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Fungi Present in the Organic and Mineral Layers of Six Broad-Leaved Tree Plantations as Assessed by the Plate Dilution Method

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Abstract: This study examined the distribution of culturable fungi and predominant genera in the organic layer and in the upper layers of the mineral soil of six broad-leaved tree plantations in autumn, after the full fall of leaves. In total, 1335 fungal isolates were recovered from an organic layer and two mineral layers (0–4 cm and 5–8 cm) of soil. The structure of fungal genera differed in the tree plantations and in the three studied soil layers. The organic layer was the layer most populated by fungi compared to the mineral layers. In the organic layer, *Penicillium* and phyllosphere fungi such as *Cladosporium* and *Phoma* dominated. Deeper in the soil, the dominance of certain genera decreased with the increase in *Trichoderma*, *Mucor*, *Mortierella*, and entomopathogenic fungi such as *Paecilomyces* and *Beauveria*. *Penicillium* was one of the most abundant fungi in all soil layers studied.

Keywords: fungi; soil; organic layer; mineral layer; tree plantation

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1. Introduction

Forest ecosystem processes are driven by interactions between above- and belowground components, and their stability and sustainability depend on nutrient conversion [1]. Trees strongly influence soil processes through their metabolism, accumulation, and decomposition of nutrients [2]. Tree litter is an important part of the biological nutrient cycling process [3,4] and, together with roots, constitutes the largest proportion of soil organic matter in forest soils and modifies soil chemistry [5]. Microorganisms play a crucial role in the biodegradation of plant residues in terrestrial ecosystems [6]. Previous studies have shown that plants can form and maintain specific communities of saprotrophic microorganisms in soil [7–9]. Along with bacteria, fungi are important as decomposers in the soil food web [10,11]. Fungi are a very diverse group of organisms and a very important part of forest ecosystems [12,13], providing a wide range of ecosystem services, such as organic matter decomposition, element cycling, plant nutrition, and plant protection [8,14]. Fungi convert hard-to-digest organic material such as cellulose and lignin into forms that other organisms can use [10,15,16]. Microscopic saprophytic fungi are dominant in forest soil, and fungal community compositions differ within tree species and soil quality [17]. The ecological importance of fungi as symbionts and decomposers is well recognized, and new research continues to increase knowledge of their role in ecosystem functioning. However, compared to their ecological and evolutionary diversity, fungi have only recently started to receive attention from the wider ecological research community [18,19]. The interactions of mycorrhizal (symbiotic) and pathogenic fungi with native and alien trees have been studied quite extensively, but still, little attention has been paid to saprotrophic fungi, which are the most abundant in the forest floor and in the soil [20,21].

An increasing number of forests, especially subtropical forests, are undergoing a long-term transformation from natural forests to plantations [22]. The soil microbial communities and functions of such plantations remain poorly understood [23]. In cities and other urbanized areas, plantations are also increasingly being established with naturally occurring and introduced tree species and homogeneous stands that are not characteristic of natural native ecosystems [24,25]. It is therefore essential to understand the structure and function of soil microbial communities in order to predict the response of such ecosystems to changes in environmental conditions. The influence of trees is most pronounced in mature stands. Changes in soil properties are evident in stands older than 40–50 years [26]. However, soil changes can be reversed or 'masked' by differences in soil taxonomy and different silvicultural practices [27].

The aim of this study was to investigate the influence of popular plantations of deciduous trees in urbanized areas on the abundance of culturable fungi and dominant fungal genera in the organic layer and in the upper layers of mineral soil.

2. Materials and Methods

2.1. Study Site

The study was carried out in central Lithuania (54°53′ N, 23°49′ E) in the hemiboreal zone of European transitional deciduous mixed forests [28]. The average annual temperature ranges from +6.5 to +7.1 °C, with an average temperature of −3.6 °C in January (the coldest month) and +17.7 °C in July (the warmest month). The average annual rainfall is 600–700 mm. Permanent snow cover lasts between 75 and 90 days [29].

The study was carried out after a mass leaf fall in October 2020, in the homogeneous plantations of deciduous native (*Quercus robur* L., *Betula pendula* Roth, *Tilia cordata* Mill.) and alien (*Quercus rubra* L., *Aesculus hippocastanum* L., *Sorbus intermedia* (Ehrh.) Pers.) monocultures in the Arboretum Park of the Agriculture Academy of Vytautas Magnus University in Kaunas district. The age of the studied plantations was 60 years and the area of the stands varied from 0.14 to 0.31 ha (Table 1). They were dominated by anthropogenic Endocalcari–Epihypogleic Cambisols.

Table 1. Species and area of investigated tree stands.

Tree Species	*Area, ha*	**Family**	**Natural Habitat**
	Native		
Quercus robur L. English oak	0.31	*Fagaceae* Dumort	Most of Europe, west of the Caucasus
Betula pendula Roth Silver birch	0.14	*Betulaceae* Grey	Europe and parts of Asia
Tilia cordata Mill. Small-leaved linden	0.29	*Tiliaceae* Juss	Europe to the Caucasus and western Asia
	Alien		
Quercus rubra L. Northern red oak	0.18	*Fagaceae* Dumort	East of North America
Aesculus hippocastanum L. Horse chestnut	0.23	*Hippocastanaceae* A. Rich	Balkan Peninsula
Sorbus intermedia (Ehrh.) Pers. Swedish whitebeam	0.29	*Rosaceae* Juss	North-western Europe

2.2. Sample Collection

The sites were visited on 30 October 2020. Composite samples of the organic layer (forest floor) and mineral topsoil with depths of 0–4 cm and 5–8 cm were taken randomly from 10 sites of each investigated tree plantation. One combined and thoroughly mixed sample of each site was produced per plot and per layer. Soil samples were sieved (<4 mm) and cleaned of visible plants, while their roots remained. For microbiological analyses, the samples were stored at 4 °C. All the microbial determinations were performed within one week of sampling.

2.3. Microbiological Analyses

The abundance of culturable fungi was determined in the organic layer and in the mineral soil layers (0–4 cm and 5–8 cm depth). The soil serial dilution method was used. Then, 10 g of the soil samples was suspended in 90 mL of sterile water in 500 mL Erlenmeyer flasks by shaking at room temperature at 150 rpm for 30 min. In total, 0.1 mL of tenfold serial dilutions of the suspensions was plated on the potato dextrose agar (PDA) (Biolife, Italy) for fungal count. Inoculated Petri plates were incubated at 21 ± 2 °C for 7–14 days. The detection limit was ten CFU g^{-1} of wet soil. Every soil sample was analyzed in three replicates.

Based on colony and cell morphology, predominant fungi were isolated from the highest dilutions of soil suspensions and purified. Fungal genera were identified using macroscopic and microscopic appearance [30].

2.4. Statistics

Data are reported as means with the standard deviation (Statistical significance was assessed with the coefficient p (if $p < 0.05$). Duncan's new multiple range test (MRT) was used. Statistical analyses were performed using Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. Distribution of Fungi

The fungal abundance in the organic layer (Figure 1A) of English oak was higher than in the other tree plantations and was 2.6 times higher than the overall average fungal number in the organic layer of all plantations (1.6×10^6 CFU g^{-1}). The fungal abundance of linden and chestnut in this layer was also among the highest (1.6 and 1.2 times higher than the average, respectively). Red oak and whitebeam had the lowest abundance of fungi ($p < 0.05$) in the organic layer of all the plantations studied.

The situation in the mineral soil layers was different. In the 0–4 cm layer of mineral soil (Figure 1B), the mean abundance of fungi was 16 times lower than in the organic layer and 26 times lower than in the 5–8 cm depth layer (Figure 1C). The highest fungal abundance was found in the linden, birch, and red oak 0–4 cm mineral soil layer and in the birch 5–8 cm soil layer. Both investigated English oak and chestnut mineral soil layers had the lowest fungal number.

3.2. Fungal Genera in Soil Layers

3.2.1. Organic Layer

In total, 634 fungal isolates were recovered from the organic layer of soil. In both oak stands, the *Penicillium* genus dominated, accounting for more than 60% of the total population of fungi found (Figure 2). *Penicillium* was also abundant in the whitebeam plantation, accounting for more than 1/3 of all fungi. *Mucor* spp. dominated the chestnut plantation, accounting for more than half of the fungi found in the organic layer. *Penicillium* and *Mucor* were absent from the organic layer of linden trees, where the *Cladosporium* genus was predominant. *Phoma* formed the majority of the fungal population in the organic layer of the birch plantation, while *Penicillium* made up only a small part. *Phoma* was common in stands of English oak and whitebeam. *Alternaria*, *Fusarium*, *Onychophora*, and *Knufia* were found in small amounts (Figure 2).

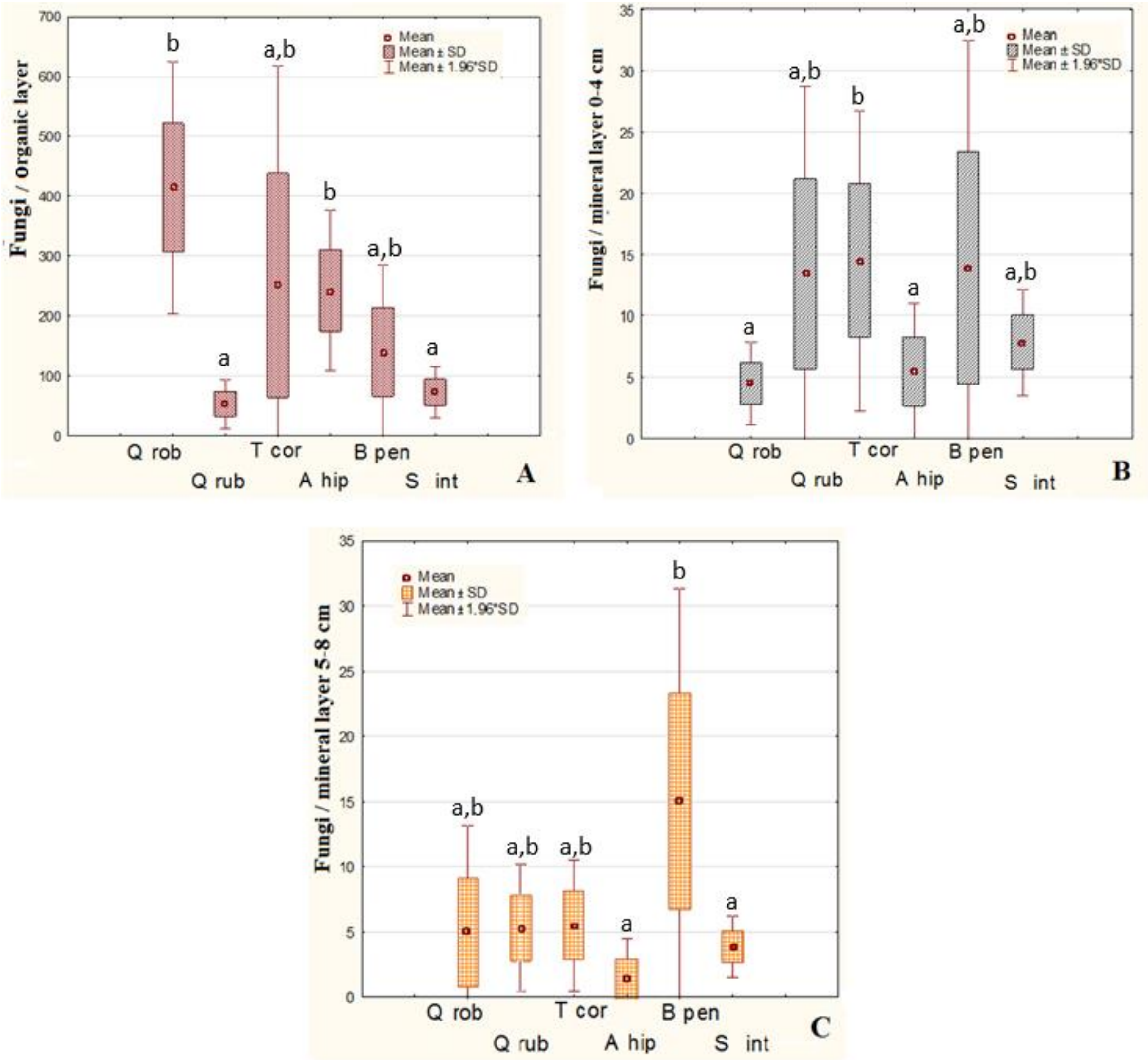

Figure 1. Total number of fungal colony-forming units ($\times$ 10^4 CFU g^{-1}) in plantation soil layers under different tree species. (**A**)—in the organic layer, (**B**)—in the mineral soil layer at 0–4 cm depth, (**C**)—in the mineral soil layer at 5–8 cm depth; Q rob—*Quercus robur*, Q rub—*Q. rubra*, T cor—*Tilia cordata*, A hip—*Aesculus hippocastanum*, B pen—*Betula pendula*, S int—*Sorbus intermedia*. Significantly different mean values in the same graph are indicated by different letters (a, b) (Duncan; $p < 0.05$).

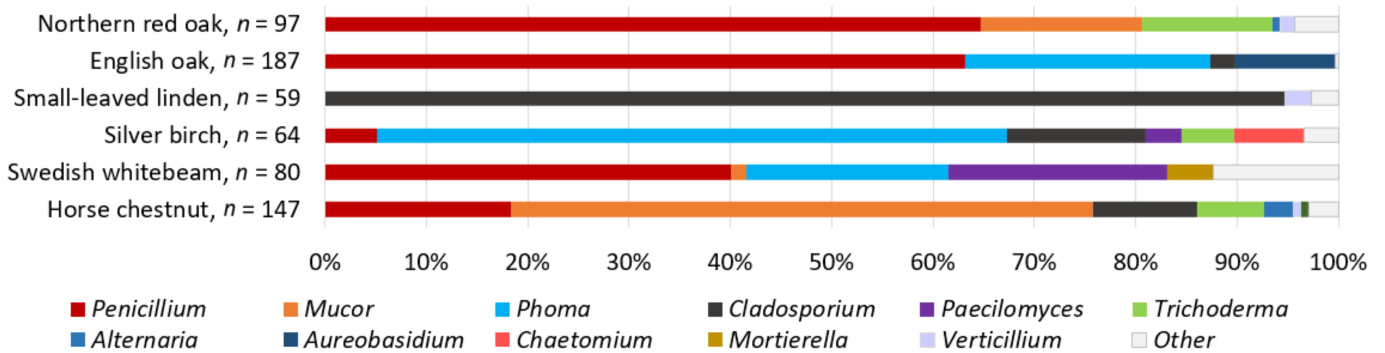

Figure 2. The structure of fungal genera in the soil organic layer in plantations of different broad-leaved trees. The number of fungal isolates (n) is given after the tree names.

3.2.2. Mineral Layer 0–4 cm

In total, 354 fungal isolates were recovered from the 0–4 cm mineral soil layer. The dominance of individual genera (Figure 3) was not as pronounced as in the organic layer. *Penicillium* was one of the dominant genera in all plantations. This genus was most dominant in the red oak mineral layer, as well as in the organic layer. *Mucor* spp. were abundant in all plantations, except red oak. In this layer, *Trichoderma* accounted for a large part of the fungal population in all the plantations. *Mortierella* was one of the predominant genera in linden, chestnut, English oak, and birch soils. This layer contained fungi of the *Beauveria* genus, which was not found only in the soil of linden and chestnut plantations.

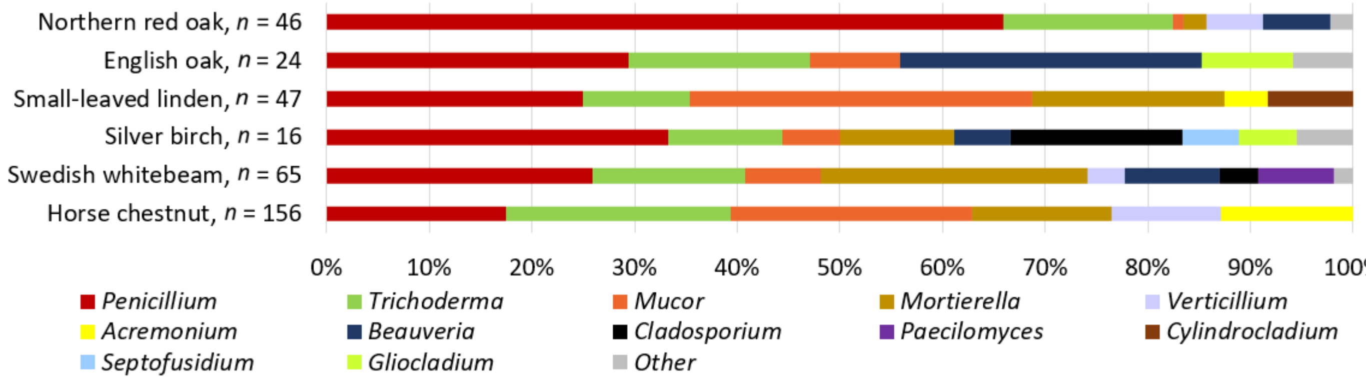

Figure 3. The structure of fungal genera in the 0–4 cm layer of mineral soil in plantations of different broad-leaved trees. The number of fungal isolates (*n*) is given after the tree names.

3.2.3. Mineral Layer 5–8 cm

In total, 347 fungal isolates were recovered from the 5–8 cm mineral soil layer. Compared to the upper layer, the occurrence of *Penicillium* decreased (Figure 4), while *Trichoderma* increased, especially in chestnut and whitebeam stands. *Beauveria* spp. were found in all plantations except linden. *Beauveria* was particularly abundant in the soil of both oaks and, to a lesser extent, in birch. As in the 0–4 cm layer, fungi of the genera *Mucor* were abundant in the soil of linden, but *Mortierella* spp. were abundant in the soil of linden and birch. Fungi of the genus *Paecilomyces* were found in significant numbers in some plantations, which were not previously found in the organic layer and in small numbers in the upper 0–4 cm of the soil layer. Fungi of the genus *Chaetomium* were also common.

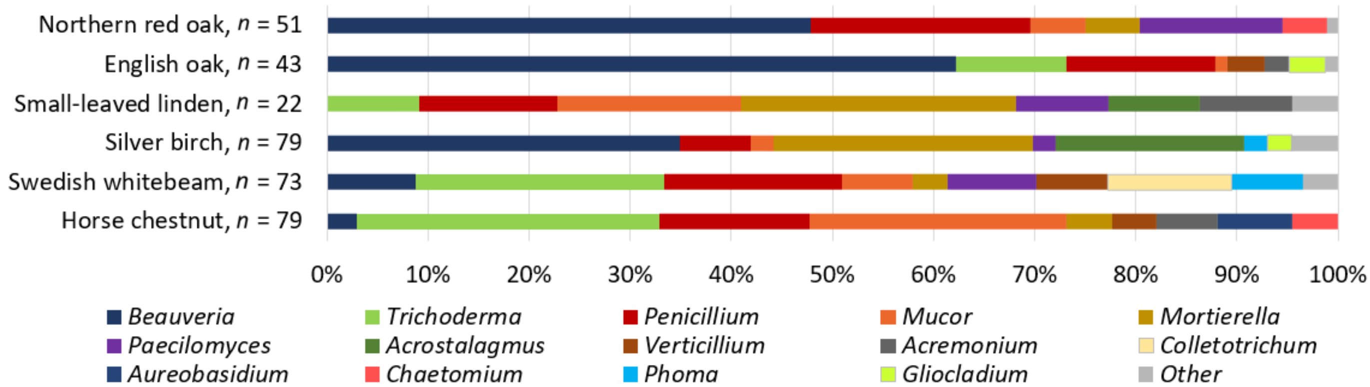

Figure 4. The structure of fungal genera in the 5–8 cm mineral soil layer in plantations of different broad-leaved trees. The number of fungal isolates (*n*) is given after the tree names.

4. Discussion

As the depth increased, the amount of fungi in the soil decreased in the plantations of all six trees. This was expected because it is known that as the proportion of organic matter decreases with depth, the abundance of saprotrophic fungi decreases, while the abundance of mycorrhizal fungi increases [31,32]. In our study, it was not possible to investigate the prevalence of mycorrhizal fungi due to methodological limitations.

Penicillium spp., carbohydrate-degrading fungi capable of degrading primary lignin [33], accounted for almost a third of all the fungi found in the study and was abundant in all the layers, especially in the organic layer (Figure 2). The most abundant fungi of the genus *Penicillium* in all layers studied were in the red oak plantation. *Mucor*, which prefers carbohydrates but does not degrade lignin [33], was the second most abundant fungal genus in both the organic layer and the mineral soil and was dominant in chestnut and linden plantations. *Trichoderma*, which is best adapted to degrade cellulose and xylan [34], unlike the fast-degrading and carbohydrate-loving *Penicillium* or *Mucor* [33,35], was abundant in all layers, especially in the 0–4 cm mineral soil layer. *Trichoderma* fungi were especially abundant in plantations of chestnut and whitebeam at a depth of 5–8 cm. Statistically significant differences ($p < 0.05$) were found between tree plantations and the frequency of *Trichoderma* fungi.

Phyllosphere fungi, such as *Phoma* and *Cladosporium*, were also among the most common in the studied plantations, especially in the organic layer of local tree stands of linden and birch. These fungi were also found in the deeper layers, but in much smaller numbers (Figures 3 and 4). According to the literature [36], tree phyllosphere fungi are specifically adapted to colonize and utilize dead host tissues, and some phyllosphere fungi with marked abilities to decompose litter components play important roles in the decomposition of structural components, nutrient dynamics, and soil organic matter accumulation.

Mortierella possess the ability to secrete lytic enzymes that hydrolyze compounds such as cellulose, while hemicellulose [37] was found abundantly only in mineral soil, especially in the 0–4 cm layer. *Mortierella* was particularly abundant in the mineral soil layer of chestnut, linden, and birch soils. According to Jayasinghe et al. [38], fast-growing genera (e.g., *Mucor*, *Penicillium*, and *Trichoderma*) are more tolerant of actinomycete antagonism than slow-growing and moderately slow-growing genera such as *Cladosporium* and *Mortierella*.

One of the most important genera of entomopathogenic fungi distributed worldwide is *Beauveria* [39,40] which was abundantly found only in mineral soil layers, especially in the 5–8 cm layer (Figure 4). A statistically significant relationship ($p < 0.05$) was found between the occurrence of these fungi and the soil layers. *Paecilomyces*, which is also entomopathogenic [41] and known for its nematophagous capacity [42], was rarely found in the organic layer of the litter, but, like *Beauveria*, was common in the mineral soil, especially in the 0–4 cm layer. *Paecilomyces* was mainly found in whitebeam mineral topsoil. There was a statistically significant relationship ($p < 0.05$) between the plantation type and the frequency of *Paecilomyces*.

Fungi of the genera *Verticillium*, *Acremonium*, and *Acrostalagmus* were found in all the layers studied, but mainly in the 5–8 cm layer of mineral soil. *Gliocladium* spp. were also found only in mineral soil, at a depth of 0–4 cm.

The biochemical characteristics of stands in deciduous tree plantations will be investigated in the future.

5. Conclusions

The structure of fungal genera differed in the tree plantations and in the three soil layers studied. The organic layer was the layer most populated by fungi compared to the mineral layers. In the organic layer, *Penicillium* and phyllosphere fungi such as *Cladosporium* and *Phoma* dominated in different plantations. Deeper in the soil, the dominance of certain genera decreased with the increase in *Trichoderma*, *Mucor*, *Mortierella*, and entomopathogenic fungi such as *Paecilomyces* and *Beauveria*. *Paecilomyces* and especially *Beauveria* were abundant in the mineral soil layers. *Penicillium* was one of the most abundant fungi in all the studied layers.

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