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The Community of Fine Root Fungi of Silver Fir (*Abies alba* Mill.) Saplings

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ABSTRACT

This study aimed to assess the biodiversity of fungi colonizing the fine roots (diameter up to 2 mm) of 3-year-old silver fir saplings from areas of Międzylesie Forest District in Poland. It was hypothesized that quantitatively and qualitatively, mycorrhizal fungi would be the dominant fungi in root communities of silver fir. DNA extraction was performed using Plant Genomic DNA purification. The internal transcribed spacer1 (ITS1) rDNA region was amplified using specific primers, and the amplicons were purified and sequenced using sequencing by synthesis (SBS) Illumina technology. The obtained sequences were compared with reference sequences in the UNITE database (https://unite.ut.ee/) using the basic local alignment search tool (BLAST) algorithm to facilitate species identification. A total of 307,511 OTUs was obtained from each sample. There were 246,477 OTUs (80.15%) of fungi known from cultures. The genera *Tuber* spp. (7.51%) and *Acephala* spp. (3.23%) accounted for the largest share of the fungal communities on the fine roots of fir trees. Hence our results indicate the dominance of mycorrhizal fungi in these communities and reflect the excellent quality of the saplings that were assessed. Pathogenic fungi constituted a much smaller share of the fungal communities.

Keywords: Lower Silesia; Międzylesie Forest District; nursery; restoration; Sudeten Mountains

INTRODUCTION

The silver fir tree (*Abies alba* Mill.) used to be one of the most important forest-forming species in the mountainous and upland regions of forest stands in Poland, in which fir is the dominant species, occupying 2% of the forest area and having a 2.7% share in the volume in the forests managed by the State Forests (Bis and Dobrowolska 2012). However, since 1998, the share of fir trees in the forests of the Sudeten Mountains in Poland has fallen below 0.4%, and the species had already lost the possibility of continued existence and self-regeneration in the competitive environment of the forests of the region. For this reason, the State Forests (Poland) undertook a program to restore fir trees to forests of the Sudeten Mountains (Barzdajn 2000, 2012, Barzdajn and Kowalkowski 2012), but with time it was noticed that fungi-related issues should not be overlooked. The studies of fungal communities inhabiting fir roots have focused mainly on mycorrhizae (within and outside their natural occurrence) (Kowalski 1982, Comandini et al. 2001, Laganà et al. 2002, Rudawska et al. 2016) and pathogenic fungi that infect their root systems (Puddu et al. 2003, Oliva and Colinas 2007, Chomicz-Zegar et al. 2016). However, no research has been undertaken in the mountainous regions of Poland. In addition to pathogenic and mycorrhizal fungi, these communities are known to include antagonists of other microorganisms and neutral organisms. Root-associated fungal communities are essential components in ecosystem processes, impacting plant growth and vigour by influencing the quality, direction, and flow of nutrients and water between plants and fungi (Unuk et al. 2019). Hence, the aim of the current study was to assess the diversity of fungi occurring in the rhizosphere of the root systems of small cuttings and fir saplings. We tested the hypothesis that mycorrhizal fungi should dominate these communities

MATERIALS AND METHODS

Fine roots of 6 saplings of silver fir from the forest nursery of Międzylesie Forest District (16°66'23"E, 50°14'86"N, south-west part of Poland) were randomly selected for the study. The samples were collected in June 2017. The research material comprised fine roots (roots up to three rows to identify all types of mycorrhizae) (McCormack et al. 2015). The fine roots were washed on sieves under running tap water and dried on sterile paper. After drying, the roots were ground in a mortar frozen to -70°C. The DNA extracted from dried roots was separated under the microscope. We composited one sample from six trees. Environmental DNA was extracted with Plant Genomic DNA Purification Kit (Thermo Fisher Scientific). The internal transcribed spacer1 (ITS1), 5.8S rDNA region was used to identify the fungal species, and the analysis was carried out with primers ITS1FI2 5′-GAACCWGCGGARGGATCA-3′ (Schmidt et al. 2013) and 5.8S 5`-CGCTGCGTT CTTCATCG-3` (Vilgalys and Gonzalez 1990). Each amplification reaction was carried out in a final volume of 25.0 µl containing 2 ml of DNA, 0.2 ml of each primer, 10.1 ml of deionized water and 12.5 mL of 2X PCR MIX (A&A Biotechnology, Gdynia, Poland). The amplification reaction was carried out in a thermocycler. This included: initial denaturation (94°C, 5 min), 35 cycles of denaturation (94°C, 30 s), annealing (56°C, 30 s), elongation (72°C, 30 s) and final elongation (72°C, 7 min). The product was then checked on a 1% agarose gel stained with Midori Green Advance DNA (Genetics, Dueren, Germany). The obtained product was purified and sequenced using sequencing by synthesis (SBS) technology from Illumina (Genomed S.A. Warsaw, Poland). The results were subjected to bioinformatic and statistical analysis according to Behnke-Borowczyk et al. (2019). The resulting sequences were compared with the reference sequences deposited in the UNITE community database (Nilsson et al. 2018, UNITE community 2020) using the basic local alignment search tool (BLAST) algorithm.

The abundance of fungi was defined as the number of OTUs in a sample. A total amount of OTUs was obtained from six samples collected from each of the 3-year-old small roots of *A. alba*. The frequency of an individual taxon was defined as the percentage (%) of OTUs in the total number of OTUs. Diversity was defined as the number of species in a sample. The trophic role of the detected fungal species in the community was determined based on literature data and listed in Appendix (Table A1).

RESULTS

A total of 307 511 OTUs were obtained. There were 246 477 OTUs (80.15%) of fungi known from cultures, 1 876 OTUs (0.61%) of non-cultured fungi, 47 572 (15.47%) OTUs of non-fungal organisms (mainly plants, including silver fir), and 1 814 OTUs (0.59%) of organisms with no reference sequence in UNITE database. The total number of taxa obtained was 1612.

The community comprised taxa belonging to Ascomycota (46.27%), Basidiomycota (33.64%), Zygomycota (2.52%), Rozellomycota (0.65%), and we also obtained some sequences that are not represented in the UNITE database (0.59%) (Table A1).

The Thelephoraceae (8.22%), *Tuber* spp. (7.51%) and *Acephala* spp. (3.23%) had the largest share of fine roots of common fir trees, therefore accounting for the largest share of the fungal community (Table A1).

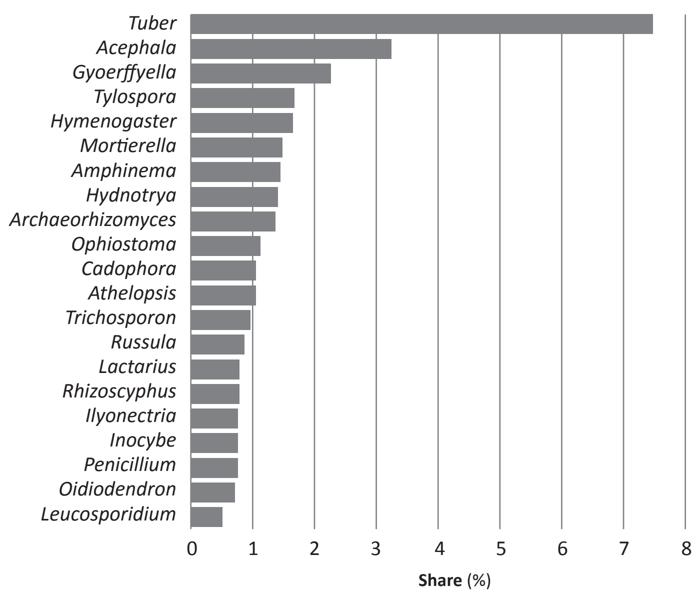


Figure 1. Percentage share (%) of the most abundant types of fungi in the community (share > 0.5%).

Mycorrhizal fungi dominated the fungal community of the fine root (57,62%). The most common taxa identified among mycorrhizal fungi were: *Hydnotrya* spp., *Tuber* spp., *Amphinema* spp., *Hymenogaster* spp., *Tylospora* spp., endophytes including *Acephala* spp., *Cadophora* spp., pathogenic fungi including *Ophiostoma* spp. and saprotrophs *Athelopsis lembospora, Archaeorhizomyces borealis* and *Mortierella* spp. (Table 1, Table A1).

DISCUSSION

The results of our analysis support our hypothesis that mycorrhizal fungi dominate the fungal communities on the fine root systems of silver fir saplings. Taxa, which belong to the fungi, forming mycorrhizal communities of silver fir, include *Tuber* spp. and *Acephala* spp., and these accounted for the largest share of the fungal communities on the fine

Table 1. Genus of ectomycorrhizal (ECM) fungi, whose frequency in the collection in fine roots of silver fir exceeded 0.03%. and which denotes the presence of a taxa in roots or soil in previous scientific reports.

Genus	Root	Soil	Reference
Amanita	+	+	Ważny (2014), Rudawska et al. (2016), Unuk et al. (2019), Behnke-Borowczyk et al. (2020)
Amphinema	+		Ważny (2014), Unuk et al. (2019)
Cenococcum	+	+	Unuk et al. (2019), Ważny (2014), Behnke-Borowczyk et al. (2020)
Cortinarius		+	Ważny (2014), DEEMY (2014-2020), Unuk et al. (2019), Behnke-Borowczyk et al. (2020)
Elaphomyces	+	+	Unuk et al. (2019), Ważny (2014), Behnke-Borowczyk et al. (2020)
Hydnotrya	+	-	Ważny (2014)
Inocybe		+	Ważny (2014), Behnke-Borowczyk et al. (2020)
Lactarius	+	+	Ważny (2014), Rudawska et al. (2016), Unuk et al. (2019), Behnke-Borowczyk et al. (2020)
Meliniomyces	+	+	Unuk et al. (2019), Behnke-Borowczyk et al. (2020)
Piloderma		+	Ważny (2014), Rudawska et al. (2016)
Russula	+	+	DEEMY (2014-2020), Ważny (2014), Rudawska et al. (2016), Unuk et al. (2019), Behnke-Borowczyk et al. (2020)
Tricholoma	+		DEEMY (2014-2020), Rudawska et al. (2016), Behnke-Borowczyk et al. (2020)
Tuber	+	+	Ważny (2014), Rudawska et al. (2016), Behnke-Borowczyk et al. (2020)
Tylospora	+	+	Ważny (2014), Rudawska et al. (2016), Unuk et al. (2019), Behnke-Borowczyk et al. (2020)
Xerocomellus	+		Rudawska et al. (2016)

root systems of fir saplings in our study. These results are slightly different from the studies of soil fungal communities in nurseries producing silver fir seedlings conducted by Behnke-Borowiczyk et al. (2020), where saprotrophic fungi dominated. However, Behnke-Borowczyk et al. (2020) also identified mycorrhizal fungi. We also identified some saprotrophs which accounted for just a small share of the fungal community (not exceeding 1.5%) on the roots of silver fir tree saplings in our study.

Smutek et al. (2010) obtained the following mycorrhizal families and species of fungi on old silver fir trees (90-105 years old): *Cortinarius sertipes, Sebacina* sp., *Amphinema byssoides, Russula puellaris, Clavulina cristata, Cortinarius sp., Tomentella sublilacina, Russula fellea, Laccaria ametistina* and *Tylospora asteropcumum*. Our results concur, confirming the occurrence of fungi belonging to all the taxa recorded by Smutek et al. (2010). Similarly, we have found the presence of some of the taxa detected by Wojewoda (2003) in the fungal community on fir trees, including *Amanita, Amphinema, Boletus, Cenococcum, Clavulina, Cortinarius, Elaphomyces, Hydnotrya, Hydnum, Inocybe, Lactarius, Leotiomycetes, Piloderma, Pseudotomentella, Russula, Sebacina, Tomentella, Tuber*, and *Tylospora*. However, unlike Ważny (2014) and Schirkonyer et al. (2013), the fungi genera *Byssocorticium, Laccaria, Paxillus, Thelephora, Tomentellopsis* and *Xerocomus* were not identified in our study. Ważny (2014) found that the mycorrhizal fungi which dominated on the youngest fir trees examined were: *Clavulina cristata* (25.2%), *Tomentella* sp. (10.5%), *Tuber puberulum* (8.9%), and *Clavulina* sp. (5.1%). While the genus *Tuber* accounted for a similar share (7.51%) of the community of fungi in our work, the presence of the other genera/species did not exceed 1%. Apart from *Geopora* sp. and *Imleria* sp., we identified similar taxa of fungi to those recorded by Rudawska et al. (2016), who studied mycorrhizal fungi of common fir trees outside their natural range, but from mature forest stands. To date, 13 species of ectomycorrhizal fungi (ECM) associated with the genus *Abies* have been included in DEEMY: *Abierhiza fascicularis, A. tomentosa, Cortinarius odorifer, Lactarius caespitosus, L. deliciosus, L. intermedius, L. salmonicolor, L. subsericatus, Polyporoletus sublividus, Russula brevipes, R. ochroleuca, R silvicola*, and *Tricholoma bufonium,* of which only *R. ochroleuca* was found in the sampled fungal community. The root community differs significantly from the fungal community associated with fir analyzed by Behnke-Borowczyk et all (2020), who isolated 13 taxa of ectomycorrhizal fungi (ECM) associated with the genus *Abies.*

The presence of *Cenococcum geophilum* and *Thelephora stuposa* was insignificant in the study, which is in opposition to the research done by Rudawska et al. (2016). The list of mycorrhizal species detected in silver fir roots include *Amphinema byssoides, Clavulina cristata, Lactarius aurantiacus, L. salmonicolor, Piloderma fallax, Tuber puberulum, T. asterophora, T. stuposa Boletus pruinatus, Cenococcum geophilum,* and *Laccaria amethystina*, which have been confirmed in other studies (Eberhardt et al. 2000). In addition, *C. geophilum, A. byssoides, T. stuposa, Amanita, Boletus, Cenococcum, Cortinarius, Inocybe, Laccaria, Lactarius*, *Russula, Sebacina, Tomentella*, and *Tuber* also form mycorrhizal compounds with other fir species (Matsuda and Hijii 1999, 2004, Ishida et al. 2007, Kranabetter et al. 2009).

Acephala applanata, which we detected in fine roots of *A. alba*, was previously almost exclusively isolated from *Picea abies* (L.) H. Karst (Grünig et al. 2006). While second species from *Acephala* genus *A. macrosclerotiorum* formed ectomycorrhizas on *Pinus sylvestris* (Münzenberger et al. 2009) was detected in a study of the community of fungi on silver fir fine roots. These results are consistent with those obtained by Behnke-Borowczyk et al. (2020) in soil research related to nurseries producing silver fir seedlings. However, the share of *Acephala* spp. in the previously studied soil was lower than in the roots. The greater share of these fungi in the root community is not surprising, because both species inhabit the roots: *A. applanata* is included in the DSE (dark septate endopyhtes) (Stroheker et al. 2021), while *A. macrosclerotiorum* is classified as ectomycorrhizal fungi (Münzenberger et al. al. 2009).

For young silver fir trees, similarly to Unuk et all (2019), we detected endophytic root-associated fungal genera *Oidiodendron, Phialocephala*, and *Rhizoscyphus*. Some consider fungi of these genera to be mycorrhizal, and therefore they are treated as fungi in Appendix A. However, their role in the silver fir root community has not yet been clearly defined.

In fungal community of silver fir fine roots identified cosmopolitan fungi from the genera *Trichoderma* and *Penicillium* as well, which are antagonists of the pathogens *Armillaria* and *Heterobasidion* (Behnke-Borowczyk and Kwaśna 2010; Grantina-Ievina et al. 2013; Baranowska et al. 2023).

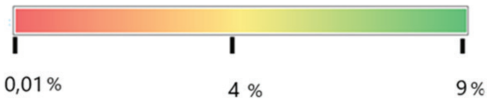
Our study also identified pathogenic fungi in the fine roots of silver fir tree saplings, namely *Ophiostoma nigrocarpum*, which, together with *O. novo-ulmi* and *Ophiostoma* sp., accounted for 1.11% of the fungal community. Fungi of the *Ophiostoma* genus are pathogens whose vectors are bark beetles on older trees (for example, *Pissodes piceae*), which cause white discolouration of fir's wood, thus reducing its economic value (Six and Bentz 2003, Kirisits 2004).

CONCLUSIONS

This study led to recognizing the spectrum of mycorrhizal, saprotrophic, and pathogenic fungi characteristic for fine roots of the 3-year-old *Abies alba.* Relatively low proportion of pathogens in these fungal communities also supports the conclusion that they were healthy trees. To fully confirm the roles and functions of the identified taxa, further identification of communities should be carried out. Parallel to the analysis of the communities of fungi inhabiting silver fir fine roots, it is necessary to study the content of nutrients and soil pH and determine their impact on these communities. In addition, research should be continued at a later stage of tree development, i.e. in young forest stands, to determine the formation of these communities and the spectrum of specific species of fungi associated with young silver fir trees.

Appendix A

Table A1. Taxa occurring in the communities of fungi in the roots of fir trees, whose frequency in the collection exceeded 0.03%. The legend of colours in the "Frequency" column:



Dominant taxa are marked in bold. Symbols of trophic groups are: M - mycorrhizal fungi, S - saprotrophic fungi, A - antagonistic fungi, P - pathogenic fungi, L - lichens, E - entomopathogenic fungi, U - unknown. The "Frequency, %" column contains the collected taxa data in the community, red representing the smallest share, and green representing the largest share of the taxon in the community (the scale is attached above).

Taxon	Order	Frequency (%)	Similarity (%)	Trophic group	Reference
Acephala applanata Grünig & T.N. Sieber + *A. macrosclerotiorum* Münzenb. & Bubner + *Acephala* sp.	Helotiales	3.231	97-100	M	Münzenberger et al. (2009)
Apiognomonia errabunda (Roberge ex Desm.) Höhn.	Diaporthales	0.063	99	P	Mańka (2005)
Archaeorhizomyces borealis Menkis, T.Y. James & Rosling + *Archaeorhizomyces* sp.	Incertae sedis	1.356	98-100		
Ascomycota		1.821	100		
Beauveria pseudobassiana S.A. Rehner & Humber + *Beauveria* sp.	Hypocreales	0.039	98-100	E	Álvarez-Baz et al. (2015)
Cadophora finlandica (C.J.K. Wang & H.E. Wilcox) T.C. Harr. & McNew + *C. orchidicola* (Sigler & Currah) M.J. Day & Currah + *Cadophora* sp.	Incertae sedis	1.049	97-100	P	Yakti et al. (2019)
Capnodiales		0.060	99		
Cenococcum geophilum Fr. + *Cenococcum* sp.	Mytilinidiales	0.355	99-100	M	Spatafora et al. (2012)
Chaetothyriales		0.271	100		
Chalara hyalocuspica Koukol + *Ch. pseudoaffinis* Koukol + *Chalara* sp.	Helotiales	0.482	97-100	S/P	Koukol (2011), Coker et al. (2019)
Cheirosporium triseriale L. Cai & K.D. Hyde	Pleosporales	0.181	99	S	Abdel-Aziz (2016)
Cladophialophora minutissima M.L. Davey & Currah + *C. chaetospira* (Grove) Crous & Arzanlou + ***Cladophialophora*** sp.	Chaetothyriales	0.211	98-100	P/S	Badali et. al. (2008)
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams + *Clonostachys* sp.	Hypocreales	0.038	98-100	A	Cota et al. (2009)
Coccomyces australis P.R. Johnst.	Rhytismatales	0.088	99	P	Johnson (1986)
Coniochaeta sp.	Coniochaetales	0.042	100	P	Damm et al. (2010)
Coniochaetaceae	Coniochaetales	0.035	100		
Dermateaceae	Helotiales	0.532	99		
Didymella dactylidis (Aveskamp, Gruyter & Verkley) Qian Chen & L. Cai + *D. protuberans* (Lév.) Qian Chen & L. Cai	Pleosporales	0.211	98-100	P/S	Chen et al. (2015)
Dothideomycetes		0.289	100		
Elaphomyces muricatus Fr. + *E. granulatus* Fr.	Eurotiales	0.176	98-99	M	Paz et al. (2017)
Erysiphe euonymicola U. Braun+ *E. hypophylla* (Nevod.) U. Braun & Cunningt.	Erysiphales	0.104	99-100	P	Sepúlveda-Chavera et al. (2013)

Table A1. (countinue) Taxa occurring in the communities of fungi in the roots of fir trees, whose frequency in the collection exceeded 0.03%. The legend of colours in the "Frequency" column:

Taxon	Order	Frequency (%)	Similarity (%)	Trophic group	Reference
Exophiala sp.	Chaetothyriales	0.033	100	S	Feng et al. (2013)
Fimetariella sp.	Sordariales	0.102	100	U	
Fusarium sp. + ***F. oxysporum*** Schltdl.	Hypocreales	0.087	99-100	S/P	Karim et al. (2016)
Geomyces asperulatus Sigler & J.W. Carmich. + *G. auratus* Traaen + *Geomyces* sp.	Helotiales	0.387	98-100		
Gyoerffyella entomobryoides (Boerema & Arx) Marvanová + *Gyoerffyella* sp.	incertae sedis	2.285	99-100	P	Jankowiak et al. (2016)
Halokirschsteiniothelia maritima (Linder) Boonmee & K.D. Hyde		0.046	100	S	Wilson (1951)
Halosphaeriaceae	Microascales	0.067	99		
Helotiaceae	Helotiales	0.685	99		
Helotiales		1.878	100		
Hydnotrya cerebriformis Harkn. + *H. michaelis* (E. Fisch.) Trappe + *H. tulasnei* (Berk.) Berk. & Broome	Pezizales	1.405	99-100	M	Hobbie et al. (2001)
Hymenoscyphus sp.	Helotiales	0.073	100	S	Gumińska and Wojewoda (1985)
Hypocreales		2.001	100		
Ilyonectria morspanacis (A.A. Hildebr.) A. Cabral & Crous + ***I. robusta*** (A.A. Hildebr.) A. Cabral & Crous	Hypocreales	0.744	99-100	P	Farh et al. (2017)
Infundichalara minuta Koukol + *Infundichalara* sp.	Helotiales	0.081	99-100	S	Koukol (2012)
Leotia lubrica (Scop.) Pers.	Helotiales	0.081	100	S	Kuo (2012)
Leotiomycetes		1.099	100		
Leptosphaeria sp.	Pleosporales	0.079	100	P	Brachaczek et. al. (2016)
Lopadostoma polynesium (Berk. & M.A. Curtis) Rappaz	Xylariales	0.033	99	P	Mehrabi and Hemmati (2015)
Lophodermium conigenum (Brunaud) Hilitzer + ***L. pinastri*** (Schrad.) Chevall. + *L. seditiosum* Minter, Staley & Millar	Rhytismatales	0.386	99-100	P	Burdekin and Phillips (1992)
Maasoglossum sp.	Geoglossales	0.182	100		Hustad and Miller (2015)
Mariannaea elegans (Corda) Samson	Hypocreales	0.032	99	U	Wang and Zabel (1990)
Meliniomyces bicolor Hambl. & Sigler + ***M. variabilis*** Hambl. & Sigler + *M. vraolstadiae* Hambl. & Sigler	incertae sedis	0.285	99-100	M	Martino et al. (2018)
Metapochonia bulbillosa (W. Gams & Malla) Kepler, S.A. Rehner & Humber		0.040	100	E	Adachi et al. (2015)
Mycosphaerellaceae	Capnodiales	0.073	99		
Nectriaceae	Hypocreales	0.055	100		
Neobulgaria pura (Pers.) Petr. + *Neobulgaria* sp.	Helotiales	0.113	99-100	S	Gumińska and Wojewoda (1985)
Neonectria sp.	Hypocreales	0.035	100	P	Kryczyński and Weber (2010)
Oidiodendron maius G.L. Barron + *O. rhodogenum* Robak + *Oidiodendron* sp.	Helotiales	0.696	99-100	M/S	Rice and Currah (2006a)
Ophiosphaerella sp.	Pleosporales	0.063	100	P	Dernoeden (2000)

Table A1. (countinue) Taxa occurring in the communities of fungi in the roots of fir trees, whose frequency in the collection exceeded 0.03%. The legend of colours in the "Frequency" column:

Taxon	Order	Frequency (%)	Similarity (%)	Trophic group	Reference
Ophiostoma nigrocarpum (R.W. Davidson) de Hoog + *Ophiostoma novo-ulmi* Brasier + ***Ophiostoma*** sp.	Ophiostomatales	1.108	98-100	P/S	Marcinkowska (2012)
Penicillium citreonigrum Dierckx + *P. penicillioides* (Delacr.) Vuill. + *P. subrubescens* Houbraken, Mansouri, Samson & Frisvad + *Penicillium* sp.	Eurotiales	0.727	98-100	A	Pitt et al. (2000)
Petriella sordida (Zukal) G.L. Barron & J.C. Gilman	Microascales	0.033	99	A	Lee and Gloer (1995)
Pezizaceae	Pezizales	0.210	100		
Phaeosphaeriaceae	Pleosporales	0.044	100	S	
Phialocephala fortinii CJK. Wang & H.E. Wilcox + *Phialocephala* sp.	Helotiales	0.414	99-100	M/U	Jumpponen et al. (1998)
Phoma boeremae Gruyter + *Phoma* sp.	Pleosporales	0.070	98-99		Chen et al. (2015)
Pleosporaceae	Pleosporales	0.033	100		
Pleosporales		0.119	100		
Pleotrichocladium opacum (Corda) Hern.0Restr., R.F. Castañeda & Gené	Pleosporales	0.248	99	S	Hernández-Restrepo et al. (2017)
Podospora appendiculata (Auersw. ex Niessl) Niessl + *Podospora* sp.	Sordariales	0.023	99-100	S	Doveri (2008)
Proliferodiscus sp.	Helotiales	0.090	100		Han et al. (2014)
Pseudogymnoascus verrucosus A.V. Rice & Currah	Incertae sedis	0.368	99	S	Rice and Currah (2006b)
Pyronemataceae	Pezizales	1.135	100		
Rhizoscyphus sp.	Helotiales	0.786	100	M	Hambleton and Sigler (2005)
Rhodoveronaea varioseptata Arzanlou, W. Gams & Crous	incertae sedis	0.053	99	M	Tedersoo et al. (2010)
Rhytismataceae	Rhytismatales	0.045	100		
Sporormiaceae	Pleosporales	0.032	100		
Stagonospora pseudovitensis Quaedvl., Verkley & Crous	Pleosporales	0.034	99	P	Quaedvlieg et al. (2013)
Strumella sp.	Pezizales	0.068	100	P/S	Zettur and Kullman (2011)
Sympodiella acicola W.B. Kendr.	incertae sedis	0.065	99	S	Shen et al. (2020)
Tetracladium setigerum (Grove) Ingold + *Tetracladium* sp.	Helotiales	0.097	99-100	S	Anderson and Marvanová (2020)
Trichoderma sp.	Hypocreales	0.120	100	A	Benítez et al. (2004)
Tuber anniae W. Colgan & Trappe + *T. cistophilum* P. Alvarado, G. Moreno, Manjón, Gelpi & Jaime Muñoz + *Tuber* sp.	Pezizales	7.508	99-100	M	(2004-2020)
Umbilicaria americana Poelt & T.H. Nash	Umbilicariales	0.032	100	L	Poelt and Nash (1993)
Venturia hystrioides (Dugan, R.G. Roberts & Hanlin) Crous & U. Braun + *Venturia* sp.	Venturiales	0.146	99-100	P	González-Domínguez et al. (2017)
Venturiaceae	Venturiales	0.076	100		
Wilcoxina sp.	Pezizales	0.049	99	M	DEEMY (2004-2020)
Xenopolyscytalum pinea Crous	Helotiales	0.368	100	P	Koukol (2019)
Frequency of Ascomycota isolates			**46.27**		
Agaricales		2.261	100		

Table A1. (countinue) Taxa occurring in the communities of fungi in the roots of fir trees, whose frequency in the collection exceeded 0.03%. The legend of colours in the "Frequency" column:

Taxon	Order	Frequency (%)	Similarity (%)	Trophic group	Reference
Agaricomycetes		0.064	100		
Amanita rubescens Pers. + *Amanita* sp.	Agaricales	0.159	99-100	M	DEEMY (2004-2020)
Amphinema byssoides (Pers.) J. Erikss. + *Amphinema* sp.	Atheliales	1.448	99-100	M	DEEMY (2004-2020)
Amphistereum leveilleanum (Berk. & M.A. Curtis) Spirin & Malysheva	Auriculariales	0.131	100	U	
Atheliaceae	Atheliales	0.078	100		
Atheliales		0.181	100		
Athelopsis lembospora (Bourdot) Oberw.	Amylocorticiales	1.030	99	U	
Auriculariales		0.444	99		
Basidiodendron caesiocinereum (Höhn. & Litsch.) Luck-Allen	Auriculariales	0.097	100	S	Kotiranta and Saarenoksa (2005)
Basidiomycota		0.565	100		
Cantharellales		0.096	100		
Ceratobasidiaceae	Cantharellales	0.351	100		
Clavariaceae	Agaricales	0.155	99		
Clavulina coralloides (L.) J. Schröt.	Cantharellales	0.245	99	S	Wojewoda (2003)
Clavulinopsis sp.	Agaricales	0.077	100	S	Kautmanová et al. (2012)
Cortinarius croceus (Schaeff.) Gray + *C. semisanguineus* (Fr.) Gillet	Agaricales	0.032	99-100	M	DEEMY (2004-2020)
Cryptococcus sp.	Tremellales	0.056	100	P/S	Springer et al. (2017)
Curvibasidium cygneicollum J.P. Samp.	incertae sedis	0.064	100	P/S	Kaitera et al. (2019)
Deconica phyllogena (Sacc.) Noordel.	Agaricales	0.061	99	S	Noordeloos (2011)
Galerina nana (Petri) Kühner + ***Galerina*** sp.	Agaricales	0.349	99-100	S	Gulden et al. (2005)
Hydnaceae	Cantharellales	0.308	100		
Hydnodontaceae	Trechisporales	0.054	100		
Hygrophoraceae	Agaricales	2.149	100		
Hymenochaetales		0.085	100		
Hymenogaster boozeri Zeller & C.W. Dodge + ***H. huthii*** Stielow, Bratek & Hensel + *H. olivaceus* Vittad.	Agaricales	1.625	99-100	M	Stielow et al. (2011)
Inocybe rufoalba Sacc. + *Inocybe* sp.	Agaricales	0.733	99-100	M	DEEMY (2004-2020)
Itersonilia perplexans Derx + *I. pannonica* (Niwata, Tornai-Leh., T. Deák & Nakase) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout	Cystofilobasidiales	0.063	99-100	P	McGovern et al. (2006)
Kockovaella sacchari M. Takash. & Nakase	Tremellales	0.032	99	U	
Lactarius sp. + *L. necator* (Bull.) Pers. + ***L. tabidus*** Fr.	Russulales	0.797	98-100	M	DEEMY (2004-2020)
Leucosporidium drummii Yurkov, A.M. Schäfer & Begerow + *Leucosporidium* sp.	Leucosporidiales	0.508	99-100	U	Sampaio et al. (2003)
Malassezia restricta E. Guého, J. Guillot & Midgley + *Malassezia* sp.	Malasseziales	0.068	99	P	Saunders et al. (2012)
Microbotryomycetes		0.050	100		
Mycena sanguinolenta (Alb. & Schwein.) P. Kumm. + *Mycena* sp.	Agaricales	0.244	99-100	S	Perry (2002)

Table A1. (countinue) Taxa occurring in the communities of fungi in the roots of fir trees, whose frequency in the collection exceeded 0.03%. The legend of colours in the "Frequency" column:

Taxon	Order	Frequency (%)	Similarity (%)	Trophic group	Reference
Phellinus castanopsidis BK. Cui, Y.C. Dai & Decock	Hymenochaetales	0.022	100	P	Cui and Decock (2012)
Piloderma sp.	Atheliales	0.216	100	M	DEEMY (2004-2020)
Piskurozyma sp.	Filobasidiales	0.016	100	U	
Pterula sp.	Agaricales	0.117	100	S	Leal-Dutra et al. (2020)
Ramariopsis sp.	Agaricales	0.021	99	S	Matouš et al. (2017)
Rhodotorula sp.	Sporidiobolales	0.034	100	A	Akhtyamova and Sattarova (2013)
Rigidoporus sanguinolentus (Alb. & Schwein.) Donk	Polyporales	0.353	100	S	Wojewoda (2003)
Russula puellaris Fr. + *R. fragilis* Fr. + *R. nigricans* Fr. + *R. veternosa* Fr. + *R. badia* Quél. + *R. ionochlora* Romagn. + *Russula* sp.	Russulales	0.859	99-100	M	DEEMY (2004-2020)
Sebacinaceae	Sebacinales	0.174	100		
Sebacinales		0.447	100		
Solicoccozyma terrea (Di Menna) Yurkov + *Solicoccozyma* sp.	Filobasidiales	0.224	99-100	A	Yurkov et al. (2019)
Sporidiobolales		0.049	100		
Stereum sanguinolentum (Alb. & Schwein.) Fr.	Russulales	0.046	100	S	Łakomy and Kwaśna (2008)
Thelephoraceae	Thelephorales	8.220	100		Richard et al. (2011)
Trechispora sp.	Trechisporales	0.069	99	S/M	Gumińska (1985), Vanegas-León et al. (2019)
Trechisporales		0.084	100		
Tremellales		0.044	100		
Tremellomycetes		0.073	100		
Tricholoma fulvum (DC.) Bigeard & H. Guill. + *Tricholoma* sp.	Agaricales	0.064	99-100	M	DEEMY 2004-(2020)
Trichosporon sp.	Tremellales	0.949	100	A	Bosqueiro et al. (2020)
Tylospora asterophora (Bonord.) Donk + *Tylospora* sp.	Atheliales	1.689	98-99	M	DEEMY (2004-2020)
Vishniacozyma victoriae (M.J. Montes, Belloch, Galiana, M.D. García, C. Andrés, S. Ferrer, Torr. -Rodr. & J. Guinea) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout + *Vishniacozyma* sp.	Tremellales	0.067	99-100	A	Gramisci et al. (2018)
Xerocomellus pruinatus (Fr. & Hök) Šutara + *X. chrysenteron* (Bull.) Šutara n	Boletales	0.032	99-100	M	Šutara (2008)
Frequency of Basidiomycota isolates			**33.64**		
Absidia sp.	Mucorales	0.034	100	S	Alastruey-Izquierdo et al. (2010)
Basidiobolus ranarum Eidam	Entomophthorales	0.064	99	S	Yang (1962)
Endogone sp.	Endogonales	0.072	100	S/M	Warcup (1990)
Jimgerdemannia lactiflua (Berk. & Broome) Trappe, Desirò, M.E. Sm., Bonito & Bidartondo	Endogonales	0.030	99-100	S/M	Desirò et al. (2018)
Mortierella horticola Linnem. + *M. angusta* (Linnem.) Linnem. + ***Mortierella*** sp.		1.484	99-100	S/A	Grantina-Ievina et al. (2014)
Mortierellaceae		0.130	100	S	
Mortierellales	Mortierellales	0.079	100	S	
Umbelopsis dimorpha Mahoney & W. Gams + *U. ramanniana* (Möller) W. Gams + *Umbelopsis* sp.	Umbelopsidales	0.241	99-100	S/A	Crowther et al. (2012), Grantina-Ievina et al. (2014)

Table A1. (countinue) Taxa occurring in the communities of fungi in the roots of fir trees, whose frequency in the collection exceeded 0.03%. The legend of colours in the "Frequency" column:

Taxon	Order	Frequency (%)	Similarity (%)	Trophic group	Reference
Frequency of zygomycota isolates			**2.52**		
Frequency of other kingdoms			**15.47**		
Not represented in UNITE database			**0.59**		
Frequency of uncultured fungi			**0.61**		

Author Contributions

WB, WK, JBB, MB conceived and designed the research, MB carried out the field measurements, JB performed laboratory analysis, JB and WB processed the data and performed the statistical analysis, WK, WB secured the research funding, AŁ, RK, JBB supervised the research and helped to draft the manuscript, MB wrote the manuscript. The main part of these results was presented at the international IUFRO Conference - Abies&Pinus 2022, "Fir and pine management in a changing environment: Risks and opportunities" held 19-22 September 2022 in Sarajevo, Bosnia and Herzegovina.

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Conflicts of Interest

The authors declare no conflict of interest.

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