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Cambisol Mycobiome in a Long-Term Field Experiment with Korean Pine as a Sole Edificator: A Case Study

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Abstract: A culture-independent mycobiome survey in Haplic Cambisol under Korean pine in a long-term field experiment in the Russian Far East was conducted using sequence analysis of the ITS region amplified with ITS3/ITS4 primers using the metagenomic DNA as a matrix. Overall 758 fungal OTUs were identified, representing 15 phyla, 47 classes, 104 orders, 183 families, and 258 genera. More OTUs represented the *Ascomycota* phylum (513) than *Basidiomycota* (113), with both phyla together comprising 95% of the relative abundance. The *Leotiomycetes* class was ultimately prevailing; apparently contributing significantly to the organic matter decomposition and microbial biomass in soil, as shown by a PCA. Only two dominant OTUs (*Pseudogymnoascus* sp. and *Hyaloscyphaceae*, both *Ascomycota*) were common in the studied samples. The presented high mycobiome diversity in soil under the monospecies artificial forest, where Korean pine had been the sole edificator for forty years, allows concluding that plant chemistry diversity is the main factor shaping the soil mycobiome in such an environment. The obtained data provide a reference for further studies of soil mycobiota, especially under Korean pine with its aesthetic, as well as nut-producing, potential. The results can be helpful in the targeted creating of a soil mycobiome beneficial for pines in afforestation and remediation contexts.

Keywords: ITS DNA diversity; soil fungi; Haplic Cambisol; Khabarovsk region; *Pinus koraiensis*

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1. Introduction

A soil microbiome census, conducted using state-of-the-art metagenomic techniques, is an indispensable initial stage for further well-integrated and more focused ecological research [1]. However, little is known about the soil mycobiome in forest ecosystems in Russia. Forests dominated by Korean pine (*Pinus koraiensis* Siebold et Zucc.), a magnificent ornamental tree, producing high quality nuts and timber [2], form large ecosystems in forest biomes, occupying vast areas in north-eastern China, the Russian Far East, Japan, and Korea [3–5]. Owing to overexploitation, the species' natural stands are fragile and diminishing, and its plantations are being extended: for instance, Korean pine is the most important plantation tree species in northeast China [3]. Breeding, selection, and seedling production, as well as pine tree growth and development, have been receiving marked research attention [2,4,6–8] due to its high productivity and aesthetics, with the main aim of introducing it into other areas, different from their natural habitats.

Soil microbial diversity in forest ecosystems is primarily determined by the dominant forest-forming tree species [9,10]. In the 1970–1980s a nationwide network of field experiments with the major forest-forming conifers was set up throughout Russia, with the main purpose of studying the adaptation of trees to a somewhat changed or totally

new environment. Currently, mature monospecies artificial pine stands provide a unique opportunity to study soil microbiome developed under the influence of sole species plant material input into soil, both via surface and root litter, as well as root exudates.

Diverse and complex soil fungal communities are extremely important for sustaining forests, as they decompose plant material, cycle nutrients, etc. Reciprocally, trees stabilize the composition of root-associated microbial communities, and not only exert selective effects on those communities, but also mitigate changes in the environment that can directly influence community composition.

The aim of this study was to reveal (a) the predominant members of fungal mycobiome in Haplic Cambisol under Korean pine as a sole edificator in a long-term field experiment in the Khabarovsk region, Russia, using ITS region DNA diversity, (b) the relationship between fungal diversity and soil basic chemical and microbiological properties.

2. Materials and Methods

2.1. The Study Site

The field experiment with Korean pine *Pinus koraiensis* was set up in the Khekhtsirsk Forestry (48°16′ N, 135°02′ E, 224 m a.s.l.) in the Khabarovsk region, Russia, in 1974. Prior to the start of the experiment, the soil was cleared of trees and ploughed, and hence nominally can be classified as anthropogenically transformed Burozem eluviated, according to the Russian classification, or Haplic Cambisol, according to the WRB classification [11]. Pine seedlings were planted out at 10,000 plants per hectare with 0.7 m between trees in a row and 1.5 m between rows in plots 30 m wide and 180 m long. After 40 years of growth and development, the trees were 9.8 ± 1.6 m high, with the yearly growth rate of 22 ± 2 cm and trunk and crown diameters averaging 13.7 ± 2.7 cm and 3.2 ± 0.8 m, respectively.

2.2. Soil Sampling and Properties

Soil was collected from two replicated experimental plots from the 0–20 cm layer (immediately below the litter layer and with maximal root density) at 60 cm from the tree row within the crone zone. Two composite samples, mixed from six individual soil cores taken randomly along the central part across a plot, were collected from each plot, and the four collected samples were brought to the laboratory. Visible roots and large particles were removed carefully by hand, and the samples were sieved through a 2-mm mesh sieve, thoroughly mixed, and divided into two subsamples for chemical analyses and DNA extraction.

2.3. Chemical Analyses

Soil carbon content was estimated by the loss of soil aliquot mass during stepwise ignition: the loss on ignition during 12 h at 500 °C was used to estimate soil organic carbon content (SOC) by multiplying the loss by 0.58. Soil organic nitrogen content (SON) was determined by the Kjeldahl method. The content of soil labile nutrients (NO_3^-, NH_4^+, and P_2O_5) and pH (H_2O) were measured using standard techniques [12]. Soil microbial biomass carbon (SMBC) and nitrogen (SMBN) contents were estimated by fumigation–extraction methods [13,14]. Basal respiration (CO_2) was estimated by measuring the CO_2 evolved by soil, without any amendments [15]; its ratio to SMBC gave the metabolic quotient (Qm, [16]). Glucose-induced CO_2 evolution was measured from soil amended with glucose or histidine at the rate of 800 $\mu gC \cdot g^{-1}$ soil. The ratio of glucose or histidine-induced respiration to the basal one are referred to as QRg and QRh, respectively. All analyses were performed in triplicates. All soil variables (Table 1) were calculated on a soil dry mass basis.

2.4. Extraction of Total Nucleic Acid from Soil

Total DNA was extracted using a DNA isolation Kit (DNA Spin Kit for Soil™, MO Bio laboratories, Inc., Carlsbad, CA, USA) as per the manufacturer's instructions. Bead-beating was performed using a mini bead-beater (Stanford, CA, USA), for 45 s at 5000 rpm. No further purification of the DNA was needed. The quality of the DNA was assessed using agarose gel electrophoresis.

Table 1. Some chemical and microbiological properties of Haplic Cambisol top 0−20 cm, from under Korean pine.

Property	Mean	S.E.M.	C.V.
pH	6.08	0.05	1
EC, $dS \cdot m^{-1}$	0.06	0.0	20
SOC,%	8.9	2.7	30
SIC,%	0.33	0.09	26
SMBC, mg/g soil	2.0	0.8	39
SMBC/SOC,%	2.1	0.3	15
SMBN, mg/g soil	0.2	0.1	57
DOC, mg/kg soil	30	30	79
Pw, mg/kg soil	0.5	0.2	47
NO_3, mg N/kg soil	6.9	2.7	40
NH_4, mg N/kg soil	2.0	1.2	59
SON, %	0.45	0.1	23
SO(C/N)	19.6	4.5	23
CO_2, μgC-CO_2/g soil·h	5.0	1.6	31
Glu, μgC-CO_2/g soil·h	14.6	0.6	4
His, μgC-CO_2/g soil·h	6.1	1.3	22
QRg	0.34	0.12	33
QRh	0.83	0.29	35
Qm, μgC-CO_2/mg SMBC	2.8	1.5	5.2

Abbreviations used: EC—electric conductivity; SOC—soil organic carbon, SON—soil organic nitrogen; SMBC—soil microbial biomass carbon, SMBN—soil microbial biomass nitrogen, DOC—dissolved organic carbon, Pw—water-extractable phosphorus, SO(C/N)—ratio in soil organic matter, Glu, His—glucose- and histidine-induced respiration, QRg, QRh—respiratory quotients with glucose and histidine, Qm—metabolic quotient of soil microbial biomass.

2.5. ITS Fragment and 16S rRNA Gene Amplification and Sequencing

The 16S rRNA gene and ITS2 regions were amplified with the primer pairs V3/V4 ITS3_KYO2/ITS4, respectively, combined with Illumina adapter sequences [17].

PCR amplification was performed as described earlier [18]. A total of 200 ng PCR product from each sample was pooled together and purified using a MinElute Gel Extraction Kit (Qiagen, Hilden, Germany). The obtained amplicon libraries were sequenced with 2 × 300 bp paired-ends reagents on MiSeq (Illumina, San Diego, CA, USA) in SB RAS Genomics Core Facility (ICBFM SB RAS, Novosibirsk, Russia). The read data reported in this study were submitted to the GenBank under the SRA accession number SRP152492 (https://www.ncbi.nlm.nih.gov/sra/?term=SRP152492; accessed on 12 July 2022).

2.6. Bioinformatic and Statistical Analyses

Raw sequences were analyzed with UPARSE pipeline [19] using Usearch v.11.0.667. The UPARSE pipeline included merging of paired reads; read quality filtering (-fastq_maxee_rate 0.005); length trimming (remove less 350 nt); merging of identical reads (dereplication); discarding singleton reads; and removing chimeras and OTU clustering using the UPARSE-OTU algorithm. The OTU sequences were assigned a taxonomy using the SINTAX [20], and as references for fungi ITS UNITE v.8.2 [21] and ITS RDP Warcup, training set v.2 [22]. Statistical analyses (descriptive statistics, principal component analysis) were performed using Statistica v.13.3, and α-biodiversity indices were calculated using PAST software v.3.17 [23].

3. Results

3.1. Mycobiome Diversity

After quality filtering, and chimera and non-fungal sequences removal, a total of 756 different operational taxonomic units (OTUs) were identified as fungal at a 97% sequence identity level, of which 610 were identified at least to a phylum level, whereas just 34 OTUs, or 4.5% of the total OTU number in the study, could not be assigned taxonomy below phylum level. Overall, the ITS sequence reads were clustered into 14 explicitly classified phyla, 47 classes, 104 orders, 183 families, and 258 genera. Of all OTUs, 193 were

classified to the species level. The individual rarefaction showed (Figure S1) that the sampling effort was close to saturation for all samples.

Overall, there were 21 genus level dominant clusters, i.e., clusters contributing ≥1% of the total number of sequence reads obtained for a sample (Table 2), altogether totaling ca. 70% of the relative abundance.

Table 2. Relative abundance of the dominant fungal genera in the top 0−20 cm of Haplic Cambisol under Korean pine.

Taxon	Mean	S.E.M.	C.V.
Amphinema	10.0	9.4	94
Sebacina	8.1	9.8	120
un.[1] *Hyaloscyphaceae*	7.9	2.9	36
un. *Helotiales*	6.9	5.8	84
Cryptococcus	4.9	5.5	113
Tomentella	4.7	3.1	65
un. *Hypocreales*	3.1	3.4	109
Pseudogymnoascus	3.1	0.5	15
Oidiodendron	2.7	2.5	92
Penicillium	2.3	1.3	57
Chalara	2.3	0.5	20
un. *Leotiomycetes*	2.2	1.0	45
Saitozyma	1.8	1.7	96
Inocybe	1.4	1.2	84
un. *Dothideomycetes*	1.4	0.7	51
Lecanicillium	1.3	2.2	176
Umbelopsis	1.2	0.6	48
Phialocephala	1.2	0.8	65
Trichoglossum	1.1	1.5	142
Tetracladium	1.0	1.0	104
Mortierella	1.0	0.3	32

[1] un. stands for unclassified.

The dominant OTUs amounted to 47 for all four soil samples and ranged from 15 to 18 OTUs in a sample. The relative abundance of the dominant OTUs after averaging all samples is shown in Table 3. The overwhelming majority (*ca*.95%) of the identified OTUs were minor or rare species.

Table 3. Relative abundance of the dominant fungal OTUs in the top 0−20 cm of Haplic Cambisol under Korean pine.

OTU's No.	Taxon	Mean	S.E.M.	C.V.
4	un.[1] *Amphinema*	8.2	9.5	116
3	un. *Sebacina*	7.6	9.4	123
5 [2]	un. *Hyaloscyphaceae*	7.0	2.1	30
7	un. *Cryptococcus*	4.9	5.5	113
6	un. *Helotiales*	3.6	5.9	162
8	un. *Pseudogymnoascus*	3.1	0.5	15
17	un. *Hypocreales*	3.0	3.4	111
10	un. *Ascomycota*	2.9	4.1	141
14	un. *Ascomycota*	2.1	3.5	163
32	un. *Tomentella*	1.8	3.0	166
16	*Saitozyma podzolica*	1.8	1.7	96
26	un. *Penicillium*	1.6	1.7	110
47	un. *Tomentella*	1.4	1.1	78
24	*Lecanicillium primulinum*	1.2	2.2	179
38	un. *Amphinema*	1.2	1.8	152
19	un. *Chalara*	1.1	1.3	116
27	*Phialocephala fortinii*	1.1	0.8	71
617	un. *Oidiodendron*	1.1	1.1	106
23	un. *Leotiomycetes*	1.0	0.3	31

[1] un. stands for unclassified. [2] denotes the dominant OTUs that were common for all four samples studied.

The main dominant phyla, i.e., *Ascomycota* and *Basidiomycota* (Figure 1a), accounted for 31 and 16 of the dominant OTUs, despite their more pronounced disparity in the total number of OTUs (513 and 113 OTUs, respectively). Several of the dominant OTUs were classified to a species level, namely *Suillus americanus*, *Saitozyma podzolica*, *Cortinarius casimiri*, *Hebeloma bruchetii*, *Inocybe_lilacina*, *Tomentella atramentaria*, *Laccaria_pumila* of *Basidiomycota*, and *Lecanicillium primulinum*, *Phialocephala fortinii*, *Leptodontidium orchidicola*, *Trichoglossum hirsutum*, *Chalara hyalocuspica*, *Oidiodendron scytaloides*, *Herpotrichia juniperi* of *Ascomycota*, and only two OTUs, namely *Pseudogymnoascus* sp. and a representative of *Hyaloscyphaceae*, both belonging to *Ascomycota*, were common to all four samples.

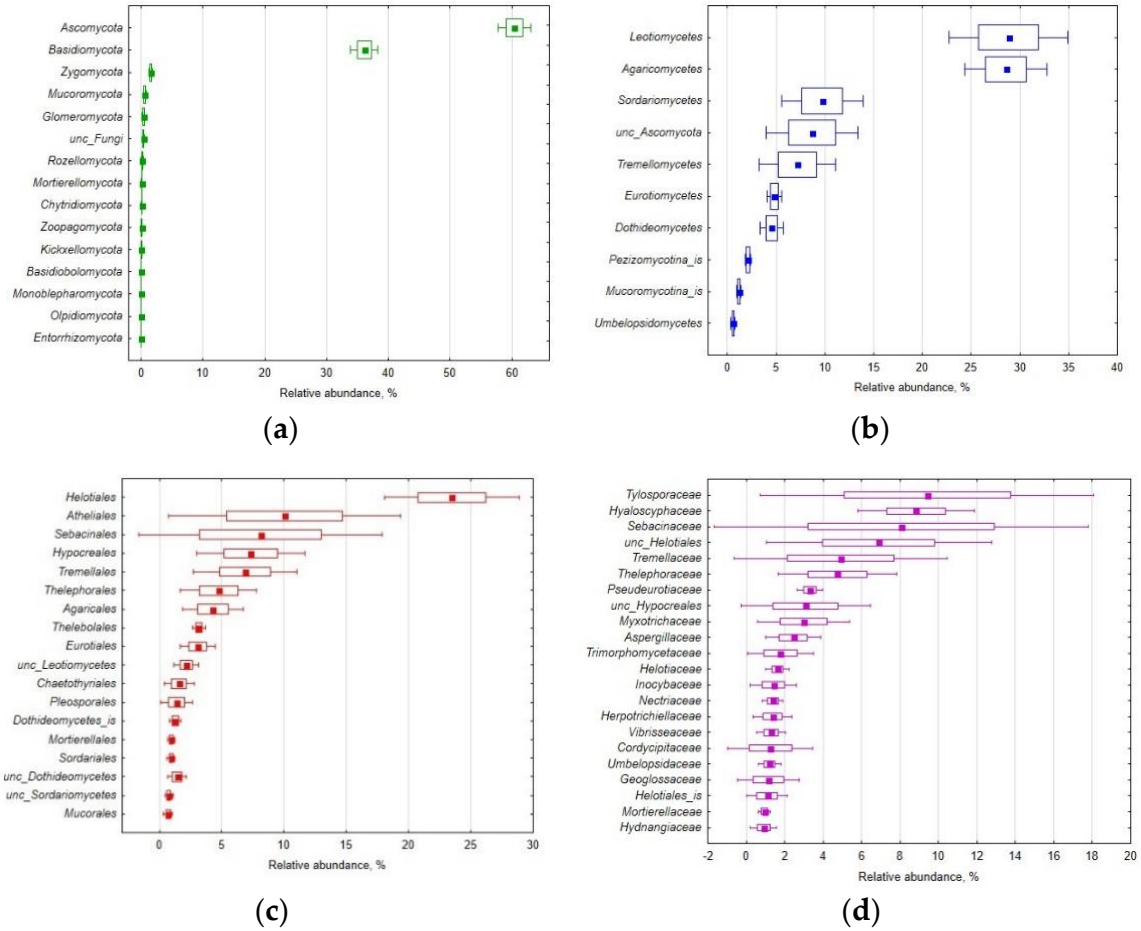

Figure 1. Relative abundance (%) of fungal phyla (**a**), classes (**b**), orders (**c**), and families (**d**) in the 0–20 cm layer of Haplic Cambisol under a Korean pine stand. The marker shows the mean (n = 4), the box shows the standard error of the mean, and the whiskers indicate the standard deviation. Abbreviations in the names of some taxa: "unc_ means" "unclassified", and "_is" denotes "incertae sedis". "*unc_Fungi*" stands for the relative abundance of sequences that could not be assigned to any taxa below the *Fungi* level and that do not represent "a phylum" in a strict sense.

Among the identified classes *Agaricomycetes*, *Tremellomycetes* (both *Basidiomycota* phylum), as well as *Sordariomycetes*, *Eurotiomycetes*, and *Leotiomycetes* (*Ascomycota* phylum) predominated in the studied soil (Figure 1b).

Alpha-biodiversity indices, calculated on the basis of the number of reads in each examined sample, showed that the mycobiome was quite diverse (Table 4). Judging by the Chao-1 index, the number of expected OTUs was only slightly (about 5% on average) in exceedance of the number of the observed OTUs, indicating a low occurrence of singletons and doubletons [24].

Table 4. Fungal OTU diversity indices in the top 0–20 cm of Haplic Cambisol under Korean pine.

Index	Mean	S.E.M.	C.V.
Richness	419	109	26
Chao-1	437	99	23
Simpson (1-D)	0.95	0.01	2
Shannon	4.0	0.4	9
Evenness	0.14	0.03	18
Equitability	0.67	0.04	6
Berger-Parker	0.16	0.03	22
Dominance (D)	0.05	0.01	28

3.2. Mycobiome Diversity and Soil Properties

The relationship between mycobiome diversity and basic soil properties, as revealed by PCA, is shown in Figure 2. The positive pole of PC 1 was defined by two dominant classes (one from *Ascomycota* and another one from *Basidiomycota*), whereas the negative pole was also associated with two classes (Figure 2a) from the dominant phyla. Two *Ascomycota* classes, namely *Leotiomycetes*, ultimately dominant in the study, and *Eurotiomycetes*, a minor dominant, were markedly separated along PC2, determining its poles.

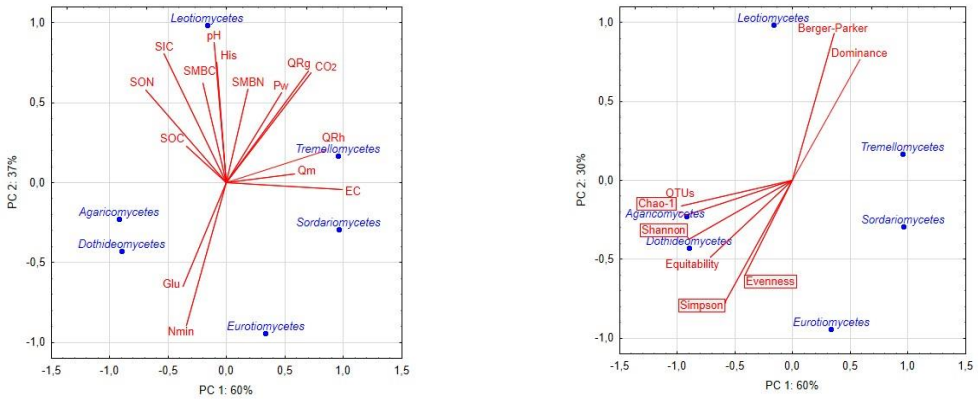

Figure 2. Principal components analysis of the data matrix with fungal classes' relative abundance as variables for analysis (blue circles) and soil properties (**a**) or α-biodiversity indices (**b**) as supplementary variables (in red): location of the variables in the plane of the first two principal components. Abbreviations: EC—electric conductivity; SOC—soil organic carbon, SON—soil organic nitrogen; SMBC—soil microbial biomass carbon, SMBN—soil microbial biomass nitrogen, Pw—water-extractable phosphorus, CO_2—soil basal respiration, Glu, His—glucose- and histidine-induced respiration, QRg, QRh—respiratory quotients with glucose and histidine, Qm—metabolic quotient of the soil microbial biomass.

The relationship of fungal classes relative abundance with α-biodiversity indices, calculated using the number of the OTU reads, revealed an association of the prevailing *Leotiomycetes* with the dominance indices, whereas richness indices were located closely to the second-ranked in abundance *Agaricomycetes* (Figure 2b).

4. Discussion

Mycobiome patterns in different forest soils revealed a high diversity some time ago [25], and since then, they have often been studied; in many cases there are more than one forest-forming tree species, and often there is a diverse and productive understory and surface vegetation. The long-term field experiment from which we collected soil samples represents an unique case, providing an opportunity to profile soil microbiome diversity under a mono-species (Korean pine) stand grown in its native environment without any surface and understory vegetation Thus, the phytomass input into soil, both above- and

belowground, has been from the pine as the sole edificator, ultimately shaping the soil microbiome over more than 40 years.

In the studied soil samples we found a diverse mycobiome; this finding strongly implies that, in the studied case, the soil mycobiome diversity is shaped primarily by the plant matter chemistry diversity. We believe the latter, i.e., plant matter quantity and quality, is the main mechanism, underlining conclusions about plant community diversity affecting soil microbiome diversity.

As compared with the soil mycobiome under Korean red pine [26], our samples had a comparable number of different taxa, except for phyla, as only *Ascomycota* and *Basidiomycota* were found under the red pine. A similar predominance of *Basidiomycota* and *Ascomycota*, with other phyla being scarce, was reported for other forest soils [27–31].

Fungi in soil are known to vary by at least four orders of magnitude in size, from single cells to gigantic single mycelial individuals [32]. Biodiversity concepts and indices were developed for a number of specimens, i.e., individual organisms of a certain species in a given location. Obviously, an individual organism of a soil fungus is extremely difficult, if at all possible, to determine and count. Thus, we cannot help but emphasize the truism that one cannot overestimate the metagenomic approach to assessing soil mycobiome by classifying diversity into taxa defined by a specific sequence similarity and obtaining inventories of the species inhabiting an ecosystem.

However, amplicon sequencing of partial regions (often ITS) of the ribosomal RNA loci, which is widely used to profile microbiomes by calculating the relative abundance of taxa-specific sequence reads in the total number of reads, is not so straightforward [33], as the rDNA copy number can vary considerably across fungi, ranging from an estimated 14 to 1442 copies [34], and can be associated with environment variation, species, and life cycle stages, i.e., between dikaryotic and monokaryotic mycelia [35]. Therefore, apparent abundance changes in a microbiome may be driven by a genomic response to the environment. This might be the case in this study as well, where variation in plant matter input may stimulate fungal decomposition and increase the rDNA copy number. This could explain the rather high variation in the relative abundance of the dominant fungal taxa among the replicated soil samples in the case of this study.

The fungal α-diversity indices in our study were rather close to the ones reported for the dark-brown soil under the tree-species rich mixed coniferous forests with Korean pine in China [30], and for Cambisols and Podzols under mixed or coniferous forests in the Italian Alps [29], where, for instance, the Shannon's index was 3.84 and 3.13–3.92, respectively, as compared with the value of our study of 3.95.

The finding of the major, i.e., with at least 5% of the relative abundance, dominant genera, such as *Amphinema*, *Sebacina*, *Cryptococcus*, unclassified *Hyaloscyphaceae,* and *Helotiales*, all known as mycorrhizal fungi or deadwood decomposers, agrees with the ecosystem studied (undisturbed pine stand with plenty of deadwood, needle, and root litter). The notable presence of *Suillus americanus* was no surprise, a well-known ectomycorrhizal fungus with a preference for pines [36], in the studied samples. The finding that *Pseudogymnoascus* sp. (*Pseudogymnoascus/Pseudeurotiaceae/Dothideomycetes/Ascomycota*) was one of the two dominant OTUs, common to the mycobiomes of all samples, is more interesting, as not many species of the genus have been isolated from different environments in the Northern Hemisphere. The genus can be found in different cold environments, being isolated from wood, root, and soil samples, and is known as the most widespread decomposer fungus in maritime Antarctic soils [37]. Another common dominant OTU, belonging to *Hyaloscyphaceae*, *Helotiales/Leotimycetes/Ascomycota*, includes various saprobes, decomposing plant matter, usually wood, and can be root symbionts as well [38].

Our finding that few OTUs dominated in each soil sample, the vast majority being minor or rare mycobiome members, suggests that the ecosystem processes performed by soil fungi rely on a very limited taxonomic diversity, at least in soil under a mono-species tree stand. This insight may be helpful in reconstructing soil microbial communities during land reclamation by forest planting or tree phytoremediation. However, some rare soil

fungi might play unique ecological roles, which may be difficult to elucidate, but should be included in the agenda for further research on the forest soil mycobiome and its role in forest sustainability and restoration.

We found only two dominant OTUs (with 2–7% relative abundance) of *Ascomycota* were common to all four soil replicates. This was somewhat surprising, as careful bulking, mixing, sieving, and aliquoting were performed during soil sampling and subsequent handling, in order to homogenize soil, hence eliminating patchiness on a smaller scale. Fungal hypha are binding agents for soil microaggregates (<250 μm), which are rather stable [39]; hyphae also occupy voids in macroaggregates, i.e., particles sized 250 μm^{-1} mm and more. Both fractions are not affected by soil sieving with the commonly used mesh size 1–2 mm. Thus, even a minor variation in soil aliquots, taken for DNA extraction, may slightly change aggregate composition and bring some variation in fungal diversity, as with DNA extraction kits 0.1–0.5 g of soil is taken for extraction. Indeed, strong variations in fungal diversity were observed between replicates of the small samples (<1 g) as compared to the larger (4 g) ones [40]. Greater microbiome diversity was found in soil microaggregates [41]. Besides, some hyphae occurring on the surfaces or in voids between bigger particles could have been removed with roots and plant debris and/or disrupted by sieving. Therefore, the fact that very few dominant OTUs were common to all four individual soil samples may be attributed, at least in part, to the soil aliquot size, aggregate heterogeneity, vulnerability to coarse particles/roots removal, and sieving. These factors can also explain why the meta-analysis did not find any globally distributed OTUs in soil fungal communities [42]; why soil fungal communities responded weakly to abiotic and biotic factors [43]; and why there was no positive correlation between plant and fungal diversity [31]. One should also bear in mind that differences in rDNA copy numbers [34] may also contributed to all of the above mentioned facts, thus making estimating mycobiome structure more challenging.

Soil pH was shown to significantly affect mycobiome composition in a broad-leaved Korean pine mixed forest [44], and our study found an association of the ultimately prevailing *Leotiomycetes* with soil pH, even within the very narrow range of just 0.07 between our soil samples. This suggests a high sensitivity of this major dominant class to this important soil property. The location of the class in the same semiplane with soil organic matter and microbial biomass characteristics (Figure 2a) strongly suggests (a) the class has a leading role in organic matter transformation in soil, and (b) its predominant contribution to the soil microbial biomass. The latter, as measured by the chloroform-fumigation extraction method, includes all microscopic soil organisms; however, microscopic fungi can account for $\frac{3}{4}$ of soil microbial biomass carbon [45]. The close association of *Leotiomycetes* relative abundance with those α-biodiversity indices (based on OTUs, Figure 2b) that relate to taxa predominance agrees with the previous finding, as pulses of plant material input into soil most likely benefit the certain OTUs of the most abundant decomposer class. Interestingly, the *Agaricomyctes* and *Dothideomycetes* classes, the most closely associated with the observed and potential OTU richness and Shannon's indices, did not reveal a close relationship with most soil properties, which may imply these fungi have a lower biomass due to partially inactive mycelium and/or spores. The close association of *Tremellomycetes* with histidine respiration quotient implies the taxon has a preference for N-enriched substrates/microhabitats, most likely close to pine roots.

5. Conclusions

The presented data provide an inventory of soil mycobiome diversity, as specifically shaped by the soil environment developed under a monospecies pine stand, i.e., by the long-term intimate relationship between soil microorganisms and the sole plant edificator, e.g., its diverse phytomass chemistry. The variation of chemical and microbial characteristics, even between a small number of individual soil replicate samples, apparently results in a soil environment dynamic enough to have very few fungal OTUs common to all replicates.

The described soil mycobiome provides a reference for further studies of soil microbial ecology, especially under Korean pine, with its aesthetic as well as nut-producing qualities.

The results of our pilot profiling of the soil mycobiome specific to the Korean pine will be helpful in the targeted creation of a soil mycobiome beneficial for pines in afforestation and remediation contexts.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/applmicrobiol2030036/s1, Figure S1: The number of fungal OTUs as dependent on the number of sequences reads in 4 individual soil samples (shown in different colours) collected from Cambisol under Korean pine stand: solid line—interpolated, dashed line—extrapolated.

Author Contributions: Conceptualization, G.K. and N.N.; methodology, N.N.; validation, G.K. and M.K.; formal analysis, T.A.; investigation, T.A.; resources, M.K.; data curation, N.N.; writing—original draft preparation, N.N.; writing—review and editing, M.K.; visualization, T.A.; supervision, M.K.; project administration, N.N.; funding acquisition, G.K. and M.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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