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Changes in Chemical Properties and Fungal Communities of Mineral Soil after Clear-Cutting and Reforestation of Scots Pine (*Pinus sylvestris* L.) Sites

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Abstract: This study aimed to assess the changes in chemical properties and fungal communities in the upper mineral soil layer in managed Scots pine (*Pinus sylvestris* L.) ecosystems. Study sites were located in the three largest *P. sylvestris* massifs in Lithuania, and six sampling sites, representing different development stages of the *P. sylvestris* forest ecosystem, were selected in each of them: mature *P. sylvestris* forest stands; clear-cuts of former *P. sylvestris* mature stand; and the *P. sylvestris* plantations aging from the 1st to 4th year. High-throughput sequencing was performed to evaluate the soil fungus community at clear-cuts, early-stage reforested sites, and mature forests in Lithuania. This study has shown that, among other chemical soil parameters, the mean concentrations of mineral nitrogen (N), total phosphorus (P), and P_2O_5 were slightly higher in the clear-cut sites, and significantly higher in the 1st year plantations compared to the mature forests. The quality filtering after PacBio sequencing showed the presence of 60,898 high-quality fungal sequences, and 1143 fungal operational taxonomic units (OTUs). The most abundant fungal OTU in our study was *Archaeorhizomyces* sp. 5425_1. In total, 70 mycorrhizal fungal OTUs were found in the soil samples at the studied sites. The most abundant ectomycorrhizal fungus identified was *Amanita fulva* (Schaeff.) Fr. The highest amount of ectomycorrhizal fungal OTUs was found in the clear-cut sites and in the mature forests. The concentrations of mineral N and P_2O_5 in the upper mineral soil layer did not significantly affect fungal OTUs diversity. Conversely, a relatively strong correlation was obtained between the number of mycorrhizal fungal OTUs and the concentrations of total N and soil organic carbon (SOC), as well as between the numbers of saprotrophic fungal OTUs and the concentration of magnesium ions (Mg^{2+}).

Keywords: *Pinus sylvestris*; high-throughput sequencing; upper mineral soil layer; soil chemistry; fungal communities; ectomycorrhizal fungi

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1. Introduction

Major parts of the world's forests are managed to achieve different goals, combining the controversial challenges of producing higher amounts of timber and simultaneously conserving biodiversity [1]. Intensive forest management often leads to drastic changes in forest ecosystems such as loss of habitats and biodiversity [2–5]. Temperate and boreal forests are increasingly affected by anthropogenic disturbances when forest biomass is harvested at various intensities. Clear-cutting is a common forest management practice to achieve maximum efficient harvesting, but it results in severe abiotic and biotic changes in the ecosystem during the reforestation stages [6,7]. Reforestation of clear-cut sites with suitable tree species is a priority for sustainable forest management, but it is also essential to maintain the stability of other live components of ecosystems during forest harvesting

and reforestation stages. The forest ecosystem components, related by common habitats, and trophic, symbiotic, and mutualistic relationships, are important for the maintenance of sustainable forest ecosystems [8–11].

Intensive harvesting of forest biomass promotes changes in chemical properties of forest soil and soil mycobiota, including mycorrhizal fungi, which are important for seedling growth [12–16]. The removal of forest biomass reduces the soil organic matter and carbon stocks. Relatively high removals of forest biomass could lead to nutrient loss, which could potentially affect soil biodiversity and reduce site productivity in the long term. Soil organic matter, providing nutrients for soil biota and plants, maintains the ability to retain soil water and air, and, therefore, it is one of the essential conditions for tree growth [17].

Mycorrhizal fungi are obligate symbionts with forest tree species, where they scavenge soil nutrients and water from the soil in exchange for photosynthate from the tree [18]. Without their fungal symbionts, the trees cannot acquire enough nutrients, and fungi have insufficient energy to carry out their life cycle without the trees. Intensive forest harvesting alters the changes in ECM fungal communities by changing the age structure and species composition of the forest trees and other plants serving as hosts.

In recent decades, numerous studies on the abundance and diversity of soil fungi and their interrelationship with plants have been conducted [19–22]. Forest soil properties, vegetation and tree species, cover, human activities, and forest management regimes affect all fungal communities. All ecophysiological groups of soil fungi, saprotrophs, symbionts, and pathogens are involved in decomposition and mineralization processes [23,24]. Ectomycorrhizal (ECM) fungi are particularly sensitive to forest harvesting [25,26] because they depend on the carbon released from tree hosts [27,28]. Intensive forest management, shortly disturbing the balance of the ecosystem, affects both the abundance and species composition of mycorrhizal fungi. Therefore, the observed changes in the structure and species composition of the mycorrhizal community may be one of the indicators caused by the impact of clear-cutting on the forest ecosystem [29].

In Lithuania, the composition of fungal communities in forest soil and the impact of forest management regimes, including clear-cutting, on soil mycobiota have not been studied extensively. Among other stands, *Pinus sylvestris* L. stands are economically valuable and cover 34.5% of the total forest area in Lithuania [30]. Due to its widespread distribution globally and regionally, *P. sylvestris* is considered a suitable species for reforestation and afforestation in the European region [31].

Recently, the differences in soil fungal communities in managed and unmanaged forests have been studied in other countries, such as Germany [32–34] and France [35]. In Poland, the comparative studies were mainly conducted on the abundance and diversity of soil mycobiota in the forest after the applications of different harvesting of woody debris and pre-planting soil preparations [36,37]. The influence of different forest litter types on tree growth and ectomycorrhizal formation of *P. sylvestris* seedlings were reported in Lithuania [38,39].

The aim of this study was to evaluate the changes of chemical properties and fungal communities in the upper mineral soil layer in the managed Scots pine (*Pinus sylvestris* L.) ecosystems, including mature forest stands, clear-cuts, and 1st, 2nd, 3rd, and 4th-year plantations. We hypothesized that changes in forest soil chemical parameters could have an impact on soil fungal communities obtained in different study sites.

This study was performed in a frame of the Lithuanian National Program (2020–2022), which was initiated based on intense debates among citizens and governmental and non-governmental organizations opposing forest management activities that may have a negative impact on biodiversity. Annually, an average of 11,968 ha of the State *P. sylvestris* forests in Lithuania are affected by forest management activities, including clear-cutting and other final felling for timber production.

Molecular methods based on PCR and ribosomal DNA sequencing have been successfully used to identify fungi at different taxonomic levels in different environmental samples and helped to elucidate the ecological conditions affecting the structure and diversity of

fungal communities [31,40–44]. To assess fungal communities in forest soil we used one of the molecular methods—high-throughput sequencing (HTS). In our study, PCR products were sequenced using a PacBio RSII platform and one SMRT cell at a SciLifeLab facility in Uppsala, Sweden. This third-generation high-throughput sequencing PacBio platform produces long reads averaging 20–25 kilobases (up to 100 kilobases). The DNA template is circularized via hairpin adaptors and recorded multiple times to offer read qualities comparable to that of traditional Sanger sequencing. The new Sequel instrument generates ~400,000 reads per SMRT cell (3.2 million reads per run), which is much fewer reads than produced by Illumina platforms, but it produces high-quality sequences of amplicons or DNA fragments of ~3 kilobases in length. This makes the platform ideal for sequencing short-to-medium length amplicons such as the full ITS region and perhaps its flanking conserved genes for precise phylogenetic placement [45]. This technology provides powerful tools to explore fungal diversity and enable identification of complex fungal communities and individual community components even at low abundances.

2. Materials and Methods

2.1. Study Sites

Study sites were in 3 different regions of Lithuania, representing the nearby locations of Alytus, Labanoras, and Varėna (Figure 1). At each location, the study sites were selected based on the forest inventory data from the Lithuanian State Forest Cadastre database. For the evaluation of the dynamic changes of chemical soil properties and fungal communities after the clear-cuttings, the study sites were selected at each region in six sampling sites representing different stages of the *P. sylvestris* forest ecosystem: (1) mature *P. sylvestris* forest stands; (2) clear-cuts of *P. sylvestris* (felled in 2019); (3) 1st year *P. sylvestris* plantations (planted in 2018); (4) 2nd year *P. sylvestris* plantations (planted in 2017); (5) 3rd year *P. sylvestris* plantations (planted in 2016); and (6) 4th year *P. sylvestris* plantations (planted in 2015). In total, 18 sampling sites were selected in May 2020 (Table 1). The study sites were selected in the clear-cuts and *P. sylvestris* plantations of different ages established after final clear-cutting of mature forest (130 years old; average tree height was 26.6–31.5 m; average tree diameter at breast height (DBH) was 37 cm). All sampling sites were described as the sites with comparable soil and climatic conditions. During the sampling period in May–September 2020, the mean air temperature was 15.8 °C, and the mean precipitation was 64 mm (data obtained from the Lithuanian Hydrometeorological Service).

Table 1. Characteristics of the sampling sites in the selected *Pinus sylvestris* L. stands according to the State Forest Cadastre (2021).

Region (Code)	Sampling Site Code *	Site Description	Geographic Coordinates	Forest Vegetation Type **	Stand Age (years)	Mean Tree DBH *** (cm)	Mean Tree Height (m)
Alytus (A)	A1	Mature forest	54°31′29.4″ N, 24°0′52.9″ E	vm	94	35.9	31.2
	A2	Clear-cut	54°32′33.4″ N, 24°8′22.1″ E	v	-	-	-
	A3	1st year plantation	54°34′13.3″ N, 23°57′59.2″ E	vm	3	-	-
	A4	2nd year plantation	54°31′31.3″ N, 24°0′50.1″ E	vm	4	-	-
	A5	3rd year plantation	54°31′26.1″ N, 23°56′2.6″ E	vm	5	-	-
	A6	4th year plantation	54°30′39.2″ N, 23°58′25.0″ E	vm	6	-	-

Table 1. *Cont.*

Region (Code)	Sampling Site Code *	Site Description	Geographic Coordinates	Forest Vegetation Type **	Stand Age (years)	Mean Tree DBH *** (cm)	Mean Tree Height (m)
Varėna (V)	V1	Mature forest	54°10′45.2″ N, 24°35′9.8″ E	vm	85	28.1	24.4
	V2	Clear-cut	54°8′32.9″ N, 24°34′36.1″ E	v	-	-	-
	V3	1st year plantation	54°11′51.9″ N, 24°35′48.7″ E	vm	3	-	-
	V4	2nd year plantation	54°9′18.4″ N, 24°31′48.3″ E	vm	4	-	-
	V5	3rd year plantation	54°10′18.7″ N, 24°35′48.9″ E	vm	5	-	-
	V6	4th year plantation	54°6′7.5″ N, 24°34′4.8″ E	vm	6	-	-
Labanoras (L)	L1	Mature forest	55°10′36.7″ N, 25°45′28.3″ E	vm	132	36.1	31.1
	L2	Clear-cut	55°9′10.8″ N, 25°47′42.8″ E	v	-	-	-
	L3	1st year plantation	55°10′35.0″ N, 25°45′11.5″ E	vm	3	-	-
	L4	2nd year plantation	55°8′35.9″ N, 25°48′37.0″ E	vm	4	-	-
	L5	3rd year plantation	55°8′17.9″ N, 25°45′28.3″ E	vm	5	-	-
	L6	4th year plantation	55°10′37.7″ N, 25°45′33.4″ E	vm	6	-	-

* A1–A6: sampling sites in Alytus region; V1–V6: sampling sites in Varėna region; L1–L6: sampling sites in Labanoras region, also shown in Figure 1. ** v: *vacciniosa*, m: *myrtilliosa*; *** DBH: tree diameter at breast height (measured at 1.3 meters above the ground).

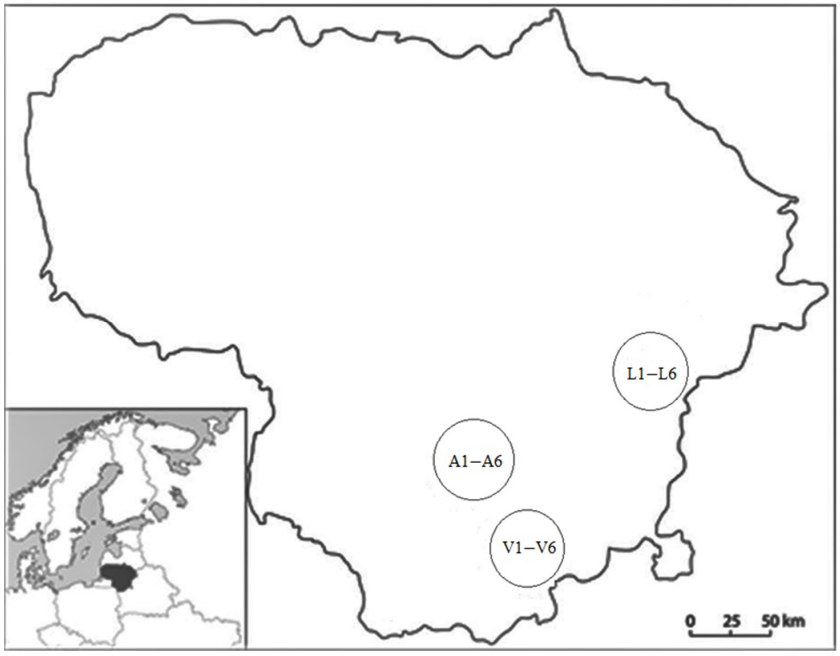

Figure 1. Location of the three study regions in Lithuania: Alytus (A, on the map), Labanoras (L), Varėna (V). In each region, the following sampling sites were included: (1) mature *Pinus sylvestris* L. forest stands; (2) clear-cuts of *P. sylvestris* (felled in 2019); (3) 1st year *P. sylvestris* plantations (planted in 2018); (4) 2nd year *P. sylvestris* plantations (planted in 2017); (5) 3rd year *P. sylvestris* plantations (planted in 2016); (6) 4th year *P. sylvestris* plantations (planted in 2015).

For the selection of three study sites, the following criteria were used: the pure *P. sylvestris* stands growing on typical forest site type prior to clear-cutting, and the adjacent sites, reforested by *P. sylvestris*, representing the annual ecosystem stages; comparable forest soil and forest vegetation type [46]. All selected study sites were characterized by the *Pinetum vacciniosa* and *P. vaccinio-myrtilliosa* forest type; the oligotrophic mineral soil of a normal moisture regime (Nbl) [47]; and nutrient-poor sandy Arenosols with a light soil texture [48]. The mean tree diameter and mean height were available for mature forest sites (Table 1). The species composition of ground vegetation was similar in all studied sites with the dominant herbs and dwarf shrubs such as *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L. and dominant mosses such as *Hylocium splendens* (Hedw.) Schimp., *Pleurozium schreberi* (Brid.) Mitt., and *Ptilium crista-castrensis* (Hedw.) De Not.

2.2. Soil Sampling and Chemical Analyses

The soil sampling was performed according to the ICP-Forests methodology [49]. For the determination of basic soil properties, soil was sampled from the upper (0–20 cm) mineral soil layer, composing three samples from five subsamples per each sampling site at each study region. Soil samples were taken at 1 m from tree stems or stumps, maintaining the same distance at all study sites. In total, 54 soil samples were taken in the field. In each sampling site, the points for soil sampling were allocated systematically in two diagonals. The soil samples were individually placed into the plastic bags, labelled, and transported to the laboratory. The following chemical parameters were analysed: pH was determined in a 0.01 M $CaCl_2$ suspension (ISO 10390:2005), organic carbon (C) was determined using a dry combustion method with a total carbon analyzer Analytic Jena multi EA 4000 Germany (ISO 10694:1995) with a Heraeus apparatus (ISO 10694, dry combustion at 900 °C), and total nitrogen (N) analyzed using the Kjeldahl method (ISO 11261). In addition, mineral N was determined in 1 M KCl extraction by the spectrometric method (ISO 14256-2); mobile potassium (K_2O) and phosphorus (P_2O_5) were determined using a solution of ammonium lactate by the Égnér-Riehm-Domingo (A-L) method. The laboratory analyses were provided by the Agrochemical Research Laboratory of the Lithuanian Research Centre for Agriculture and Forestry (LAMMC).

2.3. Fungal Sampling

The fungal core samples were sampled next to the soil samples as additional samples. The core samples were taken to represent each of the selected study sites, i.e., the clear-cut, the 1st–4th years plantation, and the mature forest with the corresponding age of the trees. The effects of clear-cutting on forest soil mycobiota (saprotrophic, mycorrhizal, pathogenic, and endophytic fungi) in *P. sylvestris* ecosystems were assessed. The soil sampling for fungal identification was conducted in May–September 2020 in all sampling sites (Table 1). Five sample plots were randomly selected in each sample site. For the assessment of forest soil mycobiota, soil samples were collected in four replicates at each sample plot, obtaining one composite sample per plot. For fungal identification, the soil (ca. 100 g) was sampled using a 2 cm diameter soil core from the 5–20 cm mineral soil layer. Between different samples, the soil core was thoroughly cleaned with ethanol. The soil samples were individually placed into the plastic bags, labelled, transported to the laboratory, and stored at −20 °C. In total, 90 soil samples were collected.

2.4. Fungal DNA Isolation, Amplification, and Sequencing

The standard CTAB (cetyltrimethylammonium bromide) method was used to isolate genomic DNA from living tissue [50]. Prior to the DNA isolation, each soil sample was freeze-dried at −60 °C for 48 h. After the freeze-drying, ca. 20–30 mg of soil was used for the DNA isolation from each sample using a NucleoSpin®Soil kit (Macherey-Nagel GmbH & Co., Duren, Germany), according to the manufacturer's recommendations. Following the DNA isolation, the DNA concentration in the individual samples was determined using a NanoDrop™ One spectrophotometer (Thermo Scientific, Rodchester, NY, USA).

The amplification of the internal transcribed spacer (ITS) rRNA region was achieved using a forward primer gITS7 (5′-GTGARTCATCGARTCTTTG-3′) [51] and a barcoded universal primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [52].

The polymerase chain reaction (PCR) was performed in 50 µL reactions and consisted of the following final concentrations: 1% DreamTaq Green Polymerase (5 µ/µL) (Thermo Scientific, Waltham, MA, USA); 11% 10X Buffer; 11% dNTPs (10 mM); 1% $MgCl_2$ (25 mM); 2% of each primer (200 nM); 72% sterile milli-Q water.

The amplifications were performed using an 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 1.5% 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 PCR products using a Qubit fluorometer 4.0 (Life Technologies, Stockholm, Sweden), they were pooled in an equimolar mix and sequenced using a PacBio RSII platform and one SMRT cell at a SciLifeLab facility in Uppsala, Sweden.

2.5. Bioinformatics

Identification of fungal species was performed by molecular method—high-throughput sequencing (HTS). The sequences were filtered and clustered using the Sequence Clustering and Analysis of Tagged Amplicons (SCATA) next-generation sequencing (NGS) pipeline, available online http://scata.mykopat.slu.se. The sequences were filtered for quality, removing short sequences (<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 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 Ribosomal Database Project (RDP) pipeline classifier (https://pyro.cme.msu.edu/index.jsp, accessed on 8 October 2022). Fungal taxa were identified using GenBank (NCBI) database and the BLASTn algorithm. The criteria used for taxonomic identification were sequence coverage > 80%; similarity to species level 98%–100%; and similarity to genus level 94%–97%. Sequences not matching these criteria were considered as unidentified and were given unique names. Representative sequences of the fungal non-singletons are available from GenBank under accession numbers ON962810–ON963944.

2.6. Statistical Analyses

The soil chemical parameters were analyzed for differences in concentrations between the study sites: mature forest stands, clear-cuts, and 1st–4th year *P. sylvestris* plantations. For the normality of the variables, the Shapiro–Wilk test was used, and the normality hypothesis was rejected with a 0.05 significance level. Therefore, the Kruskal–Wallis analysis of variance (ANOVA) test was used to ascertain the significant differences in variables between the sites.

For the identification of the diversity and composition of the fungal communities, the Shannon diversity (H) index was evaluated [53]. The assignment of ecological roles was based on FUNGuild [54]. The rarefaction analysis was performed using Analytical Rarefaction v.1.3, available at http://www.uga.edu/strata/software/index.html (accessed on 20 February 2022). Rarefaction curves showing the relationship between the cumulative number of fungal operational taxonomic units (OTUs) and the number of ITS rDNA sequences were obtained from soil samples in different study sites.

To calculate and draw a Venn diagram, we used an online program (http://bioinformatics.psb.ugent.be/webtools/Venn/ (accessed on 25 August 2022). The differences in the rela-

tive abundance of fungal OTUs between study sites were tested using the Kruskal–Wallis test. Comparison of fungal composition was calculated using the Bray–Curtis similarity coefficient, which was used in the analysis of similarity to determine whether the fungal communities differed between study sites. Nonmetric multidimensional scaling (NMDS) was used to provide a visual summary of the pattern Bray–Curtis values. Relationships between soil chemical parameters and fungal variables were examined using the Pearson correlation and canonical correspondence analysis (CCA). Statistical analyses were performed using the statistical software package XLSTAT (Addinsoft, N.Y., USA) and open access software R version 4.0.5 (R Core team, Austria (http://www.R-project.org/) (accessed on 10 June 2021).

3. Results

3.1. Chemical Properties of Mineral Topsoil

The mean pH_{CaCl2} value was slightly lower in the mineral 0–20 cm topsoil layer at the clear-cut sites than in the mature *P. sylvestris* forest stands (Figure 2A). Although no clear trend was identified in the reforested sites after clear cuttings of the mature forest stands, the highest mean pH value (pH_{CaCl2} 4.4 ± 0.2) was obtained in the 3rd year plantations. No significant differences were obtained for the mean concentrations of SOC and total N (Figure 2B,C), as well as K, Ca, and Mg (Table 2) across all studied sites. A statistically significantly higher mean concentration of mineral N was found in the 1st year plantations (Figure 2D).

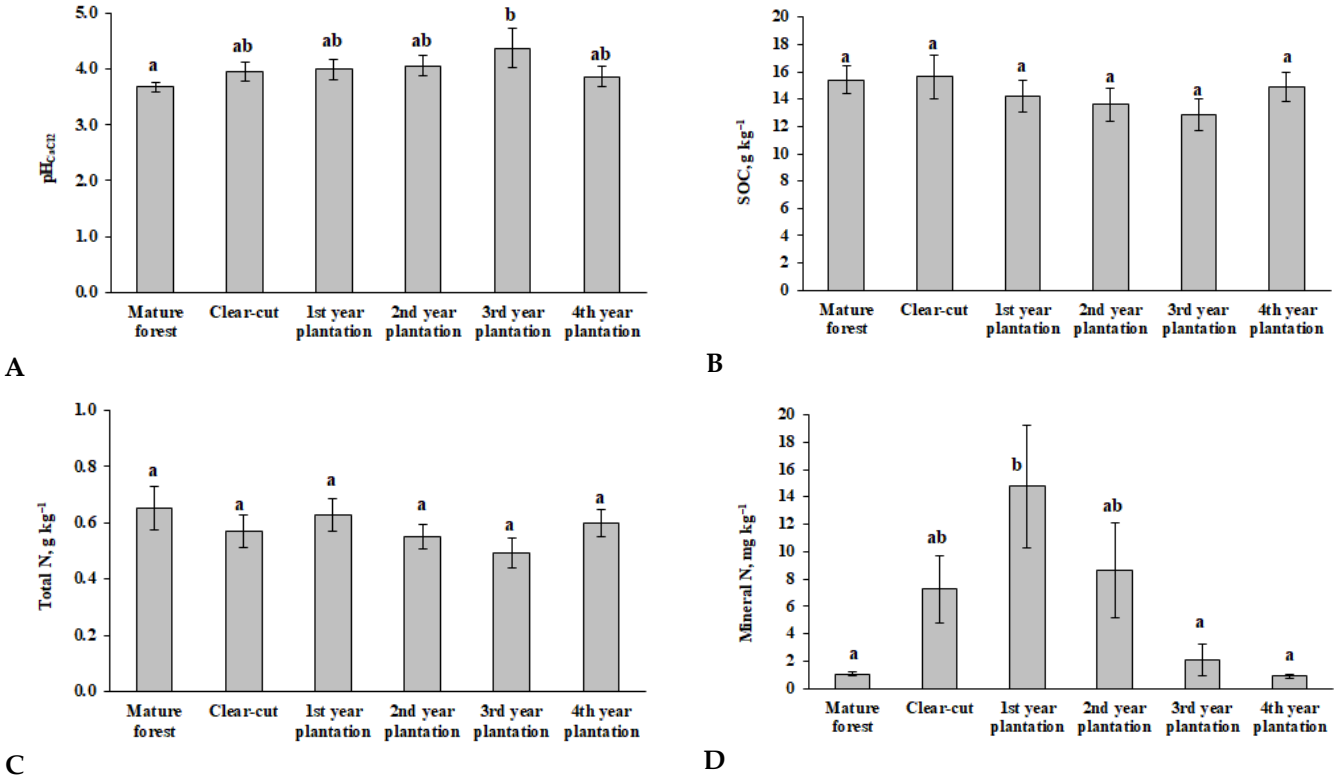

Figure 2. Mean pH_{CaCl2} (**A**), mean concentrations of soil organic carbon (SOC, g kg^{-1}) (**B**), total nitrogen (Total N, g kg^{-1}) (**C**), and mineral nitrogen (Min N, g kg^{-1}) (**D**) in the 0–20 cm topsoil layer in the mature forest, clear-cut, and reforested 1st–4th year *Pinus sylvestris* L. plantations. The data from three study sites (A, L, and V) were combined. The values followed by the same letter indicate no significant difference between the sampling sites at $p < 0.05$.

Table 2. Mean EC (mS m^{-1}), mean concentrations of total phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), chlorides (Cl^-) (mg kg^{-1}), and P_2O_5, K_2O, Ca^{2+}, and Mg^{2+} in the 0–20 cm topsoil layer in the mature forest, clear-cut, and reforested 1st–4th year *Pinus sylvestris* L. plantations. The data from three study sites (A, L, and V) were combined. The values followed by different letters a and b within each column indicate statistically significant differences between the sampling sites at $p < 0.05$.

Site Description	EC	Total P	Total K	Total Ca	Total Mg
	(mS m^{-1})	(mg kg^{-1})			
Mature forest	2.5 ± 0.1 ab	195.6 ± 19.1 a	146.3 ± 8.3 a	186.7 ± 27.3 a	242.4 ± 33.8 a
Clear-cut	2.6 ± 0.2 ab	232.3 ± 29.4 ab	137.2 ± 7.9 a	151.2 ± 9.6 a	229.0 ± 27.8 a
1st year plantation	3.6 ± 0.5 b	302.8 ± 33.9 b	145.4 ± 12.7 a	191.5 ± 29.6 a	228.8 ± 40.0 a
2nd year plantation	2.6 ± 0.2 ab	250.4 ± 14.5 ab	122.6 ± 8.8 a	179.7 ± 20.2 a	248.2 ± 48.5 a
3rd year plantation	2.3 ± 0.1 a	231.9 ± 20.1 ab	138.6 ± 9.3 a	206.2 ± 19.4 a	242.3 ± 41.1 a
4th year plantation	2.9 ± 0.3 ab	205.8 ± 11.9 a	152.0 ± 6.1 a	211.1 ± 21.0 a	263.3 ± 24.7 a
	Cl^-	P_2O_5	K_2O	Ca^{2+}	Mg^{2+}
	(mg kg^{-1})				
Mature forest	5.8 ± 0.2 a	79.7 ± 9.2 a	34.1 ± 2.9 a	132.6 ± 10.8 ab	41.8 ± 1.5 a
Clear-cut	6.1 ± 0.4 a	122.6 ± 26.2 ab	35.3 ± 4.0 a	121.3 ± 6.1 ab	39.9 ± 2.1 a
1st year plantation	6.7 ± 0.3 a	169.1 ± 27.8 b	36.6 ± 4.3 a	122.8 ± 12.5 ab	40.8 ± 3.1 a
2nd year plantation	6.7 ± 0.4 a	109.8 ± 12.1 ab	31.3 ± 4.2 a	120.4 ± 10.8 a	37.7 ± 2.9 a
3rd year plantation	6.7 ± 0.4 a	101.0 ± 11.4 ab	27.2 ± 2.4 a	161.6 ± 9.7 b	48.3 ± 5.6 a
4th year plantation	6.9 ± 0.4 a	107.9 ± 16.5 ab	32.1 ± 3.8 a	140.8 ± 12.3 ab	50.6 ± 6.7 a

The highest electrical conductivity (EC) was found in the 1st year plantation, but in other studied sites, the EC varied within a relatively narrow range from 2.3 ± 0.1 mS m^{-1} to 3.6 ± 0.5 mS m^{-1} (Table 2). A higher concentration of total P was found in the 1st year plantation, but in the 2nd, 3rd, and 4th year plantations, the total P concentration did not differ from the P concentration in the mature forest. The concentration of P_2O_5 in the mineral 0–20 cm topsoil layer was 1.5 times higher in the clear-cut, and 2.1 times higher in 1st year plantations than in the mature forest stands.

The highest concentration of Ca^{2+} was found in the 3rd year plantation. However, there were no statistically significant differences in the concentrations of K_2O and Mg^{2+} across all studied sites (Table 2). Overall, the soil nutrient concentrations in the 4th year *P. sylvestris* plantations were comparable with those obtained in the mature forest stands.

3.2. Fungal Community

The quality filtering after PacBio sequencing showed the presence of 60,898 high-quality fungal sequences representing 1143 fungal operational taxonomic units (OTUs) (Table 3 and Figure 3), and the non-fungal OTUs were excluded.

Table 3. Generated high-quality fungal sequences and detected diversity of the fungal taxa in the soil at different *Pinus sylvestris* L. sites in sampling sites. The data from the three study sites (A, L, and V) were combined.

Site Description	No. of Fungal Sequences	No. of Fungal Taxa	Shannon Diversity Index H
Mature forest	10,523	430	3.86
Clear-cut	14,534	415	3.76
1st year plantation	5846	388	4.30
2nd year plantation	7818	392	4.00
3rd year plantation	5078	345	4.30
4th year plantation	17,099	559	4.21
Total	60,898	1143	

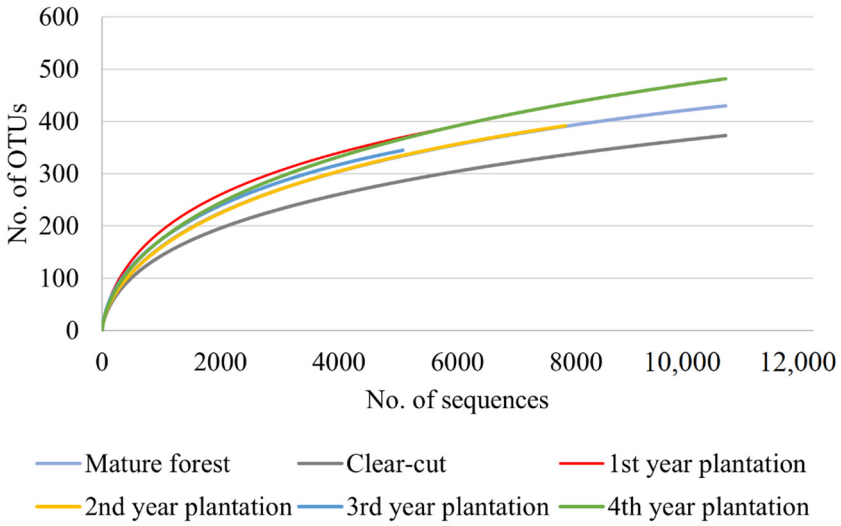

Figure 3. Rarefaction curves showing the relationship between the cumulative number of fungal operational taxonomic units (OTUs) and the number of internal transcribed spacer (ITS) rRNA sequences from corresponding sampling sites; the data obtained in the three study sites (A, L, and V) were combined to give a generalized trend for the mature forest stands, clear-cuts, and reforested 1st–4th year *Pinus sylvestris* L. plantations.

Among all the fungal OTUs, 352 OTUs (30.8%) were identified up to the species level, 161 OTUs (14.1%) up to the genus level, and 630 OTUs (55.1%) were identified only up to a higher taxonomic level. Of the 1143 fungal OTUs across all the soil samples, *Ascomycota* was the most dominant phylum, and accounted 26.4% of all the fungal OTUs, followed by *Basidiomycota* (23.3%), *Mucoromycota* (3.3%), *Chytridiomycota* (0.7%), and *Zoopagomycota* (0.1%).

The rarefaction analysis showed that the number of fungal OTUs did not reach species saturation (Figure 3), indicating that a potentially higher diversity of taxa could be detected by a deeper sequencing.

The non-metric multidimensional scaling shows that the fungal communities in the mature forest stands, clear-cuts, and the 1st–4th year *P. sylvestris* plantations did not differ significantly from each other (Figure 4).

The assignment of fungal ecological roles (trophic groups) is represented in Figure 5. The most common fungal OTUs in the soil samples were identified as of unknown ecological roles (59.8%–67.1%, representing all studied study sites), followed by saprotrophs (13.7%–19.8%), mycorrhizal fungi (2.6%–8.4%), pathogens (3.8%–7.4%), endophytes (3.0%–4.3%), and others (3.6%–5.2%).

Among all obtained fungal OTUs, 91 fungal OTUs (8.0%) were detected in all studied sites. The numbers of obtained unique and common fungal OTUs in between different sites are shown in the Venn diagrams (Figures 6 and 7). The 161 common fungal OTUs were found in the mature forest stands, clear-cuts, and 4th year plantations. A similar number of unique fungal OTUs were found in the mature forest and in the clear-cut sites: 150 and 133 OTUs, respectively). In the 4th year plantations, the number of unique fungal OTUs increased significantly. The lowest number (45 OTUs) of shared fungal OTUs was found in the mature forest stands and the clear-cuts. A similar number of shared OTUs were found in the mature forest stands and the 4th year plantations, and in the clear-cuts and the 4th year plantations (74 and 76 OTUs, respectively) (Figure 6).

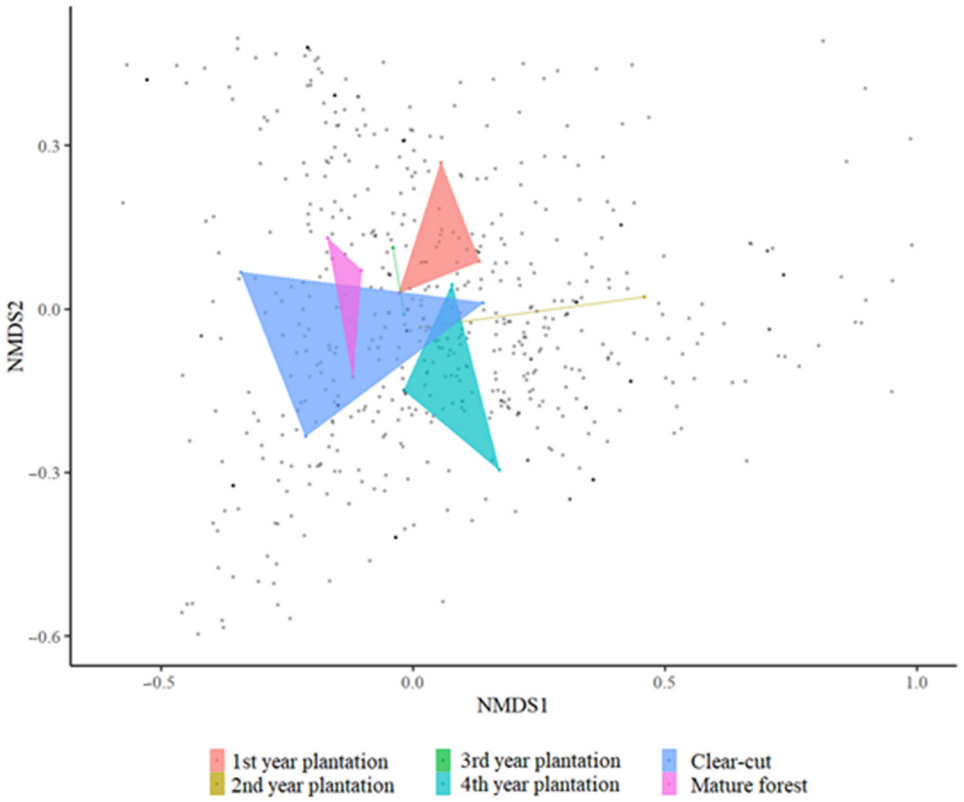

Figure 4. Nonmetric multidimensional scaling (NMDS) ordination of the fungal communities in the study sites (Bray–Curtis index). Each point represents separate fungal OTUs. The data obtained in three study sites (A, L, and V) were combined to show the overall situation for the mature forest stands, clear-cuts, and reforested 1st–4th year *Pinus sylvestris* L. plantations.

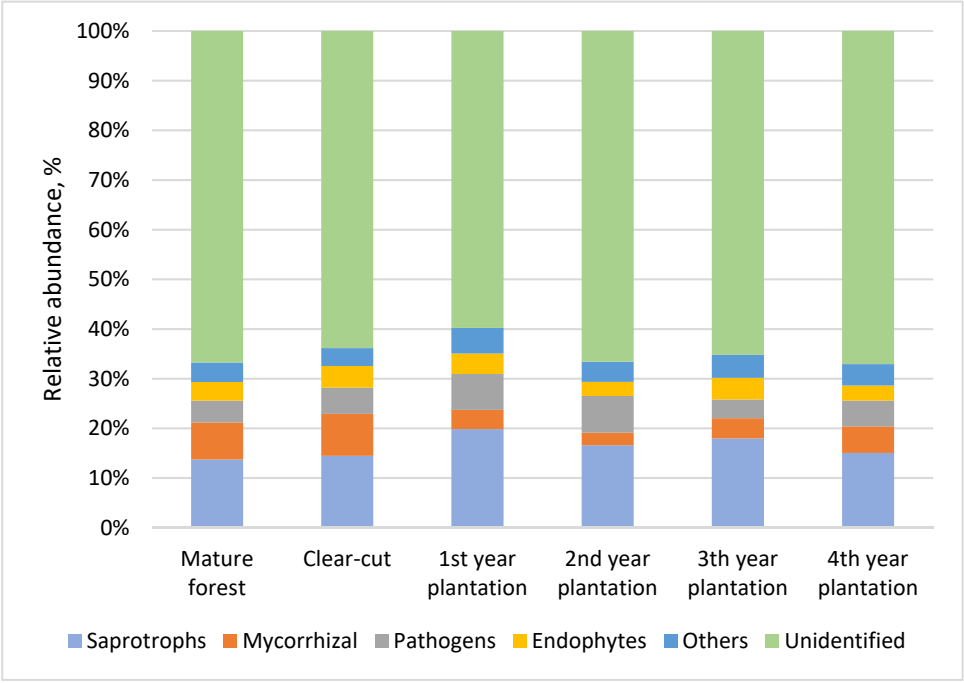

Figure 5. Ecological roles of the fungal OTUs in the soil sampled from different study sites. The data for the mature forest stands, clear-cuts, and reforested 1st–4th year *Pinus sylvestris* L. plantations were combined correspondingly from three study sites (A, L, and V).

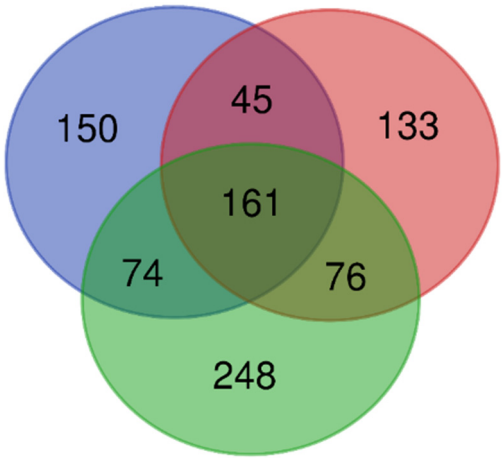

Figure 6. Venn diagram indicating the diversity of the fungal OTUs found in the mature forests, clear-cuts, and 4th year plantations, and the number of fungal OTUs shared across studied sites; the data obtained in three study sites (A, L, and V) were combined. Different colours represent different study sites: blue indicates mature forest stands, red—clear-cuts, and green—4th year plantations.

Among all fungal OTUs found in *P. sylvestris* plantations, 100 OTUs were exclusively found in the 1st year plantations, 120 OTUs in the 2nd year plantations, 77 OTUs in the 3rd year plantations, and 217 OTUs in the 4th year plantations. The highest number of OTUs was common for the 2nd year and the 4th year plantations. The lowest number of shared fungal OTUs was between the 2nd and 3rd year plantations, i.e., 17 OTUs. In total, 118 fungal OTUs were shared between all plantations (Figure 7).

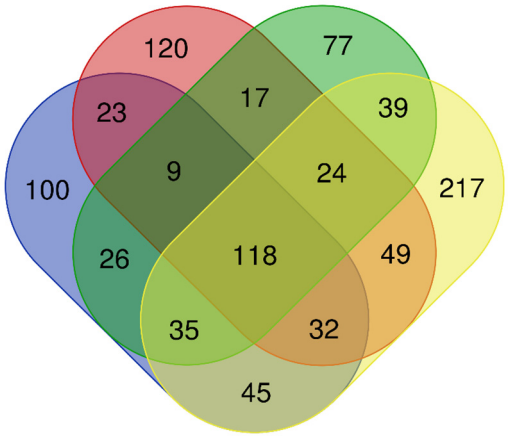

Figure 7. Venn diagram indicating the diversity of the fungal OTUs found in the 1st–4th year *Pinus sylvestris* L. plantations and the number of fungal OTUs shared across all plantations; the data obtained at three study sites (A, L, and V) were combined. Different colours represent different study sites: blue—1st year plantations, red—2nd year plantations, green—3rd year plantations, and orange—4th year plantations.

The 30 most common fungal OTUs, representing 62.03% of all high-quality fungal sequences in the soil sampled from the different study sites, are given in Table 4.

Table 4. Relative abundance and similarity of the 30 most common fungal OTUs (shown as a proportion of all the high-quality fungal sequences) in the soil samples from the *Pinus sylvestris* L. sites, representing mature forests, clear-cuts, and reforested 1st–4th year plantations; the data from three study sites (A, L, and V) were combined. The values followed by different letters within each row indicate statistically significant differences at $p < 0.05$.

OTU	Phylum [a]	References	Similarity, (%) [b]	Relative Abundance (%)						
				Mature Forest	Clear-Cut	1st Year Plantation	2nd Year Plantation	3rd Year Plantation	4th Year Plantation	Total
Archaeorhizomyces sp. *5425_1*	A	MH248043	207/207 (100)	21.59 b	18.52 ab	5.03 ab	0.97 a	2.40 ab	9.01 ab	11.49
Helotiaceae sp. 5425_2	A	MK131613	240/240 (100)	1.84 a	9.67 a	2.51 a	12.13 a	5.45 a	6.91 a	6.82
Unidentified sp. 5425_18	A	MN902668	258/258 (100)	1.22 a	4.67 a	1.54 a	3.61 a	10.73 a	8.30 a	5.16
Oidiodendron chlamydosporicum	A	MG597466	235/235 (100)	2.26 ab	6.31 b	1.66 ab	1.06 a	5.71 ab	7.94 b	4.90
Penicillium camemberti	A	MT355566	251/251 (100)	3.24 a	2.51 a	2.10 a	5.95 a	6.64 a	3.54 a	3.67
Cladosporium sp. 5425_16	A	MT645945	243/243 (100)	4.68 a	1.42 a	5.61 a	2.88 a	4.21 a	1.53 a	2.84
Amanita fulva	B	MT229863	260/260 (100)	2.14 a	7.08 a	-	-	-	-	2.06
Jackrogersella multiformis	A	MT573483	254/254 (100)	9.52 a	0.05 a	1.54 a	0.06 a	1.93 a	0.05 a	1.99
Piloderma sphaerosporum	B	MK131527	292/292 (100)	1.45 a	6.75 a	-	0.01 a	0.10 a	0.01 a	1.88
Placynthiella oligotropha	A	MK812378	248/248 (100)	0.02 a	-	0.10 a	11.61 a	0.10 a	1.08 a	1.81
Malassezia sp. 5425_17	B	MT594863	369/369 (100)	4.68 b	0.96 ab	2.82 ab	0.84 a	1.26 ab	0.87 ab	1.77
Unidentified sp. 5425_68	A	EU292507	216/217 (99)	0.29 ab	-	0.26 ab	0.43 ab	0.10 a	5.22 b	1.60
Mycena zephirus	B	MH856339	304/304 (100)	-	-	16.08	-	-	-	1.54
Phanerochaete sp. 5425_7	B	KP135164	293/293 (100)	0.45 a	0.94 ab	6.55 b	1.28 ab	2.38 ab	0.56 ab	1.45
Cladophialophora minutissima	B	MT644883	313/313 (100)	0.11 a	0.21 a	0.46 a	8.14 a	0.20 a	0.97 a	1.45

Table 4. *Cont.*

OTU	Phylum [a]	References	Similarity, (%) [b]	Relative Abundance (%)						
				Mature Forest	Clear-Cut	1st Year Plantation	2nd Year Plantation	3rd Year Plantation	4th Year Plantation	Total
Thelephora terrestris	A	MG597448	253/254 (99)	0.01 ab	0.01 a	0.65 ab	-	1.50 ab	4.49 b	1.45
Mortierella sp. 5425_53	M	HQ021936	342/344 (99)	1.59 a	0.39 a	0.33 a	0.93 a	2.58 a	1.39 a	1.12
Russula decolorans	B	MN992510	359/359 (100)	3.79 b	1.73 ab	0.03 a	-	-	0.05 ab	1.08
Unidentified sp. 5425_0	A	JQ312914	240/241 (99)	0.68 a	1.22 a	1.03 a	0.91 a	1.44 a	1.16 a	1.07
Russula vinosa	B	MK838342	366/367 (99)	-	-	0.02 a	-	-	3.63 a	1.02
Collophora sp. 5425_14	A	MW449039	244/244 (100)	1.28 a	0.72 a	2.28 a	0.69 a	1.81 a	0.45 a	0.98
Xylaria hypoxylon	A	MK577428	252/252 (100)	4.41 b	0.03 ab	0.51 ab	0.04 a	0.85 ab	0.04 ab	0.90
Alternaria infectoria	A	MT635276	253/253 (100)	0.29 a	0.24 a	1.80 a	0.52 a	2.09 a	1.35 a	0.90
Mortierella sp. 5425_47	M	MT601876	293/293 (100)	1.37 b	0.50 ab	0.19 a	0.23 ab	2.97 ab	0.34 ab	0.75
Cladophialophora sp. 5425_111	A	AB986417	268/271 (99)	-	-	-	-	-	2.54	0.71
Mortierella macrocystis	M	MH859487	330/330 (100)	0.45 ab	1.14 b	1.25 ab	0.15 a	1.34 ab	0.30 ab	0.68
Glonium pusillum	A	MT635300	249/249 (100)	0.48 a	0.43 a	0.94 a	1.91 a	0.51 a	0.32 a	0.65
Unidentified sp. 5425_67	A	HQ433092	300/300 (100)	0.13 ab	1.86 b	0.02 a	0.18 ab	0.02 a	0.51 ab	0.64
Solicoccozyma terricola	B	MH487580	329/329 (100)	0.22 a	0.52 a	0.21 a	1.04 a	0.20 a	1.06 a	0.63
Lecanoromycetes sp. 5425_115	A	MT027951	234/234 (100)	0.07 a	0.09 a	0.31 a	0.01 a	0.96 a	1.66 a	0.61
Total of OTUs				68.25	67.96	55.82	55.59	57.48	65.26	63.63

[a] Ascomycota (A), Basidiomycota (B), Mucoromycota (M). [b] Sequence similarity column shows base pairs between the query sequence and the reference at GenBank, and the percentage of sequence similarity.

The most abundant fungi in the studied sites were *Archaeorhizomyces* sp. 5425_1 (11.5% of all high-quality sequences), *Helotiaceae* sp. 5425_2 (6.8%), Unidentified sp. 5425_18 (5.2%), *Oidiodendron chlamydosporicum* Morrall (4.9%), *Penicillium camemberti* Thom (3.7%), and *Cladosporium* sp. 5425_16 (2.8%) (Table 4). Among the obtained most common fungal OTUs, five species represented ectomycorrhizal (ECM) fungi, which included *Amanita fulva* (Schaeff.) Fr. (2.1% of all high-quality sequences), *Piloderma sphaerosporum* (Peck) Julich (1.9%), *Thelephora terrestris* Ehrh. (1.5%), *Russula decolorans* Fr. (1.1%), *Russula vinosa* Lindblad (1.0%), and only one OTU represented the plant pathogen *Collophora* sp. 5425_14 (1.0%). The dominant endophytes in the studied fungal community were *Mortierella* sp. 5425_53 and *Mortierella macrocystis* W.Gams.

The non-metric multidimensional scaling of the mycorrhizal fungal communities showed a partial separation of the samples obtained from the different sites. The mycorrhizal fungal communities did not differ significantly among the mature forest stands and the 4th year plantations ($p > 0.05$). A fungal community between 1st year plantations and the clear-cut site differed significantly ($p < 0.05$) (Figure 8).

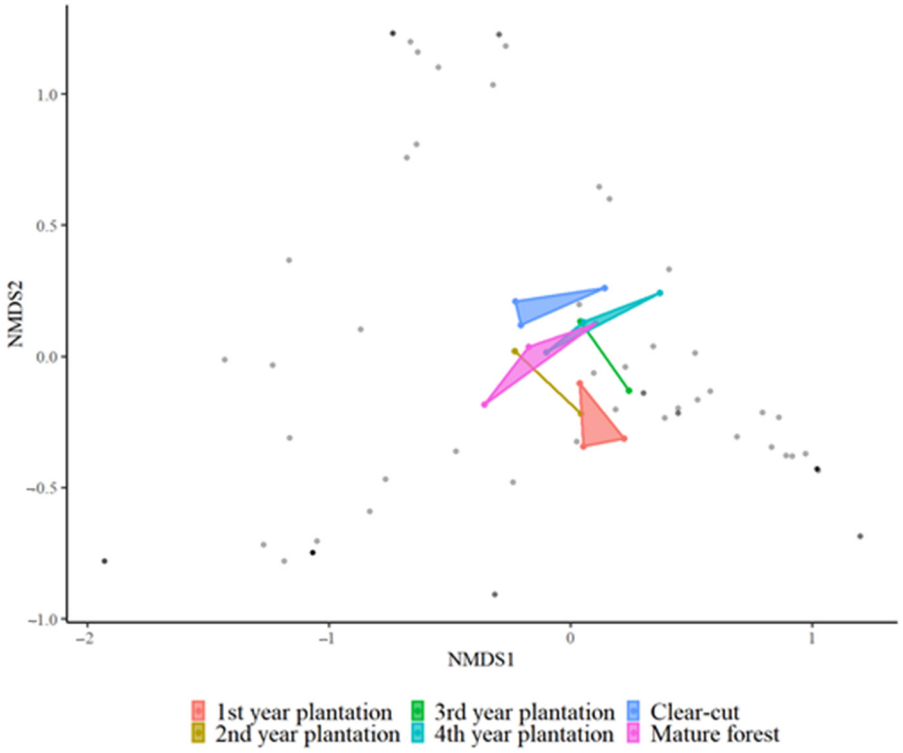

Figure 8. Nonmetric multidimensional scaling (NMDS) ordination of the mycorrhizal fungi abundance in study sites (Bray–Curtis index). Each point represents a separate mycorrhizal fungal OTU. The data obtained in three study sites (A, L, and V) were combined to show the overall situation for the mature forest stands, clear-cuts, and reforested 1st–4th year *Pinus sylvestris* L. plantations.

In total, 70 mycorrhizal fungal OTUs were found in the soil samples in the studied sites. The relative abundances of the thirty most common ectomycorrhizal (ECM) fungi are given in Table 5.

The most abundant ECM fungi were *Amanita fulva* (2.1% of all the high-quality sequences), *Piloderma sphaerosporum* (1.9%), *Thelephora terrestris* (1.5%), *Russula decolorans* (1.1%), *Russula vinosa* (1.0%), and *Pseudotomentella* sp. 5425_255 (0.6%) (Table 5).

Table 5. Relative abundance and similarity of the 30 most common ectomycorrhizal (ECM) fungal OTUs in the soil samples from the *Pinus sylvestris* L. sites, representing mature forest stands, clear-cuts, and reforested 1st–4th year plantations; the data from three study sites (A, L, and V) were combined. The values followed by different letters within each row indicate statistically significant differences at $p < 0.05$.

OTU	Phylum [a]	References	Similarity, (%) [b]	Relative Abundance (%)						
				Mature Forest	Clear-Cut	1st Year Plantation	2nd Year Plantation	3rd Year Plantation	4th Year Plantation	Total
Amanita fulva	B	MT229863	260/260 (100)	2.14 a	7.08 a	-	-	-	-	2.06
Piloderma sphaerosporum	B	MK131527	292/292 (100)	1.45 a	6.75 a	-	0.01 a	0.10 a	0.01 a	1.88
Thelephora terrestris	B	MT644883	313/313 (100)	0.01 ab	0.01 a	0.65 ab	-	1.50 ab	4.49 b	1.45
Russula decolorans	B	MN992510	359/359 (100)	3.79 b	1.73 ab	0.03 b	-	-	0.05 ab	1.08
Russula vinosa	B	MK838342	366/367 (99)	-	-	0.02 a	-	-	3.63 a	1.02
Pseudotomentella sp. 5425_119	B	HM146848	345/345 (100)	-	2.46	-	-	-	-	0.59
Elaphomyces cf. *muricatus*	A	KR029741	287/288 (99)	-	2.20	-	-	-	-	0.53
Amphinema sp. 5425_155	B	MK838190	288/288 (100)	1.43 a	0.01 a	-	0.04 a	-	-	0.25
Laccaria proxima	B	MN663149	301/301 (100)	-	-	0.43 a	-	1.75 a	0.08 a	0.21
Tricholoma portentosum	B	LC375763	304/304 (100)	0.1a	0.65 a	-	0.01 a	0.02 a	-	0.19
Amanita pantherina	B	MZ410633	310/310 (100)	0.13 a	-	-	-	-	0.59 a	0.19
Russula vesca	B	MZ410735	320/321 (99)	0.76 a	0.13 a	-	0.04 a	-	-	0.17
Russula albonigra	B	DQ422029	321/321 (100)	0.95	-	-	-	-	-	0.16
Russula adusta	B	MG687346	321/321 (100)	-	0.65 a	0.07 a	-	-	-	0.16
Piloderma bicolor	B	MH809947	283/283 (100)	-	0.65	-	-	-	-	0.15
Xerocomus badius	B	MN947388	284/284 (100)	0.04 a	-	0.03 a	-	-	0.46 a	0.14
Russula paludosa	B	LC192779	358/358 (100)	-	0.44 a	0.02 a	-	-	-	0.11
Pseudotomentella sp. 5425_255	B	MK290703	316/316 (100)	-	0.36	-	-	-	-	0.09
Tomentella stuposa	B	MK838269	313/314 (99)	-	0.30	-	-	-	-	0.07

Table 5. *Cont.*

OTU	Phylum [a]	References	Similarity, (%) [b]	Relative Abundance (%)						
				Mature Forest	Clear-Cut	1st Year Plantation	2nd Year Plantation	3rd Year Plantation	4th Year Plantation	Total
Tricholoma virgatum	B	DQ389735	302/302 (100)	-	0.30	-	-	-	-	0.07
Amanita porphyria	B	MT345267	296/296 (100)	0.01 a	0.21 a	0.02 a	-	-	-	0.05
Cortinarius sp. 5425_463	B	MG597372	303/305 (99)	0.28	-	-	-	-	-	0.05
Wilcoxina sp. 5425_54	A	MW238026	257/257 (100)	-	0.01 a	-	-	-	0.11 a	0.03
Pseudotomentella griseopergamacea	B	MG597410	321/321 (100)	-	-	-	-	-	0.11	0.03
Russula aeruginea	B	MG680182	301/301 (100)	0.13 a	0.01 a	-	0.04 a	-	-	0.03
Albatrellus sp. 5425_551	B	HQ204631	330/338 (98)	-	0.12	-	-	-	-	0.03
Inocybe sp. 5425_176	B	MK838270	303/303 (100)	-	-	-	-	-	0.10	0.03
Piloderma olivaceum	B	MH864982	281/281 (100)	0.14 a	0.01 a	-	-	-	-	0.03
Inocybe lutescens	B	MW845894	295/295 (100)	0.13 a	0.01 a	-	-	-	0.01a	0.03
Tylospora fibrillosa	B	MF926576	287/287 (100)	-	0.10	-	-	-	-	0.02
Total of OTUs				11.57	24.17	1.27	0.14	3.37	9.63	10.89

[a] Ascomycota (A), Basidiomycota (B). [b] Sequence similarity column shows base pairs between the query sequence and the reference at GenBank, and the percentage of sequence similarity.

Among all 70 mycorrhizal fungal OTUs, 9 OTUs were exclusively found in the soil of the mature forest stands, and 13 OTUs were found in the clear-cut sites. However, only three mycorrhizal fungal OTUs—*Amanita fulva*, *Piloderma olivaceum* (Parmasto) Hjortstam, and *Elaphomyces granulatus* Fr.—were identified as the common OTUs in the soil at the mature forest stands and the clear-cut sites. Among all mycorrhizal fungal OTUs, two OTUs were exclusively identified in the soil of the 1st and 3rd year plantations. No unique mycorrhizal fungal OTUs were identified in the 2nd year plantations. In total, 11 OTUs were exclusively found in the soil at the 4th year plantations. Across all 70 mycorrhizal fungal OTUs found in the soil, *Basidiomycota* was the most dominant phylum, accounting for 88.6%. The highest number of ectomycorrhizal fungal OTUs (30 OTUs) were found in the clear-cut sites, and 27 ECM fungal OTUs were found in the mature forest stands. The number of ECM fungal OTUs significantly decreased in 1st, 2nd, and 3rd year plantations (10, 7, and 8 OTUs, respectively), and their number significantly increased up to 24 OTUs in the 4th year *P. sylvestris* plantations.

3.3. Relations between Chemical Parameters and Fungal Community in the Mineral Topsoil

Relatively strong positive significant correlations were found between the number of mycorrhizal fungal OTUs and the concentrations of total N and SOC, as well as between the number of saprotrophic fungal OTUs and the Mg^{2+}concentrations (Table 6).

Table 6. Pearson's correlation between chemical soil parameters (pH_{CaCl2}, SOC, total N, P, K, Ca, and Mg, and also mobile Ca^{2+}, Mg^{2+}, and Cl^{-}) and the parameters of soil fungi in the studied sites.

Variables	pH_{CaCl2}	SOC	N	P	K	Ca	Mg	Ca^{2+}	Mg^{2+}	Cl^{-}
		g kg^{-1}					mg kg^{-1}			
No. of fungal sequences	0.050	0.041	0.114	−0.342	0.262	0.099	0.327	−0.079	−0.153	0.493
No. of fungal OTUs	−0.212	0.321	0.336	0.011	0.324	0.299	0.303	0.152	0.110	0.483
No. of mycorrhizal fungi	−0.413	0.518 *	0.498 *	−0.177	0.477	0.225	0.479	0.212	0.066	0.021
No. of pathogenic fungi	−0.028	0.064	0.087	0.263	−0.085	0.078	−0.120	−0.110	−0.181	0.389
No. of saprotrophic fungi	−0.173	0.271	0.350	0.262	0.406	0.493	0.298	0.477	0.505 *	0.206
No. of endophytes	−0.207	0.436	0.380	0.141	0.435	0.142	0.384	0.068	0.158	0.245
Shannon_H	−0.274	0.183	0.003	0.363	−0.090	−0.099	−0.200	0.192	0.383	−0.015

* Statistically significant correlations at $p < 0.05$.

The relations between the relative abundance of the 30 most common fungal OTUs and chemical soil parameters (pH_{CaCl2}, SOC, total N, Mg^{2+}, and Cl^{-}) in the study sites were evaluated using canonical correspondence analysis (CCA) (Figure 9). The relative abundance of fungi *Archaeorhizomyces* sp. 5425_1 and *Malassezia* sp. 5425_17 significantly correlated with the SOC concentration. Relative abundance of fungus *Glonium pusillum* H.Zoog correlated with the soil pH value. The relative abundance of fungi *Penicillium camemberti*, *Helotiaceaea* sp. 5425_2, and *Mortierella* sp. 5425_53 significantly correlated with the Mg^{2+} concentration (Figure 9).

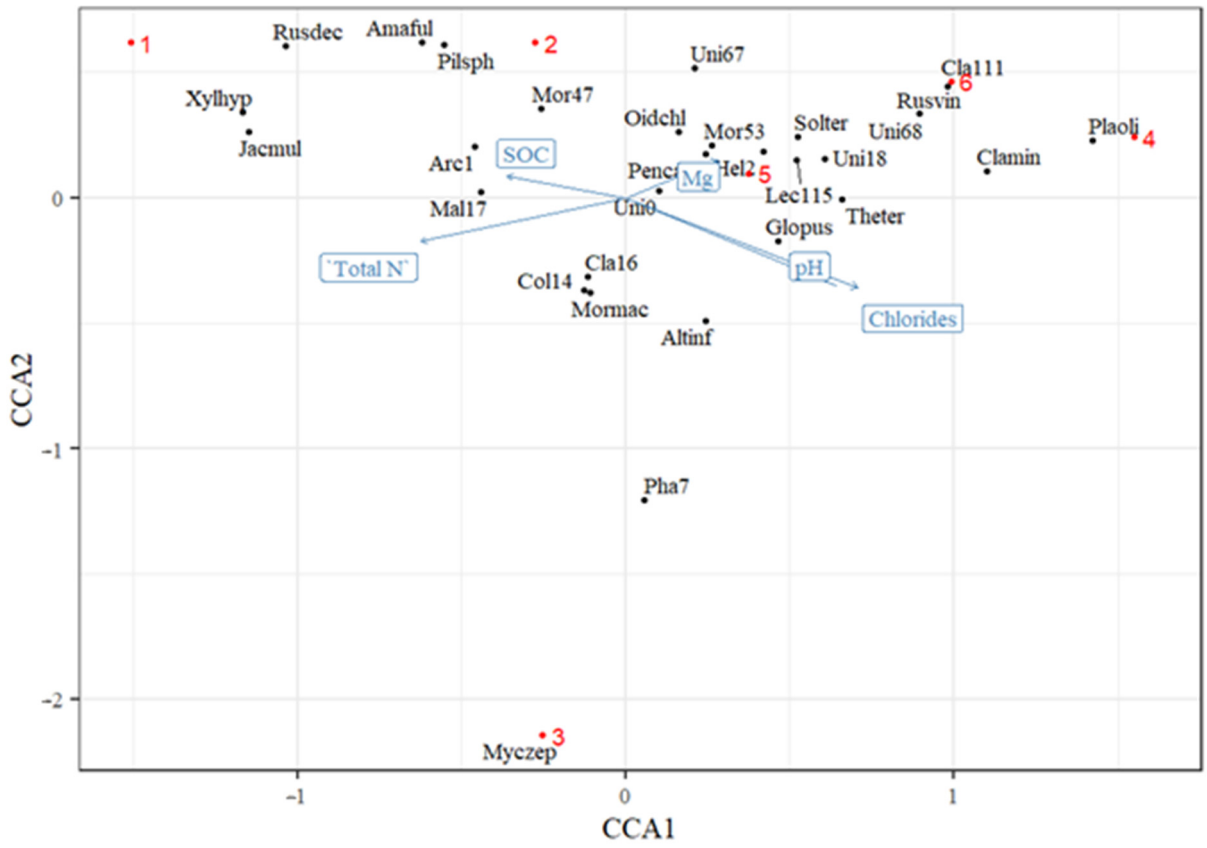

Figure 9. Canonical correspondence analysis (CCA) of the selected chemical variables (pH_{CaCl2}, SOC, total N, Mg^{2+}, and Cl^{-}) and the most common 30 fungal OTUs. Red numbers represent study sites: 1 is the mature forest stands; 2—clear-cuts; 3—the 1st year plantations; 4—the 2nd year plantations; 5—the 3rd year plantations; 6—the 4th year plantations. The first and second axes explain 32% and 30% of the variance. Abbreviations of fungal species are composed of the three-letter abbreviation of the species scientific name. If the fungal taxa were identified only to the genus level, abbreviations are composed of the three-letter abbreviation of the genus name and the last number of the identification code (full names of fungal out are given in Table 4).

4. Discussion

The current study showed significantly higher concentrations of mineral N, P_2O_5, and K_2O in the mineral 0–20 cm soil layer of the 1st and 2nd year *P. sylvestris* plantations, established on clear-cut sites. The obtained values were from 7% for K_2O, to 2.1 times higher than in the adjacent mature forest stands. In the period between 1 and 3 years after clear-cutting of mature pine forest, changes in mineral N concentrations have been observed in the previous studies [55–57]. Several studies have shown that nitrate leaching was observed to be particularly intense in the clear-cut sites, and higher concentrations of phosphates, potassium, and SOC were often obtained [56,58–60]. In the mineral soils of clear-cut sites, more intensive nitrification processes are recorded due to the changes in the site temperature and humidity, which causes the decomposition of the forest floor, the mineralization and decrease of organic matter content, as well changes in the mechanisms of plant competition for nutrients [57,61–64]. The abovementioned processes intensify the leaching of N compounds, especially nitrates, into the deeper soil layers. Therefore, a rapid increase in the concentrations of mineral N forms (NO_3-N; NO_2-N, and NH_4-N) may impair the recovery and development of the ground vegetation [65]. However, the growth of young *P. sylvestris* at the early stages of stand development is unlikely to be significantly affected. However, other studies indicated the opposite results, showing

that the concentrations of mobile P_2O_5 and K_2O in the 0–10 cm layer of mineral soils of clear-cuts were lower than in the stand [65].

The SOC concentrations depend on the balance between carbon input with plant litter and carbon loss due to the mineralization of soil organic matter [66,67]. Due to the more intensive mineralization of soil organic matter, clear-cutting has been found to enhance SOC fluxes, including the leaching of total and labile SOC [68–72]. Although in the clear-cut sites more intensive mineralization and an increase in SOC in the upper mineral soil layer could be expected, the current study did not reveal such a trend.

One of the most important indicators is the C: N ratio, which directly determines the decomposition and transformation of soil organic matter [73]. This study analyzed the C: N ratio for determining soil quality and microbiological activity, but no clear differences were found between the C: N values in the mature forest, clear-cut, and reforested sites. As reported by Siebers et al. [74], more significant changes in the C: N ratios in the upper mineral soil layer after clear-cutting of the mature forest are often recorded after 24 months, indicating the relatively slow decomposition of forest litter and felling residues, followed by the gradual migration of elements into deeper soil layers.

Changes in chemical soil parameters certainly affect the diversity and abundance of soil mycobiota. The removal of the main source of plant biomass C and the concomitant physical and chemical changes in the soil leads to a change in the composition of fungal communities towards species that can more easily spread, establish, or survive under new conditions [25,29,75]. For example, ectomycorrhizal (ECM) fungi may induce N limitation of free-living microbial decomposers, and this competition between ECM fungi and free-living decomposers for N can slow soil C cycling and increase soil C storage [76,77].

In the current study, the higher concentrations of measured chemical soil parameters, such as mineral N, P_2O_5, and K_2O in the upper mineral 0–20 cm soil layer in the plantations did not cause significant changes in fungal operational taxonomic units (OTUs) diversity. A relatively strong correlation was found between the number of mycorrhizal fungal OTUs and the concentrations of total N and SOC, as well as between the number of saprotrophic fungal OTUs and the Mg^{2+} concentrations. Some relations were evaluated between separate fungal OTUs and chemical soil parameters. The relative abundance of fungi *Archaeorhizomyces* sp. 5425_1 and *Malassezia* sp. 5425_17 significantly correlated with the SOC concentration. The relative abundance of fungus *Glonium pusillum* correlated with the soil pH value. The relative abundances of fungi *Penicillium camemberti*, *Helotiaceaea* sp. 5425_2, and *Mortierella* sp. 5425_53 significantly correlated with the Mg^{2+} concentration.

In the current study, a total of 1143 fungal OTUs were found. Although the number of species found is not small, the main dominant species are the same as in the studies conducted by other authors. The most abundant fungal OTU in our study was *Archaeorhizomyces* sp. 5425_1, which represented 11.5% of the total fungal OTUs. *Archaeorhizomycetes*, a widespread fungal class with a dominant presence in many soils, contain cryptic filamentous species forming plant–root associations whose role in terrestrial ecosystems remains unclear [78–80]. The second most abundant fungus *Helotiaceae* sp. 5425_18 represented 6.8% of the total fungal OTUs. The distribution of species of the *Helotiaceae* family is usually widespread, and therefore it is difficult to assign this fungal OTU species to any trophic group. *Oidiodendron chlamydosporicum* was also identified as one of the most common fungal OTU in our study. This fungal OTU was found in all study sites—the mature forest stands, clear-cuts, and 1st–4th year *P. sylvestris* plantations. In accordance with previous studies [81,82], the *Oidiodendron* sp. was more abundant in undisturbed soil.

In the current study, a similar number of unique fungal OTUs were detected in the mature forest stands and in the clear-cuts. This tendency was comparable for both analyses—soil fungal OTUs and mycorrhizal fungal OTUs. In the 1st–3rd year *P. sylvestris* plantations, the number of unique fungal OTUs decreased. In the 4th year plantations, the number of unique fungal OTUs significantly increased. Based on the results of our study, the number of fungal OTUs was able to recover four years after clear-cutting, but the composition of fungal communities remained different.

Mycorrhizal fungi are a very important component in assessing the reforestation process after clear-cutting [83–88]. Therefore, in our study, more attention was paid to mycorrhizal fungi and changes in relative abundance in different study sites. One of the functions of mycorrhizal fungi, especially in the early stages of forest ecosystem development, is protection against soil-borne pathogens and nematode infections in pine forests [89–91]. Furthermore, when colonized with mycorrhizal fungi, tree seedlings are known to tolerate different environmental stresses, such as drought or high temperatures, more efficiently [31,92–95]. In the temperate and boreal forests, most woody plant species are obligate partners with ectomycorrhizal fungi, which provide nutrients (for example, N) and water to host trees in exchange for photosynthetically fixed carbon (C) [75,88,90,96]. Among other forest tree species, *P. sylvestris* has been recognized as an obligate mycotroph, known as a host of 200–300 ectomycorrhizal fungal species [97–101]. In our study, a total of 70 mycorrhizal fungal OTUs were found in the soil sampled from the *P. sylvestris* study sites. The highest number of ECM fungal OTUs was found in the clear-cut sites, and the lowest number was found in the 2nd and 3rd year *P. sylvestris* plantations. The most abundant ECM fungi in our study were: *Amanita fulva*, *Piloderma sphaerosporum,* and *Thelephora terrestris*. *Amanita fulva* was found only in the mature forest stands and in the clear-cuts, while it was not detected in the 1st–4th year plantations. *T. terrestris* is considered a pioneer, multi-host mycobiont, common in nurseries, as well as young and old forests [82,102]. Among all ECM fungal OTUs, only three—*Elaphomyces* cf. *muricatus* Fr, *Wilcoxina* sp. 5425_54, and *Elaphomyces granulatus*—represented the phylum Ascomycota. According to other studies, *Wilcoxina mikolae* (Chin S. Yang & H.E. Wilcox) Chin S. Yang & Korf was the most abundant and most frequently occurring species in the forest nurseries and clear-cuts [31,103]. *Wilcoxina* species form ectendomycorrhiza and are the most common colonizers of young conifers.

According to previous studies, clear-cutting has negative short-term effects on ectomycorrhizal fungal biomass and diversity [25,104,105]. However, other findings indicated that clear-cutting has profound long-lasting effects on the abundance and composition of ectomycorrhizal fungal communities [25,103,106]. Clear-cutting may cause a complete disappearance of energy supply to mycorrhizal fungi, while thinning only reduces energy input [25,27]. However, mycorrhizal fungi may remain unchanged due to clear cuttings if the fungi are associated with the retained trees or with the tree roots, extending into the felled area from the forest edges [107–109]. According to the study by Sterkenburg [75], several ectomycorrhizal fungal species were still detected in the soil three years after clear cutting with no retained trees, although at very low abundance. This could be related to the high number of naturally grown *P. sylvestris* seedlings prior to the forest felling [75,110]. In contrast, in our study, there were no common ECM fungal species detected in the soil in the 1st–4th year plantations. Only one ECM fungus, *Thelephora terestris*, was found in all study sites, except for the 2nd year *P. sylvestris* plantations.

An important aspect of our study was that the evaluated effects reflected a period of four years after clear-cutting, and the obtained data were compared to those obtained in a mature *P. sylvestris* forest typical for the hemiboreal forest zone. This study contributes to existing knowledge on changes in soil chemistry and mycobiota during the early stages of forest regeneration, both nationally and regionally.

5. Conclusions

The study aim was to determine the fungal communities in relation to soil chemical parameters in the upper mineral soil layer of Scots pine (*Pinus sylvestris* L.) ecosystems, including mature forest stands, clear-cuts, and 1st, 2nd, 3rd, and 4th year plantations. A higher electrical conductivity (EC), higher concentrations of mineral nitrogen (N), total phosphorus (P), and P_2O_5 were obtained in the soil of the 1st year plantations than the mature forest. The highest soil pH values and Ca^{2+} concentrations were found in the 3rd year plantations. The soil chemical parameters obtained in the 4th year plantations were comparable with those obtained in the mature forest stands.

A total of 1143 fungal operational taxonomic units (OTUs) were identified in *P. sylvestris* forest soils. The most dominant phyla were *Ascomycota*, *Basidiomycota*, *Mucoromycota*, *Chytridiomycota*, and *Zoopagomycota*. The highest Shannon diversity index was obtained in the 1st and 3rd year plantations, while the lowest was found in the clear-cuts. The fungal communities identified in all studied sites of *P. sylvestris* plantations did not differ significantly. The number of common and unique fungal OTUs varied among the study sites, but a higher number of unique fungal OTUs was more typical for the 4th year plantations than other sites. The most abundant fungi were *Archaeorhizomyces* sp. 5425_1, *Helotiaceae* sp. 5425_2, Unidentified sp. 5425_18, and *Oidiodendron chlamydosporicum*. The most abundant ectomycorrhizal (ECM) fungal OTUs were found in the clear-cut and mature forest stand sites. The most abundant ECM fungi were *Amanita fulva*, *Piloderma sphaerosporum*, *Thelephora terrestris*, *Russula decolorans*, and *Russula vinosa*.

The soil of *P. sylvestris* sites was inhabited by different fungal communities during the first four years after clear-cutting. The assumption that changes in forest soil chemical parameters can affect soil fungal communities in different study sites was partially confirmed, although the identified changes in soil parameters did not reveal significant effects on fungal abundance and diversity in all cases.

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References

1. Gustafsson, L.; Baker, S.C.; Bauhus, J.; Beese, W.J.; Brodie, A.; Kouki, J.; Lindenmayer, D.B.; Lohmus, A.; Pastur, G.M.; Messier, C.; et al. Retention forestry to maintain multifunctional forests: A world perspective. *Bioscience* **2012**, *62*, 633–645. [CrossRef]
2. Kuuluvainen, T. Disturbance dynamics in boreal forests: Defining the ecological basis of restoration and management of biodiversity. *Silva Fenn.* **2002**, *36*, 5–11. [CrossRef]
3. Chazdon, R.L. Beyond deforestation: Restoring forests and ecosystem services on degraded lands. *Science* **2008**, *320*, 1458–1460. [CrossRef] [PubMed]
4. Kuuluvainen, T. Forest Management and Biodiversity Conservation Based on Natural Ecosystem Dynamics in Northern Europe: The Complexity Challenge. *Ambio* **2009**, *38*, 309–315. [CrossRef] [PubMed]
5. Bouget, C.; Nusillard, B.; Pineau, X.; Ricou, C. Effect of deadwood position on saproxylic beetles in temperate forests and conservation interest of oak snags. *Insect Conserv. Divers.* **2012**, *5*, 264–278. [CrossRef]
6. Koivula, M.J. Useful model organisms, indicators, or both? Ground beetles (Coleoptera, Carabidae) reflecting environmental conditions. *Zookeys* **2011**, *100*, 287–317. [CrossRef]
7. Sklodowski, J.J. Manual soil preparation and piles of branches can support ground beetles (Coleoptera, carabidae) better than four different mechanical soil treatments in a clear-cut area of a closed-canopy pine forest in northern Poland. *Scand. J. For. Res.* **2016**, *32*, 123–133. [CrossRef]
8. Junninen, K.; Penttila, R.; Martikainen, P. Fallen retention aspen trees on clear-cuts can be important habitats for red-listed polypores: A case study in Finland. *Biodivers. Conserv.* **2007**, *16*, 475–490. [CrossRef]
9. Paillet, Y.; Berges, L.; Hjalten, J.; Odor, P.; Avon, C.; Romermann, B.M.; Bijlsma, R.J.; De Bruyn, L.; Fuhr, M.; Grandin, U.; et al. Biodiversity Differences between Managed and Unmanaged Forests: Meta-Analysis of Species Richness in Europe. *Conserv. Biol.* **2009**, *24*, 101–112. [CrossRef] [PubMed]
10. Chaudhary, A.; Burivalova, Z.; Koh, L.P.; Hellweg, S. Impact of Forest Management on Species Richness: Global Meta-Analysis and Economic Trade-Offs. *Sci. Rep.* **2016**, *6*, 23954. [CrossRef]

11. Chandler, J.R.; Haeussler, S.; Hamilton, E.H.; Feller, M.; Bradfield, G.; Simard, S.W. Twenty years of ecosystem response after clearcutting and slashburning in conifer forests of central British Columbia, Canada. *PLoS ONE* **2017**, *12*, e0172667. [CrossRef] [PubMed]
12. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*, 2nd ed.; Academic Press: London, UK, 1997; p. 605.
13. 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] [PubMed]
14. Menkis, A.; Uotila, A.; Arhipova, N.; Vasaitis, R. Effects of stump and slash removal on growth and mycorrhization of *Picea abies* seedlings outplanted on a forest clear-cut. *Mycorrhiza* **2010**, *20*, 505–509. [CrossRef] [PubMed]
15. Thakur, M.P.; Geisen, S. Trophic Regulations of the Soil Microbiome. *Trends Microbiol.* **2019**, *27*, 771–780. [CrossRef] [PubMed]
16. Behnke-Borowczyk, J.; Kowalkowski, W.; Kartawik, N.; Baranowska, M.; Barzdajn, W. Soil fungal communities in nurseries producing Abies alba. *Balt. For.* **2020**, *26*, 5–12. [CrossRef]
17. Clarke, N.; Podenphant, L.K.; Kjonaas, O.J.; Barcena, T.G.; Vesterdal, L.; Stupak, I.; Finer, L.; Jacobson, S.; Armolaitis, K.; Lazdina, D.; et al. Effects of intensive biomass harvesting on forest soils in the Nordic countries and the UK: A meta-analysis. *For. Ecol. Manag.* **2021**, *482*, 118877. [CrossRef]
18. Simard, S.W.; Austin, M.E. The Role of Mycorrhizas in Forest Soil Stability with Climate Change. In *Climate Change and Variability*; Simard, S.W., Austin, M.E., Eds.; IntechOpen: Rijeka, Croatia, 2010; Chapter 15; pp. 275–303.
19. Kucerava, B.; Dobrovolny, L.; Remes, J. Responses of *Abies alba* seedlings to different site conditions in *Picea abies* plantations. *Dendrobiology* **2013**, *69*, 49–58. [CrossRef]
20. Kobala, M.; Grčman, H.; Zupan, M.; Levanič, T.; Simončič, P.; Kadunc, A.; Hladnik, D. Influence of soil properties on silver fir (*Abies alba* Mill.) growth in the Dinaric Mountains. *For. Ecol. Manag.* **2015**, *337*, 77–87. [CrossRef]
21. Kormanek, M.; Banach, J.; Lenczuk, D. Influence of soil compaction on the growth of silver fir (*Abies alba* Mill.) under a forest canopy. *Ecol. Quest.* **2015**, *22*, 47–54. [CrossRef]
22. Delgado, E.F.; Valdez, A.T.; Covarrubias, S.A.; Tosi, S.; Nicola, L. Soil Fungal Diversity of the Aguarongo Andean Forest (Ecuador). *Biology* **2021**, *10*, 1289. [CrossRef] [PubMed]
23. Cairney, J.W.G.; Meharg, A.A. Interactions between ectomycorrhizal fungi and soil saprotrophs: Implications for decomposition of organic matter in soils and degradation of organic pollutants in the rhizosphere. *Can. J. Bot.* **2002**, *80*, 803–809. [CrossRef]
24. Hobbie, E.A.; Macko, S.A.; Shugart, H.H. Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. *Oecologia* **1999**, *118*, 353–360. [CrossRef]
25. Jones, M.D.; Durall, D.M.; Cairney, J.W.G. Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. *N. Phytol.* **2003**, *157*, 399–422. [CrossRef] [PubMed]
26. Teste, F.P.; Karst, J.; Jones, M.D.; Simard, S.W.; Durall, D.M. Methods to control ectomycorrhizal colonization: Effectiveness of chemical and physical barriers. *Mycorrhiza* **2006**, *17*, 51–65. [CrossRef] [PubMed]
27. Luoma, D.L.; Eberhart, J.L.; Molina, R.; Amaranthus, M.P. Response of ectomycorrhizal fungus sporocarp production to varying levels and patterns of green-tree retention. *For. Ecol. Manag.* **2004**, *202*, 337–354. [CrossRef]
28. Nehls, U. Mastering ectomycorrhizal symbiosis: The impact of carbohydrates. *J. Exp. Bot.* **2008**, *59*, 1097–1108. [CrossRef] [PubMed]
29. Byrd, K.B.; Parker, V.T.; Vogler, D.R.; Cullings, K.W. The influence of clear-cutting on ectomycorrhizal fungus diversity in a lodgepole pine (*Pinus contorta*) stand, Yellowstone National Park, Wyoming, and Gallatin National Forest, Montana. *Can. J. Bot.* **2000**, *78*, 149–156.
30. Lithuanian State Forest Survey Service. In *Lithuanian Statistical Yearbook of Forestry*; Lithuanian State Forest Survey Service: Kaunas, Lithuania, 2020; p. 25.
31. Rudawska, M.; Leski, T. Ectomycorrhizal Fungal Assemblages of Nursery-Grown Scots Pine are Influenced by Age of the Seedlings. *Forests* **2021**, *12*, 134. [CrossRef]
32. Wubet, T.; Christ, S.; Schöning, I.; Boch, S.; Gawlich, M.; Schnabel, B.; Fischer, M.; Buscot, F. Differences in soil fungal communities between European Beech (*Fagus sylvatica* L.) dominated forests are related to soil and understory vegetation. *PLoS ONE* **2012**, *7*, e47500. [CrossRef]
33. Yurkov, A.M.; Kemler, M.; Begerow, D. Assessment of yeast diversity in soils under different management regimes. *Fungal Ecol.* **2012**, *5*, 24–35. [CrossRef]
34. Goldmann, K.; Schöning, I.; Buscot, F.; Wubet, T. Forest management type influences diversity and community composition of soil fungi across temperate forest ecosystems. *Front. Microbiol.* **2015**, *6*, 1–11. [CrossRef] [PubMed]
35. Buee, M.; Reich, M.; Murat, C.; Morin, E.; Nilsson, R.H.; Uroz, S.; Martin, F. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytol.* **2009**, *184*, 449–456. [CrossRef] [PubMed]
36. Kwasna, H.; Walkowiak, L.; Lakomy, P.; Behnke-Borowczyk, J.; Gornowicz, R.; Mikicinski, A.; Gałązka, S.; Szewczyk, W. Effect of silvicultural techniques on the diversity of microorganisms in forest soil and their participation in biological control of *Armillaria* and *Heterobasidion*. *J. Plant Prot. Res.* **2015**, *55*, 241–253. [CrossRef]
37. Malecka, M.; Kwasna, H.; Szewczyk, W. Fungal communities in barren forest soil after amendment with different wood substrates and their possible effects on trees pathogens, insects and nematodes. *J. Plant Prot. Res.* **2015**, *55*, 301–311. [CrossRef]
38. Aučina, A.; Rudawska, M.; Leski, T.; Skridaila, A.; Riepšas, E.; Iwanski, M. Growth and mycorrhizal community structure of *Pinus sylvestris* seedlings following the addition of forest litter. *Appl. Environ. Microbiol.* **2007**, *73*, 4867–4873. [CrossRef]

39. Aučina, A.; Rudawska, M.; Leski, T.; Skridaila, A.; Pašakinskiene, I.; Riepšas, E. Forest litter as the mulch improving growth and ectomycorrhizal diversity of bare-root Scots pine (*Pinus sylvestris*) seedlings. *Iforest* **2015**, *8*, 394–400. Available online: http://www.sisef.it/iforest/contents/?id=ifor1083-008 (accessed on 25 August 2022).
40. Horton, T.R.; Bruns, T.D. The molecular revolution in ectomycorrhizal ecology: Peeking into the black-box. *Mol. Ecol.* **2001**, *10*, 1855–1871. [CrossRef]
41. 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]
42. Banchi, E.; Ametrano, C.G.; Tordoni, E.; Stankovi, D.; Ongaro, S.; Tetiach, M.; Pallavicini, A.; Muggia, L.; Verardo, P.; Tassan, F.; et al. Environmental DNA assessment of airborne plant and fungal seasonal diversity. *Sci. Total Environ.* **2020**, *738*, 140249. [CrossRef]
43. Marčiulynas, A.; Marčiulyniene, D.; Lynikiene, J.; Gedminas, A.; Vaičiukyne, M.; Menkis, A. Fungi and oomycetes in the irrigation water of forest nurseries. *Forests* **2020**, *11*, 459. [CrossRef]
44. Marčiulyniene, D.; Marčiulynas, A.; Lynikiene, J.; Vaičiukyne, M.; Gedminas, A.; Menkis, A. DNA-Metabarcoding of Belowground Fungal Communities in Bare-Root Forest Nurseries: Focus on Different Tree Species. *Microorganisms* **2021**, *9*, 150. [CrossRef] [PubMed]
45. Nilsson, R.H.; Anslan, S.; Bahram, M.; Wurzbacher, C.; Baldrian, P.; Tedersoo, L. Mycobiome diversity: High-throughput sequencing and identification of fungi. *Nat. Rev. Microbiol.* **2019**, *17*, 95–109. [CrossRef] [PubMed]
46. Karazija, S. *Lietuvos Miškų Tipai. (Forest tipes of Lithuania)*; Mokslas: Vilnius, Lithuania, 1988; p. 210. (In Lithuanian)
47. Vaičys, M.; Karazija, S.; Kuliešis, A.; Rutkauskas, A. *Miškų Augavietės (Forest Sites)*; Lutute: Kaunas, Lithuania, 2006; p. 95. (In Lithuanian)
48. IUSS Working Group WRB. *World Reference Base for Soil Resources 2014: International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*; World Soil Resources Reports No. 106; FAO: Rome, Italy, 2015; p. 192.
49. Sampling and Analysis of Soil. 2010. Available online: https://www.icp-forests.org/pdf/manual/2010/Manual_2010_soil.pdf (accessed on 25 August 2022).
50. Rosling, A.; Landeweert, R.; Lindah, B.D.; Larsson, K.H.; Kuyper, T.W.; Taylor, A.F.S.; Finlay, R.D. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. *New Phytol.* **2003**, *159*, 775–783.
51. Ihrmark, K.; Bodeker, I.T.M.; Cruz-Martinez, K.; Friberg, H.; Kubartova, A.; Schenck, J.; Strid, Y.; Stenlid, J.; Brandstrom-Durling, M.; Clemmensen, K.E.; et al. New primers to amplify the fungal ITS2 region-evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol. Ecol.* **2012**, *82*, 666–677. [CrossRef] [PubMed]
52. 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.
53. Magurran, A.E. *Ecological Diversity and Its Measurement*, 1st ed.; Princeton University Press: Princeton, NJ, USA, 1988; p. 192.
54. 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]
55. Jussy, J.H.; Ranger, J.; Bienaimé, S.; Dambrine, E. Effects of a clear-cut on the in situ nitrogen mineralisation and the nitrogen cycle in a 67-year old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantation. *Ann. For. Sci.* **2004**, *61*, 397–408. [CrossRef]
56. Gundersen, P.; Schmidt, I.K.; Raulund-Rasmussen, K. Leaching of nitrate from temperate forests—Effects of air pollution and forest management. *Environ. Rev.* **2006**, *14*, 1–57. [CrossRef]
57. Hedwall, P.O.; Grip, H.; Linder, S.; Lövdahl, L.; Nilsson, U.; Bergh, J. Effects of clear-cutting and slash removal on soil water chemistry and forest floor vegetation in a nutrient optimised Norway spruce stand. *Silva Fenn.* **2013**, *47*, 16. [CrossRef]
58. Palviainen, M. Logging residues and ground vegetation in nutrient dynamics of a clear-cut boreal forest. *Diss. For.* **2005**, *12*, 1–38. [CrossRef]
59. Wall, A. Effect of removal of logging residue on nutrient leaching and nutrient pools in the soil after clearcutting in a Norway spruce stand. *For. Ecol. Manag.* **2008**, *256*, 1372–1383. [CrossRef]
60. Armolaitis, K.; Varnagirytė-Kabašinskienė, I.; Stupak, I.; Mikšys, V.; Kukkola, M.; Wójcik, J. Carbon and nutrients of Scots pine stands on sandy soils in Lithuania in relation to bioenergy sustainability. *Biomass Bioenergy* **2013**, *54*, 250–259. [CrossRef]
61. Smolander, A.; Kitunen, V.; Mälkönen, E. Dissolved soil organic nitrogen and carbon in a Norway spruce stand and adjacent clear-cut. *Biol. Fertil. Soils* **2001**, *33*, 190–196. [CrossRef]
62. Palviainen, M.; Finér, L.; Laurén, A.; Mattsson, T.; Högbom, L. A method to estimate the impact of clear-cutting on nutrient concentrations in boreal headwater streams. *Ambio* **2015**, *44*, 521–531. [CrossRef] [PubMed]
63. Stuiver, B.; Wardle, D.; Gundale, M.; Nilsson, M. Seedling responses to changes in canopy and soil properties during stand development following clear-cutting. *For. Ecol. Manag.* **2016**, *378*, 31–43. [CrossRef]
64. Rappe George, M.O.; Hansson, L.J.; Ring, E.; Jansson, P.E.; Gärdenäs, A.I. Nitrogen leaching following clear-cutting and soil scarification at a Scots pine site—A modelling study of a fertilization experiment. *For. Ecol. Manag.* **2017**, *385*, 281–294. [CrossRef]
65. Česonienė, L.; Daubaras, R.; Tamutis, V.; Kaškonienė, V.; Kaškonas, P.; Stakėnas, V.; Zych, M. Effect of clear-cutting on the understory vegetation, soil and diversity of litter beetles in scots pine-dominated forest. *J. Sustain. For.* **2019**, *38*, 791–808. [CrossRef]

66. Six, J.; Conant, R.T.; Paul, E.A. Stabilization mechanisms of soil organic matter: Implications for C—Saturation of soil. *Plant Soil* **2002**, *241*, 155–176. [CrossRef]
67. Lal, R. Sequestering carbon in soils of agro-ecosystems. *Food Policy* **2011**, *36*, 33–39. [CrossRef]
68. Qualls, R.G.; Haines, B.L.; Swank, W.T.; Tyler, S.W. Soluble organic and inorganic nutrient fluxes in clearcut and mature deciduous forests. *Soil Sci. Soc. Am. J.* **2000**, *64*, 1068–1077. [CrossRef]
69. Piirainen, S.; Finer, L.; Mannerkoski, H.; Starr, M. Effects of forest clear-cutting on the carbon and nitrogen fluxes through podzolic soil horizons. *Plant Soil* **2002**, *239*, 301–311. [CrossRef]
70. Schelker, J.; Eklöf, K.; Bishop, K.; Laudon, H. Effects of forestry operations on dissolved organic carbon concentrations and export in boreal first-order streams. *J. Geophys. Res.* **2012**, *117*, G01011. [CrossRef]
71. Schelker, J.; Öhman, K.; Löfgren, S.; Laudon, H. Scaling of increased dissolved organic carbon inputs by forest clear-cutting—What arrives downstream? *J. Hydrol.* **2014**, *508*, 299–306. [CrossRef]
72. Nieminen, M.; Sarkkola, S.; Laurén, A. Impacts of forest harvesting on nutrient, sediment and dissolved organic carbon exports from drained peatlands: A literature review, synthesis and suggestions for the future. *For. Ecol. Manag.* **2017**, *392*, 13–20. [CrossRef]
73. Cools, N.; Vesterdal, L.; De Vos, B.; Vanguelova, E.; Hansen, K. Tree species is the major factor explaining C:N ratios in European forest soils. *For. Ecol. Manag.* **2014**, *311*, 3–16. [CrossRef]
74. Siebers, N.; Kruse, J. Short-term impacts of forest clear-cut on soil structure and consequences for organic matter composition and nutrient speciation: A case study. *PLoS ONE* **2019**, *14*, e0220476. [CrossRef]
75. Sterkenburg, E.; Clemmensen, K.E.; Lindahl, B.D.; Dahlberg, A. The significance of retention trees for survival of ectomycorrhizal fungi in clear−cut Scots pine forests. *J. Appl. Ecol.* **2019**, *56*, 1367–1378. [CrossRef]
76. Orwin, K.H.; Kirschbaum, M.U.F.; St John, M.G.; Dickie, I.A. Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: A model-based assessment: Organic nutrient uptake enhances soil C. *Ecol. Lett.* **2011**, *14*, 493–502. [CrossRef]
77. Averill, C.; Hawkes, C.V. Ectomycorrhizal fungi slow soil carbon cycling. *Ecol. Lett.* **2016**, *19*, 937–947. [CrossRef]
78. Rosling, A.; Timling, I.; Taylor, D.L. Archaeorhizomycetes: Patterns of distribution and abundance in soil. In *Genomics of Soil-and Plant-Associated Fungi*; Horwitz, B.A., Mukherjee, P.K., Mukherjee, M., Kubicek, C.P., Eds.; Springer: Berlin/Heidelberg, Germany, 2013; Volume 3, pp. 333–349. [CrossRef]
79. Menkis, A.; Urbina, H.; James, T.Y.; Rosling, A. *Archaeorhizomyces borealis* sp. nov. and a sequence-based classification of related soil fungal species. *Fungal Biol.* **2014**, *118*, 943–955. [CrossRef]
80. Choma, M.; Bárta, J.; Šantrucková, H.; Urich, T. Low abundance of Archaeorhizomycetes among fungi in soil metatranscriptomes. *Sci. Rep.* **2016**, *6*, 38455. [CrossRef]
81. Lee, E.H.; Eom, A.H. Ectomycorrhizal fungal communities of red pine (*Pinus densiflora*) seedlings in disturbed sites and undisturbed old forest sites. *Mycobiology* **2013**, *41*, 77–81. [CrossRef] [PubMed]
82. Kwasna, H.; Behnke-Borowczyk, J.; Gornowicz, R.; Lakomy, P. Effects of preparation of clear-cut forest sites on the soil mycobiota with consequences for Scots pine growth and health. *For. Path.* **2019**, *49*, 1–12. [CrossRef]
83. Gardes, M.; Bruns, T.D. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: Above and below ground views. *Can. J. Bot.* **1996**, *74*, 1572–1583. [CrossRef]
84. Dahlberg, A.; Jonsson, L.; Nylund, J.E. Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in South Sweden. *Can. J. Bot.* **1997**, *75*, 1323–1335. [CrossRef]
85. Teste, F.P.; Lieffers, V.J.; Strelkov, S.E. Ectomycorrhizal community responses to intensive forest management: Thinning alters impacts of fertilization. *Plant Soil* **2012**, *360*, 333–347. [CrossRef]
86. Hartmann, M.; Howes, C.G.; VanInsberghe, D.; Yu, H.; Bachar, D.; Christen, R.; Nilsson, R.H.; Hallam, S.J.; Mohn, W.W. Significant and persistent impact of timber harvesting on soil microbial communities in Northern coniferous forests. *ISME J.* **2012**, *6*, 2199–2218. [CrossRef]
87. Hawkins, B.J.; Jones, M.D.; Kranabetter, J.M. Ectomycorrhizae and tree seedling nitrogen nutrition in forest restoration. *New For.* **2015**, *46*, 747–771. [CrossRef]
88. Policelli, N.; Horton, T.R.; Hudon, A.T.; Patterson, T.R.; Bhatnagar, J.M. Back to Roots: The Role of Ectomycorrhizal Fungi in Boreal and Temperate Forest Restoration. *Front. For. Glob. Chang.* **2020**, *3*, 97. [CrossRef]
89. Futai, K.; Taniguchi, T.; Kataoka, R. Ectomycorrhizae and their importance in forest ecosystems. In *Mycorrhizae: Sustainable Agriculture and Forestry*; Siddiqui, Z., Akhtar, M.S., Futai, K., Eds.; Springer: Dordrecht, The Netherlands, 2008; pp. 241–285.
90. Smith, S.; Read, D. *Mycorrhizal Symbiosis*, 3rd ed.; Academic Press: London, UK, 2008; p. 768.
91. Karst, J.; Erbilgin, N.; Pec, G.J.; Cigan, P.W.; Najar, A.; Simard, S.W.; Cahill, J.F. Ectomycorrhizal fungi mediate indirect effects of a bark beetle outbreak on secondary chemistry and establishment of pine seedlings. *N. Phytol.* **2015**, *208*, 904–914. [CrossRef]
92. Baath, E. Soil fungal biomass after clear-cutting of a pine forest in central Sweden. *Soil Biol. Biochem.* **1980**, *12*, 495–500. [CrossRef]
93. Newman, S.E.; Davies, F.T.J. High root-zone temperatures mycorrhizal fungi water relations and root hydraulic conductivity of container-grown woody plants. *J. Am. Soc. Hortic. Sci.* **1988**, *113*, 138–146. [CrossRef]
94. Boyle, C.D.; Hellenbrand, K.E. Assessment of the effect of mycorrhizal fungi on drought tolerance of conifer seedlings. *Can. J. Bot.* **1991**, *69*, 1764–1771. [CrossRef]
95. Perry, D.A.; Amaranthus, M.P.; Borchers, J.G.; Borchers, S.L.; Brainerd, R.E. Bootstrapping in ecosystems. *Bioscience* **1989**, *39*, 230–237. [CrossRef]

96. Lang, C.; Polle, A. Ectomycorrhizal fungal diversity, tree diversity and root nutrient relations in a mixed Central European forest. *Tree Physiol.* **2011**, *31*, 531–538. [CrossRef]
97. Jonsson, L.; Dahlberg, A.; Nilsson, M.C.; Karen, O.; Zackrisson, O. Continuity of ectomycorrhizal fungi in self-regenerating boreal *Pinus sylvestris* forests studied by comparing mycobiont diversity on seedlings and mature trees. *N. Phytol.* **1999**, *142*, 151–162. [CrossRef]
98. Dahlberg, A.; Schimmel, J.; Taylor, A.F.S.; Johannesson, H. Post-fire legacy of ectomycorrhizal fungal communities in the Swedish boreal forest in relation to fire severity and logging intensity. *Biol. Conserv.* **2001**, *100*, 151–161. [CrossRef]
99. Urban, A.; Puschenreiter, M.; Strauss, J.; Gorfer, M. Diversity and structure of ectomycorrhizal and co-associated fungal communities in a serpentine soil. *Mycorrhiza* **2008**, *18*, 339–354. [CrossRef]
100. Rudawska, M.; Wilgan, R.; Janowski, D.; Iwanski, M.; Leski, T. Shifts in taxonomical and functional structure of ectomycorrhizal fungal community of Scots pine (*Pinus sylvestris* L.) underpinned by partner tree ageing. *Pedobiologia* **2018**, *71*, 20–30. [CrossRef]
101. Aučina, A.; Rudawska, M.; Wilgan, R.; Janowski, D.; Skridaila, A.; Dapkuniene, S.; Leski, T. Functional diversity of ectomycorrhizal fungal communities along a peatland–forest gradient. *Pedobiologia* **2019**, *74*, 15–23. [CrossRef]
102. Colpaert, J.V. Thelephora. In *Ectomycorrhizal Fungi. Key Genera in Profile*; Cairney, J.W.G., Chambers, S.M., Eds.; Springer: Berlin/Heidelberg, Germany, 1999; pp. 245–325.
103. Bzdyk, R.M.; Sikora, K.; Studnicki, M.; Aleksandrowicz-Trzcinska, M. Communities of Mycorrhizal Fungi among Seedlings of Scots Pine (*Pinus sylvestris* L.) Growing on a Clearcut in Microsites Generated by Different Site-Preparation Methods. *Forests* **2022**, *13*, 353. [CrossRef]
104. Kropp, B.R.; Langlois, C.G. Ectomycorrhizae in reforestation. *Can. J. For. Res.* **1990**, *20*, 438–451. [CrossRef]
105. Grebenc, T.; Christensen, M.; Vilhar, U.; Čater, M.; Martin, M.P.; Simončič, P.; Kraigher, H. Response of ectomycorrhizal community structure to gap opening in natural and managed temperate beech-dominated forests. *Can. J. For. Res.* **2009**, *39*, 1375–1386. [CrossRef]
106. Varenius, K.; Karen, O.; Lindahl, B.; Dahlberg, A. Long-term effects of tree harvesting on ectomycorrhizal fungal communities in boreal Scots pine forests. *For. Ecol. Manag.* **2016**, *380*, 41–49. [CrossRef]
107. Simard, S.W.; Durall, D.M. Mycorrhizal networks: A review of their extent, function, and importance. *Can. J. Bot.* **2004**, *82*, 1140–1165. [CrossRef]
108. Douhan, G.W.; Vincenot, L.; Gryta, H.; Selosse, M.A. Population genetics of ectomycorrhizal fungi: From current knowledge to emerging directions. *Fungal Biol.* **2011**, *115*, 569–597. [CrossRef]
109. Varenius, K.; Lindahl, B.D.; Dahlberg, A. Retention of seed trees fails to lifeboat ectomycorrhizal fungal diversity in harvested Scots pine forests. *FEMS Microbiol. Ecol.* **2017**, *93*, 1–11. [CrossRef]
110. Teste, F.P.; Simard, S.W.; Durall, D.M. Role of mycorrhizal networks and tree proximity in ectomycorrhizal colonization of planted seedlings. *Fungal Ecol.* **2009**, *2*, 21–30. [CrossRef]