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The community of soil fungi associated with the western red cedar (*Thuja plicata* Donn ex D. Don, 1824)

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Abstract

The western red cedar (*Thuja plicata* Donn ex D. Don), an important forest-forming species in the Western part of the North American continent, is an alien species naturalised in Europe. It is popular and highly valued in horticulture. While considering the progressing climate change, it may also be a potential alternative to native species in European forests. The community of soil fungi associated with the western red cedar in forested areas of Europe has not been fully determined. Thus, this study is aimed to identify the community of soil fungi associated with the western red cedar. The experimental plots are located in the Kościan Forest District (51°98'87″ N; 16°23'54″ E). All soil samples were taken from the topsoil layer at a depth of 25 cm with a trowel, three from the centre of natural regeneration (1G, 2G, 5G) and three from the centre stand under the canopy of old-growth western red cedar (3G, 4G, 6G). Fungi were identified directly from the soil based on the ITS1 rDNA region. The derived product was sequenced using Illumina's sequencing by synthesis (SBS) technology. Sequences were referred to the National Center for Biotechnology Information (NCBI) database applying the BLAST algorithm. The fungal counts were defined based on the number of operational taxonomic units (OTU) in the sample. The OTU number was 835 206, with fungal isolates accounting for 683 095 (81.79%). A total of 8 591 taxa belonging to the Kingdom Fungi were identified. The species with the greatest shares in the community included *Mortierella* spp. (10.5%), *Russula* spp. (5.6%), *Hydnum* spp. (3.44%), *Solicoccozyma* spp. (3.1%) and *Penicillium* spp. (2.2%). Results showed that saprotrophs and mycorrhizal fungi predominated in the community. The dominance of ectomycorrhizal fungi over arbuscular ones, quite impressive natural regeneration was shown in *T. plicata* stands in Kościan. Subsequent research should take into account tree stands in Poland in which natural regeneration does not occur or occurs sporadically.

Key words

alien species, Illumina system, mycorrhizal fungi, the Kościan Forest District, saprotrophs, western red cedar

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Introduction

For over thirty years, intensive studies have been conducted worldwide on alien tree species (Pyšek et al. 2020). They were also carried out in Central Europe (Białobok and Chylarecki 1965; Tumiłowicz 1968, 1988; Panka 2012, 2016). They have provided information on the biology of alien species, methods of their introduction and expansion onto new areas, effects of their presence and control methods (Richardson 2011; Simberloff et al. 2013; Pyšek et al. 2020). One of the non-invasive-alien species in the forests in Poland is the western red cedar (*Thuja plicata* Donn ex D. Don), which is a major forest-forming species in the Pacific Northwest. In the 1980s, the total area covered by western red cedars in Polish forests was estimated at slightly less than 8 ha (Tumiłowicz 1988). These surfaces are remnants of Prussian attempts to introduce alien species (Tumiłowicz 1988). Cultivation of the western red cedar was located in the area of 22 forest districts, some of which are now in Poland (including Nowe Ramuki, Karsko, Kąty, Łopuchówko and Trzciel). However, not all of the stands with the share of the western red cedar were described (Tumiłowicz 1988). In 1901, Schwappach wrote that surplus of *T. plicata* seed or planting material was transferred to other forest districts, municipal forests or private forests. This explains according to Tumiłowicz (1988) the existence of old-growth stands of similar age in other forest districts in Poland, as for example, the stands of western red cedar in the forest districts of Kościan or Podanin, the description of which was not considered in Tumiłowicz's 1988 monograph.

The currently observed climate change and the resulting dieback of native tree species (e.g. Scots pine) may lead to timber shortages (Panka 2016; Sierota et al. 2019). In times of climate change and increasing threats for native tree species in Sweden and other European countries, exotics such as *T. plicata* might be considered alternatives to native tree species (Wilson et al. 2016). As a result, it might prove necessary to use exotic species in forest regeneration, for example western red cedar, Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) or black locust (*Robinia pseudoacacia* L.). It has been established that when appropriate habitat and tending operations are provided, these species could be grown almost throughout the country (Bellon et al. 1977). It was also indicated as a valuable admixture in stands (Tumiłowicz 1988; Panka 2012, 2016). In addition to forests, the western red cedar is widely used in horticulture. Its share outside forests, in backyard gardens, is growing, especially in the surroundings of new buildings. *Thuja occidentalis* L. and *T. plicata* (and their hybrids) belong to alien trees most frequently cultivated in Europe (Zieliński et al. 2019). For many plants, including trees, the formation of a mycorrhiza is an essential condition required for their growth (Brundrett 2002). Species of fungi of mycorrhizas frequently prove to be host-specific and are sometimes transferred from the natural range of the host plant (Pringle et al. 2009; Nuñez and Dickie 2014).

A community of soil fungi associated with the western red cedar is currently unidentified. Nevertheless, Damszel et al. (2020) carried out a research of macroscopic fungi associated with Douglas fir, white pine (*Pinus strobus* L.) and western red cedar in Warmia.

This study is aimed to identify the fungal community associated with the rhizosphere of western red cedar from the Kościan Forest District. These thuja stocks, which are unique in the study area due to the age of the thuja (Tab. 1), were probably established by foresters from the Prussian research institutes. We assumed that cosmopolitan fungi, including saprotrophs and mycorrhizal fungi, would predominate in this community.

Material and methods

A total of six soil samples for analyses were collected in January 2018 from three clusters of western red cedars, each of approximately 0.1 ha (Tab. 1). The research area is in the Kościan Forest District, the Olejnica Forest Unit (51°98"87" N; 16°23'54" E) in sub-compartment 238c (LMśw = mesic mixed deciduous forest) and one cluster in sub-compartment 217s (Lśw = mesic deciduous forest).

All soil samples were taken from the topsoil layer at a depth of 25 cm with a trowel, three from the centre of the natural regeneration (1G, 2G, 5G) and three from the centre stand under the canopy of old-growth western red cedar (3G, 4G, 6G). Each sample's collection site was 15 m apart (Fig. 1). Each sample was packed separately.

Table 1. The share of functional groups of fungi in the communities of fungi in different variants of the experiment. Natural regeneration – 1G, 2G, 5G, under old-stands canopy – 3G, 4G, 6G

Functional groups	Variants of experiments					
	1G	2G	5G	4G	3G	6G
Pathogens	7.63	6.90	10.93	5.37	8.89	6.56
Mycorrhizal fungi	11.34	19.40	19.33	14.99	6.39	24.18
Saprotrophs	14.11	12.73	18.82	9.10	13.62	13.47
Others	66.93	60.97	50.92	70.54	71.11	55.79
Share of Ascomycota of mycorrhizal fungi [%]	9.97	11.20	8.45	23.23	14.68	26.61
Share of Basidiomycota of mycorrhizal fungi [%]	85.77	86.47	87.94	71.07	77.51	71.59
Share of Glomeromycota of mycorrhizal fungi [%]	4.26	2.33	3.61	5.69	7.81	1.80

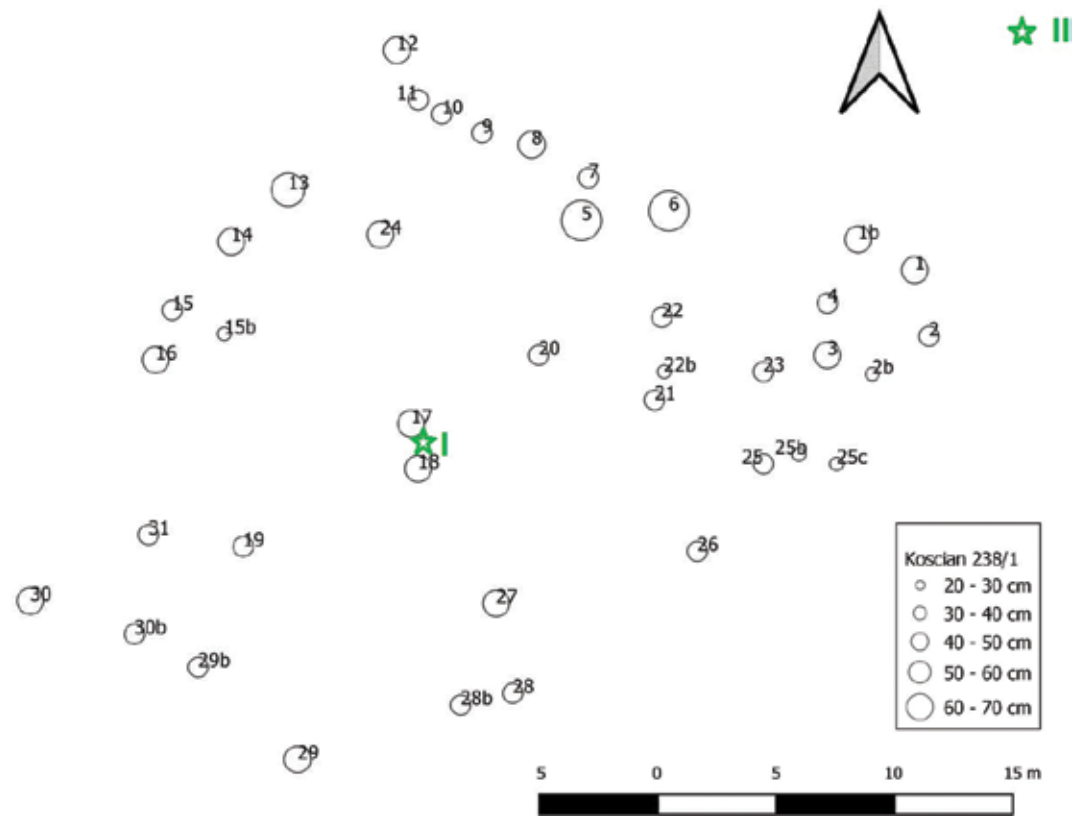

Figure 1. Kościan 238/1 – stem distribution map. In the diagram, the star (No. I and II) indicates examples of sample collection sites

DNA extraction was performed using the plant genomic DNA purification kit (Thermo Scientific) following the manufacturer's protocol. Fungi were identified to species based on the ITS1 rDNA region. Analysis was conducted using the ITS1F2 (5` GAA CCW GCG GAR TCA 3`) and 5.8S (5` CGC TGC GTT CTT CAT

CG 3`) primers (Schmidt et al. 2013). The reaction mixture consisted of 2.5 µl DNA, 0.2 µl each primer, 10.6 µl deionised water and 12.5 µl 2X PCR MIX (A&A Biotechnology). Amplification was run in a thermocycler. The process comprised initial denaturation (94°C, 5 min), 35 cycles of denaturation (94°C, 30 s), annealing (56°C, 30 s), extension (72°C, 30 s) and final extension (72°C 7 min). Next, the product was verified in 1% agarose gel stained by Midori Green Advance DNA (Genetics). The obtained product was purified and sequenced using the sequencing by symbiosis (SBS) technology by Illumina (Genomed S.A. Warszawa). The sequences were referred to the National Center for Biotechnology Information (NCBI) database (GenBank), applying the BLAST algorithm. Fungal counts were defined as the number of operational taxonomic units (OTU). The frequency of a single taxon was determined as the OTU percentage in the total OTU number. Latin names of identified fungi were adopted following the Index Fungorum (http://www.indexfungorum.org).

Results were subjected to bioinformatic analysis according to Behnke-Borowczyk et al. (2020). Obtained sequences were compared using the BLAST algorithm with the reference sequences from the NCBI database (https://www.ncbi.nlm.nih.gov). The functions of fungi in the community were determined based on literature data.

The statistical analysis of biodiversity (based on the analysis of taxa) was conducted using five indexes: Margalef index (Mg), Shannon diversity index (H), which is used to determine the species richness of the communities. Moreover, Shannon evenness index (E), and Berger-Parker index (d) were used as well. The dominance of a single taxon was analysed with Simpson index (D) (Magurran 1988). The number of obtained sequences in the studied sample was treated as the abundance of organisms.

Results

The number of obtained isolates (as OTU) was 835 206, of which the number of fungal isolates was 683 095 (81.79%). A total of 8 591 taxa belonging to Kingdom Fungi were identified. The detected taxa belonged to the following phyla: Ascomycota (18.474–28.452%), Basidiomycota (19.894–29.659%), Mucoromycota (10.584–18.533%), Chytridiomycota (0.366–0.666%), Glomeromycota (0.357–0.785%) Rozellomycota (0.000–0.028%) and Oomycota (0.072–0.612%; Tab. 2).

The greatest shares in the community of soil fungi were recorded for the following taxa: saprotrophic (9.10–14.11%; Tab. 2): *Mortierella macrocystis* (1.109–5.501%), *Archaeorhizomyces borealis* (0.140–5.080%), *Mortierella* sp. (3.149–4.448%), *Ramariopsis flavescens* (0.305–3.418%), *Mortierella humilis* (0.539–2.607%), *Saitozyma podzolica* (1.111–2.320%), *Mortierella longigemmata* (0.034–2.049%), *Pseudogymnoascus* sp. (0.104–1.939%), *Mortierella hyalina* (0.012–1.214%), *Ceriporiopsis mucida* (0.000–1.035%), mycorrhizal fungi (6.39–24.18%; Tab. 2): *Russula amoenolens* (0.006–11.903%), *Hydnum repandum* (0.129–6.597%), *Russula emetica* (0.000–4.363%), *Cenococcum geophilum* (0.012–2.809%), *Russula puellaris* (0.178–4.249%) and *Russula nigricans* (0.002–1.400%). The mycorrhizal fungi were dominated by those from the Basidiomycota phylum (71.07–86.47%), with the share of this group being higher in the samples from the old trees. The share of Glomeromycota among mycorrhizal fungi ranged from 1.8% to 7.81%.

The presence of pathogens (5.37–10.93%; Tab. 2) was also detected, whose share in the investigated community was lower than saprotrophic and mycorrhizal fungi. Pathogens including *Ilyonectria destructans* (0.471–1–3.77%), *Cadophora* sp. (0.006–2.427%) and *Puccinia* sp. (0.282–1.350%), as well as antagonists in relation to pathogens of forest trees: *Solicoccozyma terricola* (0.726–7.666%), *Aspergillus inflatus* (0.500–1.984%), *Solicoccozyma terrea* (0.124–1.745%), *Penicillium citreonigrum* (0.003–1.373%) and other fungi, for example *Basidiobolus ranarum* (0.012–1.821%).

Similar to the case of Shannon's diversity index (H), higher values of the indices of Shannon's evenness index E were obtained for the fungal community of natural regeneration. In turn, Simpson's index expresses the probability of finding two specimens belonging to the same species in a random variant. The highest probability was recorded for the fungal community in soil in simple 6G (under old-stands canopy), while it was lowest in simple 1G (natural regeneration). Lower values of this index were obtained for the fungal community of natural regeneration compared to that of soil under old-stands canopy. The highest values of the dominance index were obtained for the fungal community in soil

Table 2. Frequency of taxa in the fungal community of the rhizosphere of the western red cedar, the share of which in the community exceeded 0.1%. Natural regeneration – 1G, 2G, 5G, under old stands canopy – 3G, 4G, 6G. Taxa, the share of which in the genus was dominant are given in bold. M – mycorrhizal fungus. A – antagonist forest tree pathogens. e.g. *Heterobasidion* or *Armillaria*. S – saprotroph, Y – yeast, P – pathogen, U – unknown

Taxa	Trophic group	Order	Samples					
			1G	2G	3G	4G	5G	6G
1	2	3	4	5	6	7	8	9
Archaeorhizomyces borealis Menkis. T.Y. James & Rosling	M	Archaeorhizo-mycetales	0.38	0.69	0.21	0.28	0.14	5.08
Arthroderma multifidum C.O. Dawson	S	Onygenales	0.72	0.14	0.19	0.09	0.10	0.04
Aspergillus inflatus (Stolk & Malla) Samson. Frisvad. Varga. Visagie & Houbraken	A	Eurotiales	0.98	1.40	1.98	0.79	0.50	1.67
Cadophora sp.	P	Helotiales	0.72	1.17	2.43	0.90	0.56	0.01
Cenococcum geophilum Fr.	M	Hysteriales	0.37	0.99	0.68	2.81	0.78	0.01
Ilyonectria destructans (Zinssm.) Rossman. L. Lombard & Crous	P	Hypocreales	0.75	1.01	1.38	0.63	1.37	0.47
Infundibulomyces cupulatus Plaingam. Somrith. & E.B.G. Jones	S	Incertae sedis	0.07	0.24	0.52	0.15	0.09	0.00
Leptodontidium sp.	S	Helotiales	0.01	0.00	0.00	0.01	0.01	0.56
Penicillium citreonigrum Dierckx	A	Eurotiales	0.78	1.37	0.40	0.89	0.41	0.00
Penicillium daleae K.W. Zaleski	A	Eurotiales	0.94	0.57	1.02	0.34	0.60	0.35
Penicillium nodositatum Valla	A	Eurotiales	0.09	0.26	0.69	0.06	0.11	0.01
Penicillium sp.	A	Eurotiales	0.52	0.55	0.45	0.43	0.58	0.08
Periconia pseudodigitata Kaz. Tanaka & K. Hiray.	S	Pleosporales	0.58	0.79	0.70	0.70	0.79	0.50
Phialocephala fortinii C.J.K. Wang & H.E. Wilcox	P	heliotiales	0.25	0.40	0.60	0.45	0.46	0.54
Pseudocamaropycnis pini Crous	S	Mytilinidiales	0.02	0.09	0.07	0.24	0.66	0.00
Pseudogymnoascus pannorum (Link) Minnis & D.L. Lindner	S	Incertae sedis	0.57	0.37	0.39	0.49	0.30	0.47
Pseudogymnoascus sp.	S	Incertae sedis	0.71	0.78	1.94	0.24	0.35	0.10
Trichocladium sp.	A	Sordariales	0.07	0.16	0.26	0.19	0.60	0.73
Frequency of Ascomycota			20.74	23.69	24.56	18.47	20.07	28.45
Aecidium sp.	P	Pucciniales	0.18	0.25	0.61	0.11	0.11	0.05
Ceriporiopsis mucida (Pers.) Gilb. & Ryvarden	S	Polyporales	0.05	0.14	0.11	0.29	1.04	0.00
Derxomyces wuzhishanensis F.Y. Bai & Q.M. Wang	U	Tremellales	0.09	0.21	0.06	0.16	0.09	0.58
Galerina unicolor (Vahl) Singer	S	Agaricales	0.07	0.26	0.70	0.07	0.09	0.00
Ganoderma sp.	P/S	Polyporales	0.37	0.38	0.58	0.31	0.35	0.08
Geminibasidium hirsutum H.D.T. Nguyen. N.L. Nick. & Seifert	U	Geminibasi-diales	0.17	0.17	0.12	0.17	0.47	0.94
Grammatus semis (Spirin & Malysheva) H.S. Yuan & Decock	S	Auriculariales	0.10	0.25	0.19	0.79	0.20	0.00
Hydnum repandum L.	M	Cantharellales	3.32	6.60	1.84	1.74	4.44	0.13
Hydnum vagabundum Swenie. Ovrebo & Matheny	M	Cantharellales	0.21	0.52	0.41	0.20	0.56	0.00
Minimedusa polyspora (Hotson) Weresub & P.M. LeClair	A	Cantharellales	1.30	0.28	0.09	0.29	0.17	0.00

1	2	3	4	5	6	7	8	9
Oxyporus schizoporoides Zmitr. & Spirin	P	Incertae sedis	0.03	0.07	0.07	0.18	0.05	1.45
Puccinia sp.	P	Pucciniales	0.28	0.69	1.33	0.35	0.85	1.35
Ramariopsis crocea (Pers.) Corner	S	Agaricales	0.05	0.18	0.16	0.29	0.94	0.40
Ramariopsis flavescens R.H. Petersen	S	Agaricales	0.37	1.30	3.42	0.30	0.45	0.45
Russula amoenolens Romagn.	M	Russulales	0.01	0.02	0.01	0.07	0.01	11.90
Russula emetica (Schaeff.) Pers.	M	Russulales	0.47	1.40	0.98	4.36	1.03	0.00
Russula nigricans Fr.	M	Russulales	0.15	0.41	0.31	1.40	0.33	0.00
Russula puellaris Fr.	M	Russulales	1.85	4.25	0.69	0.18	0.56	1.32
Russula turci Bres.	M	Russulales	0.01	0.00	0.01	0.17	0.73	0.00
Saitozyma podzolica (Babeva & Reshetova) Xin Zhan Liu. F.Y. Bai. M. Groenew. & Boekhout	Y	Tremellales	1.52	1.38	1.37	1.23	2.32	1.11
Solicoccozyma terrea (Di Menna) Yurkov	A	Filobasidiales	0.20	0.59	1.74	0.19	0.33	0.12
Solicoccozyma terricola (T.A. Pedersen) Yurkov	A	Filobasidiales	1.78	3.20	7.67	1.25	1.77	0.73
Trechispora invisitata (H.S. Jacks.) Liberta	S	Trechisporales	0.67	0.12	0.06	0.14	0.09	0.00
Tylospora asterophora (Bonord.) Donk	M	Atheliales	0.01	0.00	0.00	0.17	0.95	0.00
Frequency of Basidiomycota			19.89	29.66	29.07	25.21	25.14	24.89
Frequency of Chytridiomycota			0.46	0.40	0.67	0.37	0.50	0.46
Frequency of Glomeromycota			0.37	0.38	0.50	0.78	0.43	0.36
Frequency of Rozellomycota			0.02	0.03	0.00	0.01	0.02	0.00
Basidiobolus ranarum Eidam.	S	Basidiobolales	1.82	0.40	0.18	0.45	1.01	0.01
Mortierella epicladia W. Gams & Emden	A	Mortierellales	0.54	0.16	0.07	0.36	0.23	0.00
Mortierella gamsii Milko	A	Mortierellales	0.54	0.18	0.10	0.28	0.14	0.02
Mortierella humilis Linnem.	A	Mortierellales	2.61	1.77	1.50	1.52	2.18	0.54
Mortierella hyalina (Harz) W. Gams	A	Mortierellales	1.21	0.20	0.11	0.06	0.13	0.01
Mortierella longigemmata Linnem.	A	Mortierellales	2.05	0.29	0.11	0.08	0.19	0.03
Mortierella macrocystis W. Gams	A	Mortierellales	1.13	2.24	1.11	2.11	5.50	1.56
Mortierella sclerotiella Milko	A	Mortierellales	0.85	0.21	0.18	0.06	0.17	0.00
Mortierella sp.	A	Mortierellales	3.80	3.66	4.45	4.20	3.15	4.39
Schizangiella serpentis J. Dwyer. B. Burwell. Humber. C. Mcleod. M. Fleetwood & T. Johnson bis	P	Basidiobolales	0.75	0.19	0.12	0.15	0.28	0.13
Umbelopsis changbaiensis Y.N. Wang. X.Y. Liu & R.Y. Zheng	S	Mucorales	0.19	0.27	0.54	0.14	0.22	0.11
Umbelopsis dimorpha Mahoney & W. Gams	S	Mucorales	0.16	0.24	0.19	0.14	0.35	0.68
Umbelopsis isabellina (Oudem.) W. Gams	S	Mucorales	0.10	0.17	0.25	0.09	0.18	0.83
Frequency of Zygomycota			18.53	12.26	11.03	11.88	17.12	10.58
Frequency of Oomycota			0.61	0.35	0.45	0.34	0.35	0.07
Uncultured Fungi			16.64	18.60	17.31	29.41	15.39	17.45
No sequence in the UNITE database			15.33	9.97	10.77	8.96	15.64	11.61

in 3G (soil under old-stands canopy), while they were lowest, too, in soil under old-stands canopy in simple 6G (Tab. 3).

Thuja plicata in the examined stands was over a hundred years old. The description of the tested surfaces is included in Table 4.

Table 3. Biodiversity of fungal communities determined based on the biodiversity indices in individual variants of the experiment. The lowest values are marked with light grey colour, and the highest with dark grey colour

Index	Samples					
	natural regeneration			under old stands canopy		
	1G	2G	5G	3G	4G	6G
D-Mg	221.862	227.483	259.862	199.153	215.932	77.592
Shannon`s diversity -H	4.723	4.445	4.711	4.364	4.550	0.667
Shannon`s evenness -E	0.603	0.565	0.589	0.566	0.584	0.096
Simpson	0.019	0.029	0.023	0.031	0.027	0.063
Berger-Parker Dominance	0.063	0.099	0.086	0.116	0.076	0.021

Table 4. Western red cedar experimental plots – yield characteristics of the stands

Experimental plots	Area [ha]	Site	Age [years]	Site height [m]	N [trees/ha]	H100 [m]	D100 [cm]	HG [m]	DG [cm]	G [m²/ha]	VD [m³/ha]
238c/1	0.10	LMśw	110	24.4	310	27.3	50.9	26.5	42.1	43.24	414.9
238c/2	0.10	LMśw	110	25.0	440	27.8	55.4	27.1	44.3	67.69	654.9
217s/4	0.08	Lśw	106	26.4	612	31.6	55.7	29.0	39.0	73.32	778.2

Note: Site height – according to the growth model for Norway spruce (Wenk et al. 1984), M-System; N – number of stems per ha; H100 – top height; D100 – top diameter; HG – mean height; DG – mean diameter; G – basal area per ha; VD – volume of the wood of an over-bark diameter ≥7 cm (standing crop)

Discussion

Few studies of *T. plicata* are concerned with fungi communities (Weber et al. 2005; Lim et al. 2007; Fodor and Hâruta 2014; Adnan et al. 2018). Our analyses are the first research in Poland concerned with the communities of soil fungi associated with the western red cedar growing in forests; however, other fungi colonising the trees of this species have been checked (Dominik and Grzywacz 1998; Damszel et al. 2020). Identification showed, according to the assumption that saprotrophs and mycorrhizal fungi predominated in the association. Basidiomycota taxa did not dominate in every fungal community in the soil. However, in most trials, the Basidiomycota group's fungi were dominant, confirmed by Behnke-Borowczyk et al. (2020).

Hydnum repandum is an ectomycorrhizal (ECM) fungus associated with various species of Pinaceae and Fagaceae (Agerer 2006; Tedersoo et al. 2010; Feng et al. 2016). It is widely distributed in Europe, but has also been recorded in North America (Swenie et. al. 2018). Its proportion in the community associated with the natural regeneration of western red cedar was higher than under the old-growth forest. Introduced tree species can form mycorrhizae with cosmopolitan fungal taxa (found both in the natural range of the tree species as well as at the site of its introduction) and taxa foreign to them (Pringle et al. 2009). Hence, the formation of non-specific mycorrhizae can enhance the potential for natural regeneration and affect the ability of mycorrhizae to establish themselves in a new area of occurrence. A similar role in the fungal community could be played by the mycorrhizal fungi *R. amoenolens* and *R. puellaris*, which also forms non-specific mycorrhizae (Horton and Bruns 1998; Kaur et al. 2011; Agerer and Rambold 2004–2014).

Pestalotiopsis sp. (Jarecka-Boncela et al. 2019) was considered the main perpetrator of the dieback of the *Thuja* in nurseries in Central Poland. Two species belonging to this genus were identified in the studied community*: P. chamaeropis* and *P. hollandica*, the share of these pathogens in the studied community was not relevant (less than 0.03%). Share of *Phytophthora* sp. species reported as a factor limiting tree cultivation (Webber et al. 2010) and occasional infestation of *T. plicata* (Green and Webber 2012) was also slightly below

(0.01). Two species were identified: *P. infestans* and *P. melonis*. Moreover, fungi from the genus *Chalara* were also detected, at present being some of the most common pathogens in the soil (Werner 2012; Lazreg et al. 2014; Theelen et al. 2018; Orr and Nelson 2018). Additionally, similar to Damszel et al. (2021), pathogens from the genera *Heterobasidion* and *Armillaria* were reported. Despite numerous *Heterobasidion* fruiting bodies observed in the root collar area, its share was below 0.1%.

Moreover, analyses identified cosmopolitan fungi from the genera *Trichoderma* and *Penicillium*, which are antagonists of the pathogens mentioned earlier (Behnke-Borowczyk and Kwaśna 2010; Grantina-Ievina et al. 2013).

Identification of the fungal community to the level of taxa needs to be the first step in inventory works, which results at the successive stage of the study will provide information on their role in a given community (Frąc et al. 2018). Inventory works do not yield a comprehensive answer to whether the identified microorganisms are viable and active (Blagodatskaya and Kuzyakov 2013), and they also fail to describe their functions (Prosser 2015).

Many fungal species are distinguished by phenotypic plasticity, and depending on the conditions, they adopt various life strategies ranging from saprotrophs to endophytes, while both these functions in the community do not have to be mutually exclusive (Vasiliauskas et al. 2007; Jaber et al. 2014). Some taxa were identified, whose function to date has not been fully identified, for example *Periconia pseudodigitata*, *Geminibasidium donianum* and *Muscinupta laevis*. Because of the information mentioned earlier, the results of our study may be treated as an introduction to further research on communities of soil fungi associated with the western red cedar, and it should be continued. Further research should consider different locations, different age, different habitats and comparison with native tree species.

Although taxa belonging to Glomeromycota did not have any significant share in a given community (accounted for less than 1%), they still need to be focused on. Fungi from that phylum are able to form arbuscular mycorrhizae (AM), entering into symbiotic associations with 70–90% terrestrial plant species (Parniske et al. 2008). Among them, *Acaulospora* spp. was detected (0.114%). *Acaulospora trappei* is a fungus-forming mycorrhizae with the western red cedar found within its natural range (Kough et al. 1985). Moreover, *Acaulospora polonica* was described in 1988 as a fungus-forming mycorrhizae with *Thuja occidentalis* (Blaszkowski 1988). Owing to the identification of many fungal taxa including *Acaulospora* spp., in order to obtain more specific data the genus-level research needs to be continued.

Weber et al. (2005) showed that in North Vancouver, Canada, where the western red cedar grows, the withdrawal might be a consequence of the soil's low inoculum content of arbuscular fungi. These fungi also affect the growth rate and growth of these trees. Their share depends on the amount of insolation (Weber et al. 2005). Therefore, for better host development, it should be cared for. In fact, a more significant share of AM was recorded in the case of two samples collected from an old forest, where more light reached the soil than in the place of vital natural regeneration. In the northern hemisphere, arbuscular mycorrhizal plants dominate in relatively mild climates and low phosphorus soils, whereas ectomycorrhizal plants dominate in colder climates and soils of high organic matter and low nitrogen (Allen et al. 1995). The western red cedar forms obligatory relationships with AM (Curran and Dunsworth 1988). It has responded more positively to AM inoculation than other closely related tree species (Kough et al. 1985). Therefore, it is likely that the settlement of the western red cedar is severely limited, where there is no compatible inoculum AM (Weber et al. 2005). Despite the dominance of ectomycorrhizal fungi over arbuscular ones, quite impressive natural regeneration was observed in *T. plicata* stands in Kościan. Subsequent research should take into account tree stands in Poland in which natural regeneration does not occur, or occurs sporadically.

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