **2012**, *10*, 135–143. [CrossRef]
29. Yakabe, L.E.; Blomquist, C.L.; Thomas, S.L.; MacDonald, J.D. Identification and frequency of *Phytophthora* species associated with foliar diseases in California ornamental nurseries. *Plant. Dis.* **2009**, *93*, 883–890. [CrossRef] [PubMed]
30. Bienapfl, J.C.; Balci, Y. Movement of *Phytophthora* spp. in Maryland's nursery trade. *Plant. Dis.* **2014**, *98*, 134–144. [CrossRef]
31. Parke, J.L.; Knaus, B.J.; Fieland, V.J.; Lewis, C.; Grünwald, N.J. *Phytophthora* community structure analyses in Oregon nurseries inform systems approaches to disease management. *Phytopathology* **2014**, *104*, 1052–1062. [CrossRef]
32. Reeser, P.W.; Sutton, W.; Hansen, E.M.; Goheen, E.M.; Fieland, V.J.; Grunwald, N.J. First report of *Phytophthora occultans* causing root and collar rot on Ceanothus, boxwood, rhododendron, and other hosts in horticultural nurseries in Oregon, USA. *Plant. Dis.* **2015**, *99*, 1282. [CrossRef]
33. Rooney-Latham, S.; Blomquist, C.; Swiecki, T.; Bernhardt, E.; Frankel, S.J. First detection in the USA: New plant pathogen, *Phytophthora tentaculata*, in native plant nurseries and restoration sites in California. *Native Plants J.* **2015**, *16*, 23–25. [CrossRef]
34. Rooney-Latham, S.; Blomquist, C.L.; Kosta, K.L.; Gou, Y.Y.; Woods, P.W. Phytophthora species are common on nursery stock grown for restoration and revegetation purposes in California. *Plant. Dis.* **2019**, *103*, 448–455. [CrossRef]
35. Dobbelaere, S.; Vanderleyden, J.; Okon, Y. Plant growth-promoting effects of diazotrophs in the rhizosphere. *Crit. Rev. Plant. Sci.* **2003**, *22*, 107–149. [CrossRef]
36. Duffy, B.; Keel, C.; Defago, G. Potential role of pathogen signaling in multitrophic plant-microbe interactions involved in disease protection. *Appl. Environ. Microbiol.* **2004**, *70*, 1836–1842. [CrossRef]
37. Morgan, J.A.W.; Bending, G.D.; White, P.J. Biological costs and benefits to plant-microbe interactions in the rhizosphere. *J. Exp. Bot.* **2005**, *56*, 1729–1739. [CrossRef] [PubMed]
38. Reinhart, K.O.; Callaway, R.M. Soil biota and invasive plants. *New Phytol.* **2006**, *170*, 445–457. [CrossRef] [PubMed]
39. Batten, K.M.; Scow, K.M.; Davies, K.F.; Harrison, S.P. Two invasive plants alter soil microbial community composition in serpentine grasslands. *Biol. Invasions* **2006**, *8*, 217–230. [CrossRef]
40. Innes, L.; Hobbs, P.J.; Bardgett, R.D. The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility. *Biol. Fertil. Soils* **2004**, *40*, 7–13. [CrossRef]
41. Priha, O.; Grayston, S.J.; Pennanen, T.; Smolander, A. Microbial activities related to C and N cycling and microbial community structure in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings in an organic and mineral soil. *FEMS Microbiol. Ecol.* **1999**, *30*, 187–199. [CrossRef] [PubMed]
42. Kowalchuk, G.A.; Hol, W.H.G.; Van Veen, J.A. Rhizosphere fungal communities are influenced by *Senecio jacobaea* pyrrolizidine alkaloid content and composition. *Soil Biol. Biochem.* **2006**, *38*, 2852–2859. [CrossRef]
43. Klavina, D.; Zaluma, A.; Pennanen, T.; Velmala, S.; Gaitnieks, T.; Gailis, A.; Menkis, A. Seed provenance impacts growth and ectomycorrhizal colonisation of *Picea abies* seedlings. *Balt. For.* **2015**, *21*, 184–191.

44. Mougel, C.; Offre, P.; Ranjard, L.; Corberand, T.; Gamalero, E.; Robin, C.; Lemanceau, P. Dynamic of the genetic structure of bacterial and fungal communities at different developmental stages of *Medicago truncatula* Gaertn. cv. Jemalong line J5. *New Phytol.* **2006**, *170*, 165–175. [CrossRef]
45. Weisskopf, L.; Tomasi, N.; Santelia, D.; Martinoia, E.; Langlade, N.B.; Tabacchi, R.; Abou-Mansour, E. Isoflavonoid exudation from white lupin roots is influenced by phosphate supply, root type and cluster-root stage. *New Phytol.* **2006**, *171*, 657–668. [CrossRef]
46. Stenström, E.; Ndobe, N.E.; Jonsson, M.; Stenlid, J.; Menkis, A. Root associated fungi of healthy-looking *Pinus sylvestris* and *Picea abies* seedlings in Swedish forest nurseries. *Scand. J. For. Res.* **2014**, *29*, 12–21. [CrossRef]
47. Bzdyk, R.M.; Olchowik, J.; Studnicki, M.; Oszako, T.; Sikora, K.; Szmidla, H.; Hilszczańska, D. The impact of effective microorganisms (em) and organic and mineral fertilizers on the growth and mycorrhizal colonization of *Fagus sylvatica* and *Quercus robur* seedlings in a bare-root nursery experiment. *Forests* **2018**, *9*, 597. [CrossRef]
48. Beyer-Ericson, L.; Damm, E.; Unestam, T. An overview of root dieback and its causes in Swedish forest nurseries. *Eur. J. For. Pathol.* **1991**, *21*, 439. [CrossRef]
49. Galaaen, R.; Venn, K. *Pythium sylvaticum* Campbell & Hendrix and other fungi associated with root dieback of 2-0 seedlings of *Picea abies* (L.) Karst. in Norway. *Meddelerser Norsk Inst. Skogforsk.* **1979**, *34*, 221–228.
50. Venn, K. Rotavdoing hos bartreplanter i skogplanteskoler (Root dieback of coniferous seedlings in forest nurseries). In *Norwegian, English Summary*; Research Paper 3/85; Norwegian Forest Research Institute: Ås, Norway, 1985; Volume 11.
51. Venn, K.; Sandvik, M.; Langerud, B. Nursery routines, growth media and pathogens affect growth and root dieback in Norway spruce seedlings. *Meddelelser Norsk Inst. Skogforsk.* **1986**, *34*, 314–328.
52. Lilja, A.; Lilja, S.; Poteri, M.; Ziren, L. Conifer seedling root fungi and root dieback in Finnish nurseries. *Scand. J. For. Res.* **1992**, *7*, 547–556. [CrossRef]
53. Lilja, A. The occurrence and pathogenicity of uni- and binucleate *Rhizoctonia* and *Pythiaceae* fungi among conifer seedlings in Finnish forest nurseries. *Eur. J. Plant. Pathol.* **1994**, *24*, 181–192. [CrossRef]
54. Santini, A.; Ghelardini, L.; De Pace, C.; Desprez-Loustau, M.L.; Capretti, P.; Chandelier, A.; Cech, T.; Chira, D.; Diamandis, S.; Gaitniekis, T.; et al. Biogeographical patterns and determinants of invasion by forest pathogens in Europe. *New Phytol.* **2013**, *197*, 238–250. [CrossRef]
55. Cram, M.M.; Hansen, E.M. *Phytophthora* Root Rot. In *Forest Nursery Pests*; Agriculture Handbook 680 Rev.; Cram, M.M., Frank, M.S., Mallams, K.M., Eds.; Department of Agriculture, Forest Service: Washington, DC, USA, 2012; pp. 126–128.
56. Goheen, E.M.; Kanaskie, A.; Navarro, S.; Hansen, E. Sudden oak death management in Oregon tanoak forests. *For. Phytophthoras* **2017**, *7*, 45–53. [CrossRef]
57. Stenlid, J.; Oliva, J.; Boberg, J.B.; Hopkins, A.J.M. Emerging diseases in European forest ecosystems and responses in society. *Forests* **2011**, *2*, 486–504. [CrossRef]
58. Vaičys, M. Miško dirvožemių klasifikacija. In *Lietuvos Dirvožemiai [Forest Site Types. Lithuanian Soils]*; Mokslas Publishers: Vilnius, Lithuania, 2001; pp. 1040–1043. (In Lithuanian)
59. Marčiulynas, A.; Marčiulynienė, D.; Lynikienė, J.; Gedminas, A.; Vaičiukynė, M.; Menkis, A. Fungi and oomycetes in the irrigation water of forest nurseries. *Forests* **2020**, *11*, 459. [CrossRef]
60. Cooke, D.E.L.; Drenth, A.; Duncan, J.M.; Wagels, G.; Brasier, C.M. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genet. Biol.* **2000**, *30*, 17–32. [CrossRef] [PubMed]
61. 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.
62. Magurran, A.E. *Ecological Diversity and Its Measurement*; Princeton University Press: Princeton, NJ, USA, 1988; p. 192.
63. Sokal, R.R.; Rohlf, F.J. *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd ed.; W.H. Freeman and Company: New York, NY, USA, 1995.
64. Shannon, C.E. A mathematical theory of communication. *Bell Syst. Tech. J.* **1948**, *27*, 379–423. [CrossRef]
65. Nguyen, D.; Boberg, J.; Ihrmark, K.; Stenstrom, E.; Stenlid, J. Do foliar fungal communities of Norway spruce shift along a tree species diversity gradient in mature European forests? *Fungal Ecol.* **2016**, *23*, 97–108. [CrossRef]
66. Menkis, A.; Burokienė, D.; Stenlid, J.; Stenström, E. High-throughput sequencing shows high fungal diversity and community segregation in the rhizospheres of container-grown conifer seedlings. *Forests* **2016**, *7*, 44. [CrossRef]
67. Mickan, B.S.; Hart, M.M.; Solaiman, Z.M.; Jenkins, S.; Siddique, K.H.M.; Abbott, L.K. Molecular divergence of fungal communities in soil, roots and hyphae highlight the importance of sampling strategies. *Rhizosphere* **2017**, *4*, 104–111. [CrossRef]
68. Pascale, A.; Proietti, S.; Pantelides, I.S.; Stringlis, I.A. Modulation of the root microbiome by plant molecules: The basis for targeted disease suppression and plant growth promotion. *Front. Plant. Sci.* **2020**, *10*, 1741. [CrossRef]
69. Aponte, C.; García, L.V.; Marañón, T. Tree species effects on nutrient cycling and soil biota: A feedback mechanism favouring species coexistence. *For. Ecol. Manage.* **2013**, *309*, 36–46. [CrossRef]
70. Klaubauf, S.; Inselsbacher, E.; Zechmeister-Boltenstern, S.; Wanek, W.; Gottsberger, R.; Strauss, J.; Gorfer, M. Molecular diversity of fungal communities in agricultural soils from Lower Austria. *Fungal Divers.* **2010**, *44*, 65–75. [CrossRef]
71. Jiang, Y.M.; Chen, C.R.; Xu, Z.H.; Liu, Y.Q. Effects of single and mixed species forest ecosystems on diversity and function of soil microbial community in subtropical China. *J. Soils Sediments* **2012**, *12*, 228–240. [CrossRef]

72. Han, L.L.; Wang, J.T.; Yang, S.H.; Chen, W.F.; Zhang, L.M.; He, J.Z. Temporal dynamics of fungal communities in soybean rhizosphere. *J. Soils Sediments* **2016**, *17*, 491–498. [CrossRef]
73. Petrini, O. Fungal Endophytes of Tree Leaves. In *Microbial Ecology of Leaves*; Andrews, J.H., Hirano, S.S., Eds.; Springer: New York, NY, USA, 1991; pp. 179–197.
74. King, B.C.; Waxman, K.D.; Nenni, N.V.; Walker, L.P.; Bergstrom, G.C.; Gibson, D.M. Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi. *Biotechnol. Biofuels* **2011**, *4*, 1–14.
75. Lang, C.; Seven, J.; Polle, A. Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed Central European forest. *Mycorrhiza* **2011**, *21*, 297–308. [CrossRef] [PubMed]
76. Mayerhofer, M.S.; Kernaghan, G.; Harper, K.A. The effects of fungal root endophytes on plant growth: A meta-analysis. *Mycorrhiza* **2013**, *23*, 119–128. [CrossRef]
77. Austin, A.T.; Vivanco, L.; Gonzalez-Arzac, A.; Perez, L.I. There's no place like home? An exploration of the mechanisms behind plant litter-decomposer affinity in terrestrial ecosystems. *New Phytol.* **2014**, *204*, 307–314. [CrossRef]
78. Jumpponen, A.; Egerton-Warburton, L.M. Mycorrhizal fungi in successional environments—A community assembly model incorporating host plant, environmental and biotic filters. In *The Fungal Community*; Dighton, J., White, J.F., Oudemans, P., Eds.; CRC Press: New York, NY, USA, 2005; pp. 139–180.
79. Ishida, T.A.; Nara, K.; Hogetsu, T. Host effects on ectomycorrhizal fungal communities: Insight from eight host species in mixed conifer–broadleaf forests. *New Phytol.* **2007**, *174*, 430–440. [CrossRef]
80. Menkis, A.; Vasaitis, R. Fungi in roots of nursery grown *Pinus sylvestris*: Ectomycorrhizal colonisation, genetic diversity and spatial distribution. *Microb Ecol* **2011**, *61*, 52–63. [CrossRef]
81. Vrålstad, T.; Myhre, E.; Schumacher, T. Molecular diversity and phylogenetic affinities of symbiotic root-associated ascomycetes of the *Helotiales* in burnt and metal polluted habitats. *New Phytol.* **2002**, *155*, 131–148. [CrossRef]
82. Sugita, T.; Boekhout, T.; Velegraki, A.; Guillot, J.; Hađina, S.; Cabañes, F.J. Epidemiology of *Malassezia*-related skin diseases. In *Malassezia and the Skin: Science and Clinical Practice*, 1st ed.; Boekhout, T., Guého-Kellermann, E., Mayser, P., Velegraki, A., Eds.; Springer: Berlin/Heidelberg, Germany, 2010; pp. 65–120.
83. Lai, X.; Cao, L.; Tan, H.; Fang, S.; Huang, Y.; Zhou, S. Fungal communities from methane hydrate-bearing deep-sea marine sediments in South China Sea. *ISME J.* **2007**, *1*, 756–762. [CrossRef]
84. Le Calvez, T.; Burgaud, G.; Mahe, S.; Barbier, G.; Vandenkoornhuyse, P. Fungal diversity in deep-sea hydrothermal ecosystems. *Appl. Environ. Microbiol.* **2009**, *75*, 6415–6421. [CrossRef]
85. Amend, A.S.; Barshis, D.J.; Oliver, T.A. Coral-associated marine fungi form novel lineages and heterogeneous assemblages. *ISME J.* **2012**, *6*, 1291–1301. [CrossRef] [PubMed]
86. Arenz, B.E.; Held, B.W.; Jurgens, J.A.; Farrell, R.L.; Blanchette, R.A. Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. *Soil Biol. Biochem.* **2006**, *38*, 3057–3064. [CrossRef]
87. Fell, J.W.; Scorzetti, G.; Connell, L.; Craig, S. Biodiversity of micro-eukaryotes in Antarctic Dry Valley soils with. *Soil Biol. Biochem.* **2006**, *38*, 3107–3119. [CrossRef]
88. Renker, C.; Alphei, J.; Buscot, F. Soil nematodes associated with the mammal pathogenic fungal genus *Malassezia* (*Basidiomycota*: *Ustilaginomycetes*) in Central European forests. *Biol. Fertil Soils* **2003**, *37*, 70–72. [CrossRef]
89. Roy, M.; Watthana, S.; Stier, A.; Richard, F.; Vessabutr, S.; Selosse, M.A. Two mycoheterotrophic orchids from Thailand tropical dipterocarpacean forests associate with a broad diversity of ectomycorrhizal fungi. *BMC Biol.* **2009**, *7*, 51. [CrossRef]
90. Klavina, D.; Menkis, A.; Gaitnieks, T.; Pennanen, T.; Lazdiņš, A.; Velmala, S.; Vasaitis, R. Low impact of stump removal on mycorrhization and growth of replanted *Picea abies*: Data from three types of hemiboreal forest. *Balt For.* **2016**, *22*, 16–24.
91. Renseigné, N.; Rudawska, M.; Leski, T. Mycorrhizal associations of nursery grown Scots pine (*Pinus sylvestris* L.) seedlings in Poland. *Ann. For. Sci.* **2006**, *63*, 715–723.
92. Zwiazek, J.J.; Equiza, M.A.; Karst, J.; Senorans, J.; Wartenbe, M.; Calvo-Polanco, M. Role of urban ectomycorrhizal fungi in improving the tolerance of lodgepole pine (*Pinus contorta*) seedlings to salt stress. *Mycorrhiza* **2019**, *29*, 303–312. [CrossRef]
93. Lazarevic, J.; Menkis, A. Fungi inhabiting fine roots of *Pinus heldreichii* in the Montenegrin montane forests. *Symbiosis* **2018**, *74*, 189–197. [CrossRef]
94. Smaill, S.J.; Walbert, K. Fertilizer and fungicide use increases the abundance of less beneficial ectomycorrhizal species in a seedling nursery. *Appl. Soil Ecol.* **2013**, *65*, 60–64. [CrossRef]
95. Jones, M.D.; Grenon, F.; Peat, H.; Fitzgerald, M.; Holt, L.; Philip, L.J.; Bradley, R. Differences in N-15 uptake amongst spruce seedlings colonized by three pioneer ectomycorrhizal fungi in the field. *Fungal Ecol.* **2009**, *2*, 110–120. [CrossRef]
96. Chu-Chou, M.; Grace, L.J. Mycorrhizal fungi of radiata pine in different forests of the North and South Islands in New Zealand. *Soil Biol. Biochem.* **1988**, *20*, 883–886. [CrossRef]
97. Menkis, A.; Lygis, V.; Burokienė, D.; Vasaitis, R. Establishment of ectomycorrhiza-inoculated *Pinus sylvestris* seedlings on coastal dunes following a forest fire. *Balt For.* **2012**, *18*, 33–40.
98. James, R.L.; Dumroese, R.K. Investigations of Fusarium diseases within Inland Pacific Northwest forest nurseries. In *Proceedings of the 53rd Western International Forest Disease Work Conference, Jackson, WY, USA, 26–29 August 2005*; Guyon, J.C., Ed.; USDA Forest Service, Intermountain Region: Ogden, UT, USA, 2007; pp. 3–11.
99. Adesemoye, A.; Eskalen, A.; Faber, B.; O'Connell, N. Current nowledge on *Fusarium* dry root rot of citrus. *Citrograph* **2011**, *2*, 29–33.

100. Adesemoye, A.O.; Mayorquin, J.S.; Peacock, B.B.; Moreno, K.; Hajeri, S.; Yokomi, R.; Eskalen, A. Association of *Neonectria macrodidyma* with dry root rot of citrus in California. *J. Plant. Pathol. Microbiol.* **2016**, *8*, 391.
101. Hamelin, R.C.; Berube, P.; Gignac, M.; Bourassa, M. Identification of root rot fungi in nursery seedlings by nested multiplex PCR. *Appl. Environ. Microbiol.* **1996**, *62*, 4026–4031. [CrossRef]
102. Menkis, A.; Burokienė, D. Distribution and genetic diversity of the root-rot pathogen *Neonectria macrodidyma* in a forest nursery. *For. Pathol.* **2012**, *42*, 79–83. [CrossRef]
103. Derevnina, L.; Petre, B.; Kellner, R.; Dagdas, Y.F.; Sarowar, M.N.; Giannakopoulou, A.; De la Concepcion, J.C.; Chaparro-Garcia, A.; Pennington, H.G.; van West, P.; et al. Emerging oomycete threats to plants and animals. Philosophical transactions of the Royal Society of London. *Proc. R. Soc. B* **2016**, *371*, 20150459.
104. Lazerg, F.; Belabid, L.; Sanchez, J.; Gallego, E. Root rot and damping-off of Aleppo pine seedlings caused by *Pythium spp.* in Algerian forest nurseries. *For. Sci.* **2016**, *62*, 322–328. [CrossRef]
105. Paulitz, T.C.; Adams, K. Composition and distribution of *Pythium* communities in wheat fields in eastern washington state. *Ecol. Popul. Biol.* **2003**, *93*, 867. [CrossRef]
106. Jiménez, J.J.; Sánchez, J.E.; Romero, M.A.; Belbahri, L.; Trapero, A.; Lefort, F.; Sánchez, M.E. Pathogenicity of *Pythium spiculum* and *P. sterilum* on feeder roots of *Quercus rotundifolia*. *Plant. Pathol.* **2008**, *57*, 369. [CrossRef]
107. Serrano, M.D.; de Vita, P.; Fernández-Rebollo, P.; Coelho, A.C.; Belbahri, L.; Sánchez, M.E. *Phytophthora cinnamomi* and *Pythium spiculum* as main agents of *Quercus* decline in southern Spain and Portugal. *IOBC/WPRS Bull.* **2012**, *76*, 97–100.
108. de Vita, P.; Serrano, M.S.; Belbahri, L.; García, L.V.; Ramo, C.; Sánchez, M.E. Germination of hyphal bodies of *Pythium spiculum* isolated from declining cork oaks at Doñana National Park (Spain). *Phytopathol. Mediterr.* **2011**, *50*, 478–481.
109. Paul, B.; Bala, K.; Belbahri, L.; Calmin, G.; Sánchez, E.; Lefort, F. A new species of *Pythium* with ornamented oogonia: Morphology, taxonomy, ITS region of its rDNA, and its comparison with related species. *FEMS Microbiol. Lett.* **2006**, *254*, 317–323. [CrossRef] [PubMed]
110. Matsumoto, C.; Kageyama, K.; Suga, H.; Hyakumachi, M. Intraspecific DNA polymorphisms of *Pythium irregulare*. *Mycol. Res.* **2000**, *104*, 1333–1341. [CrossRef]
111. Thines, M.; Choi, Y.J. Evolution, diversity, and taxonomy of the Peronosporaceae, with focus on the genus *Peronospora*. *Phytopathology* **2016**, *106*, 6–18. [CrossRef]
112. Scanu, B.; Jones, B.; Webber, J.F. A new disease of Nothofagus in Britain caused by *Phytophthora pseudosyringae*. *New Dis. Rep.* **2012**, *25*, 27. [CrossRef]
113. Scheewe, P. Identification of pathogenic races of *Phytophthora fragariae* Hickman in Germany. In *Progress in Temperate Fruit Breeding*, *Developments in Plant Breeding*; Schmidt, H., Kellerhals, M., Eds.; Springer: Dordrecht, Germany, 1994; Volume 1, pp. 67–71.
114. Jung, T.; Nechwatal, J.; Cooke, D.E.; Hartmann, G.; Blaschke, M.; Oßwald, W.F.; Duncan, J.M.; Delatour, C. *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. *Mycol Res.* **2003**, *107*, 772–789. [CrossRef]