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REGULAR ARTICLE

The communities of ectomycorrhizal fungal species associated with *Betula pendula* ROTH and *Pinus sylvestris* L. growing in heavy-metal contaminated soils

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Abstract

Aims Pioneer tree species such as *Betula pendula* and *Pinus sylvestris* encroach soils contaminated with heavy metals (HMs). This is facilitated by ectomycorrhizal fungi colonizing tree roots. Thus, we evaluated the ectomycorrhizal fungal (EMF) communities of *B. pendula* and *P. sylvestris* growing in HM-contaminated soils compared to non-contaminated soils. We also studied the effect of HMs and soil properties on EMF communities and soil fungal biomass.

Methods Roots of *B. pendula* and *P. sylvestris* were collected from three HM-contaminated sites and from two non-contaminated sites located in Poland. EMF species were identified using DNA barcoding. Soil fungal biomass was determined by soil ergosterol.

Results B. pendula and *P. sylvestris* growing in HM-contaminated soils had similar EMF communities, where *Scleroderma*, *Rhizopogon* and *Russula* as well as ectomycorrhizae of the long-distance exploration type dominated. Among all of the examined soil factors studied, toxicity index (TI_{Total}) was the most significant factor shaping the composition of EMF communities. Despite significant differences in the structure of the EMF communities of trees growing in HM-contaminated sites compared to control sites, no differences in overall diversity were observed.

Conclusions Only well-adapted EMF species can survive toxic conditions and form ectomycorrhizal symbiosis with encroaching trees facilitating the forest succession on contaminated soils.

Keywords Ectomycorrhiza · Next generation sequencing (NGS) · Heavy metal pollution · Pioneer tree species · Silver birch · Scots pine

Abbreviations

ROS	Reactive Oxygen Species
HM	Heavy Metal
GSH	Glutathione
MTs	Metallothioneins
EMF	Ectomycorrhizal Fungi
ECM	Ectomycorhiza
OTU	Operational Taxonomic Unit
HPLC	High-Performance Liquid Chromatography
DAD	Diode-Array Detector

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TI	Toxicity Index
NMDS	Non-metric Multidimensional Scaling
CCA	Canonical Correspondence Analysis

Introduction

The southern part of Poland has been a centre of the lead and zinc industry since the Middle Ages (Grodzińska et al. 2000). This region has been ranked as one of the most polluted regions in Europe (Piekut et al. 2019). Several smelters, associated industrial plants, and ore mines are still in use resulting in the accumulation of high levels of toxic heavy metals (HMs) in soils, particularly in the upper soil layers (Augustyniak et al. 2014), where the highest density of fine root (Jackson et al. 1996) and ectomycorrhizal root tips (Rosling et al. 2003) occurs.

The uptake and accumulation of heavy metals in plant tissues causes various adverse morphological, physiological and biochemical responses (Doğanlar and Atmaca 2011). HMs induce the production of reactive oxygen species (ROS) which hinder cell metabolism and have multiple toxic effects, including lipid peroxidation and damage to proteins and DNA (Pongrac et al. 2009). HMs also block and displace essential functional groups in biomolecules (Schützendübel and Polle 2002). As a consequence, HMs can inhibit photosynthesis and diminish water and nutrient uptake, resulting in chlorosis, growth inhibition, browning of root tips, and eventual plant death (Seregin and Ivanov 2001).

Several pioneer tree species occur naturally or are intentionally introduced in HMs polluted areas. The most common trees in polluted soils in Eastern Europe are silver birch (*Betula pendula* Roth.) (Prach and Pyšek 2001) and Scots pine (*Pinus sylvestris* L.) (Picon-Cochard et al. 2006). Long-lived organisms such as trees can overcome high (i.e. toxic) concentrations of HMs in soils through resistance (sensu Levitt 1980) mechanisms including ectomycorrhizal symbiosis (Wilkinson and Dickinson 1995). Jourand et al. (2010) and Gonçalves et al. (2009) postulated that HM-resistant EMF ecotypes strongly enhance the growth of host plants on nickel-contaminated areas. Elevated concentrations of HMs in ectomycorrhizal root tips indicate that the fungal mantle is an effective barrier that prevents transport of these elements to roots of higher branch order, shoots, and leaves (Leyval et al. 1997; Turnau et al. 2002). For example, Blaudez et al. (2000) stated that binding Cd in the cell walls of fungal hyphae and HM accumulation in the vacuolar compartment of fungal cells should be considered as two essential HM-detoxification mechanisms in ectomycorrhizal fungi (EMF). Courbot et al. (2004) reported an increase in glutathione (GSH) concentration in *Paxillus involutus* mycelia growing under Cd-stress conditions as glutathione can form highly stable complexes with Cd and other thiophilic HMs such as Hg, Cu, and Zn (Rubino 2015). Additionally, metallothioneins (MTs), cysteine-rich heavy metal-binding peptides, have also been found in EMF tissues (Courbot et al. 2004; Ramesh et al. 2009). Moreover, ectomycorrhizal fungi can secrete several compounds into the soil that can immobilize HMs outside the mycelium (Ahonen-Jonnarth et al. 2000). Although EMFs possess many mechanisms responsible for HM resistance, Pennanen et al. (1996) observed a reduction in the fungal biomass in HM-contaminated soils.

Several studies have investigated the EMF communities occurring in HM-contaminated sites (Huang et al. 2012; Krpata et al. 2008; López-García et al. 2018; Regvar et al. 2010) so far. However, none of these studies has compared the EMF species communities and associated fungal biomass between two naturally growing tree species. Therefore, this study characterized and compared the EMF communities of *Betula pendula* (silver birch) and *Pinus sylvestris* (Scots pine) growing around zinc-lead smelters and compared them with non-contaminated (control) sites. Additionally, the effects of HMs and associated soil properties on EMF communities and soil fungal biomass were also investigated.

We hypothesized that EMF communities associated with *B. pendula* and *P. sylvestris* growing in HM-contaminated soils should be more similar to each other than EMF communities associated with the same tree species growing in non-contaminated areas. The expected similarity of EMF communities of silver birch and Scots pine growing in HM-contaminated sites would be due to an increased contribution of specific, probably HM-resistant, EMF species.

Material and methods

Study sites

The study was carried out in three HM-contaminated sites located in the close vicinity of large non-ferrous

metal smelters in southern Poland (Fig. 1): Szopienice (50° 15′ 46”N, 19° 04′ 23″E; 256 m asl), Miasteczko Śląskie (50° 30′ 12”N, 18° 56′ 08″E; 301 m asl), and Bukowno (50° 16′ 22” N, 19° 28′ 31″E; 346 m asl). The area around the Szopienice smelter has been exposed to HM pollution for over 170 years (Augustyniak et al. 2014). Miasteczko Śląskie and Bukowno sites have been under constant and increasing heavy metal pollution since 1967 when both smelters were established (Azarbad et al. 2015). The control sites (non-contaminated with heavy metals) were located in Kórnik (52° 14′ 22”N, 17° 04′ 46″E; 85 m asl), central Poland, and in Złoty Potok (Parkowe Reserve) (50° 42′ 14”N, 19° 25′ 24″E; 316 m asl), southern Poland. We chose these control sites according to their well-known management history and their use in other similar studies (e.g. Łukasik 2006: Rudawska et al. 2011).

Three plots of *B. pendula* and three of *P. sylvestris*, ranging in age from 25 to 35 years old, were established in the Bukowno and Miasteczko Śląskie sites. Two plots of *B. pendula* and two of *P. sylvestris*, ranging in age from 20 to 25 years old were established at the Szopienice site. Similarly, two plots of *B. pendula* and two plots of *P. sylvestris* ranging between 20 and 35 years old were established at each of the control sites, Kórnik and Złoty Potok. Each of the established plots was approximately 100 m^2 and was located from 10 to 300 m from each other. The soils of Miasteczko Śląskie, Kórnik and Złoty Potok are characterised as Podzols (Jaśniska-M’Bodj 2015; Misiorny 2009; Wierzbicki 2012), while soils from Bukowno and Szopienice are described as Technosols (Górniak 2010; Szeremeta 2010).

Sample collection and processing

Five soil samples (approximately 500 g each) were collected randomly in September 2015 and 2016 from each plot at the study sites for a total of 120 samples. Soil samples were collected from a depth of 0–15 cm after removing litter, stored in closed plastic bags, and refrigerated for no longer than 24 h until processed. Two subsamples (100 g) were taken from each of the collected samples, homogenized (mixed and sieved to <2 mm), and either air-dried for chemical analysis or stored at −20 °C for fungal biomass assessment.

Simultaneously, other soil samples (20 cm long × 20 cm wide × 15 cm deep) were collected and stored in sealed plastic bags and placed at −20 °C for ectomycorrhizal evaluation. Roots in thawed samples were gently extracted from the soil with tweezers and washed under tap water over a 1 mm sieve. Tightly adhering materials were removed manually with forceps under a stereomicroscope (Olympus, zoom 0.67–40). The number of healthy-looking ectomycorrhizal root tips of *B. pendula* and *P. sylvestris* were recorded separately. Exploration types of ectomycorrhizae were classified based on Agerer (2001). Ectomycorrhizae of *Russula*, *Lactarius*, and *Hygrophorus*, of which their species-specific information was unavailable, were grouped together and described as a separate category (contact/short/medium-smooth, c/s/m_s).

Approximately 50 g of fresh *B. pendula* leaves and *P. sylvestris* needles were collected from five randomly selected trees three times during the growing season in 2015 and 2016. Leaves and needles were used to assess the degree of plant HM-contamination in each site.

Chemical analyses

Soil pH was measured using a 1:2.5 soil to 1 M KCl solution ratio. Organic matter content expressed as carbon (C) content was measured using the loss of ignition method. Total nitrogen (N) content was determined by the Kjeldahl method. The content of available phosphorus (P) was measured using the Egner-Riehm method. The total concentrations of HMs (Cd, Pb, Zn, Cu, Ni) and Ca were analyzed according to the methodology proposed by Sastre et al. (2002) and was obtained by mineralization of 0.25 g of soil in 8 ml of concentrated HNO_3 and 2 ml of 30% H_2O_2 in a microwave oven (Milestone Ethos One, Italy) at 190 °C. After mineralization, samples were diluted to 25 ml with deionized water and filtered. The fraction of available HMs was obtained by shaking a soil sample (1:10 soil to solution ratio) with 0.01 M $CaCl_2$ for 2 h (Wójcik et al. 2014). The content of HMs was measured using flame absorption spectrometry (Thermo Scientific iCE 3500, USA). All of the analyses included blank samples (distilled water) and certified reference materials (NCS DC 77302, China National Analysis Centre for Iron and Steel) to ensure the quality of the analyses.

Leaves and needles were rinsed three times with distilled water, dried at 105 °C, and homogenized in an agate mill (Retsch, Germany). Powder samples were then divided into three 0.25 g subsamples and each were mineralized in 8 ml of 65% HNO_3 and 2 ml of 30% H_2O_2 in a microwave oven (Milestone Ethos One, Italy)

Fig. 1 General geographical map describing the location of the study sites

at 190 °C. After mineralization, samples were diluted to 25 ml with deionized water and filtered. The HM content in each sample was determined using flame absorption spectrometry (Thermo Scientific iCE 3500, USA). Only Cd, Pb, Zn, and Cu were determined in plant material. Ni was not included because its concentration in leaves and needles was below the detection limit. All of the analysis procedures included blank samples (distilled water) and certified reference material (Oriental Basma Tobacco Leaves [INCT-OBTL-5], Institute of Nuclear Chemistry and Technology, Poland) to ensure the quality of the analyses.

DNA extraction and next generation sequencing

A total of 15,800 root tips collected from *B. pendula* and 9744 from *P. sylvestris* were analyzed (Table S1). Before molecular analysis, ECM root tips were pooled by plot and analyzed separately. Ectomycorrhizal root tips collected from each plot were placed in 2 ml tubes containing Lysing Matrix A (MP Biomedicals, USA). After the addition of 450 μl of ATL lysis buffer (Qiagen, Germany), samples were homogenized three times for 40 s at a speed of 6.5 m s^{-1} using a FastPrep-24 homogenizer (MP Biomedicals). Proteinase K (Bio Basic, Canada) was added to the solution to a final concentration of 0.2 mg ml^{-1} and samples were then incubated for 24–48 h at 56 °C. Subsequently, 100 μl of the lysate from each sample was used to isolate total genomic DNA using the ZR-69 Quick-gDNA MiniPrep Kit (Zymo Research).

The Internal transcribed spacer 1 (ITS1) was PCR-amplified using ITS1 (TCCGTAGGTGAACCTGCGG) and ITS2 (GCTGCGTTCTTCATCGATGC) primers (White et al. 1990) and fused with Ion Torrent double indexed adapters. PCRs were prepared in two technical replicates, each in a reaction volume of 5 μl containing Type-it Microsatellite Kit (Qiagen), 2.5 μM of each primer, and 1 μl of DNA template. The amplification program was as follows: 95 °C for 5 min, followed by 35 cycles of 30 s at 95 °C, 60 s at 50 °C and 30 s at 72 °C with a final extension step at 72 °C for 7 min.

Technical replicates were merged and diluted with 10 μl of deionized water. 5 μl of the diluted PCR mixture was electrophoresed on a 1.5% agarose gel to check amplification efficiency. Then, all amplicons were pooled in equimolar amounts, size-selected on a 3% agarose gel and purified using QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's

protocol. DNA concentration and fragment length distribution of the library was established with the use of a High Sensitivity D1000 Screen Tape assay on 2200 Tape Station system (Life Technologies, USA). Clonal template amplification was performed using the Ion Torrent One Touch System II and the Ion PGM Hi-Q View OT2 Kit (Life Technologies, USA) according to the manufacturer's instructions. The library was sequenced using an Ion PGM Hi-Q View Sequencing Kit, Ion 318 chip and the Ion PGM system (Life Technologies).

Bioinformatics analysis

Sequence reads <200 bp were removed from the dataset in Geneious R11.1.5 (Biomatters Ltd.). Then, the Fastx toolkit (Hannon 2010) was used to extract sequences having a minimum of 50% of bases with a quality score ≥ 25. In Geneious R11.1.5, quality filtered sequences were separated into individual combinations of indices representing each site. Chimeras were removed using two approaches: the default settings in UCHIME version 4.2.40 (Edgar 2016), and the SILVA database for ARB for small subunit ribosomal RNAs version 132 (Glöckner et al. 2017) as implemented in Geneious R11.1.5 (Biomatters Ltd.). Operational taxonomic unit (OTU) clustering was performed at 97% similarity in USEARCH version 11.0.667 (Edgar 2010). Singletons (<5 reads) were removed, and OTUs were then clustered using the –cluster_OTUs algorithm (Edgar 2013). Fungal OTU consensus sequences were compared to the Unite (Kõljalg et al. 2013) and MycoBank (Robert et al. 2013) databases to identify EMF species using a 97% identity threshold (Table S2).

Fungal biomass in soils

Ergosterol concentration was used as an indicator of total living fungal (saprophytic and mycorrhizal) biomass in soils (Olsson et al. 1996). This indicator accounts also for both Ascomycetes and Basidiomycetes (Olsson et al. 2003). Analysis was performed according to the method previously described by Gong et al. (2001) with a few modifications proposed by de Ridder-Duine et al. (2006). One gram of soil and 6 ml of methanol was added to a 20 ml scintillation vial containing 2 g of acid-washed glass beads (1 g of 500 μm diameter and 1 g of 1000 μm diameter) and then intensively shaken for 1 h. Subsequently, a 1.5 ml aliquot from each sample was transferred into a plastic tube and centrifuged for 15 min at 14000 rpm. Ergosterol concentration was measured by HPLC (Merck-Hitachi, Germany) with a LiChrospher® RP-18 HPLC Column (4 × 250 mm) using pure methanol (HPLC grade) as a mobile phase at a flow rate of 1 ml min^{-1}. The DAD detection (Merck-Hitachi, Germany) was set at 282 nm. As a standard ≥95.0% Ergosterol HPLC grade (Sigma-Aldrich) was used.

Statistics

The level of HM contamination was characterized using the toxicity indices for both total (TI_{Total}) and available (TI_{Bio}) concentrations of heavy metals (Stefanowicz et al. 2008):

$$TI = \sum_{i}^{n} (C_i / EC50_i)$$

where C_i is the concentration of heavy metal i in soil and $EC50_i$ is the concentration of that heavy metal causing a 50% reduction in dehydrogenase activity ($EC50_{Cd} = 90$, $EC50_{Cu} = 35$, $EC50_{Ni} = 100$, $EC50_{Pb} = 652$, $EC50_{Zn} = 115$ mg kg^{-1}) according to Welp (1999), where EC (effective concentration) values were estimated with the Levenberg-Marquardt algorithm and a Weibull distribution of data to calculate nonlinear regressions. Experiment was performed with soil material from the plough layer (0–30 cm) of a loess soil (FAO: haplic luvisol), passed through a 2-mm sieve.

Rarefaction curves were generated using PAST ver. 3.25 (Hammer et al. 2001) in order to evaluate the representativeness of our sampling effort. Diversity of EMF communities was expressed using taxa richness, Shannon's, dominance ($D = \Sigma(p_i)^2$, where p_i is the relative number of sequences of taxon i), evenness and Margalef diversity, indices were determined using PAST ver. 3.25 (Hammer et al. 2001). Taxonomic diversity and taxonomic distinctness was analyzed according to Warwick and Clarke (1998), using taxonomic division, class, order and family as group information. The relative abundance (%) of EMF species were calculated based on the number of sequence reads of each particular EMF species out of all sequence reads at each plot, or site, expressed as 100%. These calculations were done separately for silver birch and Scots pine.

The normality of distribution of analyzed data was studied using the Kolomogorov-Smirnov test.

Homogeneity of variance between the studied groups (contaminated vs. non-contaminated sites; *B. pendula* vs. *P. sylvestris*) was analyzed with the Levene test. In the case of a lack of normal distribution of raw data, the Box-Cox transformation was performed. Differences in soil chemical parameters, the EMF diversity indices, and soil ergosterol concentration between both tree species and contaminated vs. non-contaminated sites were analyzed using a one-way ANOVA followed by a Tukey's post-hoc test at $\alpha = 0.05$. To verify the effect of locality a two way nested ANOVA test was performed with factors "tree", "contamination" plus the factor "locality" nested in the factor "tree". The above statistical analyses were performed using Statistica v. 13.0 (Dell Inc.).

Differences among EMF communities were tested using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity index was used to visualize the similarity between the EMF communities. The above multivariate analyses were carried out in PAST ver. 3.25 (Hammer et al. 2001).

Canonical Correspondence Analysis (CCA) was performed to study the relationships between the EMF species as well as exploration types and soil parameters (only variables with Variance Inflation Factor (VIF) > 20 were included). Scaling was based on inter-species distances using a biplot scaling type. The relative importance and statistical significance of each soil parameter in the ordination model was assessed by a forward selection procedure and the Monte Carlo permutation test ($n = 999$ permutations), and only significant parameters were used for the CCA. Analyzed data were standardized (log(n + 1)) prior to analysis. The multivariate analyses were performed using CANOCO ver. 4.5 (ter Braak and Šmilauer 2002).

Results

Soil and leaf/needle chemistry

The soils in the HM contaminated sites ranged from acidic (Miasteczko Śląskie) through neutral (Bukowno) to alkaline (Szopienice), while the soils in the control sites (Kórnik and Złoty Potok) were acidic (Table S3). Soils in the contaminated sites had both a total concentration of HMs and a toxicity index (TI_{Total}) higher than soils in the control sites. Only soils in the Bukowno and Miasteczko Śląskie sites had significantly higher concentrations in both the bioavailable fraction of HMs and the TI_{Bio} than those in the control sites. The concentrations of Cd, Pb, and Zn were 41 to 107, 29 to84, and 38 to173 times higher, respectively, in the contaminated sites then those in the control sites. The concentrations of Cu, Mn, and Ni were 7 to 28, 0.4 to 5 and 2 to 9 times higher, respectively, in the soils of contaminated sites compared to soils from the control sites. Most of the sites were macroelement-poor as evidenced by relatively low C, N and P levels (Table S3). Differences between concentration of HMs in soils from plots of *B. pendula* and *P. sylvestris* were not statistically significant according to nested ANOVA (Table 1). However, there were differences in Cu_{Bio}, C, N, and P concentrations and soil pH. Moreover, nested ANOVA indicated that the concentrations of HMs and other soil properties differed significantly between *B. pendula* and *P. sylvestris* within location.

A strong correlation, ranging from 0.5 to 0.8, was observed between the concentration of heavy metal contaminations in soils and the concentration of heavy metals in leaves/needles (data not shown). Leaves of *B. pendula* and needles of *P. sylvestris* collected in the Miasteczko Śląskie site contained the highest concentration of HMs (Table S4). The lowest concentration of HMs was found in leaves and needles collected from trees in both control sites.

Ectomycorrhizal fungal communities

Analysis of the rarefaction curve showed that the read depth was sufficient to recover all EMF species in the tested sites (Fig. S1). A total of 65 EMF species were identified of which 64 were found in *B. pendula* and 54 in *P. sylvestris* (Table 2). In total, 25 EMF species were found in the contaminated soils, while 49 were identified in the non-contaminated (control) sites. A total of 53 EMF species were members of the Basidiomycota, while 12 were Ascomycota. The greatest number of identified species belonged to the family Russulaceae (17 species in total) with 13 species identified in the contaminated sites and 11 species in the control sites.

Scleroderma citrinum, *Scleroderma* sp.01, Rhizopogon sp.01, *Russula decolorans,* and *Russula depallens* were the most abundant among EMF communities associated with *B. pendula* in contaminated sites, while *R. decolorans* and *Lactarius tabidus* were

Table 1 Results of two-way nested ANOVA; Tree – tree species (*Betula pendula* vs. *Pinus sylvestris*), Contamination – type of site (contaminated vs. non-contaminated), Tree (Locality) – effect of the site nested in tree species (within site effect)

	Tree		Contamination		Tree (Locality)	
	F	*p*	F	*p*	F	*p*
Total metals (mg kg^{-1})						
Cd	0.689	0.410	1642.078	0.000*	12.464	0.000*
Pb	0.697	0.407	2006.752	0.000*	26.829	0.000*
Zn	1.605	0.210	5030.055	0.000*	69.559	0.000*
Cu	0.461	0.500	1040.092	0.000*	18.469	0.000*
Mn	2.087	0.154	33.138	0.000*	70.121	0.000*
Ni	3.965	0.051	742.764	0.000*	23.763	0.000*
TI_{Total}	1.340	0.252	4510.340	0.000*	49.260	0.000*
Bioavailable metals (mg kg^{-1})						
Cd	0.146	0.704	5135.326	0.000*	11.426	0.000*
Pb	0.035	0.853	201.468	0.000*	36.349	0.000*
Zn	3.739	0.058	5684.195	0.000*	30.084	0.000*
Cu	7.681	0.007 *	1.601	0.211	35.754	0.000*
TI_{Bio}	0.526	0.471	5702.127	0.000*	12.698	0.000*
C (%)	5.534	0.022*	22.550	0.000*	18.770	0.000*
N (%)	15.794	0.000*	5.901	0.018*	17.750	0.000*
C/N	4.575	0.037*	13.033	0.001*	48.367	0.000*
P (mg P_2O_5 kg^{-1})	52.021	0.000*	9.741	0.003*	48.141	0.000*
Ca (g kg^{-1})	18.354	0.000*	328.679	0.000*	96.678	0.000*
pH	20.470	0.000*	916.590	0.000*	167.69	0.000*
Ergosterol (μg g^{-1})	1.761	0.190	47.845	0.000*	6.005	0.000*

Significant differences ($p \leq 0.05$) are marked with *

dominant in *B. pendula* growing in the control sites (Table 2). Similarly, *Rhizopogon* sp.01 and *S. citrinum* dominated in *P. sylvestris* growing in the contaminated sites, while *Suillus* sp.01 and *Russula vinosa* dominated in *P. sylvestris* roots growing in the control sites.

A higher EMF richness was found in *B. pendula* and *P. sylvestris* growing in control sites than in the contaminated sites (Table 3). However, Shannon's diversity and dominance indices, did not differ significantly between contaminated and non-contaminated sites for either *B. pendula* ($F = 1.174$, $p = 0.4$ and $F = 1.212$, $p = 0.385$ respectively) and *P. sylvestris* ($F = 1.732$, $p = 0.247$ and $F = 1.186$, $p = 0.395$ respectively). Only the EMF community of *B. pendula* in the Bukowno site had a significantly higher evenness index ($F = 4.337$, $p = 0.04$) and lower Margalef diversity index ($F = 10.437$, $p = 0.004$). Moreover, we have found that taxonomic diversity and taxonomic distinctness did not differ significantly between contaminated and non-contaminated sites for either *B. pendula* ($F = 1.424$, $p = 0.418$ and $F = 1.82$, $p = 0.338$ respectively) and *P. sylvestris* ($F = 0.741$, $p = 0.593$ and $F = 0.862$, $p = 0.531$ respectively) (Table 4).

The NMDS ordination clearly separated the EMF communities of contaminated from non-contaminated sites, irrespective of tree species (Fig. 2). More precisely, EMF communities of *B. pendula* ($F = 1.685$, $p = 0.024$) and *P. sylvestris* ($F = 1.607$, $p = 0.047$) differed significantly between contaminated and non-contaminated sites based on the PERMANOVA test. The EMF communities also differed between *B. pendula* and *P. sylvestris* growing in the control sites ($F = 1.584$, $p = 0.031$), but no significant difference was observed between *B. pendula* and *P. sylvestris* growing in the contaminated sites ($F = 0.919$, $p = 0.528$).

The soil parameters (TI_{Total}, TI_{Bio}, C/N ratio, Ca and P content, pH, and ergosterol concentration) used in the ordination (canonical correspondence analysis, CCA)

Table 2 Relative abundance (%) of ectomycorrhizal fungal species observed in *Betula pendula* and *Pinus sylvestris* from contaminated (Bukowno, Miasteczko Śląskie and Szopienice) and control (Kórnik and Złoty Potok) sites

Species name	Abbreviation	Family	Phylum	Contaminated sites						Control sites			
				Bukowno		Miasteczko Śląskie		Szopienice		Złoty Potok		Kórnik	
				Betula pendula	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*
Acephala macrosclerotiorum	Ace_mac	Vibrisseaceae	Ascomycota				5,1				7,3		
Amanita muscaria	Ama_mus	Amanitaceae	Basidiomycota								1,5		3,3
Amanita sp.	Ama_sp.	Amanitaceae	Basidiomycota								0,1		
Boletus badius	Bole_bad	Boletaceae	Basidiomycota			9,7						0,4	
Boletus edulis	Bole_edu	Boletaceae	Basidiomycota							6,5			0,1
Cenococcum sp.01	Ce_sp.01	Gloniaceae	Ascomycota		4,9	2,8	0,8	0,3	0,3	7,7	5,1	0,2	1,4
Cenococcum sp.02	Ce_sp.02	Gloniaceae	Ascomycota								0,9		
Cenococcum sp.03	Ce_sp.03	Gloniaceae	Ascomycota		8,6					4,0			0,7
Cenococcum sp.04	Ce_sp.04	Gloniaceae	Ascomycota										
Cortinarius coleoptera	Cor_col	Cortinariaceae	Basidiomycota								0,6		
Cortinarius neofurvolaesus	Cor_neo	Cortinariaceae	Basidiomycota		0,5					6,8			
Cortinarius sp.01	Co_sp.01	Cortinariaceae	Basidiomycota									0,1	2,6
Cortinarius sp.02	Co_sp.02	Cortinariaceae	Basidiomycota						1,3				
Cortinarius sp.04	Co_sp.04	Cortinariaceae	Basidiomycota					0,5	0,1				
Cortinarius sp.05	Co_sp.05	Cortinariaceae	Basidiomycota										
Cortinarius sp.06	Co_sp.06	Cortinariaceae	Basidiomycota							0,3			
Genea hispidula	Gen_his	Pyronemataceae	Ascomycota									0,1	
Hygrocybe purpureofolia	Hyg_pur	Hygrophoraceae	Basidiomycota										
Inocybe jacobi	Ino_jac	Inocybaceae	Basidiomycota								0,4		
Inocybe sp.	Ino_sp.	Inocybaceae	Basidiomycota										
Lactarius mammosus	Lac_mam	Russulaceae	Basidiomycota										
Lactarius rufus	Lac_ruf	Russulaceae	Basidiomycota									0,1	3,5
Lactarius sp.	La_sp.	Russulaceae	Basidiomycota		0,2			2,2	0,1	45,0		16,3	

Table 2 (continued)

Species name	Abbreviation	Family	Phylum	Contaminated sites						Control sites			
				Bukowno		Miasteczko Śląskie		Szopienice		Złoty Potok		Kórnik	
				Betula pendula	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*
Lactarius tabidus	Lac_tab	Russulaceae	Basidiomycota										0,1
Piloderma fallax	Pil_fal	Atheliaceae	Basidiomycota							1,2			
Piloderma sp.01	Pi_sp.01	Atheliaceae	Basidiomycota				1,1	0,1					0,1
Rhizopogon roseolus	Rhi_ros	Rhizopogonaceae	Basidiomycota	4,5	60,6	1,1	9,1	67,2	74,2	0,1	0,1		
Rhizopogon sp.01	Rh_sp.01	Rhizopogonaceae	Basidiomycota						0,4				
Russula decolorans	Rus_dec	Russulaceae	Basidiomycota		0,3	1,3	7,8					0,5	5,3
Russula depallens	Rus_dep	Russulaceae	Basidiomycota									0,3	
Russula sp.01	Ru_sp.01	Russulaceae	Basidiomycota		0,1		0,6	0,1	0,9			0,1	0,4
Russula sp.02	Ru_sp.02	Russulaceae	Basidiomycota		0,2	0,8				0,4	0,6		
Russula sp.03	Ru_sp.03	Russulaceae	Basidiomycota				0,7	0,4					
Russula sp.04	Ru_sp.04	Russulaceae	Basidiomycota					1,8	1,7				
Russula sp.05	Ru_sp.05	Russulaceae	Basidiomycota					0,5					
Russula sp.06	Ru_sp.06	Russulaceae	Basidiomycota						0,9				
Russula sp.07	Ru_sp.07	Russulaceae	Basidiomycota										
Russula sp.08	Ru_sp.08	Russulaceae	Basidiomycota	65,3	13,7	5,4	1,6		1,5	20,0	0,2	81,6	4,2
Russula sp.09	Ru_sp.09	Russulaceae	Basidiomycota					8,0	1,1				
Russula velenovskyi	Rus_vel	Russulaceae	Basidiomycota		0,1					0,1	18,1		
Russula versicolor	Rus_ver	Russulaceae	Basidiomycota			15,6	0,6		0,1	41,4	0,1	0,2	0,2
Russula vinosa	Rus_vin	Russulaceae	Basidiomycota	18,9	1,5	9,1	2,5	3,5	0,5	5,1			1,0
Scleroderma citrinum	Scl_cit	Sclerodermataceae	Basidiomycota		5,6				0,4				
Scleroderma sp.01	Sc_sp.01	Sclerodermataceae	Basidiomycota			2,9							
Scleroderma sp.03	Sc_sp.03	Sclerodermataceae	Basidiomycota			66,8	45,8	12,8	16,1		0,1		
Scleroderma sp.04	Sc_sp.04	Sclerodermataceae	Basidiomycota										0,7
Sistotrema sp.	Sis_sp.	Hydnaceae	Basidiomycota					0,2	0,2		5,8		52,8
Suillus bovinus	Sui_bov	Suillaceae	Basidiomycota				8,7			1,7			
	Sui_pse	Suillaceae	Basidiomycota								0,8		6,2

Table 2 (continued)

Species name	Abbreviation	Family	Phylum	Contaminated sites						Control sites			
				Bukowno		Miasteczko Śląskie		Szopienice		Złoty Potok		Kórnik	
				Betula pendula	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*	*Betula pendula*	*Pinus sylvestris*
Suillus pseudobrevipes													
Suillus sp.01	Su_sp.01	Suillaceae	Basidiomycota		1,5								
Suillus sp.02	Su_sp.02	Suillaceae	Basidiomycota				0,1	1,3	0,1		0,3		10,1
Suillus sp.03	Su_sp.03	Suillaceae	Basidiomycota	8,4				0,4					
Tomentella atramentaria	Tom_atr	Thelephoraceae	Basidiomycota								14,0		
Tomentella botryoides	Tom_bot	Thelephoraceae	Basidiomycota							0,2			
Tomentella cinerascens	Tom_cis	Thelephoraceae	Basidiomycota							1,7	0,2	6,4	6,4
Tomentella sp.	Tom_sp.	Thelephoraceae	Basidiomycota		0,2								
Tomentellopsis echinospora	Tom_ech	Thelephoraceae	Basidiomycota	2,8					0,3		0,2		
Tricholoma terreum	Tri_ter	Tricholomataceae	Basidiomycota								0,3		
Tuber anniae	Tub_ann	Tuberaceae	Ascomycota										
Tuber sp.01	Tu_sp.01	Tuberaceae	Ascomycota		1,9								
Tuber sp.02	Tu_sp.02	Tuberaceae	Ascomycota								0,1		0,5
Tuber sp.03	Tu_sp.03	Tuberaceae	Ascomycota								0,3		0,2
Tylospora sp.	Tyl_sp.	Atheliaceae	Ascomycota										0,1
Wilcoxina sp.	Wil_sp.	Pyronemataceae	Ascomycota						0,1	0,1			
Xerocomellus cisalpinus	Xer_cis	Boletaceae	Basidiomycota						0,7				

explained 77.25% of the total EMF variation (Fig. 3). The only significant factor was $TI_{Total,}$ which explained 17.86% ($F = 1.76$, $p = 0.001$) of the variability. The CCA analysis further indicated that *Scleroderma citrinum*, *Scleroderma* sp.01, *Rhizopogon* sp.01, *Rhizopogon roseolus*, *Russula* sp.05, *Russula* sp.06, *Russula* sp.07, *Russula* sp.08, *Cortinarius* sp.06, were associated with high HM concentrations in the soil.

Exploration types of ectomycorrhizae

The composition of the ECM exploration types expressed as their relative abundance (%) varied among sites (Fig. 4). Long-distance exploration type was the most abundant in the contaminated sites (from 70.9% to 92.2%), except *B. pendula* in Bukowno where $c/s/m_s$ exploration type dominated (65.3%). In the control sites, the most abundant were ectomycorrhizae of the $c/s/m_s$ type (from 70.0% to 99.1%) except *P. sylvestris* in Kórnik where long-distance exploration type dominated (72.0%). The first two axes of the CCA ordination based on soil parameters (TI_{Total}, TI_{Bio}, C/N ratio, Ca and P content, pH, and ergosterol concentration) and ECM exploration types explained 93.66% of the species variance (Fig. 5). The only significant soil factors were $TI_{Total,}$ which explained 54.81% ($F = 9.80$, $p = 0.002$) of the variability, and the P content, which explained 17.05% ($F = 4.18$, $p = 0.020$). The relative abundance of long-distance type was correlated with the TI_{Total}, soil pH and Ca concentration.

Soil fungal biomass

CCA analysis (Fig. 3) indicated that the concentration of ergosterol was negatively correlated with TI_{Bio} ($R = -0.73$). However, no clear evidence was obtained, that indicated that the concentration of ergosterol was significantly lower in contaminated sites than in control sites (Fig. S2). Only in the case of *B. pendula* in Bukowno and *P. sylvestris* in Miasteczko Śląskie was the concentration of ergosterol significantly lower than in the other sites ($F = 9.222$, $p < 0.000$ and $F = 18.132$; $p < 0.000$, respectively).

Discussion

To the best of our knowledge, this is the first study that has comprehensively examined the effect of heavy metals (as measured by toxicity index, TI) and associated soil properties on the composition of fungal communities associated with naturally established, pioneer *B. pendula* and *P. sylvestris* trees. Toxicity index was used because it most accurately reflects the combined effect of multiple HMs present in different proportions on soil microorganisms (Deng et al. 2015). In our study TI coefficients well reflected the influence of heavy metals on microorganisms. The results showed a statistically significant negative correlation between dehydrogenase activity and TI_{Total} ($R = -0.534$, $p < 0.05$) and TI_{Bio} ($R = -0.478$, $p < 0.05$) (data unpublished). This is also confirmed by Stefanowicz et al. (2008), Azarbad et al. (2015) in studies conducted on similar sites.

The availability of HMs to microorganisms and higher plant species depends to a large extent on the soil properties and in particular on soil pH (e.g. Bąba et al. 2016). As the alkalinity of soil increases, the mobility and thus bioavailability of HMs decreases due to increased precipitation and displacement from the sorption complex by alkaline ions e. g. Ca and Mg (e.g. Ragnarsdottir and Hawkins 2006). For this reason, relatively high soil pH (7.5) at the HM contaminated Szopienice site resulted in TI_{Bio} being comparable to that of the control sites. Nested ANOVA indicated that locality had a significant influence on differences of soil properties between *B. pendula* and *P. sylvestris*, which suggests that our results may be to some extent dependent on site selection.

The TI_{Total} coefficient was found to be the most important determinant shaping EMF communities. However, other factors, like soil pH, may also have an influence on EMF communities. Trees growing at the contaminated sites have been constantly exposed to high concentrations of HMs for at least 20 years. Although an overall decline in EMF diversity was not observed (Table 3), significant differences in the structure of EMF communities between HM contaminated and control sites were found irrespective of the tree species, while such differences were evident when these tree species were growing in the control (non-contaminated) sites as shown by the PERMANOVA test. The similarity of EMF communities of *B. pendula* and *P. sylvestris* growing in the contaminated sites can be associated with the adaptation of some EMF species to high concentrations of HMs in soil (Colpaert et al. 2011). Other researchers postulate that sharing the same soil conditions also contributes significantly to the establishment of similar EMF species communities (Cline

Table 3 Means (± SE) of species richness, dominance, Shannon-diversity, evenness and Margalef diversity indices of EMF communities associated with *Betula pendula* and *Pinus sylvestris* at contaminated (Bukowno, Miasteczko Śląskie, and Szopienice) and non-contaminated (Kórnik and Złoty Potok) sites; values within each column followed by the same letter are not significantly different according to Tukey's test at the $\alpha = 0.05$ significance level

Site	Species richness	Dominance	Shannon diversity	Evenness	Margalef diversity
Betula pendula					
Bukowno	5	0.55 (0.10) a	0.78 (0.19) a	0.76 (0.01) a	0.32 (0.07) a
Miasteczko Śląskie	9	0.47 (0.03) a	1.06 (0.06) a	0.55 (0.10) b	0.81 (0.10) ab
Szopienice	17	0.23 (0.02) a	1.73 (0.07) a	0.49 (0.11) b	1.45 (0.12) b
Kórnik	17	0.63 (0.36) a	0.85 (0.78) a	0.25 (0.14) b	1.05 (0.21) ab
Złoty Potok	16	0.43 (0.20) a	1.26 (0.44) a	0.32 (0.10) b	1.39 (0.30) b
Pinus sylvestris					
Bukowno	16	0.52 (0.11) a	0.94 (0.17) a	0.38 (0.11) a	0.86 (0.17) a
Miasteczko Śląskie	14	0.58 (0.16) a	0.83 (0.31) a	0.57 (0.13) a	0.61 (0.29) a
Szopienice	17	0.58 (0.29) a	0.93 (0.52) a	0.41 (0.30) a	1.22 (0.27) a
Kórnik	20	0.35 (0.16) a	1.51 (0.39) a	0.39 (0.17) a	1.55 (0.01) a
Złoty Potok	28	0.23 (0.05) a	1.82 (0.20) a	0.38 (0.12) a	1.79 (0.08) a

et al. 2005; Trocha et al. 2012) as the soil is a strong filter for ECM fungi as proposed by Jumpponen and Egerton-Warburton (2005). This may also explain the differences between EMF communities observed in both *B. pendula* and *P. sylvestris* growing in contaminated compared with control sites. In contrast to our results, Huang et al. (2014) reported a low similarity in the EMF communities of *Pinus massoniana* and *Quercus fabri* growing in Mn contaminated mine wasteland, which can be explained by host identity, one of the strongest factors shaping EMF communities (Ishida et al. 2007). More studies comparing EMF communities associated with different tree species on HM contaminated sites are needed to better understand the impact of soil factors as opposed to tree identity.

Table 4 Means (± SE) of taxonomic diversity and taxonomic distinctness of EMF communities associated with *Betula pendula* and *Pinus sylvestris* at contaminated (Bukowno, Miasteczko Śląskie, and Szopienice) and non-contaminated (Kórnik and Złoty Potok) sites; values within each column followed by the same letter are not significantly different according to Tukey's test at the $\alpha = 0.05$ significance level

Site	Taxonomic diversity	Taxonomic distinctness
Betula pendula		
Bukowno	1.44 (0.40) a	3.19 (0.21) a
Miasteczko Śląskie	1.39 (0.16) a	2.42 (0.06) a
Szopienice	1.44 (0.52) a	2.47 (0.07) a
Kórnik	2.24 (0.92) a	3.16 (0.93) a
Złoty Potok	0.31 (0.26) a	1.99 (0.76) a
Pinus sylvestris		
Bukowno	1.56 (0.50) a	3.14 (0.30) a
Miasteczko Śląskie	1.31 (0.19) a	2.43 (0.33) a
Szopienice	1.67 (0.78) a	2.86 (0.57) a
Kórnik	2.54 (0.16) a	3.23 (0.05) a
Złoty Potok	1.75 (0.82) a	2.46 (0.68) a

No significant differences were observed in the diversity of EMF communities between contaminated and non-contaminated soils in either *B. pendula* or *P. sylvestris*. Results of similar studies in sites contaminated with HMs are inconclusive. Some studies, like ours, indicate that there is no reduction in biodiversity of EMF communities. For example Hrynkiewicz et al. (2008) found that naturally established *Salix caprea* trees on former ore-bearing sites rich in Zn and Cu had EMF communities with a similar level of biodiversity as the fungal communities of *S. caprea* trees growing in soils not contaminated with HMs. Additionally, no significant changes in the diversity and richness of EMF communities associated with *Pinus massoniana* were observed at Pb-Zn mine sites in central-south China (Huang et al. 2012). Other in situ studies revealed a significant reduction in the biodiversity of ectomycorrhizal fungi. For example, Staudenrausch et al. (2005) reported low diversity in EMF communities

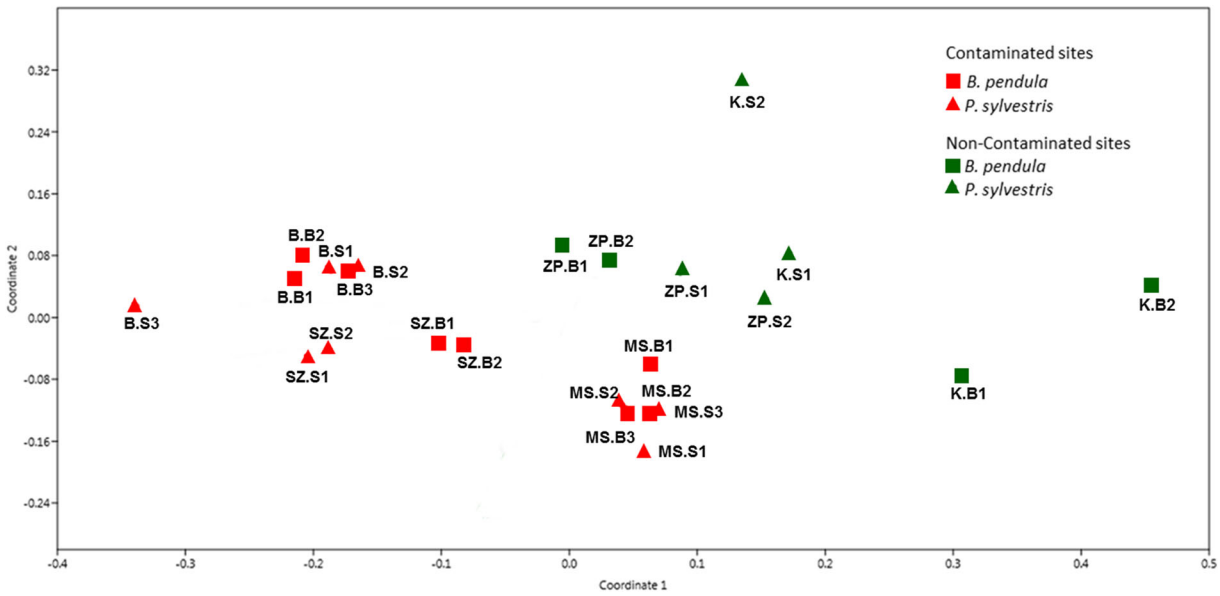

Fig. 2 Non-metric multidimensional scaling (NMDS) of the EMF communities associated with *Betula pendula* (Bp) and *Pinus sylvestris* (Ps) at contaminated (red marks) and non-contaminated (green marks) sites. K – Kórnik; ZP – Złoty Potok; B – Bukowno; MS – Miasteczko Śląskie; SZ – Szopienice

associated with *Betula pendula* growing at a site that was a former bare uranium heap. On the other hand,

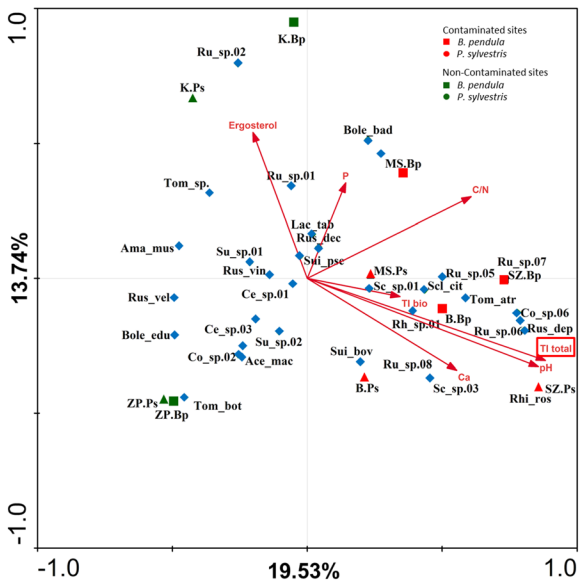

Fig. 3 CCA diagram of EMF species (green diamonds) associated with *Betula pendula* (Bp) and *Pinus sylvestris* (Ps) driven by environmental variables (red arrows). The red box indicates a statistically significant variable. EMF taxa whose relative abundance was <5% were excluded from the analysis. The abbreviations of species names are expanded in Table 2. K – Kórnik; ZP – Złoty Potok; B – Bukowno; MS – Miasteczko Śląskie; SZ – Szopienice

Populus tremula growing around a former Pb/Zn smelter was reported to have EMF communities that were highly diverse ($H' = 2$) (Krpata et al. 2008). We, also postulate, similar to Huang et al. (2014), that some EMF species are well-adapted to high concentrations of HMs in soils and significantly impact the level of diversity of EMF communities in contaminated soils. However, as there is no consistency in the results obtained by different studies, there is an urgent need for further, more comprehensive empirical research.

The EMF communities associated with *B. pendula* and *P. sylvestris* growing in the contaminated sites studied were mainly composed of Russulaceae, *Scleroderma* spp., and *Rhizopogon* sp. (Table 2; Fig. 3). CCA analysis also indicated that the occurrence of some species belonging to the genus *Russula* is associated with high TI_{Total} and high soil pH. Higher relative abundance of *Russula* species may reflect a higher HM resistance threshold of these fungi. *Russula* spp. have the ability to accumulate large amounts of HMs (Borovička and Řanda 2007; Busuioc and Elekes 2013). One of the mechanisms that enables *Russula* fungi to accumulate HMs is the synthesis of proteins similar to metallothioneins (low molecular mass intracellular peptides rich in cysteine that are capable of binding heavy metals) (Coyle et al. 2002) and the cysteine- and histidine-rich proteins (Leonhardt et al.

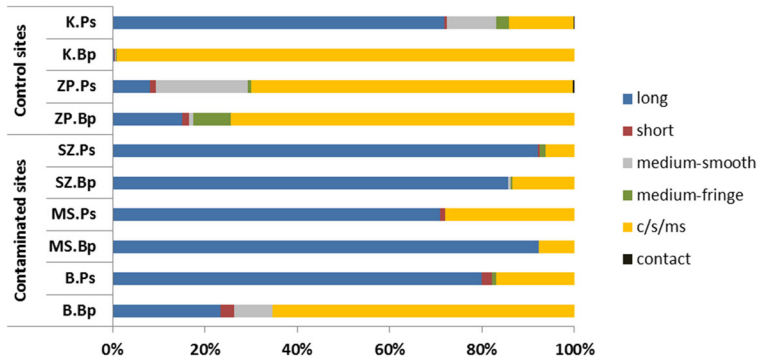

Fig. 4 Relative abundances (%) of different exploration types of ectomycorrhizae collected in the study sites. K – Kórnik; ZP – Złoty Potok; B – Bukowno; MS – Miasteczko Śląskie; SZ – Szopienice; Bp – *Betula pendula*; Ps – *Pinus sylvestris*; c/s/m_s- *Russula*, *Lactarius*, and *Hygrophorus* can be either contact (c), short (s),or medium-smooth (m_s) depending on the species

2014). Karpati et al. (2011), investigating ectomycorrhizae of *Quercus rubra* growing in urban soils, reported that *Russula* species are able to tolerate a wide range of environmental conditions, including high levels of HMs and salts.

Results of our study also indicated *Scleroderma citrinum*, *Scleroderma* sp.01, and *Scleroderma* sp.03 were associated with high levels of HMs (Fig. 3). *Scleroderma* species are ubiquitous in temperate forests and their fruiting bodies are often found in disturbed habitats (Gonzalez-Chavez et al. 2009; Krupa and Kozdrój 2007), including mine tailings containing high concentrations of HMs (Howe et al. 1997). We also observed ectomycorrhizae of *Rhizopogon* spp. abundant on contaminated sites in our study (Table 2). Turnau et al. (1996) demonstrated that *R. roseolus* has several mechanisms to tolerate HMs, such as binding HMs to polyphosphate granules and other amorphous or granular structures containing phosphorus, present in vacuole production of metal-binding pigments. Turnau et al. (1996) suggested that oxalate and carbonate crystals found on the surface of the *R. roseolus* mantle could also bind toxic metals, which was confirmed by Ahonen-Jonnarth et al. (2000).

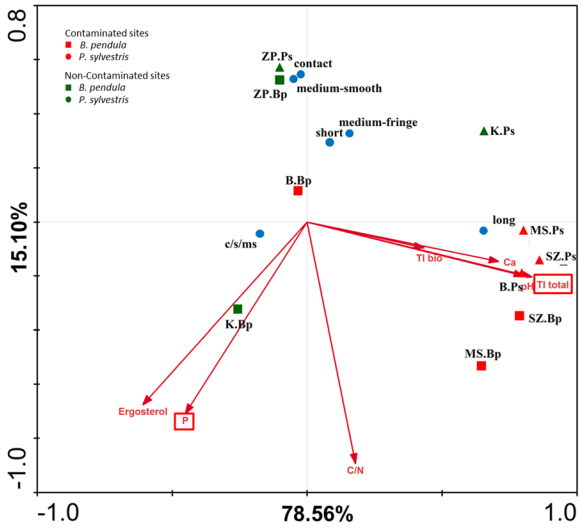

Fig. 5 CCA diagram of ECM exploration types (blue dots) driven by environmental variables (red arrows). The red boxes indicate statistically significant variables. K – Kórnik; ZP – Złoty Potok; B – Bukowno; MS – Miasteczko Śląskie; SZ – Szopienice; Bp – *Betula pendula*; Ps – *Pinus sylvestris*; c/s/m_s- *Russula*, *Lactarius*, and *Hygrophorus* can be either contact (c), short (s) or medium-smooth (m_s) depending on the species

The exploration types of ectomycorrhizae, based on the amount of emanating hyphae and the presence and differentiation of rhizomorphs, may mirror EMF ecological function (Agerer 2001). Results of our study indicated that the long-distance ectomycorrhizae dominated in HM-contaminated sites. Long-distance ectomycorrhizae, due to large amounts of emanating hyphae, may explore and take up nutrients as well as HMs from a vast soil volume. This ability may allow metal–tolerant EMF species to act like natural filters preventing toxic metals transfer to the host tree and helping them with nutrient supply (Colpaert et al. 2011). However, medium-fringe and contact exploration types were found dominating in other HM-contaminated sites (Rudawska et al. 2011; Hrynkiewicz et al. 2008, respectively). Rudawska et al. (2011) suggested that ectomycorrhizae of a medium-fringe exploration type were able to absorb vast amounts of toxic metals thus preventing their entrance into the host plant. On the other hand, Hrynkiewicz et al. (2008) postulated that ectomycorrhizae of contact

exploration type, having small absorption surface area, were able to significantly reduce heavy-metals uptake.

The concentration of ergosterol was negatively correlated with the soil toxicity index, soil pH and Ca content (Fig. 3), which is in agreement with previous research (Bååth et al. 2005; Rousk et al. 2009). One of the reasons that soil fungal biomass decreased in soils containing high concentrations of HMs could be due to a deficiency of living fine roots, an essential factor in forming an ectomycorrhizal symbiotic relationship. Also, a lower biomass of saprotrophic fungi could result from lower amounts of good quality leaf litter and soil contamination. The negative correlation between soil fungal biomass and soil toxicity levels, pH and Ca content suggests that despite the tolerance of some EMF species to HM contaminated soils, high levels of HMs combined with specific soil conditions (high soil pH and Ca content in Bukowno and Szopienice), significantly reduces fungal biomass (Rousk et al. 2009). On the other hand, the presence of HMs in the soil may lead to changes in the community structure of soil microorganisms, causing an increase of saprophytic fungi over bacterial biomass, which is manifested by an increased concentration of ergosterol (Chander et al. 2001; Khan and Joergensen 2006).

Conclusions

Although species of tree is considered to be the major factor shaping the EMF community, phylogenetically distant tree species, such as *B. pendula* and *P. sylvestris*, growing in HM contaminated soils share similar EMF communities. This finding suggests that only well-adapted EMF species with specific functional traits, like long-distance extrametrical mycelium, can survive toxic conditions and form ectomycorrhizal relationships with resident plant hosts. Among all of the examined soil factors studied, toxicity index (TI_{Total}) was the most significant factor shaping the composition of EMF communities and ECM functional traits. Despite significant differences in the structure of the EMF community of trees growing in HM-contaminated sites compared to control sites, as well as differences in the amount of soil fungal biomass in the two different types of sites, no differences in overall diversity were observed. Results of the present study contribute to our understanding of the ecological response of ectomycorrhizal fungi and their hosts to heavy metal contamination, which is still a serious problem in historically industrialized areas and could benefit our ability to remediate these sites.

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Authors contribution WB, LKT, and RC designed the experiment; WB, LKT, and KB collected and WB, KB, AT and IG analyzed the samples; WB, AT, MD, and LKT analyzed the data; WB wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

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