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Addition of Organic Matter to Pine Plantations on Agricultural Land Positively Alters the Mycobiome of Agricultural Soils

Tadeusz Malewski [1], Piotr Borowik [2], Ireneusz Olejarski [3], Artur Rutkiewicz [2], Adam Okorski [4] and Tomasz Oszako [2,*]

[1] Museum and Institute of Zoology, Polish Academy of Science, ul. Wilcza 64, 00-679 Warszawa, Poland; tmalewski@miiz.waw.pl
[2] Departement of Forest Protection, Forest Research Institute, ul. Braci Leśnej 3, 05-090 Sękocin Stary, Poland
[3] Departement of Ecology, Forest Research Institute, ul. Braci Leśnej 3, 05-090 Sękocin Stary, Poland; i.olejarski@ibles.waw.pl
[4] Department of Entomology, Phytopathology and Molecular Diagnostics, Faculty of Agriculture and Forestry, University of Warmia and Mazury in Olsztyn, Pl. Łódzki 5, 10-727 Olsztyn, Poland
* Correspondence: t.oszako@ibles.waw.pl

Abstract: Afforestation of former agricultural land poses a real challenge for foresters because soil life is often severely limited by the loss of natural soil fungal diversity. In addition, former agricultural soils have low levels of fungal species typical of forest soils, which have a unique microbiome that plays a protective role (antagonists, plant growth promoters, mycorrhizal fungi, etc.). This study aimed to determine the effect of using organic material in the form of bark compost, wood waste, and sawdust to improve the soil mycobiome of soils that have been damaged by their agricultural use. This study used experimental plots established 20 years ago, and we compared the biodiversity of the treated soils with that of the control soils by analysing soil samples with powerful molecular methods. Next-generation sequencing analysis of DNA extracted from soil samples and subsequent analysis of their species composition and biodiversity showed that the mycobiome of soil fungi has been altered by the addition of various forms of organic material. The proportion of fungi belonging to the Ascomycota decreased in favour of species from the Basidiomycota and Mucoromycota. The dominant fungal groups in the soil of the control area were *Sagenomella*, *Wilcoxina*, *Oidiodendron*, *Meliniomyces*, and *Penicillium*. Enrichment with organic matter by adding bark compost under the roots led to an increase in *Penicillium*, *Inocybe*, and *Amphinema*. The application of bark compost on the surface led to an increase in the dominance of *Inocybe* fungi in the soil. The mycobiome of the plant to which woody debris was applied was characterised by a marked dominance of fungi of the genera *Russula*, *Oidiodendron*, and *Penicillium*. Similar ratios were found in the plant to which sawdust was applied, where the fungi *Meliniomyces*, *Penicillium*, *Oidiodendron*, and *Russula* dominated. A comparative analysis of fungal diversity with the Shannon diversity index showed that the most diverse fungal communities were found in the sawdust plant (6.56), while the control sample (a soil sample from an agricultural area where no organic material was applied) had an index of 5.71. After the treatments, more potential antagonists against pine pathogens and mycorrhizal fungi were found to form beneficial symbiotic relationships with them. In our opinion, the results of this study show that it is worthwhile to introduce different forms of organic matter to post-agricultural land to improve soil biodiversity and mycorrhizal associations of pine roots with fungi to ensure the sustainability of the first generation of forests created.

Keywords: species diversity; pathogens; saprobes; mycorrhizae associations; NGS; relative abundance; Venn diagrams; OTUs; Shannon diversity; Bray-Curtis dissimilarity index

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1. Introduction

Ecological restoration, when carried out effectively and sustainably, contributes to protecting biodiversity, improving human health and well-being, and enhancing food

and water security, to the provision of goods, services, and economic prosperity, and to supporting climate change mitigation, resilience, and adaptation [1]. At the UN, ecosystem restoration assumptions related to forest ecosystem degradation and conservation have been issued for the next decade (2021–2030). They go in two directions: the issue of global forest degradation (which includes the problem of deforestation) and the analysis of the theoretical basis of forest ecosystem restoration, taking into account application successes.

This research is part of environmental policy and offers opportunities to counteract land degradation through long-term agricultural use and to restore biodiversity from the perspective of forest landscape restoration (FLR) and to mitigate climate change. The use of organic material demonstrates the need for a comprehensive approach to restoring and protecting natural ecosystems and strengthening their resilience [2].

The development of post-agricultural land through afforestation carries the risk that the first generation of Scots pine *Pinus sylvestris* L. will not survive to fell age due to the threat of the fungus *Heterobasidion annosum* [3–5]. Its spores are ubiquitous and cause primary infection when they fall on the stumps produced during the rearing of the first thinning. As it has no natural counterparts (as in the forest ecosystem), it can multiply uncontrollably and cause secondary infections through root contacts between diseased and healthy trees [6–8]. As a result, gaps appear and, after a certain time, usually at the age of 40, the stand loses its compactness and is only suitable for removal and restoration [6].

The prevention methods developed involve the removal of tree stumps, which are a niche used by root pathogens. However, they are labor-intensive (mechanical) or environmentally damaging (chemical), and even if they are environmentally friendly (biological), they are expensive, which has recently become important in forestry in the face of strong competition from different employers. The rising costs of forest prevention and protection, as well as ecological considerations, make it necessary to look for other methods, which include increasing the natural resistance of the soil to pathogens.

Restoring organic levels that ensure the circulation of elements (macro- and micronutrients), the presence of natural antagonists (fungi such as *Trichoderma* spp. or *Penicillium* spp.) against pathogens, and fungi that form symbiotic mycorrhizal relationships (e.g., *Amanita* or *Rusula* genus) seems to be the most appropriate direction in the current economic and social situation [9]. For this reason, an attempt was made to use various organic substrates that are often a by-product of timber harvesting, such as wood waste or sawdust (in sawmills) or wood processing (debarking), and that can be used to produce compost (bark) that promotes the number and diversity of beneficial fungi present in the soil [10]. This approach is in line with the concept of the circular economy and European Union policy [11].

To underline the importance and uniqueness of the study started 20 years ago, we have tried to review its implications and applicability to the field of forestry. Primarily, the study aimed to determine whether the introduction of organic material into degraded soils (after agricultural use) can restore mycobiota characteristic of forest soils.

2. Materials and Methods

In autumn 2001, as part of soil preparation, a furrow was ploughed on an experimental plot (0.3 ha each) in the forest district of Bielsk (eastern Poland) with the habitat typical of pine plantations. Subsequently, various organic substances were introduced into the furrows at a rate of 1.5 mp/acre to restore the organic level (Figure 1). On each experimental plot (in three repetitions), the following substrates were applied separately: bark compost on the surface (BCS) and under the roots (BCR), wood waste (WW), and sawdust (S). No treatments were applied to the control plot (C). Organic matter (BCS, WW, and S) was distributed in the rows, while (BCR) was applied under the roots at a rate of 5 L/plant during planting. The addition of each organic matter was repeated in three experimental plots, which were spatially randomised to exclude the random effect of soil conditions.

In spring 2002, the plots were planted with 2-year-old seedlings of *Pinus sylvestris* L. After 20 years, in 2021, in the designated experimental plots, the restoration of the soil's

organic horizon and changes in its biological properties and the identification of the fungi in the rhizosphere were carried out using the Next Generation Sequencing method (NGS). To better understand environmental factors, e.g., extreme climatic factors such as droughts, rainfall and temperature data were downloaded from a nearby weather station. The effects of biotic factors, e.g., pathogenic fungi, could only be compared with controls, and genetic analyses were very helpful in this case.

Figure 1. (**a**) On the trial plot in the forest district of Bielsk in 2001, row upon row of scattered remains of wood (WW). (**b**) Experimental plot in the Bielsk forest district in 2007, control conditions (C), on the left the variant with wood remains (WW), on the right the variant with sawdust (S), in front the variant with bark compost (BCS). (**c**) Control plot (C) in the Bielsk forest district with dead trees. (**d**) Drone photo from 2019, visible gaps in the control plots (C) in the Bielsk forest district, Strabla forestry enterprise.

2.1. Analysis of Mycobiome

For the genetic analyses, 12 cylinders of 100 cm^3 soil were collected, which were bulk samples from the Bielsk forest areas. They were taken after the organic layer of the soil had been removed from a depth of about 5 cm. Three replicates of each sample were made, resulting in a total of 36 samples. The soil samples were thoroughly mixed before about 1 g was collected. DNA extraction, libraries preparation, processing, and analysis of sequencing data were performed as previously described [9]. Obtained sequence reads were filtered and low-quality reads were discarded. High-quality reads were checked for chimera and the chimera was also removed. The obtained high-quality, free-of-chimera reads were assigned to an Operational Taxonomic Unit (OTU). OTUs with reads numbers below 100 were discarded from further analysis. Sequences were submitted to the NCBI Sequence Read Archive (SRA) under the study accession number PRJNA954760.

2.2. Analysis of Fungal Biodiversity

The taxonomic information and the community composition were obtained and analysed at kingdom, phylum, class, order, family, genus, and species taxonomic levels. Alpha diversity of the fungal communities was estimated using the Shannon diversity index. Confidence intervals for the Shannon index were calculated using the resampling

method [12,13]. Evaluation of beta diversity was performed using the Brey–Curtis dissimilarity index. For the calculations, we used the scikit-bio 0.5.8 [14] software package and custom codes in Python 3.8. The assignment of ecological roles was based on FUNGuild [15].

3. Results

3.1. Precipitation and Temperature in the Vicinity of the Observation Plots

In the years when the soil was prepared and pine seedlings were planted, and in the years that followed, there was a severe drought (Figure A3 in Appendix B). An index of annual precipitation, especially during vegetation season, dropped far below average.

3.2. Validation of the Measurements Results

The curated records comprised 701,927 reads. The reads per sample ranged from 130,408 to 178,138. The first analysis we performed was to assess whether a particular sample was sufficiently sequenced to represent the biodiversity of the soil. The rarefaction curve [16] was created by randomly resampling the sample pool several times and then plotting the average number of species found in each sample (Figure 2). In the studies conducted, more than 120,000 reads were collected for each of the soil conditions studied. The data presented in Figure 2 show that, with a sample size of 50,000 reads, about 99% of OTUs were detected, confirming the validity of the approach used.

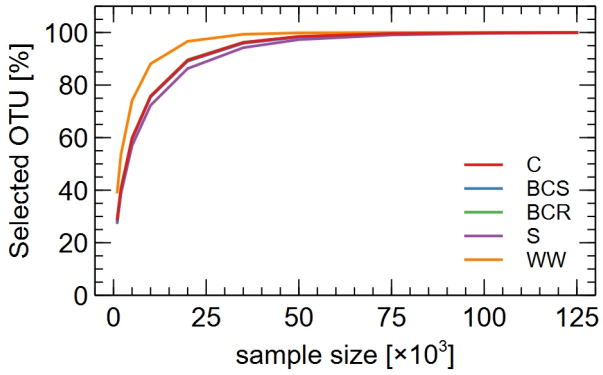

Figure 2. Rarefaction analysis of coverage of OTU detected for different soil conditions studied: C—control, BCR—bark compost under the roots, BCS—bark compost on the surface, WW—wood waste and S—sawdust. The rarefaction curves for each sample are represented by different colours. The x-axis represents the sequencing depth in number of reads and the y-axis represents the estimate of OTU richness determined at species level.

3.3. Mycobione Abundance Analysis

The amplicons were assigned to 373 OTUs. Two-hundred-and-one genera and two-hundred-and-ninety-one species were found. Most of the fungi belonging to the *Ascomycota* group were found in the control group, and the fewest after mulching the plots with the pine bark compost. On the other hand, the growth of fungi belonging to the *Basidiomycota* group was stimulated by applying the same compost under the pine roots (Figure 3, Appendix A). It is also noteworthy that a considerable proportion of the fungi could not be identified.

Of the group *Ascomycota*, most of those found belong to the *Leotiomycetes* (Figure 4).

Fungi of the class *Eurotiomycetes* were also highly represented.

The *Sordariomycetes* were much less represented and were mainly found after soil treatments with sawdust and wood residues left after fellings (S and WW).

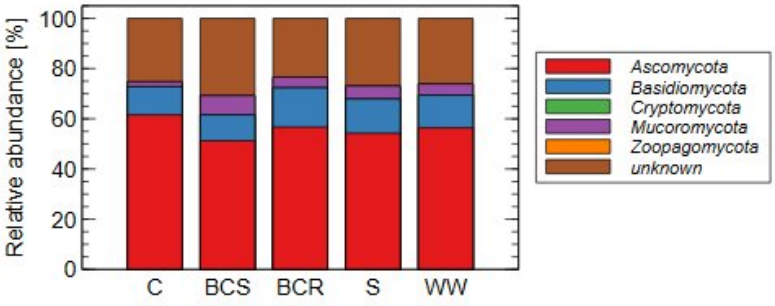

Figure 3. Relative abundance of detected fungi phylum versus various studied soil conditions: C—control, BCR—bark compost under the roots, BCS—bark compost on the surface, S—sawdust, and WW—wood waste.

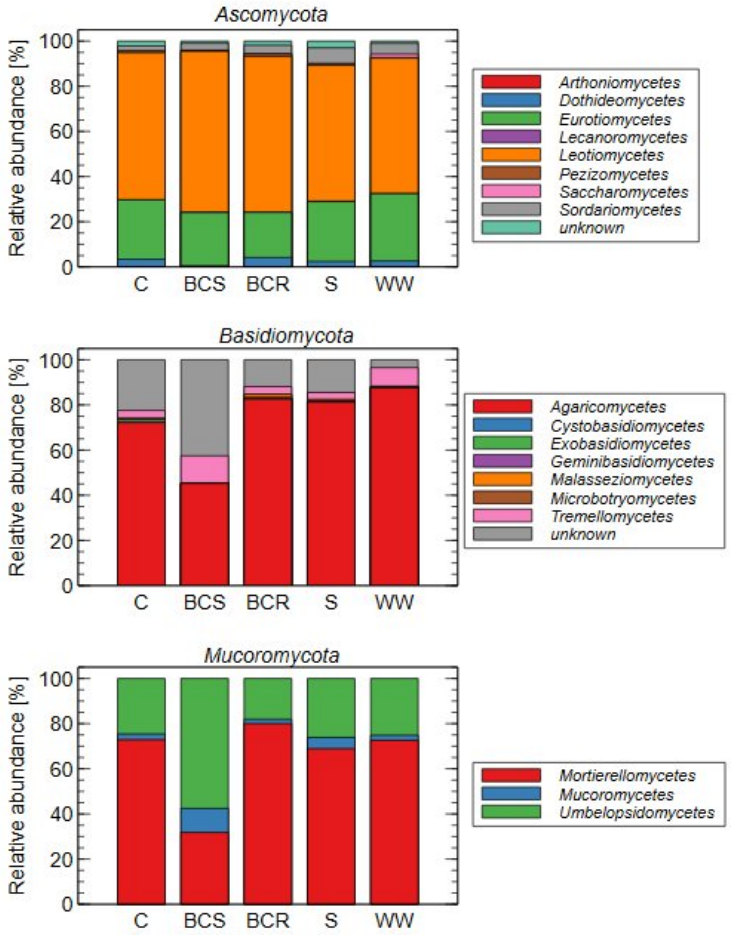

Figure 4. Relative abundance of detected fungi class versus various studied soil conditions: C—control, BCR—bark compost under the roots, BCS—bark compost on the surface, S—sawdust, and WW—wood waste. Three most abundant phylum are plotted. Fungi phylum is indicated in the charts.

Among the *Basidiomycota*, however, the largest number of fungi was found in the *Agaricomycetes* present both in treatments (less in BCS than BCR, S, and WW) and the control.

The second abundant class of fungi in treatments (BCS and WW) was *Tremellomycetes*; in this case, they were more frequent in BCS.

From the *Mucoromycota* group of fungi, most belonged to *Mortierellomycetes* (Figure 4). They were found in both control and treatments, but the least amount was found in BCS.

Among *Mucoromycota*, the *Umbelopsidomycetes* were particularly common in BCS in contrast to *Mortierellomycetes*.

The analysis carried out shows that OTUs belonging to 18–22 genera account for between 32% and 41% of all reads (Table 1, Figure 5). Fungi, which are characteristic of forest communities, such as fungi of the genus *Russula* (which form mycorrhizal associations with tree roots), were found after S and especially WW treatments as well as other mycorhizal fungi (Appendix A). Fungi of the genus *Penicillium*, which are antagonistic to the pathogen *H. annosum*, also colonised these variants and especially BCS.

Fungi of the genus *Inocybe* preferred BCS as a substrate, and BCR even more. They also formed mycorrhizal associations with tree roots, similar to *Tricholoma* spp., which were also abundant in BCR.

Control was dominated by the mycorrhizal fungi genus *Wilcoxina*.

Table 1. Dominance of fungi groups for various soil conditions studied: C—control, BCS—bark compost on the surface, BCR—bark compost under the roots, WW—wood waste and S—sawdust. Yellow—dominants (>5%), blue—subdominants (1–5%), grey—accessory (<1%).

Groups of Fungi	Control	BCS	BCR	WW	S
Sagenomella	6.21	2.20	0.84	0.73	2.01
Wilcoxina	6.06	0.13	2.05	0.04	0.13
Oidiodendron	6.03	5.04	2.14	7.12	5.06
Meliniomyces	6.02	0.41	2.02	1.04	8.10
Penicillium	5.10	10.02	4.03	6.14	7.03
Tricholoma	2.13	1.06	4.12	1.07	1.04
Cortinarius	2.04	1.14	0.05	0.01	1.06
Mortierella	2.03	2.11	0.52	0.81	1.02
Geomyces	1.14	0.31	2.07	0.81	0.09
Solicoccozyma	1.04	0.41	0.41	0.31	0.82
Hyaloscypha	1.02	4.05	0.21	0.23	0.72
Inocybe	0.91	5.03	7.02	2.04	0.02
Exophiala	0.70	0.32	0.21	1.08	1.03
Hydnum	0.31	0.24	0.04	2.04	0.00
Trichoderma	0.21	0.92	0.32	0.90	0.41
Russula	0.12	0.13	1.03	9.13	5.07
Cenangium	0.12	0.09	2.03	0.40	0.14
Collarina	0.06	0.00	0.00	1.08	0.00
Apiotrichum	0.06	0.11	1.03	0.08	0.07
Hygrophorus	0.02	0.13	1.14	1.06	0.00
Amphinema	0.02	5.01	0.00	0.00	0.00
Cenococcum	0.01	0.01	0.32	0.00	2.12

Some fungi, such as the genus *Amphinema*, only appeared or dominated in the variant of compost applied to the soil surface (BCS), e.g., *Hyaloscypha* (Figure 5). If, on the other hand, the same compost was spread under the roots of the trees, fungal genera specific to this variant (BCR) only occurred, e.g., *Apiotrichum*, or were present there in the greatest quantities (*Cenangium*, *Geomyces*, *Inocybe*, and *Tricholoma*). The genus *Peniclillium* occurred everywhere but was strongly represented in the case of BCS, while the genus *Rusula* dominated after WW wood residues were used. Overall, the fewest taxa were found in the control (C-14) and in sawdust (S-13), while the most were found after the application of compost (BCR and BCS, 18 each) and wood residues (WW-17).

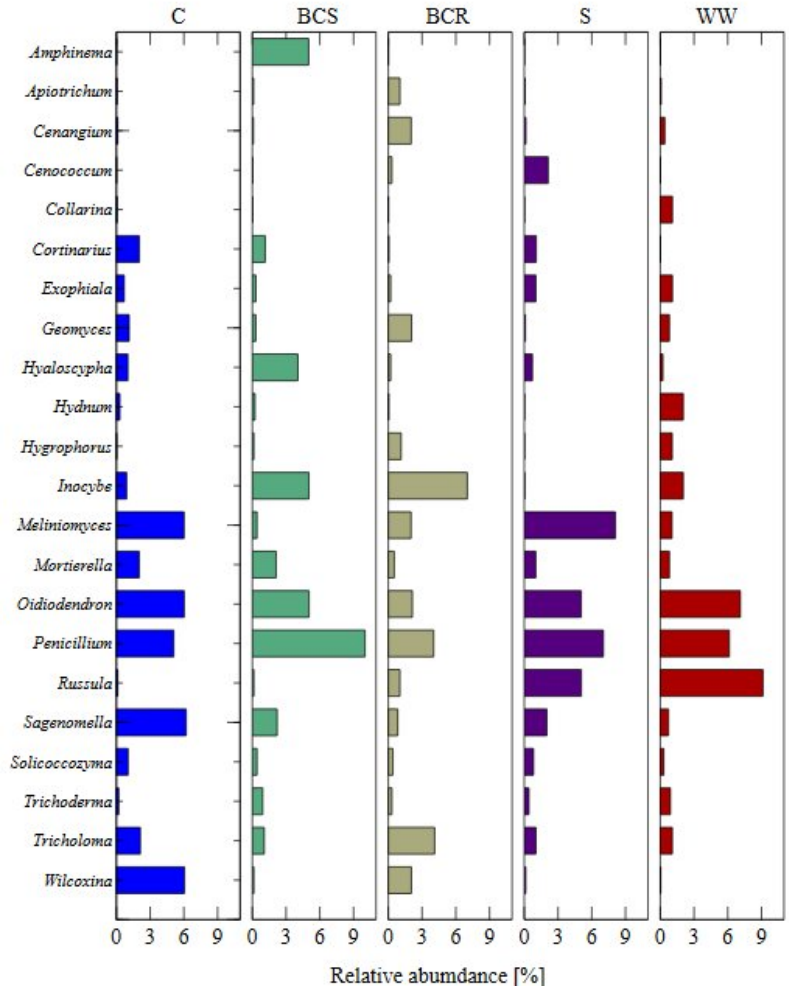

Figure 5. Relative abundance of fungi for various soil conditions studied: C—control, BCR—bark compost under the roots, BCS—bark compost on the surface, S—sawdust, and WW—wood waste.

3.4. Species Diversity Analysis

3.4.1. Venn Diagram Analysis

Venn diagrams show the differences between the control and the treatment with organic material supplied 20 years ago (Figure 6). Significant quantitative differences in favour of the treatment were found for BCR and S, e.g., S and WW in the combinations BCS/S and BCR/S and BCS/WW. The statistical significance of the biological diversity level difference between studied soil conditions was confirmed by the analysis of the Shannon index presented in the following subsection.

The addition of BCR resulted in the largest mycobiome reconstruction associated with the appearance of 213 new OTUs. Similarly, the addition of sawdust, which enriched the soil with organic material consisting mainly of cellulose and lignin, led to the appearance of 202 new OTUs. The addition of these organic materials is mainly associated with the appearance of new OTUs and the disappearance of a relatively small number of OTUs that were present in the control (80 and 91, respectively). The opposite is true for the addition of BCS and WW. The addition of these organic substances also increased the number of OTUs (171 and 160, respectively), but significantly decreased the number of OTUs in the control (122 and 160, respectively).

The changes in OTUs are related to genera and species with low abundance. Only 18 to 22 genera have more than 1% of the readings in at least one experimental condition (Table 1, Figure 5). The addition of organic material increased the number of *Apiotrichum* reads from 1.2 to 17.2 times, *Russula*-from 1.1 to 76.1, and *Trichoderma* from 1.5 to 4.4 times.

Organic material reduced the abundance of *Cortinarius* from 1.8 to 204, *Sagenomella* from 2.8 to 8.5, *Solicoccozyma* from 1.3 to 3.4, and *Wilcoxina* from 3.0 to 151.5-fold.

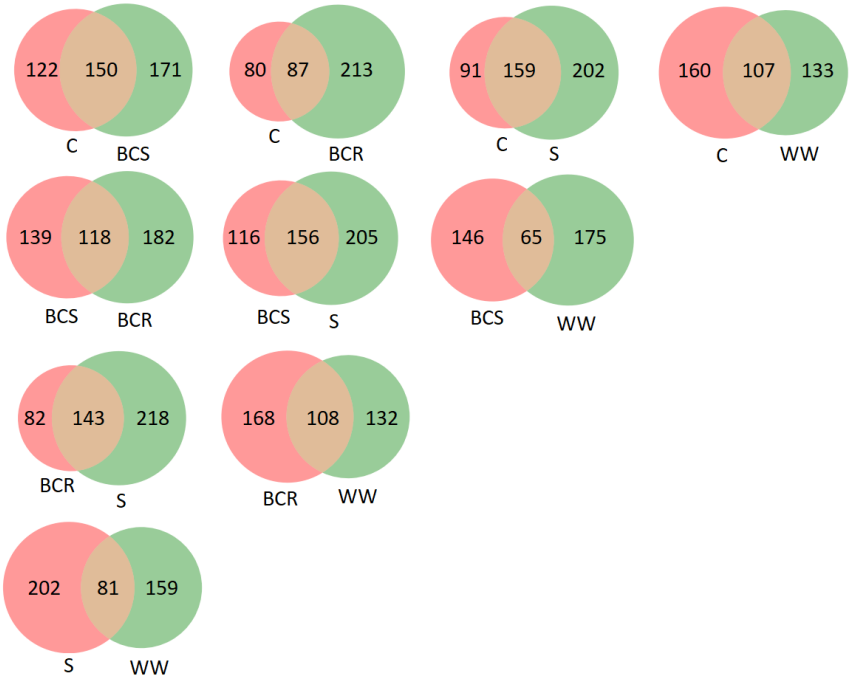

Figure 6. Venn diagrams showing the number of common OTUs between the soil conditions studied: C—control, BCR—bark compost under the roots, BCS—bark compost on the surface, S—sawdust, and WW—wood waste.

3.4.2. Shannon Alpha-Diversity Index

The Shannon index showed the highest species diversity when sawdust (S) and wood waste (WW) were added to the soil (Figure 7). The treatment with bark compost (BCS and BCR) also performed better compared to the control. As one can notice in Figure 7, the whiskers representing 95% confidence intervals do not overlap when we compared various studied soil conditions, and especially do not overlap for comparison with the control treatment. That is equivalent to a statistically significant difference at $p < 0.05$ between the studied treatments.

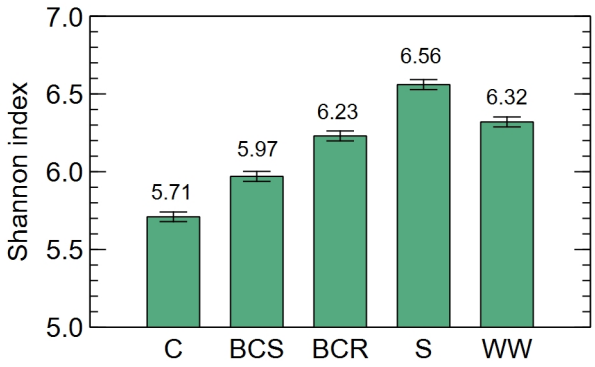

Figure 7. Shannon index for various soil conditions studied: C—control, BCR—bark compost under the roots, BCS—bark compost on the surface, S—sawdust, and WW—wood waste. Whiskers represent 95% confidence intervals.

3.4.3. Bray-Curtis Dissimilarity

The Bray–Curtis dissimilarity index shows that the control differs most from woody debris (WW) application and least from compost thrown under the pine roots. Similarly, the other treatment options BCS, BCR, and S differ from WW (Figure 8).

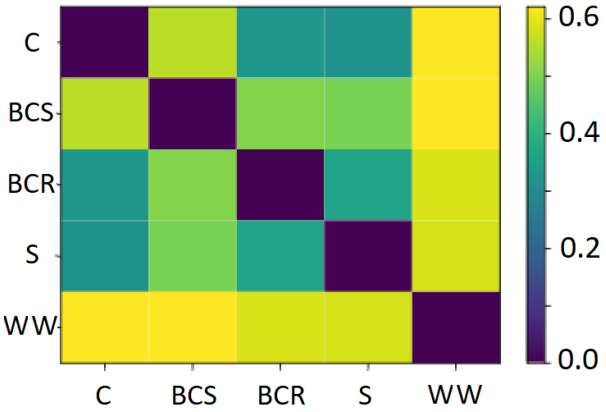

Figure 8. Bray–Curtis dissimilarity index is bounded between 0 and 1, where 0 means the two sites have the same composition (that is, they share all the species) and 1 means the two sites do not share any species. Comparison of species detected in studied soil conditions: C—control, BCR—bark compost under the roots, BCS—bark compost on the surface, S—sawdust, and WW—wood waste.

3.4.4. Hierarchical Grouping Analysis

The addition of different organic substrates to the soil led to clear differences between the different variants of our experiment even after 20 years (Figure 9). The control differs most from the other treatments, while WW and S as well as BCS and BCR show similarities.

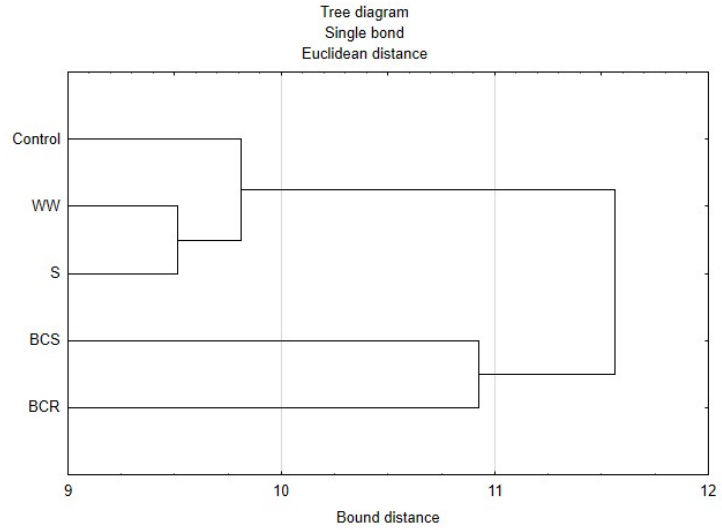

Figure 9. Dendrogram of similarities between treatments and controls, various soil conditions studied: C—control, BCR—bark compost under the roots, BCS—bark compost on the surface, S—sawdust, and WW—wood waste.

4. Discussion

4.1. Possible Influence of Precipitation and Temperature on Pine Seedlings

Severe drought (2001–2004) probably caused high mortality of young pines (Figure A3 in Appendix B). Seedlings with open root systems planted into the soil and not covered with organic material suffered especially. In the treatment variants, the organic material protected the soil from both extreme weather conditions (high temperatures) due to high insolation and accumulated moisture after infrequent rainfall. The decomposition of

organic matter also provided plants with nutritional elements and, along with wood residues harvested from logging, spores, and shreds of fungi—including mycorrhizal fungi—were probably transferred. As is known, mycorrhizae increase the surface area of root systems up to 1000 times, hence the survival rate of seedlings in treatment plots was much higher than in control plots.

4.2. Successful Afforestation Problem-Oriented Solution Approach

Land designated for afforestation in Poland includes 2.3 million hectares of marginal land. These include infertile soils, soils contaminated with chemicals, damaged or mechanically transformed soils, and fallow land or land with unfavourable natural and topographic conditions [17]. The specific physico-chemical and biological conditions of former agricultural soils pose a risk of infection with root pathogens such as *Heterobasidion* spp. [18–20]. In Poland, biological control has been successfully introduced on a large scale [4], but nowadays the creation of natural soil resistance would probably be the best prevention method. The integrated approach to soil health assumes that soil is a living system whose equilibrium is determined by interactions between physical, chemical, and biological properties, with the activity of the soil microbiota playing an important role [21]. Fungi are an important component of soil health, with a high adaptability to habitat requirements [22]. They fulfil various ecological functions and occur as saprotrophs that transform dead organic matter and influence the carbon and nitrogen cycles of the ecosystem [22], as mycorrhizal fungi that form symbiotic relationships with plants [23], and as pathogens that cause major damage to forest stands [24].

The diversity and activity of soil fungi are influenced by various abiotic factors such as soil pH, moisture, salinity, soil structure, or temperature conditions [25]. Soil fungal populations are also influenced by targeted soil use, e.g., agricultural crops [26]. Optimising yields of agricultural crops requires the use of plant protection products, including fungicides. These can have negative impacts on the environment through leaching of their residues into the soil [27]. According to Wightwick et al. [28], the use of fungicides in agroecosystems negatively affects soil microorganisms, which have important functions in the soil as they are involved in organic matter degradation and nutrient metabolism [29]. In a study by Baćmaga et al. [30], it was shown that the excessive use of triazole fungicides has harmful effects on both the population of microorganisms and the activity of their soil enzymes.

Long-term nitrogen fertilisation, which is necessary for the production of high-quality agricultural products, leads to unfavourable changes in fungal populations in the soil. A study by Zhou et al. [31] showed that the most common classes of fungi in all the soils studied were *Dothideomycetes, Eurotiomycetes, Leotiomycetes, Sordariomycetes*, and *Agaricomycetes*. The results of the study confirmed that long-term application of nitrogen and phosphorus fertilisers reduces fungal biodiversity and significantly alters fungal community composition. In addition, higher fertiliser concentrations were found to have a greater impact on fungal communities than lower concentrations [31]. Our observations revealed a lower abundance of *Sordariomycetes* in the control than in the treatments.

A study by Morugán-Coronado et al. [26] demonstrated that the application of sustainable management practices, mainly based on the use of organic fertilisers and reduced tillage, has a positive impact on the total number of microorganisms in the soil, including fungi and bacteria. Under our experimental conditions, the abundance of *Agaricomycetes* in BCR, S, and WW and of *Tremellomycetes* in BCS and WW, in particular, increased compared to C.

The identified group of *Ascomycota* forms ascocarps, usually in the form of a perithecium, rarely a cleistothecium, and the sacs are usually 8-spored, lack a lid (operculum), and are arranged at the base or margin of the hymenium. They are widespread and occur in virtually all ecosystems. They can be pathogens of plants (e.g., *Cryphonectria parasitica*, which causes chestnut blight, and *Magnaporthe grisea*, which attacks rice), arthropods or

mammals, and parasites or saprotrophs. One fungus belonging to this class, *Neurospora crassa*, is a model organism for molecular and genetic studies [32].

The fungi of the *Leotiomycetes* are characterised by sacs that usually have a single thin wall (similar, for example, to the *Sodariomycetes*) and have a perforation at the tip through which the spores can be ejected. *Leotiomycetes* is a class of genera that are diverse in both morphology and habitat [33].

Fungi of the class *Eurotiomycetes* form ascocarps in the form of cleistothecia. This fungal class includes many human pathogenic fungi (especially in the orders *Eurotiales, Onygenales*, and *Chaetothyriales)* as well as filamentous fungi [34].

The *Sordariomycetes* include numerous species that were only known in the form of anamorphs. In former times, these species were counted among the group of imperfect fungi. Many species of the orders *Hypocreales, Ophiostomatales*, and *Chaetosphaeriales* have two and sometimes even more anamorphs [32].

In summary, *Ascomycota* includes many pine needle pathogens, so their decline after the treatments carried out (BCS, BCR, S and WW) can be considered a success in favour of *Basidiomycota*. The latter group includes fungi that form symbiotic mycorrhizal relationships with trees. Compared to the control, an acceleration towards forest-specific mycobiomes can thus be observed. The threat of root and stump rot has also decreased, as at least *Mucorymocota* is a potential group of fungi that counteract the pathogen *H. annosum*. The importance of Nau is also underlined by the fact that about 20% of the fungi have not yet been identified, which means that there could be new species among them.

Among the *Basidiomycota*, however, the largest number of fungi was found in the *Agaricomycetes* Doweld, of which the fungus (*Agaricus*) is the nomenclatural species. This taxon replaced the previously defined *Homobasidiomycetes* (single basidiomycetes), with additional orders included [35].

The second most abundant in the treatment (BCS and WW) class of *Tremellomycetes* was also compiled by Alexander Doweld [36]. The range of this group is similar to that of the *Gelimycetes*, which were separated in earlier systems and are often found in forests on dead trees.

Of the phylum *Zygomycota* group of fungi, most belonged to *Mortierellales* (Figure 4). They were found in both control and treatments, but the least amount was found in BCS. They are a monotypic fungal order [37,38] within and the monotypic division of *Mortierellomycota* [39]. They contain only one known family, *Mortierellaceae* Luerss, and six genera and about 129 species.

Mucoromycota produces asexual sporangiospores and forms zygospores by way of the fusion of morphologically similar gametangia. *Pilobolus, Mucor*, and *Rhizopus* are common filamentous fungi. They form a mycelium consisting of branched filaments without septa [40]. When two sexually different hyphae (+) and (−) come into contact, their walls dissolve and the contents of the cytoplasm fuse together (plasmogamy). Mitotic divisions occur in the nuclei of both sex cells, followed by the fusion of the nuclei of different sex cells (karyogamy). A multinucleate zygospore with a diploid chromosome number is formed. It surrounds itself with a thick wall of dark color and enters a dormant phase. During this time, it behaves similarly to a spore. When it begins to germinate, meiosis immediately takes place in its nuclei and haploid nuclei are formed. Most species, however, spread mainly via sporangiospores. *Pilobolus*, under the influence of the explosive hydrostatic pressure at the end of the sporangiophore, expels the entire sporangium at a distance of up to 7 m. It is covered with a sticky mass that adheres to the grass, where it is eaten by the herbivores and then excreted in the faeces. At this point, the spores begin to grow and feed on the faeces. The ability to carry the spores away from the faecal pile is important for taxa that enter the faeces by feeding, as herbivorous mammals do not usually feed near the faecal pile [40].

The *Umbelopsidaceae*, which were particularly common in BCS, are a family of fungi belonging to the order *Mucorales*. Members of this family (currently in the single genera

Umbelopsis) are widely distributed [41]. The name *Mucoromycetes* Doweld (numerous in BCS) was created in 2001 [42].

Genus *Wilcoxina* belongs to the family *Pyronemataceae*. The species have a cosmopolitan distribution and have been found together with host plants in a variety of environments, e.g., nursery soils with high pH, mining areas with low pH and heavy metal pollution, natural forests and plantations, urban areas, and peat soils [43]. *Wilcoxina* species are mycorrhizal fungi and frequently infest a variety of coniferous and deciduous trees such as *Pinus, Betula* and *Quercus* [44].

Some species have been shown to produce the siderophore compound ferricin [45]. *Wilcoxina* was isolated in 1985 by Chin Yang and Richard Korf [46].

4.3. Observed Positive Changes in Soil Mycobiomes after Treatments

The Venn diagram shows that the control contained fewer OTUs, especially compared to BCR (2.6 times more) and S (about 2 times more). These two variants also dominated in terms of the number of OTUs compared to other organic matter. Compost and sawdust also had the highest number of common organisms, e.g., BCS/S—156 and BCR/S—163. The fewest OTUs were detected in wood waste (WW) or its combinations with other treatments. This is probably since wood waste, unlike compost, was colonised by lignin- and cellulose-degrading fungi, but these are also the most characteristic of the forest environment. Hydrolysis of cellulose requires synergism of several organisms, including bacteria, actinobacteria, filamentous fungi, and plants [47]. Among these organisms, filamentous fungi stand out, with the genera *Penicillium* and *Trichoderma* known as models for cellulase production at laboratory and industrial scales [48].

Under our experimental conditions, the expected species of forest mycorrhizal fungi occurred in WW and S, such as *Russula* and *Inocybe* in BCS and BCR (Table 1, marked in yellow). Other fungi antagonistic to the pathogen *Heterobasidion*, such as *Penicillium*, were more frequent or even twice as frequent in WW or S than in the control group. On the other hand, filamentous fungi of the *Ascomycota* group of the genus *Sagenomella*, which cause diseases in animals (e.g., *Sagenomella chlamydospora*, which causes systemic diseases in dogs), were predominant in the soils after agricultural use. In our study, *Penicillum* accounted for between 4% and 10% of sequence reads, and the highest abundance was found in soils enriched with bark compost under the roots (10%) (Table 1).

Soil enrichment also increased the abundance of *Trichoderma* from 0.2% in the control to 0.9% in bark compost under the roots and wood waste groups. Coarse woody debris consisting of pits, stumps, root mounds, and logs, that are formed as a result of tree fall, also supported the development of ectomycorrhizae [49].

The control after 20 years of the experiment differed from the treatment variants, as shown by the Bray–Curtis dissimilarity index (Figure 8). Thus, the addition of organic matter accelerated the changes in the forest soil environment. Sawdust (S) and logging residues (WW) were particularly favourable concerning the Shannon index (Figure 7). The latter certainly contained fungal spores, including mycorrhizal spores, and were covered with the mycelium of wood decomposing fungi, which are thus antagonists of *H. annosum*. The forest habitat, assessed as fresh coniferous forest, is not very fertile but is suitable for growing pines. By adding organic material, we created an organic horizon (characteristic of forest soils) and restored the natural interactions between the fungi so that none of them dominates.

The above research findings are in line with the studies of Szewczyk et al. [17]. As we did, they investigated the effects of pine sawdust, composted bark, or coarse woody post-harvest waste from conifers on the abundance and diversity of cultivable fungi to increase soil suppression of *Armillaria* and *Heterobasidion*. The soil was found to be colonised by saprotrophic fungi of *Ascomycota* and *Zygomycota*, including species known to be potential antagonists of *Armillaria* or *H. annosum* (e.g., *Clonostachys* + *Trichoderma* spp., *Penicillium commune*, *P. daleae*, *P. janczewskii*) or to stimulate *Armillaria* (e.g., *Pseudogymnoascus roseus, Trichocladium opacum*). Three years after treatment, fungal abundance and diversity, the

abundance of *P. commune* and, locally, *P. janczewskii* increased, while *Clonostachys* + *Trichoderma* spp. and, locally, *P. daleae* and *T. opacum* decreased [50]. The addition of organic matter to soils established 10 years ago after agricultural use had a positive effect on the growth of bare-root pine seedlings. The larger annual increases in shoot, needle length, and biomass (compared to control plants) were clearly visible. The addition of wood residues after clear-cutting seems to have the strongest positive effect on pine plantations established on former agricultural land.

At all sites, the important role of representatives of the genus *Penicillium* was established. According to Park et al. [51], these fungi are widespread in both aquatic and terrestrial environments, where they play an important ecological role. These species are very common in the rhizosphere of plants [52]. Some *Penicillium* produce soluble phosphorus, siderophores, and phytohormones such as indoleacetic acid and gibberellic acid, which are important for plant health [53]. These fungi are extensively studied for the production of secondary metabolites capable of producing substances important to the pharmaceutical industry, such as antimicrobial agents or metabolites that could be used as immunosuppressants, cholesterol-lowering agents, and anti-HIV and anti-tumor agents [54].

In our study, the genus *Inocybe* sensu lato (s.l.) was found to be abundant in the BCS treatment. This is an extremely diverse genus of ectomycorrhizal fungi, comprising about 1000 species [55]. In addition, species of the genus *Oidiodendron* were represented in this variation. These species are isolated in large numbers from soils and decaying plant material worldwide [56]. Representatives of this genus belong to the mycorrhizal fungi [57]. Our investigations revealed that representatives of *Hyaloscypha* were abundantly represented in the BCS group. In agreement with recent studies, it can be concluded that they are various saprobes with small apothecia that form on decaying plant material, usually wood, and form ectomycorrhizae, ericoid mycorrhizae, and mycothiol, and also grow endophytically in plant roots [58].

Kubiak et al. [59] concluded that sawdust and wood residues can be considered stimulators of microbiological changes in the soil and can be used in afforestation plots.

5. Conclusions

- The introduction of organic material is able to positively change the soil mycobiome, which is characteristic of agricultural land and approaches the typical forest ecosystem. The proportion of fungi belonging to the Ascomycota decreased in favour of species from the Basidiomycota and Mucoromycota. Beneficial mycorrhizal fungi such as *Russula* and *Amanita* appeared after enriching the soil with wood residues or sawdust.
- The application of wood residues, sawdust, or compost to the soil surface increased soil biodiversity on agricultural land 20 years later, e.g., by promoting the beneficial genus *Inocybe*, which increased after supplementation with compost added to the roots or spread on the soil surface. A comparative analysis of fungal diversity with the Shannon diversity index showed that the most diverse fungal communities were found in the sawdust plant. Undesirable fungi causing diseases in animals from the genus *Sagenomella*, such as *Sagenomella chlamydospora*, were prevalent in the soils after agricultural use (control).
- Ploughed strips, where seedlings of forest-forming species were planted after enrichment of the soil with organic matter, seem to be the right management for the establishment of the first generation of pines on post-agricultural land.
- When an antagonistic myobiome against forest pathogens has developed in agricultural soils, the threat from root and butt rot diseases is lower because the high species diversity does not allow any of the organisms to develop en masse and dominate. Antagonistic fungi against the pathogen *Heterobasidion*, such as *Penicillium*, were more frequent or even twice as frequent as in the control group after the addition of wood residues or sawdust.

- The enrichment of soils with organic matter after agricultural use can be regarded as a preventive method for sustainable forest management and with regard to integrated pest management (IPM).
- Based on the results of the analysis of data on the occurrence and diversity of species and functional groups of fungi in soils converted from agricultural to forestry use, it was confirmed that the methods of investigation used, as well as DNA analyses of the mycobiome, are very well suited to assessing the degree of transformation of the soil mycobiome and the need for the support of soil processes with regard to the conversion of the mycobiome to forestry use.

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Appendix A. Fungal Fruiting Bodies Found in the Treatment Plots

Figure A1. (**a**) *Sparasis crispa*, (**b**) *Lycopedron* sp. (young), (**c**) *Coprinus comatus*, (**d**) *Rusula* sp., (**e**) *Teleophora terrestris*, (**f**) *Lepiota* sp., (**g**) *Xerocomus chrysenteron*, (**h**) *Siulus* sp., (**i**) *Russula* sp., (**j**) *Laccaria amthystina*, (**k**) *Lycoperdon perlatum* (old), (**l**) *Amanita muscaria*.

Appendix B. Meteorological Conditions during the Experiment

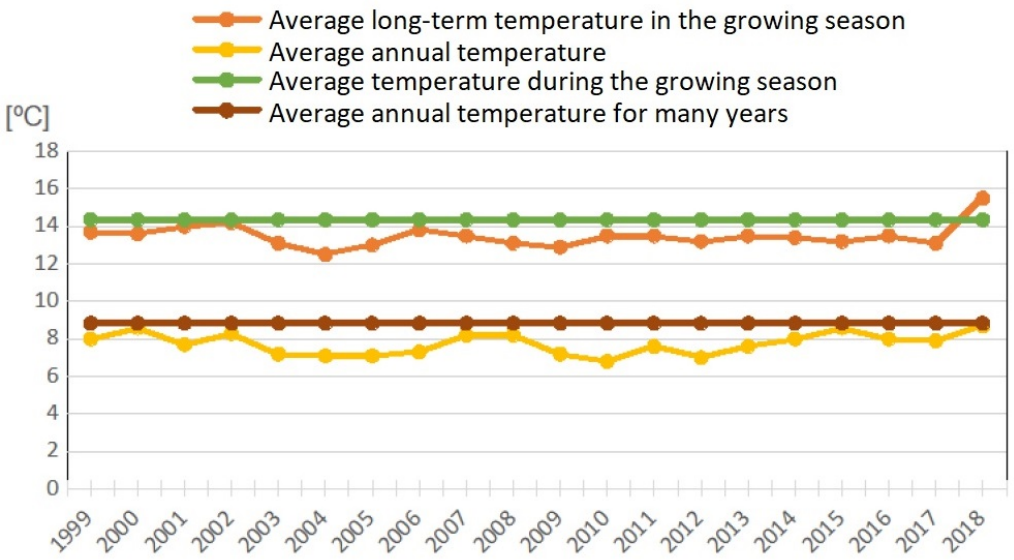

Figure A2. Measured temperatures during the experiment.

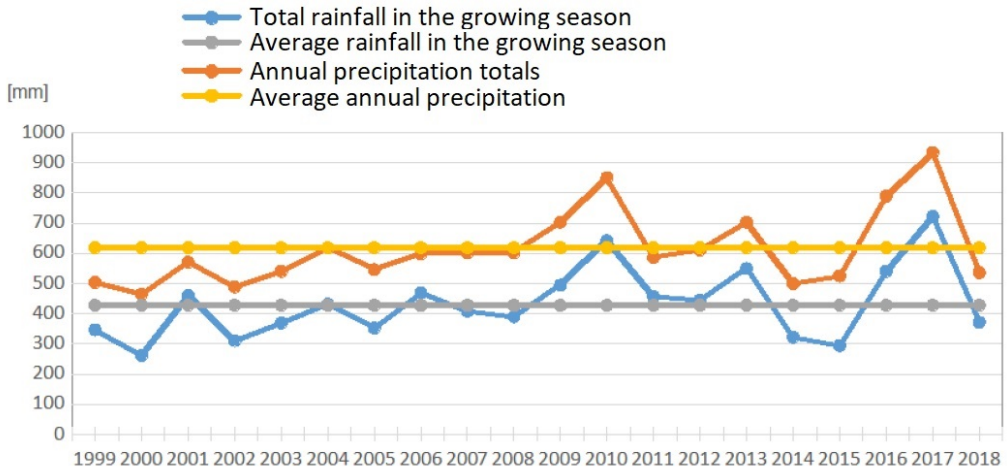

Figure A3. Measured precipitation during the experiment.

References

1. Gann, G.D.; McDonald, T.; Walder, B.; Aronson, J.; Nelson, C.R.; Jonson, J.; Hallett, J.G.; Eisenberg, C.; Guariguata, M.R.; Liu, J.; et al. International principles and standards for the practice of ecological restoration. *Restor. Ecol.* **2019**, *27*, S1–S46. [CrossRef]
2. Bożętka, B. Przywracanie ekosystemów leśnych–cele i założenia działań globalnych w kontekście Dekady Przywracania Ekosystemów (2021–2030) ONZ= Forest ecosystem restoration–assumptions and aims of global activities in the context of the United Nations Decade of Ecosystem Restoration (2021–2030). *Przegląd Geogr.* **2022**, *94*, 471–501.
3. Piri, T.; Vainio, E.J.; Nuorteva, H.; Hantula, J. High seedling mortality of Scots pine caused by Heterobasidion annosum ss. *Forests* **2021**, *12*, 1289. [CrossRef]
4. Sierota, Z. Heterobasidion root rot in forests on former agricultural lands in Poland: Scale of threat and prevention. *Sci. Res. Essays* **2013**, *8*, 2298–2305.
5. Oszako, T.; Kukina, O.; Dyshko, V.; Moser,W.K.; ´Slusarski, S.; Okorski, A.; Borowik, P. Afforestation of Land Abandoned by Farmers Poses Threat to Forest Sustainability due to Heterobasidion spp. *Forests* **2023**, *14*, 954. [CrossRef]
6. Sierota, Z. Rola grzyba Phlebiopsis gigantea [Fr.: Fr.] Julich w ograniczaniu huby korzeni w drzewostanach sosny zwyczajnej [*Pinus sylvestris* L.] na gruntach porolnych. *Pr. Inst. Badaw. Leśnictwa Ser. A* **1995**, *810*, 1–180.
7. Rishbeth, J. Stump protection against Fomes annosus: III. Inoculation with Peniophora gigantea. *Ann. Appl. Biol.* **1963**, *52*, 63–77. [CrossRef]
8. Gunulf, A.; Wang, L.; Englund, J.E.; Rönnberg, J. Secondary spread of Heterobasidion parviporum from small Norway spruce stumps to adjacent trees. *For. Ecol. Manag.* **2013**, *287*, 1–8. [CrossRef]

9. Malewski, T.; Borowik, P.; Olejarski, I.; Berezovska, D.; Dyshko, V.; Behnke-Borowczyk, J.; Pusz, W.; Matic, S.; Oszako, T. Mycobiome of Post-Agricultural Soils 20 Years after Application of Organic Substrates and Planting of Pine Seedlings. *Forests* **2022**, *14*, 36. [CrossRef]
10. Setälä, H.; McLean, M.A. Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. *Oecologia* **2004**, *139*, 98–107. [CrossRef]
11. Mhatre, P.; Panchal, R.; Singh, A.; Bibyan, S. A systematic literature review on the circular economy initiatives in the European Union. *Sustain. Prod. Consum.* **2021**, *26*, 187–202. [CrossRef]
12. Good, P.I. *Resampling Methods. A Practical Guide to Data Analysis*; Birkhäuser: Boston, MA, USA, 2006. [CrossRef]
13. Bonamente, M. *Statistics and Analysis of Scientific Data*; Springer: New York, NY, USA, 2017. [CrossRef]
14. Scikit-Bio Software Package Project. Available online: http://scikit-bio.org/docs/0.5.8/ (accessed on 15 December 2022).
15. Nguyen, D.; Boberg, J.; Ihrmark, K.; Stenström, 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]
16. Hurlbert, S.H. The Nonconcept of Species Diversity: A Critique and Alternative Parameters. *Ecology* **1971**, *52*, 577–586. [CrossRef]
17. Małecka, M.; Kwaśna, 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]
18. Sierota, Z. An analysis of the root rot spread in a Scots pine stand growing in post-agricultural land. *Folia For. Pol. Ser. A Leśnictwo* **1997**, *39*, 27–37.
19. Szewczyk, W. Occurrence of Heterobasidion annosum (Fr.) Bref. in the roots of blown down trees in Scots pine stands growing on post-agricultural soil of the experimental forest district Zielonka. *Zielonka Acta Sci. Pol. Silv. Colendar Rat. Ind. Lignar* **2007**, *6*, 89–95.
20. Mareš, R. The extent of root rot damage in Norway spruce stands established on fertile sites of former agricultural land. *J. For. Sci.* **2010**, *56*, 1–6. [CrossRef]
21. Frąc, M.; Hannula, S.E.; Bełka, M.; Jędryczka, M. Fungal biodiversity and their role in soil health. *Front. Microbiol.* **2018**, *9*, 707. [CrossRef]
22. Tedersoo, L.; Bahram, M.; Põlme, S.; Kõljalg, U.; Yorou, N.S.; Wijesundera, R.; Ruiz, L.V.; Vasco-Palacios, A.M.; Thu, P.Q.; Suija, A.; et al. Global diversity and geography of soil fungi. *Science* **2014**, *346*, 1256688. [CrossRef]
23. Hilszczańska, D.; Ciesielska, A.; Sierota, Z. Enzymatic activity of thelephora terrestris and Hebeloma crustuliniforme in cultures and mycorrhizal association with Scots pine seedlings. *Pol. J. Environ. Stud.* **2008**, *17*, 881–886.
24. Davydenko, K.; Nowakowska, J.A.; Kaluski, T.; Gawlak, M.; Sadowska, K.; García, J.M.; Diez, J.J.; Okorski, A.; Oszako, T. A Comparative Study of the Pathogenicity of Fusarium circinatum and other Fusarium Species in Polish Provenances of *P. sylvestris* L. *Forests* **2018**, *9*, 560. [CrossRef]
25. Rouphael, Y.; Franken, P.; Schneider, C.; Schwarz, D.; Giovannetti, M.; Agnolucci, M.; De Pascale, S.; Bonini, P.; Colla, G. Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. *Sci. Hortic.* **2015**, *196*, 91–108. [CrossRef]
26. Morugán-Coronado, A.; Pérez-Rodríguez, P.; Insolia, E.; Soto-Gómez, D.; Fernández-Calviño, D.; Zornoza, R. The impact of crop diversification, tillage and fertilization type on soil total microbial, fungal and bacterial abundance: A worldwide meta-analysis of agricultural sites. *Agric. Ecosyst. Environ.* **2022**, *329*, 107867. [CrossRef]
27. Zubrod, J.P.; Bundschuh, M.; Arts, G.; Brühl, C.A.; Imfeld, G.; Knäbel, A.; Payraudeau, S.; Rasmussen, J.J.; Rohr, J.; Scharmüller, A.; et al. Fungicides: an overlooked pesticide class? *Environ. Sci. Technol.* **2019**, *53*, 3347–3365. [CrossRef]
28. Wightwick, A.M.; Reichman, S.M.; Menzies, N.W.; Allinson, G. Industry wide risk assessment: a case study of Cu in Australian vineyard soils. *Water Air Soil Pollut.* **2013**, *224*, 1–8. [CrossRef]
29. Roman, D.L.; Voiculescu, D.I.; Filip, M.; Ostafe, V.; Isvoran, A. Effects of triazole fungicides on soil microbiota and on the activities of enzymes found in soil: A review. *Agriculture* **2021**, *11*, 893. [CrossRef]
30. Baćmaga, M.; Wyszkowska, J.; Kucharski, J. The effect of the Falcon 460 EC fungicide on soil microbial communities, enzyme activities and plant growth. *Ecotoxicology* **2016**, *25*, 1575–1587. [CrossRef]
31. Zhou, J.; Jiang, X.; Zhou, B.; Zhao, B.; Ma, M.; Guan, D.; Li, J.; Chen, S.; Cao, F.; Shen, D.; et al. Thirty four years of nitrogen fertilization decreases fungal diversity and alters fungal community composition in black soil in northeast China. *Soil Biol. Biochem.* **2016**, *95*, 135–143. [CrossRef]
32. Zhang, N.; Castlebury, L.A.; Miller, A.N.; Huhndorf, S.M.; Schoch, C.L.; Seifert, K.A.; Rossman, A.Y.; Rogers, J.D.; Kohlmeyer, J.; Volkmann-Kohlmeyer, B.; et al. An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycologia* **2006**, *98*, 1076–1087. [CrossRef]
33. Maddison, D.R.; Schulz, K.S.; Maddison, W.P. The tree of life web project. *Zootaxa* **2007**, *1668*, 19–40. [CrossRef]
34. Geiser, D.M.; Gueidan, C.; Miadlikowska, J.; Lutzoni, F.; Kauff, F.; Hofstetter, V.; Fraker, E.; Schoch, C.L.; Tibell, L.; Untereiner, W.A.; et al. Eurotiomycetes: Eurotiomycetidae and chaetothyriomycetidae. *Mycologia* **2006**, *98*, 1053–1064. [CrossRef]
35. Hibbett, D.S.; Binder, M.; Bischoff, J.F.; Blackwell, M.; Cannon, P.F.; Eriksson, O.E.; Huhndorf, S.; James, T.; Kirk, P.M.; Lücking, R.; et al. A higher-level phylogenetic classification of the Fungi. *Mycol. Res.* **2007**, *111*, 509–547. [CrossRef]
36. Doweld, A. *Prosyllabus Tracheophytorum: Tentamen Systematis Plantarum Vascularium Tracheophy*; LXXX + 110 pp.; Geos: Moscow, Russia, 2001.
37. Voigt, K.; Wöstemeyer, J. Phylogeny and origin of 82 zygomycetes from all 54 genera of the Mucorales and Mortierellales based on combined analysis of actin and translation elongation factor EF-1α genes. *Gene* **2001**, *270*, 113–120. [CrossRef]

38. Schmidt, S.K.; Wilson, K.; Meyer, A.; Gebauer, M.; King, A.J. Phylogeny and ecophysiology of opportunistic "snow molds" from a subalpine forest ecosystem. *Microb. Ecol.* **2008**, *56*, 681–687. [CrossRef]
39. Tedersoo, L.; Sánchez-Ramírez, S.; Koljalg, U.; Bahram, M.; Döring, M.; Schigel, D.; May, T.; Ryberg, M.; Abarenkov, K. High-level classification of the Fungi and a tool for evolutionary ecological analyses. *Fungal Divers.* **2018**, *90*, 135–159. [CrossRef]
40. Spatafora, J.W.; Chang, Y.; Benny, G.L.; Lazarus, K.; Smith, M.E.; Berbee, M.L.; Bonito, G.; Corradi, N.; Grigoriev, I.; Gryganskyi, A.; et al. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* **2016**, *108*, 1028–1046. [CrossRef]
41. Cannon, P.F.; Kirk, P.M. *Fungal Families of the World*; Cabi International: Wallingford, UK, 2007.
42. Index Fungorum. Available online: https://www.indexfungorum.org/ (accessed on 24 December 2020).
43. Mikola, P. Ectendomycorrhiza of conifers. *Silva Fenn.* **1988**, *22*, 19–27. [CrossRef]
44. Nakas, J.P.; Hagedorn, C. *Biotechnology of Plant-Microbe Interactions*; McGraw-Hill: New York, NY, USA, 1990; pp. 287–317.
45. Prabhu, V.; Biolchini, P.F.; Boyer, G.L. Detection and identification of ferricrocin produced by ectendomycorrhizal fungi in the genus Wilcoxina. *Biometals* **1996**, *9*, 229–234. [CrossRef]
46. Yang, C.; Korf, R. Monograph of the genus Tricharina and of a new, segregate genus, Wilcoxina (Pezizales). *Mycotaxon* **1985**, *24*, 467–531.
47. Kuhad, R.C.; Gupta, R.; Singh, A. Microbial cellulases and their industrial applications. *Enzym. Res.* **2011**, *2011*, 280696. [CrossRef]
48. de França Passos, D.; Pereira Jr, N.; de Castro, A.M. A comparative review of recent advances in cellulases production by Aspergillus, Penicillium and Trichoderma strains and their use for lignocellulose deconstruction. *Curr. Opin. Green Sustain. Chem.* **2018**, *14*, 60–66. [CrossRef]
49. Olchowik, J.; Hilszczańska, D.; Bzdyk, R.M.; Studnicki, M.; Malewski, T.; Borowski, Z. Effect of deadwood on ectomycorrhizal colonisation of old-growth oak forests. *Forests* **2019**, *10*, 480. [CrossRef]
50. Oszako, T.; Olejarski, I. Inicjowanie procesow przeksztalcenia gleb porolnych w gleby lesne poprzez wykorzystanie pozostalosci zrebowych, kompostow i trocin. *Pr. Inst. Badaw. Leśnictwa Ser. A* **2003**, *1*, 76–79.
51. Park, M.S.; Lee, J.W.; Kim, S.H.; Park, J.H.; You, Y.H.; Lim, Y.W. Penicillium from rhizosphere soil in terrestrial and coastal environments in South Korea. *Mycobiology* **2020**, *48*, 431–442. [CrossRef]
52. Elias, F.; Woyessa, D.; Muleta, D. Phosphate solubilization potential of rhizosphere fungi isolated from plants in Jimma Zone, Southwest Ethiopia. *Int. J. Microbiol.* **2016**, *2016*, 5472601. [CrossRef]
53. Altaf, M.M.; Imran, M.; Abulreesh, H.H.; Khan, M.S.; Ahmad, I. Diversity and applications of *Penicillium* spp. in plant-growth promotion. In *New and Future Developments in Microbial Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 261–276.
54. Koolen, H.H.F.; Soares, E.R.; da Silva, F.M.A.; de Almeida, R.A.; de Souza, A.D.L.; de Medeiros, L.S.; Rodrigues Filho, E.; de Souza, A.Q.L. An antimicrobial alkaloid and other metabolites produced by *Penicillium* sp. An endophytic fungus isolated from *Mauritia flexuosa* L. f. *Química Nova* **2012**, *35*, 771–774. [CrossRef]
55. He, M.Q.; Zhao, R.L.; Hyde, K.D.; Begerow, D.; Kemler, M.; Yurkov, A.; McKenzie, E.H.; Raspe, O.; Kakishima, M.; Sanchez-Ramirez, S.; et al. Notes, outline and divergence times of Basidiomycota. *Fungal Divers.* **2019**, *99*, 105–367. [CrossRef]
56. Rice, A.V.; Currah, R.S. Oidiodendron: A survey of the named species and related anamorphs of Myxotrichum. *Stud. Mycol.* **2005**, *53*, 83–120. [CrossRef]
57. Leopold, D.R. Ericoid fungal diversity: Challenges and opportunities for mycorrhizal research. *Fungal Ecol.* **2016**, *24*, 114–123. [CrossRef]
58. Vohník, M.; Figura, T.; Réblová, M. Hyaloscypha gabretae and Hyaloscypha gryndleri spp. nov.(Hyaloscyphaceae, Helotiales), two new mycobionts colonizing conifer, ericaceous and orchid roots. *Mycorrhiza* **2022**, *32*, 105–122. [CrossRef]
59. Kubiak, K.; Tkaczyk, M.; Małecka, M.; Sierota, Z. Pine sawdust as stimulator of the microbial community in post-arable afforested soil. *Arch. Agron. Soil Sci.* **2017**, *63*, 427–441. [CrossRef]