

Soil fungal communities in nurseries producing *Abies alba*

JOLANTA BEHNKE-BOROWCZYK^{1*}, WOJCIECH KOWALKOWSKI², NATALIA KARTAWIK¹, MARLENA BARANOWSKA² AND WŁADYSŁAW BARZDAJN²

¹ Department of Forest Pathology, Poznan University of Life Sciences, Wojska Polskiego 71c, 60-625 Poznan, Poland

² Department of Silviculture, Poznan University of Life Sciences, Wojska Polskiego 71a, 60-625 Poznan, Poland

* Corresponding author: jbehnke@up.poznan.pl, phone: +48 618487771

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Abstract

Silver fir is an important forest-forming species in the mountain and upland parts of Poland. It was determined that fir lost the possibility of spontaneous return to the forests of the region. That is why the Polish State Forests has launched a programme to restore its resources in the Sudetes. Until now, fungi and their relationship with fir have not been fully addressed in the Sudetes Restitution Programme, therefore this study was undertaken. The aim of this study was to determine the species composition of soil fungi from quarters producing silver fir seedlings. The research material was rhizosphere soil collected from 3-year-old saplings produced in forest nurseries of the Międzyzylesie Forest District. Fungal composition was determined by Next-Generation sequencing (Illumina NextSeq 500). The total number of OTUs obtained (638 435) included sequences of cultivated horculturated fungi (433 412 OTUs = 52.7%), non-cultivable fungi, that is which cannot be cultured (97 108 OTUs = 22.41%) and potential fungi with no reference sequence in UNITE (73 419 OTUs = 16.93%) and non-fungal organisms (34 496 = 7.96%). The following taxa as *Mortierella* sp. (8.23%), *Tylospora* sp. (6.68%) and *Russula* sp. (3.76%) had the largest share in the soil fungal communities. The results of the study indicate the dominance of saprotrophic and mycorrhizal fungi in the communities of fir roots, which proves that saplings are of good quality. Small share of pathogens sequences suggests that soil-born fungal pathogens are not a major threat to fir seedlings in nurseries.

Keywords: mycorrhizal fungi, nursery, *Abies alba*, seedlings, Międzyzylesie Forest District

Introduction

Fungi constitute a significant part of the soil microorganism community. It is estimated that they take approximately 1.33% of the soil volume and 0.2% of its weight, and that in 1 g of soil there are up to 10 million microorganism cells, including 75–1500.000 fungal spores (Watanabe 2002, Behnke-Borowczyk and Kwaśna 2010). The taxa of fungi that live in the soil are present in the roots and seeds. The most common soil fungi are species of the following genera: *Absidia* Tiegh., *Acremonium* Link, *Alternaria* Nees, *Aspergillus* P. Micheli ex Haller, *Botrytis* P. Micheli ex Haller, *Chaetomium* Kunze, *Cladosporium* Link, *Cylindrocarpon* Wollenw., *Fusarium* Link, *Mucor* Fresen., *Penicillium* Link, *Rhizopus* Ehrenb. and *Stemphylium* Wallr. (Waksman 1916, 1917, Behnke-Borowczyk and Kwaśna 2010, Frąc et al. 2018). Considering the relationship with plants, pathogenic, antagonistic to plant pathogenic, mycorrhizal, saprotrophic and parasitic fungi can be distinguished (Behnke-Borowczyk and Kwaśna 2010, Frąc et al. 2018).

The soil fungal biodiversity has a great impact on given tree species, especially at the nursery production stage, where it influences quality of the planting material. Silver fir (*Abies alba* Mill.) is an important forest-forming species in the mountain and upland parts of Poland. In 1998, its share in the forests of the Polish part of the Sudetes was below 0.4% and it was resolved that fir lost the possibility of spontaneous return to the forests of the region (Barzdajn 2000, 2012, Barzdajn and Kowalkowski 2012). It was why the Polish State Forests have launched a forest restoring programme in the Polish part of the Sudetes. Until now, fungi and their relationship with fir has not been fully addressed in the Sudetes Restitution Program, therefore this study was undertaken (Barzdajn 2000, 2012, Barzdajn and Kowalkowski 2012). Previous studies of the fir fungal communities were mainly focused on mycorrhizae (Kowalski 1982, Comandini et al. 2001, Laganà et al. 2002, Kowalski 2008, Ważny 2011, 2014, Rudawska et al. 2016, Terhonen et al. 2019) and pathogens (Puddu et al. 2003, Oliva and Colinas 2007, Łakomy et

al. 2007, Niemtur et al. 2014, Chomicz-Zegar et al. 2016, Terhonen et al. 2019). On the other hand, soil fungal communities accompanying fir in nursery production were not investigated. The aim of this study was to determine the species composition of soil fungi from quarters producing silver fir seedlings. It was assumed that mycorrhizal fungi and saprotrophs will have a dominant share in such communities.

Materials and methods

In June 2017 thirty soil samples were taken from nurseries located in Międzyzlesie Forest District, where 3-year-old fir trees grew. The soil samples (in five repetitions) were collected as follows: 1) from under Scots pine canopy, 2) from under spruce canopy, 3) from under fir canopy (natural regeneration), 4) from Kosturkiewicz's containers, in which fir grew in nursery fields, 5) from open bed, and 6) from under fir trees that have been replanted after two seasons. Samples were taken randomly, from the topsoil to a depth of 25 cm (every 20 m). Thirty individual samples were placed in a container, mixed thoroughly and approx. 0.5 kg of such prepared soil was taken to the laboratory. Six samples of approximately 300 mg were taken from the later sample for further analysis.

DNA extraction was performed using DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The ITS1, 5.8S rDNA region was used to identify the fungal species, and the analysis was carried out with ITS1FI2 5'-GAACCGCGGARGGATCA-3' (Schmidt et al. 2013) and 5.8S 5'-CGCTGCGTT CTTCATCG-3' primers (Vilgalys et al. 1990). Each amplification reaction was carried out in a final volume of 25.0 µL containing 2 µL DNA, 0.2 µL of each primer, 10.1 µL deionized water and 12.5 µL 2X PCR MIX (A&A Biotechnology, Gdynia, Poland). Initial denaturation was carried out at 94°C for 5 min. Cycling conditions were: primer annealing at 56°C for 30 s elongation at 72°C for 30 s and final elongation at 72°C for 7 min. 35 cycles were carried out. The PCR product was then checked on a 1% agarose gel stained with Midori Green Advance DNA (Genetics, Dueren Germany). The resulting PCR product was purified and sequenced with the use of Illumina SBS technology (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 (<https://unite.ut.ee/>, Nilsson et al. 2018) using BLAST algorithm.

Abundance of fungi was defined as the number of OTUs in a sample. Frequency of an individual taxon was defined as percentage (%) of OTUs in the total number of OTUs. Diversity was defined as the number of species in a sample.

The role of the detected fungal species in the community was determined based on literature data, and the Information System for Characterization and DEtermination of EctoMYcorrhizae (DEEMY) (Agerer and Rambold 2004–2014, Rudawska et al. 2016).

Results

The total number of OTUs obtained (638 435) included sequences of cultivated or cultured fungi (433 412 OTUs = 52.7%), non-culturable fungi that is which cannot be cultured (97 108 OTUs = 22.41%) and potential fungi with no reference sequence in UNITE (73 419 OTUs = 16.93%) and non-fungal organism (34 496 = 7.96%). Taxa belonging to the following clusters were identified in the study: Ascomycota (15.54%), Basidiomycota (24.16%), Mucoromycota (9.48%), Zoopagomycota (1.995%), Chytridiomycota (0.22%), Glomeromycota (0.08%), Rozellomycota (1.14%), non-cultivated fungi (22.41%) and representatives of the other Kingdoms (7.96%). Sequences that were not represented in the NCBI database (16.94%) were also present (Table 1).

The *Mortierella* sp. (8.23%), *Tylospora* sp. (6.68%) and *Russula* sp. (3.76%) had the largest share in the different soil fungal communities (Table 1).

The soil fungal communities were dominated by saprotrophic (28.6%), mycorrhizal (19.11%) and those unrecognized (18.97%) fungi. Pathogens (4.9%), antagonists in relation to forest tree pathogens (3.19%) and a small percentage of parasitic and lichen-forming fungi were also present. Among the mycorrhizal fungi and endophytes that were isolated *Tylospora* sp. (6.68%), *Russula* sp. (3.76%), *Amanita* sp. (1.95%), *Tricholoma* sp. (1.2%), and *Piloderma* sp. (1.11%) were identified. The most common mycorrhizal fungi and endophytes in the studied fungal community were: *Tylospora* sp. (6.68%). The most common pathogens were: *Chalara* (Corda) Rabenh. (0.82%), *Malassezia* Baill. (0.51%), *Fusarium* Link (0.41%) and antagonists in relation to pathogens: *Solicocozyma* Xin Zhan Liu, F.Y. Bai, M. Groenew. et Boekhout (0.62 %).

Discussion

In the last two decades, numerous studies on the diversity of soil microorganisms, e.g. fungi or bacteria and their association with plants have been conducted. The abundance and diversity of microorganisms in soil from several common plant species, 'model plants', and their wild relatives were studied. Many of them play an important role in agriculture and forestry (Terhonen et al. 2019). However microorganisms associated with silver fir have not been fully studied and recognized, especially in Poland. So far, stand regeneration (Filipiak and Komisarek 2005), eutrophication (Lasota et al. 2015), effects of soil properties, and preparation on the growth of trees (Kuceřová et al. 2013, Kobala et al. 2015, Kormanek et al. 2015)

Table 1. Taxa occurring in the communities of soil fungi, which share in the collection exceeded 0.01%

Dominant taxa are marked in bold. Symbols of trophic groups M – mycorrhizal fungi; S – saprotrophic fungi; A – antagonistic fungi; P – pathogenic fungi; L – lichens according to DEEMY (Agerer and Rambold 2004–2014, Rudawska et al. 2016). Colors of the last column contain the collected taxa data in the community: red – the smallest share; green – the largest share of the taxon in the community (the scale is attached below).

Taxons	Trophic group	Order	Similarity [%]	Frequency [%]
Ascomycota				
<i>Acephala</i> sp. + <i>A. applanata</i> Grünig & T.N. Sieber + <i>A. macrosclerotiorum</i> Münzenb. & Bubner	M	Helotiales	99–100	0.103
<i>Acremonium</i> sp. + <i>A. alternatum</i> + <i>A. rutilum</i> W. Gams	S	Hypocreales	98–99	0.047
<i>Alatospora acuminata</i> Ingold + <i>A. flagellata</i> (Gönczöl) Marvanová	S	Leotiales	99–100	0.052
<i>Apodus deciduus</i> Malloch & Cain	S	Sordariales	98	0.013
<i>Archaeorhizomyces</i> sp. + <i>A. borealis</i> Menkis. T.Y. James & Rosling	S	Archaeorhizomycetales	98–99	0.362
Archaeorhizomycetes			100	0.014
<i>Arthrobotrys conoides</i> Drechsler + <i>A. oligosporus</i> Fresen.	A	Orbiliiales	99	0.035
<i>Ascobolus</i> sp.	S	Pezizales	98	0.214
Ascomycota			100	1.509
<i>Aspergillus</i> sp. + <i>A. costiformis</i> H.Z. Kong & Z.T. Qi + <i>A. inflatus</i> (Stolk & Malla) Samson. Frisvad. Varga. A. Visagie & Houbraken + <i>A. kassunensis</i> Baghd. + <i>A. parvulus</i> G. Sm. + <i>A. pseudodeflectus</i> Samson & Mouch. + <i>A. tardus</i> Bissett & Widden	A	Eurotiales	98–100	0.146
<i>Byssonectria fusispora</i> (Berk.) Rogerson & Korf		Pezizales	99	0.058
<i>Cadophora</i> sp. + <i>C. finlandica</i> (C.J.K. Wang & H.E. Wilcox) T.C. Harr. & McNew + <i>C. orchidicola</i> (Sigler P & Currah) M.J. Day & Currah	P	Helotiales	98–99	0.096
<i>Caloplaca obscurella</i> (J. Lahm) Th. Fr.		Teloschistales	98	0.015
Capnodiales			100	0.046
<i>Cenococcum</i> sp. + <i>C. geophilum</i> Fr.	M	Mytilinidiales	98–99	0.393
Chaetomiaceae	S	Sordariales	98	0.107
<i>Chaetomium</i> sp. + <i>Ch. grande</i> Asgari & Zare	S	Sordariales	99–100	0.057
<i>Chaetosphaeria</i> sp. + <i>Ch. vermicularioides</i> (Sacc. & Roum.) W. Gams & Hol. Jech.	S	Chaetosphaeriales	98–99	0.026
Chaetosphaeriaceae		Chaetosphaeriales	100	0.016
Chaetothyriales			100	0.063
<i>Chalara</i> sp. + <i>Ch. hyalocuspica</i> Koukol + <i>Ch. longipes</i> (Preuss) Cooke + <i>Ch. piceaeabietis</i> Hol. Jech.	P	Microascales	99–100	0.819
<i>Chloridium</i> sp.	S	Chaetosphaeriales	100	0.012
<i>Ciliophora</i> sp.	S	Incertae sedis	100	0.191
<i>Citeromyces siamensis</i> Nagats. H. Kawas. Limtong. Mikata & T. Seki		Saccharomycetales	98	0.041
<i>Cladophialophora</i> sp. + <i>C. chaetospira</i> (Grove) Crous & Arzanlou + <i>C. minutissima</i> M.L. Davey & Currah	P	Chaetothyriales	98–100	0.086
<i>Cladorrhinum brunnescens</i> W. Gams + <i>C. bulbiliosum</i> W. Gams & Mouch + <i>C. flexuosum</i> Madrid. Cano. Gené & Guarro	A	Sordariales	98–100	0.028
<i>Colletotrichum antirrhinicola</i> Damm + <i>C. cliviae</i> Yan L. Yang. Zuo Y. Liu. K.D. Hyde & L. Cai	S	Incertae sedis	99–100	0.028
<i>Crocicreas</i> sp.	S	Helotiales	100	0.013
<i>Cryptosporiopsis</i> sp.	P	Helotiales	100	0.016
<i>Dendryphon nanum</i> (Nees) S. Hughes	S	Pleosporales	98	0.059
Dermateaceae		Helotiales	100	0.308
<i>Devriesia</i> sp.	S	Incertae sedis	100	0.016
<i>Didymella dactylidis</i> (Aveskamp. Gruyter & Verkley) Qian Chen & L. Cai + <i>D. protuberans</i> (Lév.) Qian Chen & L. Cai	S	Pleosporales	99	0.203
Dothideomycetes			100	0.272
<i>Elaphomyces granulatus</i> Fr. + <i>E. muricatus</i> Fr.	M	Elaphomycetales	99–100	0.198
<i>Fusarium</i> sp. + <i>F. asiaticum</i> O'Donnell. T. Aoki. Kistler & Geise + <i>F. concolor</i> Reinking + <i>F. oxysporum</i> Schltld.	P	Hypocreales	98–100	0.413
<i>Geomyces</i> sp. + <i>G. asperulatus</i> Sigler & J.W. Carmich. + <i>G. auratus</i> Traaen	S	Helotiales	99	0.142
<i>Gyoerffyella</i> sp. + <i>G. entomobryoides</i> (Boerema & Arx) Marvanová	S	Incertae sedis	98–99	0.961
<i>Halokirschsteiniotelia maritima</i> (Linder) Boonmee & K.D. Hyde	S	Mytilinidiales	99	0.044
<i>Harzia camerounensis</i> Crous & Jol. Roux + <i>H. sphaerospora</i> (Matsush.) D.W. Li & N.P. Schultes	S	Melanosporales	98–99	0.030
Helotiaceae		Helotiales	100	0.203
Helotiales			100	1.176
Herpotrichiellaceae		Chaetothyriales	99	0.033
Hyaloscyphaceae		Helotiales	99	0.116
<i>Hymenoscyphus</i> sp.	S	Helotiales	98	0.026
Hypocreales			100	0.528
<i>Hypomyces</i> sp. + <i>H. microspermus</i> Rogerson & Samuels + <i>H. ochraceus</i> (Pers.) Tul. & C. Tul	A	Hypocreales	98–99	0.052
<i>Ilyonectria morspanacis</i> (A.A. Hildebr.) A. Cabral & Crous + <i>I. robusta</i> (A.A. Hildebr.) A. Cabral & Crous	P	Hypocreales	99–100	0.044
<i>Knufia peltigerae</i> (Fuckel) Réblová & Unter.	L	Incertae sedis	99	0.017
Lasiosphaeriaceae		Sordariales	99	0.105
<i>Lecanicillium</i> sp. + <i>L. primulinum</i> Kaifuchi. Nonaka & Masuma	A	Hypocreales	99–100	0.145
Lecanoromycetes	A		100	0.033
<i>Lecytophora fasciculata</i> (J.F.H. Beyma) E. Weber. Görke & Begerow	P	Coniochaetales	99	0.032
<i>Leotia lubrica</i> (Scop.) Pers.	L	Helotiales	99	0.061
Leotiomyces sp.	L		100	0.213
<i>Leptodontidium</i> sp. + <i>L. trabinellum</i> (P. Karst.) Baral. Platas & R. Galán	P	Incertae sedis	98–99	0.020
<i>Leptosphaeria</i> sp.		Pleosporales	98	0.051
<i>Lophodermium conigenum</i> (Brunaud) Hilitzer + <i>L. piceae</i> (Fuckel) Höhn. + <i>L. pinastri</i> (Schrad.) Chevall. + <i>L. seditiosum</i> Minter. Staley & Millar	P	Rhytismatales	98–100	0.069
<i>Maasoglossum</i> sp.	S	Helotiales	99	0.012
<i>Mariannaea elegans</i> (Corda) Samson	S	Hypocreales	99	0.025
<i>Meliniomyces bicolor</i> Hambl. & Sigler + <i>M. variabilis</i> Hambl. & Sigler + <i>M. vraolstadiae</i> Hambl. & Sigler	S	Incertae sedis	98–99	0.013
<i>Metapochonia bulbiliosa</i> (W. Gams & Malla) Kepler. S.A. Rehner & Humber	S	Hypocreales	99	0.082
<i>Metarhizium anisopliae</i> (Metschn.) Sorokin + <i>M. carneum</i> (Duché & R. Heim) Kepler. S.A. Rehner & Humber		Hypocreales	98–99	0.061
<i>Microascus restrictus</i> Sand. Den. Gené & Deanna A. Sutton	S	Microascales	98	0.015
<i>Minutisphaera parafimbriatospira</i> Raja. Oberlies. Shearer & A.N. Mill.	A	Minutisphaerales	98	0.040
<i>Mycopappus</i> sp.		Helotiales	98	0.010
Mycosphaerellaceae		Incertae sedis	100	0.015
<i>Nadsonia commutata</i> Golubev	S	Saccharomycetales	99	0.012
<i>Nectria</i> sp.	P	Hypocreales	100	0.070

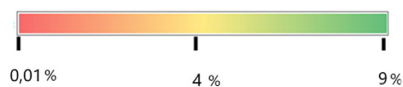
Table 1. Taxa occurring in the communities of soil fungi, which share in the collection exceeded 0.01% (continued)

Taxons	Trophic group	Order	Similarity [%]	Frequency [%]
Nectriaceae	P	Hypocreales	99	0.098
<i>Neobulgaria</i> sp. + <i>N. pura</i> (Pers.) Petr	S	Helotiales	98–99	0.072
<i>Neocosmospora solani</i> (Mart.) L. Lombard & Crous	S	Hypocreales	98	0.020
<i>Neonectria</i> sp. + <i>N. candida</i> (Ehrenb.) Rossman. L. Lombard & Crous	P	Hypocreales	98–99	0.010
<i>Ocellularia punctulata</i> (Leight.) Zahlbr.		Ostropales	99	0.219
<i>Ochroconis</i> sp.	S	Venturiales	100	0.010
<i>Oidiodendron</i> sp. + <i>O. chlamydozporicum</i> Morrall + <i>O. echinulatum</i> G.L. Barron + <i>O. majus</i> G.L. Barron + <i>O. pilicola</i> Kobayasi + <i>O. rhodogenum</i> Robak	S	Incertae sedis	98–100	0.763
Orbiliaceae	S	Orbiliiales	99	0.014
<i>Penicillium amaliae</i> Visagie. Houbraken & K. Jacobs + <i>P. arianae</i> Visagie. Houbraken & K. Jacobs + <i>P. atrovenetum</i> G. Sm. + <i>P. bialowiezense</i> K.W. Zaleski + <i>P. brasilianum</i> Bat. + <i>P. cairnsense</i> Houbraken. Frisvad & Samson + <i>P. caperatum</i> Udagawa & Y. Horie + <i>P. chrysogenum</i> Thom + <i>P. citreonigrum</i> Dierckx + <i>P. glandicola</i> (Oudem.) Seifert & Samson + <i>P. lanosum</i> Westling + <i>P. lapidosum</i> Raper & Fennell + <i>P. nodositatum</i> Valla + <i>P. onobense</i> C. Ramirez & A.T. Martinez + <i>P. penicillioides</i> (Delacr.) Vuill. + <i>P. riverlandense</i> Visagie & K. Jacobs + <i>P. senticosum</i> D.B. Scott + <i>P. simplicissimum</i> (Oudem.) Thom + <i>P. singorense</i> Visagie. Seifert & Samson + <i>P. subrubescens</i> Houbraken. Mansouri. Samson & Frisvad + <i>P. vasconiae</i> C. Ramirez & A.T. Martinez	A	Eurotiales	98–100	0.363
Pezizaceae			100	0.011
Pezizales			100	0.043
<i>Phacidium grevilleae</i> Crous & M.J. Wingf.	S	Phacidiales	100	0.124
<i>Phialocephala fortinii</i> C.J.K. Wang & H.E. Wilcox + <i>P. fusca</i> W.B. Kendr.	P	Helotiales	98–100	0.023
<i>Plectosphaerella cucumerina</i> (Lindf.) W. Gams	P	Glomerellales	98	0.022
Pleosporales			100	0.166
<i>Pleotrichocladium opacum</i> (Corda) Hern.Restr. R.F. Castañeda & Gené	S	Pleosporales	99	0.449
<i>Pleuroascus nicholsonii</i> Massee & E.S. Salmon	S	Incertae sedis	97	0.020
<i>Pleurophoma ossicola</i> Crous. Krawczynski & H.G. Wagner	S	Xylariales	99	0.096
<i>Pochonia</i> sp. + <i>P. cordycipiticonsociata</i> H. Huang. Mu Wang & L. Cai	S	Hypocreales	98–99	0.021
<i>Podospora</i> sp. + <i>P. appendiculata</i> (Auerw. ex Niessl) Niessl	S	Sordariales	99–100	0.043
<i>Pseudogymnoascus verrucosus</i> A.V. Rice & Currah	S	Incertae sedis	100	0.151
<i>Pseudopenidiella pini</i> (P.M. Kirk & Minter) P.M. Kirk	S	Incertae sedis	99	0.071
<i>Pseudosigmoidea ibarakiensis</i> Diene & Narisawa	P	Incertae sedis	98	0.014
Pyrenomataceae			98	0.042
<i>Rhodoveronaea varioseptata</i> Arzanlou. W. Gams & Crous	S	Incertae sedis	100	0.011
<i>Robillarda sessilis</i> (Sacc.) Sacc.	R	Incertae sedis	99	0.066
<i>Saccharomyces cerevisiae</i> (Desm.) Meyen	A	Saccharomycetales	99	0.035
Saccharomycetales	A		100	0.014
<i>Sagenomella diversispora</i> (J.F.H. Beyma) W. Gams + <i>S. striatispora</i> (Onions & G.L. Barron) W. Gams	S	Eurotiales	98	0.023
Sordariales			99	0.014
Sordariomycetes			99	0.179
<i>Sphaerodes fimicola</i> (E.C. Hansen) P.F. Cannon & D. Hawksw.	S	Hypocreales	98	0.070
<i>Sphaerostilbella aureonitens</i> (Tul. & C. Tul.) Seifert. Samuels & W. Gams	A	Hypocreales	99	0.224
Sporormiaceae			100	0.026
<i>Sporothrix</i> sp. + <i>S. brunneoviolacea</i> Madrid. Gené. Cano & Guarro + <i>S. dimorphospora</i> (Roxon & S.C. Jong) Madrid. Gené. Cano & Guarro	P	Ophiostomatales	98–100	0.018
<i>Tetracladium</i> sp. + <i>T. setigerum</i> setigerum (Grove) Ingold	S	Helotiales	99	0.196
Trichocomaceae	A	Eurotiales	100	0.023
<i>Trichoderma</i> sp. + <i>T. alcalifuscens</i> (Overton) Jaklitsch & Voglmayr + <i>T. asperellum</i> Samuels. Lieckf. & A Nirenberg + <i>T. crassum</i> Bissett + <i>T. cremeum</i> P. Chaverri & Samuels + <i>T. foliicola</i> (Jaklitsch & Voglmayr) Jaklitsch & Voglmayr + <i>T. fomiticola</i> Jaklitsch + <i>T. longipilis</i> Bissett + <i>T. martiale</i> Samuels + <i>T. neokoningii</i> Samuels & Soberanis + <i>T. nothescens</i> Samuels & Jaklitsch + <i>T. pachypallidum</i> Jaklitsch + <i>T. parapiluliferum</i> (B.S. Lu. Druzhin. & Samuels) Jaklitsch & Voglmayr + <i>T. pleuroti</i> S.H. Yu & M.S. Park + <i>T. pubescens</i> Bissett + <i>T. rossicum</i> Bissett. C.P. Kubicek & Szakács + <i>T. spirale</i> Bissett + <i>T. stellatum</i> (B.S. Lu. Druzhin. & Samuels) Jaklitsch & Voglmayr + <i>T. theobromicola</i> Samuels & H.C. Evans		Hypocreales	97–100	0.320
<i>Tuber</i> sp.	M	Pezizales	99	0.036
<i>Valdensinia heterodoxa</i> Peyronel	P	Helotiales	100	0.013
<i>Venturia</i> sp. + <i>V. hystrioides</i> (Dugan. R.G. Roberts & Hanlin) Crous & U. Braun	P	Venturiales	99–100	0.085
Venturiaceae	P	Venturiales	100	0.080
<i>Verrucaria</i> sp. + <i>V. elaeomelaena</i> (A. Massal.) Arnold	L	Verrucariales	99	0.015
<i>Verticillium leptobactrum</i> W. Gams	P	Glomerellales	98	0.014
<i>Xenopolyscytalum pinea</i> Crous	P	Helotiales	97	0.057
Xylariales			100	0.021
Frequency of Ascomycota isolates				15.5411
Basidiomycota				
Agaricales			100	0.841
Agaricomycetes sp.		Agaricales	100	0.145
<i>Amanita</i> sp. + <i>A. beckeri</i> Huijsman + <i>A. fulva</i> Fr. + <i>A. muscaria</i> (L.) Lam. + <i>A. rubescens</i> Pers.	M	Agaricales	97–100	1.954
<i>Apiotrichum dulcitum</i> (Berkhout) Yurkov & Boekhout	S	Trichosporonales	99	0.022
Atheliaceae			99	0.061
Auriculariales			100	0.163
Basidiomycota				0.715
Boletaceae	M	Boletales	100	0.013
<i>Boletus edulis</i> Bull.	M	Boletales	100	0.107
Cantharellales	M		100	0.183
<i>Cantharellus decolorans</i> Eyssart. & Buyck	M	Cantharellales	99	0.102
Ceratobasidiaceae	S	Cantharellales	100	0.173
Clavariaceae			99	0.052
<i>Clavulina</i> sp. + <i>C. coralloides</i> (L.) J. Schröt.	M	Cantharellales	98–99	0.455
<i>Clavulinopsis</i> sp.	M	Agaricales	97	0.034
<i>Conocybe echinata</i> (Velen.) Singer + <i>C. semiglobata</i> Kühner & Watling	M	Agaricales	99–100	0.029
<i>Coprinopsis narcotica</i> (Batsch) Redhead. Vilgalys & Moncalvo	S	Agaricales	100	0.017
<i>Cortinarius</i> sp.+ <i>C. croceus</i> (Schaeff.) Gray + <i>C. fulvoconicus</i> M.M. Moser + <i>C. semisanguineus</i> (Fr.) Gillet	M	Agaricales	98–100	0.053
<i>Cronartium pini</i> (Willd.) Jørst.	P	Pucciniales	98	0.040
<i>Entoloma byssisedum</i> (Pers.) Donk + <i>E. conferendum</i> (Britzelm.) Noordel. + <i>E. juncinum</i> (Kühner & Romagn.) Noordel. + <i>E. sericeum</i> Quéf.	M	Agaricales	98–100	0.023

Table 1. Taxa occurring in the communities of soil fungi, which share in the collection exceeded 0.01% (continued)

Taxons	Trophic group	Order	Similarity [%]	Frequency [%]
Erythrobasidiales				
<i>Flagelloscypha minutissima</i> (Burt) Donk	S	Agaricales	100	0.011
<i>Galerina</i> sp. + <i>G. atkinsoniana</i> A.H. Sm. + <i>G. fallax</i> A.H. Sm. & Singer + <i>G. nana</i> (Petri) Kühner + <i>G. pseudocamerina</i> Singer + <i>G. stylifera</i> (G.F. Atk.) A.H. Sm. & Singer	S	Agaricales	97–100	0.045
<i>Geminibasidium</i> sp.		Geminibasidiales	99	0.903
<i>Gymnopilus decipiens</i> (Sacc.) P.D. Orton + <i>G. penetrans</i> (Fr.) Murrill	S	Agaricales	99	0.055
<i>Hygrocybe</i> sp. + <i>H. appalachianensis</i> (Hesler & A.H. Sm.) Kronaw. + <i>H. intermedia</i> (Pass.) Fayod	M	Agaricales	99–100	0.141
Hygrophoraceae				
<i>Hymenogaster boozeri</i> Zeller & C.W. Dodge + <i>H. huthii</i> Stielow. Bratek & Hensel + <i>H. olivaceus</i> Vittad.	M	Agaricales	99	0.050
<i>Hypochnicium</i> sp. + <i>H. geogenium</i> (Bres.) J. Erikss. + <i>H. punctulatum</i> (Cooke) J. Erikss.	S	Agaricales	98–100	0.355
<i>Inocybe</i> sp. + <i>I. curvipes</i> P. Karst. + <i>I. flocculosa</i> Sacc. + <i>I. napipes</i> J.E. Lange + <i>I. nitidiuscula</i> (Britzelm.)M	M	Agaricales	97–100	0.068
Lapl. + <i>I. rimosa</i> (Bull.) P. Kumm. + <i>I. rufoalba</i> Sacc.		Polyporales	98–100	0.018
<i>Lactarius musteus</i> Fr. + <i>L. necator</i> (Bull.) Pers. + <i>L. quietus</i> (Fr.) Fr. + <i>L. rufus</i> (Scop.) Fr. + <i>L. tabidus</i> Fr. M	S	Agaricales	98–100	0.312
<i>Lepiota echinella</i> Quél. & G.E. Bernard + <i>L. fuscovineacea</i> F.H. Møller & J.E. Lange	S	Russulales	98–99	0.772
Leucosporidiales				
<i>Leucosporidium</i> sp. + <i>L. drummii</i> Yurkov. A.M. Schäfer & Begerow + <i>L. fasciculatum</i> Babeva & Lisichk. + <i>L. fellii</i> Gim. Jurado & Uden + <i>L. golubevii</i> Gadanho. J.P. Samp. & R. Bauer		Agaricales	100	0.018
<i>Lycoperdon nigrescens</i> Pers. + <i>L. perlatum</i> Pers. + <i>L. pratense</i> Pers. + <i>L. subumbrinum</i> Jeppson & E. Larss.	S	Agaricales	99–100	0.016
<i>Malassezia</i> sp. + <i>M. cuniculi</i> J. Cabañes & G. Castellá + <i>M. globosa</i> Midgley. E. Guého & J. Guillot + <i>M. restricta</i> E. Guého. J. Guillot & Midgley + <i>M. sympodialis</i> R.B. Simmons & E. Guého	P	Agaricales	98–100	0.507
Microbotryomycetes				
<i>Minimedusa polyspora</i> (Hotson) Weresub & P.M. LeClair		Incertae sedis	100	0.057
<i>Russula</i> sp. + <i>R. amethystina</i> Quél. + <i>R. badia</i> Quél. + <i>R. chloroides</i> (Krombh.) Bres. + <i>R. firmula</i> Jul. Schäff. + <i>R. fragilis</i> Fr. + <i>R. ionochlora</i>	M	Cantharellales	99	0.082
<i>Romagn.</i> + <i>R. nigricans</i> Fr. + <i>R. ochroleuca</i> Fr. + <i>R. paludosa</i> Britzelm. + <i>R. puellaris</i> Fr. + <i>R. sapinea</i> Samari + <i>R. vetermosa</i> Fr.		Russulales	100	3.760
Russulaceae	M	Russulales	100	0.040
<i>Sebacina</i> sp.	M	Sebacinales	100	0.020
Sebacinaceae	M	Sebacinales	100	0.013
Sebacinales	M	Sebacinales	100	0.016
<i>Sistotrema</i> sp.	S	Cantharellales	99	0.014
<i>Solicoccozyma fuscescens</i> (Golubev) Yurkov + <i>S. phenolica</i> (Á. Fonseca. Scorzetti & Fell) Yurkov + <i>S. terrea</i> (Di Menna) Yurkov + <i>S. terricola</i> (T.A. Pedersen) Yurkov	A	Filobasidiales	99	0.621
Sporidiobolales				
Thelephoraceae	M	Thelephorales	100	0.168
<i>Tomentella</i> sp.	M	Thelephorales	99	0.392
<i>Trechispora</i> sp. + <i>T. hymenocystis</i> (Berk. & Broome) K.H. Larss. + <i>T. invisitata</i> (H.S. Jacks.) Liberta + <i>T. stevensonii</i> (Berk. & Broome) K.H. Larss.	S	Trechisporales	98	0.079
Trechisporales	S	Trechisporales	98–100	0.147
Tremellales	S	Trechisporales	100	0.066
<i>Tremellomyces</i> sp.	S	Tremellales	99	0.027
<i>Tricholoma</i> sp. + <i>T. fulvum</i> (DC.) Bigeard & H. Guill. + <i>T. saponaceum</i> (Fr.) P. Kumm.	M	Agaricales	99	0.071
<i>Trichosporon</i> sp.	A	Agaricales	99–100	1.195
<i>Tubulicrinis</i> sp.	S	Tremellales	99	0.157
<i>Tylospora</i> sp. + <i>T. asterophora</i> (Bonord.) Donk	M	Hymenochaetales	98	0.011
<i>Paraglomus</i> sp.	M	Atheliales	98–99	6.681
<i>Scutellospora alterata</i> Oehl. J.S. Pontes. Palenz. I.C. Sánchez & G.A. Silva	M	Paraglomerales	99	0.002
	M	Diversisporales	99	0.002
Frequency of Glomeromycota isolates				0.082
Rozellomycota				1.142
Frequency of Rozellomycota isolates				1.142
Mucoromycota				
<i>Absidia</i> sp. + <i>Absidia caerulea</i> Bainier + <i>A. cylindrospora</i> Hagem + <i>A. glauca</i> Hagem	S	Mucorales	97–100	0.037
<i>Mortierella</i> sp.	S	Mucorales	100	8.226
Mortierellaceae	S	Mucorales	99	0.384
Mortierellales	S	Mucorales	100	0.610
<i>Mucor abundans</i> Povah + <i>M. bainieri</i> B.S. Mehrotra & Baijal + <i>M. circinelloides</i> Tiegh. + <i>M. hiemalis</i> Wehmer + <i>M. moelleri</i> (Vuill.) Lendn.	S	Mucorales	99	0.040
<i>Umbelopsis</i> sp.	S	Umbelopsidiales	100	0.183
Frequency of Mucoromycota isolates				9.480
Zoopagomycota				
Basidiobolaceae	S		100	0.009
Basidiobolales	S		100	0.01
<i>Basidiobolus ranarum</i> Eidam	S	Basidiobolales	99	1.79
<i>Piptocephalis graefenhanii</i> H.M. Ho + <i>P. tieghemiana</i> Matr.	S	Zoopagales	98	0.013
<i>Syncephalis</i> sp.	S	Zoopagales	98	0.173
Frequency of Zoopagomycota isolates				1.995
uncultured fungus				22.4055
No sequence in the UNITE database				16.9346
Number of isolates				577013
Number of fungus isolates				433 412

Scale:



have been studied, taking soil from fir nursery beds into consideration.

Functional groups of fungi in forest soils include free-living saprotrophs and mycorrhiza (Smith and Read 2008). Functional groups of fungi inhabiting forest soils include mycorrhizal fungi and free-living saprotrophs (Smith and Read 2008). It is often difficult to determine the function of the latter. Many fungal species show phenotypic plasticity and depending on the prevailing conditions, adopt different life strategies – from saprotrophs to endophytes. Their role in the community is not always clear (Vasiliauskas et al. 2007, Kowalski 2008, Kuo et al. 2014, Jaber et al. 2014, Unuk et al. 2019). For example, the classification of the *Mycena* genus is very ambiguous. According to Unuk et al. (2019), *Mycena* spp. are saprotrophs however Kowalski (2008) classified *Mycena galopus* (Pers.) P. Kumm. as an ectomycorrhizal species. In this study *Mycena* were grouped with mycorrhizal fungi.

In soil of silver fir nursery, the fungal communities were dominated by saprotrophs involved in the processing of decayed organic matter.

Fungal communities determined in the study were dominated by saprotrophs that play an important role in the processing of decayed organic matter. *Rozellomycota* with saprotrophic *Mortierella* species accounted for 8.23% of the whole community. Mucoromycota and Ascomycota (*Archaeorhizomyces* sp., *Ascobolus* sp., *Ciliophora* sp., *Didymella* sp., *Geomyces* sp., *Gyoerffyyella* sp., *Oidiodendron* sp., *Phacidium* sp., *Pleotrichocladium* sp., *Pseudogymnoascus* sp., *Tetracladium* sp., *Gyoerffyyella* spp., *Oidiodendron* spp., and *Pleotrichocladium opacum* (Corda) Hern. Restr., R.F. Castañeda et Gené.) accounted for 15.54%, whereas Basidiomycota accounted for 24.16% of the whole community. The results of this research partly coincide a study by Sun et al. (2016), in which authors have detected 86 OTUs belonging to the Ascomycota phylum, and accounted for 3% of the total sequence reads; 234 OTUs from the Basidiomycota phylum (56% of the reads). The frequency of Basidiomycota in this study was much higher than Ascomycota. Among other Basidiomycota fungi many mycorrhizal species were detected, for example *Amanita*, *Boletus*, *Cantharellus*, *Clavulina*, *Cortinarius*, *Elaphomyces*, *Hygrophoraceae*, *Lactarius*, *Phellodon*, *Piloderma*, *Russula*, *Sebacina*, *Thelephora*, *Tomentella*, *Tricholoma*, *Tylospora* and *Xerocomellus*. Also, usually widely spread decay fungi from *Amphinema* genera were found. This taxon was earlier described by Smutek et al. (2010) in their study.

The above-mentioned species are common in boreal forests and temperate zones in Europe, including Poland (Kennedy et al., 2003, Nara 2006, Kowalski 2008). Some of these species (*Russula* and *Piloderma*) were however found in warmer climate – in Mexico (Argüelles-Moyao and Garibay-Orijel 2018). The fungi often form mycorrhiza with *Abies* (Matsuda and Hijii 1999, 2004, Eberhardt et al. 2000, Ishida et al. 2007, Cremer et al. 2009, Kranabet-

ter et al. 2009, Smutek et al. 2010, Argüelles-Moyao and Garibay-Orijel 2018) and occur in silver fir nursery soil, younger and older fir stands (Korkama et al. 2006, Kowalski 2008, Wallander et al. 2010, Kyaschenko et al. 2017).

To date 13 species of ectomycorrhizal fungi (ECM) associated with the genus *Abies* have been included in DEEMY: *Abierhiza fascicularis* Haug et R. Weber, *A. tomentosa* Haug et R. Weber, *Cortinarius odorifer* Britzelm., *Lactarius caespitosus* Hesler et A.H. Sm., *L. deliciosus* (L.) Gray, *L. intermedius* Krombh. ex Berk. et Broome, *L. salmonicolor* R. Heim et Leclair, *L. subsericatus* Kühner et Romagn. ex Bon, *Polyporoletus sublividus* Snell, *Russula brevipes* Peck, *R. ochroleuca* Pers., *R. silvicola* Shaffer and *Tricholoma bufonium* (Pers.) Gillet, of which only *R. ochroleuca* was found in the sampled soil fungal community. The presence of *Cenococcum geophilum* and *Thelephora stuposa* was not significant 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 soils includes: *Amanita*, *Amphinema*, *Boletus*, *Cenococcum*, *Clavulina* J. Schröt., *Cortinarius*, *Elaphomyces*, *Hydnum* L., *Inocybe* (Fr.) Fr., *Lactarius*, *Leotiomyces* O.E. Erikss. et Winka, *Piloderma*, *Pseudotomentella* Svrček, *Russula*, *Sebacina*, *Thelephora* and *Tylospora* that were earlier described in Poland by Wojewoda (2003). *Byssocorticium* Bondartsev et Singer ex Singer, *Laccaria* Berk. et Broome, *Paxillus* Fr., *Tomentellopsis* Hjortstam and *Xerocomus* Quéf were not detected in the study. The above-mentioned species were found in fir nursery soil by Schirkonyer et al. (2013), Ważny (2014) and Unuk et al. (2019).

Glomeromycota and Ascomycota (*Acephala*, *Cenococcum*) mycorrhizal or root-associated fungi were not as abundant as Basidiomycota. Members of Glomeromycota form arbuscular mycorrhizas (AMS) with mosses and roots of land vascular plants (Parniske 2008). They form symbioses with the roots of most plant species (>80%). *Cenococcum* is one of the most common ectomycorrhizal fungal species encountered in forest ecosystems. *Cenococcum geophilum* and *Thelephora stuposa* were not significantly frequent in the studied soil, and this again is in opposition with Rudawska et al. (2016).

A few phytopathogens, e.g. *Chalara*, *Malassezia* and *Fusarium* were detected in the study. The above-mentioned pathogens are typical examples of soil-borne pathogens (Werner 2012, Lazreg et al. 2014, Orr and Nelson 2018, Theelen et al. 2018, Kowalska et al. 2017). Their frequency was low, and several ones, e.g. *Malassezia* (common pathogen of mammals, Theelen et al. 2018) are not dangerous for trees. In sampled soils fungi antagonistic to phytopathogens, including species of *Penicillium* (0.36%) and *Trichoderma* (0.32%) were also present. The low frequency of pathogens and relatively high frequency of their antagonists, apart from forming the high frequency of mycorrhizal fungi, gave the chance to produce the healthy and good-quality planting material.

Using the Illumina sequencing technique 3,072 fungal taxa were found in the material sampled in this study. Argüelles-Moyao and Garibay-Orijel (2018) obtained 1,746 species from *Abies religiosa* (Kunth) Schltdl. et Cham. soil in central Mexico. The results of mycological studies still do not present the actual microbial situation in soil. Despite the advanced techniques used, the detection and identification of many taxa is still impossible. This affects taxon sequences that are not deposited in databases, and fungi non-cultivated on artificial media. In this study, the frequency of the former and the latter was 17% and 20%, respectively.

Conclusion

This study led to recognising the spectrum of saprotrophic, mycorrhizal, and pathogenic fungi characteristic for the soil, in which 3 year old *Abies alba* grew.

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