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# Diversity and Spatial Structure of Soil Fungi and Arbuscular Mycorrhizal Fungi in Forest Litter Contaminated with Copper Smelter Emissions

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**Abstract** The diversity and spatial structure of soil fungi (SF) and arbuscular mycorrhizal fungi (AMF) communities in the southern taiga forest litter were studied in sites with two contrasting contamination levels with copper smelter emissions. The operational taxonomic unit richness and evenness in the communities of both target groups decreased under contamination. The community structure of contaminated and control areas differed for SF, whereas they were similar for AMF. According to spatial structure analysis results on a scale of tens of meters, a gradual change of composition with distance was revealed for the SF community within 30-m intervals in the control sites. No spatial autocorrelation was found for AMF in the control sites. However, pronounced patchiness was characteristic of both SF and AMF communities within 10 m of contaminated sites. In the contaminated area, no specific spatial structure determinants of the studied communities was found among environmental factors such as water content, heavy metal concentrations in the forest litter, sample plot localization relative to canopy density, and herb vegetation diversity and abundance. However, in the control sites, AMF richness depended on herb abundance and litter chemistry.

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## 1 Introduction

Because of the primary role of soil microorganisms in ecosystem functioning, the relationships between their diversity and terrestrial ecosystem stability are rightly considered a foreground direction in ecology (Torsvik and Ovreas 2002; Nannipieri et al. 2003). The areas surrounding point polluters can be used as a convenient model for solving ecosystem stability problems (Vorobeichik and Kozlov 2012), wherein heavy metal excess usually is the main stressor.

The description of the soil community in a certain biotope is complicated by the spatial variability of its composition and structure. Spatial variability factors include topology, vegetation parameters, plant roots abundance, soil animal burrows, soil humidity, and chemistry. Polluted territories are far more spatially heterogeneous than unpolluted areas on the scale of a few centimeters to kilometers (Fritsch et al. 2011; Trubina and Vorobeichik 2012), although this aspect is insufficiently studied. The high spatial variability of soil biota activity is usual for impact regions (Vorobeichik 2007). We are unaware of studies of the soil microbial diversity of polluted territories in which spatial variability and structure (autocorrelation) were assessed, although such studies are numerous for unpolluted areas (Klironomos et al. 1999; Kadowaki et al. 2013;

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Lilleskov et al. 2004). A growing number of ecologists share the idea that spatial structure is a primary and immanent attribute of natural systems that demands special attention (Ettema and Wardle 2002; Fortin and Dale 2005; Kadowaki et al. 2013; Klironomos et al. 1999; Legendre 1993). The spatial structure of soil microbial communities and pollution effects on this structure are still the disjoint fields of soil biology. Additionally, an essential methodological aspect, i.e., spatial autocorrelation assessment in sampling schemes, is missing from microbial diversity studies in impacted regions, leading to possible biases in the study parameter estimations (Franklin and Mills 2007; Klironomos et al. 1999).

This study aimed to assess soil fungi (SF, mainly the phyla *Ascomycota* and *Basidiomycota*) and arbuscular mycorrhizal fungi (AMF, the phylum *Glomeromycota*) diversity in boreal forests exposed to long-term severe pollution from a large point polluter (copper smelter). SF were chosen as a target group because of their essential functions in boreal ecosystems, in which they are tightly intertwined with conifers through mycorrhizal symbiosis. SF, considered the main decomposers of plant debris, are most abundantly found in the litter layer (Baldrian et al. 2012). Interest in AMF was aroused by their vital role in the functioning of most temperate forest herb species (Akhmetzhanova et al. 2012), i.e., mycobionts regulate the flux of nutrients and toxic elements into host plants, prevent infection of roots with pathogens (Smith and Read 2008), and eventually determine diversity and productivity of herbal communities (Öpik et al. 2008). The early succession of plant communities, specifically in industrially polluted areas, is considered to begin with the establishment of nonmycotrophic or weakly mycotrophic plant species, which are gradually replaced by mycotrophic ones. The outcome of competition between mycotrophic and nonmycotrophic species depends either on the presence or absence of AMF in the soil or on plant and AMF specificity. Moreover, the influence of AMF on plant uptake of metals is both metal- and species-specific, which also suggests a competitive advantage of some plant species but not of others (Brundrett and Abbott 2002; Toler et al. 2005a, b).

We hypothesized that (1) the richness and evenness of SF and AMF communities in impacted (polluted) areas are lower than those in control (unpolluted) territories, (2) the composition of SF and AMF communities differ between the impacted and control areas, and (3) the spatial structure (i.e., distance and autocorrelation strength) of SF and AMF communities is less pronounced in the impacted areas than that in the control areas.

## 2 Material and Methods

### 2.1 Study Area

The study area was located in the Middle Ural Copper Smelter's surrounding area (Sverdlovsk region, city of Revda, 50 km from Ekaterinburg). The smelter has been in operation since 1940. The total emission from the smelter exceeded 140,000 t/year in 1990–2000 but decreased to 3000–5000 t/year in 2013 (Vorobeichik et al. 2014). The main ingredient of the emission is gas (90 %, which includes $SO_2$, HF, and $NO_x$) and dust particles with sorbed heavy metals (mainly Cu, Fe, Zn, Pb, and Cd) and As. The smelter emissions formed a pronounced 20–30-km pollution gradient and transformed different ecosystem parameters, resulting in increased soil acidity and heavy metal content in the soil and biota and decreased nutritional element concentrations (Kaigorodova and Vorobeichik 1996).

We chose two zones with contrasting degrees of ecosystem damage, control (30 km west of the pollution source; 56° 47.9′ N, 59° 25.6′ E) and impact zones (2.5 km; 56° 50.3′ N, 59° 52.4′ E). In each zone, the study sites were located in similar biotopes (spruce-fir forests with an admixture of pine, birch, and aspen) with similar soil groups (umbric- and umbric-gleyic albeluvisols). The recreational load on the study territory was negligible because of its significant distances from settlements.

### 2.2 Sampling

Three study sites (25×25 $m^2$, each located 30–150 m from the others) were chosen in each zone. The spatial distribution of samples is listed in Table S1 (Supplementary information). Forest litter samples up to the A horizon were taken from four to eight sample plots (0.5×0.5 $m^2$) in each site (21 in the control zone and 15 in the impact zone) on August 8, 2013. In each sample plot, five litter subsamples were taken and combined into a bulk sample. After coarse root fragments (larger than 0.5 mm in diameter) were removed from the sample, 10 g of the litter was isolated for molecular

analyses. Samples were either air-dried for chemical analysis or stored at −20 °C for molecular analyses. We measured the target groups' diversity, mass water content, and heavy metal concentrations in each sample. In each sample plot, we registered the litter horizon's thickness, position relative to canopy density (under canopy or in a canopy gap), and herb and moss layers' projection coverage.

For mycorrhizal colonization assessment intact roots of 3–15 individuals of the most frequent and abundant herbaceous species were sampled in each pollution zone in October 2014. *Linnaea borealis* L. (10 individuals), *Oxalis acetosella* L. (13), and *Calamagrostis arundinacea* (L.) Roth. (11) were collected in the control sites, and *Agrostis capillaris* L. (15), *Brachipodium pinnatum* (L.) Beauv. (12), *C. arundinacea* (5), and *Sanguisorba officinalis* L. (3) in the impact sites. After washing, fine lateral roots were cut off, cleared in 10 % KOH, and stained in 0.07 % trypan blue. Root segments were examined with a compound microscope for the presence of AMF. Mycorrhizal intensity and percentage of arbuscules and vesicles was estimated as the proportion of microscope fields of view with AMF structures from all observed fields (27–90 for each plant individual). Mycorrhizal intensity was converted into percentage of root length colonized by AMF according to Akhmetzhanova et al. (2012).

Authors had all permits and approvals for the conducted field work. Sampling procedures were designed to minimize the impact on the study sites and followed bio-ethic codex of IPAE. No endangered or protected species were sampled or damaged.

### 2.3 Chemical Analysis

The mass water content in the litter samples was measured using a UW2200H laboratory balance (Shimadzu, Japan). Air-dried samples were ground into 1–2-mm powder. The mobile form concentrations of Cu, Cd, Pb, and Zn extracted using 5 % $HNO_3$ (with a litter/acid ratio of 1:10 and an extraction period of 24 h after 1 h of shaking) were measured with an AAS Vario 6 atomic absorption spectrometer (Analytic Jena AG, Germany). The pH of the litter in deionized water extract (1:10) was measured with an inoLab 740 ionometer (WTW, Germany). The analytical laboratory is technically certified (certificate ROSS.RU0001.515630).

### 2.4 Molecular Analysis

The fungal community structure was assessed using terminal restriction fragment length polymorphism (T-RFLP) analysis. DNA was extracted from forest litter samples (0.25 g) using a Soil Microbe DNA MiniPrep (Zymo Research, USA). Sample homogenization was conducted using MM 400 Mixer Mill (Retsch GmbH, Germany). The isolated DNA's yield and quality were assessed using a BioSpec-nano micro-volume spectrophotometer (Shimadzu, Japan). The absence of PCR inhibitors was verified by agarose gel electrophoresis of ITS1 fragments. We used hot start polymerase (HotStarTaq Plus DNA Polymerase, Qiagen, USA) in conjunction with the touchdown PCR technique (Korbie and Mattick 2008) to reduce nonspecific primer binding and DNA amplification. PCR was performed on the GeneAmp 2720 thermal cycler (Applied Biosystems, USA) in a 10-μl reaction volume containing genomic DNA (25–50 ng). PCR conditions are described in Table S2 (Supplementary information). The internal transcribed spacer (ITS) region of SF was amplified using the high-coverage primers ITS1-F_KYO1 (Toju et al. 2012) and ITS4 (White et al. 1990). The small-subunit rRNA gene of AMF was amplified using primers AML1 and AML2 (Lee et al. 2008). The ITS1-F_KYO1 primer was labeled with FAM fluorescent dye, and AML2 was labeled with ROX. PCR products were digested with restriction endonucleases (SibEnzyme, Russia) ErhI and AspLEI for SF and AMF, respectively. Before T-RFLP analysis, digests were purified using an AxyPrep PCR Cleanup kit (Axygen, USA). Capillary electrophoresis with fluorescence detection was performed using an ABI-3130 automated Genetic Analyzer (Applied Biosystems). Restriction fragment lengths were determined with GeneMapper v.3.7 (Applied Biosystems) using S450 (Syntol, Russia) as internal size standard.

### 2.5 Electropherogram Analysis

The identification of "true" peaks resulting from fluorescently labeled DNA fragments in electropherograms was performed using an approach based on the statistical noise level determination (Abdo et al. 2006). This method possesses high sensitivity for small peaks and considers differences in noise level between different sample profiles. To compensate for the analytical errors in the assessment of profile diversity, we aligned and

binned DNA fragments with similar lengths. Peaks with a distance between centers <0.95 bp were considered the same operational taxonomic unit (OTU). Therefore, OTU is defined as DNA fragments compared with the other fragments in a dataset and binned into a group based on their similarity (Schloss and Westcott 2011). Although OTU profiling usually proved to be a convenient tool for detecting changes in microbial communities, each OTU can be complex and correspond to several species.

We used peak areas as estimates of OTU abundance, which was standardized by dividing by the total peak area in a sample profile. Fragment sizes <30 bp and any rare OTU with its maximum area among all samples constituting <1 % of the total in each sample were excluded from further analyses. The results obtained are highly reproducible. Differences between same DNA extract profiles did not exceed 3–4 % for the independent setup of PCR and subsequent restriction.

### 2.6 Diversity Analysis

OTU accumulation curves were used to explore the relationship between OTU richness and sampling effort in each pollution zone (Gotelli and Colwell 2001). Accumulation curves were built with resampling without replacement (1000 permutations per point).

For the analysis of differences in the structure and variability of fungal communities between different zones, we built ecological dissimilarity matrices for each target group based on the Bray–Curtis (*BC*) index (Legendre and Legendre 2012). Between- and within-zone differentiation of the communities' structure was evaluated using principal coordinate analysis in a multidimensional Euclidian space. Differences in community structure were tested using one-way multivariate analysis of variance (ANOVA; Anderson 2001). We also assessed community variance homogeneities between different zones using the procedure of Anderson (2006). Permutation testing with 10,000 permutations was performed to test the null hypothesis of no difference in community structure or dispersion between zones.

The relationship between OTU number and environmental parameters was evaluated by linear regression. Correlations between the different metal concentrations prompted us to use a toxicity index ($I_{tox}$) as the integral parameter of the litter toxicity in each sample. $I_{tox}$ is expressed as the sum of ratios between each metal concentration in the sample to its minimal concentration divided by the minimal observed value of this sum. This index represents a factor by which a sample's toxicity is increased compared with the most uncontaminated sample.

### 2.7 Spatial analysis

The distance and strength of the spatial autocorrelation of the communities' composition in each zone were estimated using Mantel's correlogram (Legendre and Legendre 2012). The null hypothesis ($rM=0$) was tested by 10,000 permutations. Environmental matrix similarity between samples was built by $I_{tox}$, herb species composition, and litter thickness of each sample plot. Relationships between SF or AMF community matrices and environmental similarities were evaluated using simple Mantel tests. All statistical analyses were performed using R v.3.0.2 (R Core Team 2014) and vegan package v.2.0-10 (Oksanen et al. 2014).

## 3 Results

### 3.1 Environmental Parameters

Forest litter in the control zone was loose and thin. Litter in the impact zone was twice as thick as in the control area and consisted of unchanged coniferous needles partially covered with moss layer. It was dark-brown and peaty with an admixture of dust in the lower part. "Burials" of undecomposed wood and herb litter were found in microdepressions.

Thirty-four herbaceous plant species typical for boreal forests were registered within the sample plots in the control sites (Table S3, Supplementary information), in which the herb layer coverage was more than 10-fold higher than that in the impact sites (Table 1). Ten species comprised approximately 80 % of the total herb abundance in the control zone. In the impact zone, only five species were registered within the sample plots (*A. capillaris*, *B. pinnatum*, *S. officinalis*, *Vaccinium myrtillus* L., *V. vitis-idaea* L.) and four additional species (*Atragene sibirica* L., *Equisetum sylvaticum* L., *Lathyrus vernus* (L.) Bernh., and *C. arundinacea* (L.) Roth.) were found aside from the sample plots. No herbaceous vegetation was registered in 6 of 15 sample plots of the impact sites. Therefore, the herb layer's

alpha diversity was higher in the control zone (10.67± 0.74 vs 1.93±0.28 species per sample plot).

The litter water content was similar between the impact and control sample plots. The moveable form concentrations of Zn, Cd, and Pb in the impact zone were 5-, 10-, and 30-fold higher, respectively, than those in the control sites, and Cu levels were 100-fold higher in the impact zone (Table 1). Therefore, $I_{tox}$ of samples from impact sites were approximately 35-fold higher than those in the control samples. The heightened acidity enhanced heavy metal toxicity in the impact samples.

Root length colonized by AMF was four times lower in the impact sites than in control sites (Table 1). In the control territory up to 100 % of *O. acetosella* and *L. borealis* and 92 % of *C. arundinacea* root length was occupied by fungi (Fig. S3, Supplementary information). In the impact area, *S. officinalis*, which inhabits forest gaps, was the most abundantly colonized (up to 98 %), while maximum root colonization of the other species comprised only 34 %. Mycorrhizal colonization of *C. arundinacea* roots (the only species met in both zones) was ten times lower in the impact sites than in the control sites.

### 3.2 Diversity of SF and AMF

The total numbers of OTUs in the study territory (i.e., in both zones) after deleting rare fragments were 204 for SF and 78 for AMF (382 and 223 with rare OTUs, respectively). Species accumulation curves built for each target group in each zone almost reached the plateau. According to the inflection point positions on the curves, the OTU accumulation rate decreased after five to seven samples (Fig. 1). Therefore, we assume that SF and AMF diversity is sufficiently covered for a reliable inference regarding their community structure in the studied biotopes.

The gamma (total number of OTUs in a zone) and alpha diversity of both SF and AMF were higher in the control zone [$F(1; 34)=39.58$, $p<< 0.001$ for SF and $F(1; 34)=7.97$, $p=0.008$ for AMF; Table 2]. Shannon's

**Table 1** Environmental parameters and root colonization with AMF in the control and impact zones

| Parameter | Control zone ($n$=21) | | | Impact zone ($n$=15) | | |
|---|---|---|---|---|---|---|
| | Mean±SE | Min–max | CV, % | Mean±SE | Min–max | CV, % |
| Herb coverage (%) | 69.5±5.4 | 15–100 | 35.5 | 6.4±2.4 | 0–30 | 147.6 |
| Moss coverage (%) | 36.2±7.7 | 0–95 | 98.0 | 30.5±8.1 | 0–95 | 102.3 |
| Litter coverage (%) | 74.7±7.4 | 10–100 | 44.1 | 78±7.6 | 10–100 | 37.5 |
| Stand density (stems/ha)[a] | 1157.3±89.7 | 1024–1328 | 13.4 | 1312.0±92.4 | 1152–1472 | 12.2 |
| Basal area ($m^2$/ha)[a] | 44.2±4.8 | 35.4–51.9 | 18.8 | 28.5±1.7 | 26.2–31.8 | 10.2 |
| Mass water content | 1.6±0.1 | 1.2–2.2 | 18.9 | 1.5±0.1 | 0.7–2 | 21.3 |
| Litter thickness (cm) | 2.4±0.3 | 0.5–5 | 56.3 | 4.8±0.7 | 1.5–9.5 | 53.1 |
| Concentration (μg/g) | | | | | | |
| Cu | 34.7±4.0 | 14.6–87.3 | 52.3 | 3659.3±350.7 | 1607.6–6638.4 | 37.1 |
| Zn | 168.7±12.4 | 97.7–299.8 | 33.7 | 882.1±66.0 | 394.4–1214.9 | 29.0 |
| Cd | 2.4±0.2 | 1.3–4.6 | 32.2 | 22.4±1.9 | 9.7–32.9 | 32.5 |
| Pb | 78.0±5.7 | 35.0–135.4 | 33.8 | 2302.7±159.9 | 1038.2–3397.3 | 26.9 |
| $I_{tox}$ | 1.6±0.1 | 1.0–2.8 | 26.6 | 54.9±3.8 | 31.8–87.8 | 26.7 |
| pH[b] | 5.1±0.1 | 4.7–5.8 | 6.6 | 4.4±0.1 | 4.1–5.0 | 6.5 |
| Root length colonized by AMF (%)[c] | 55.3±7.2 | 0–99.6 | 76.3 | 13.6±3.5 | 0–97.75 | 150.9 |
| Percentage of arbuscules[c] | 14.3±2.5 | 0–38.8 | 100.9 | 3.5±1.6 | 0–52.9 | 275.6 |
| Percentage of vesicles[c] | 18.3±2.9 | 0–57.14 | 94.6 | 3.9±1.3 | 0–33.3 | 202.8 |

*CV* coefficient of variation, $I_{tox}$ toxicity index

[a] For each zone, $n$=3 plots 25×25 m [data by Bergman (2011)]

[b] For each zone, $n$=15 samples

[c] $n$=34 plant individuals for control zone, $n$=35 for impact zone

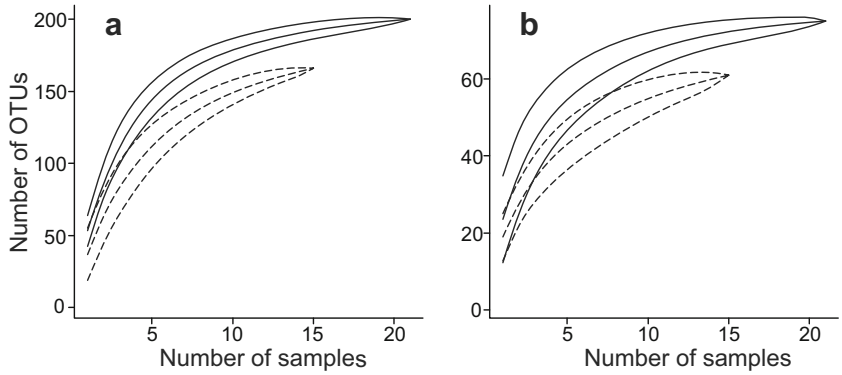


**Fig. 1** Relationship between SF (**a**) and AMF (**b**) OTU accumulation and sampling effort in the control (*solid lines*) and impact (*dashed lines*) zones. Means and standard deviations are shown

index of SF in the control zone was significantly higher than that in the impact sites [$F(1; 34)=25.26$, $p<<0.001$], whereas it is similar between the zones for AMF [$F(1; 34)=3.01$, $p=0.092$]. The between-zone difference in OTU effective numbers comprised 8.67 for SF and 1.47 for AMF.

We found only four SF OTUs and three AMF OTUs unique for the impact territory (Fig. S1, Supplementary information). As a rule, their relative frequency was low (0.125–0.20) excluding one frequent OTU in the AMF group (0.67).

The evenness of SF and AMF communities was lower in impact zone than that in control zone. In total, 33 dominating OTUs of SF, which comprised 53 % of the total SF abundance, were distinguished in the control sites versus 30 SF OTUs in the impact area, comprising 76 % of the total abundance (Fig. 2). In the AMF group, eight OTUs comprising 59 % of the total abundance and four OTUs comprising 67 % of the total abundance were found in the control and impact sites, respectively. Seven SF OTUs shared between zones were found among the dominants. Three AMF OTUs were the most abundant in both zones.

Multivariate ANOVA based on *BC* dissimilarity matrices revealed strong between-zone differentiation of the SF community composition [$F(1; 34)=2.42$, $p<<0.001$; Fig. 3a]. However, no between-zone differentiation was found for AMF [$F(1; 34)=1.50$, $p=0.14$; Fig. 3b]. *BC* between samples from different zones was 0.94 (range 0.77–0.99) for SF and 0.72 (0.20–0.95) for AMF (Fig. S2, Supplementary information).

### 3.3 Relationship Between OTU Richness and Environment

We uncovered a significant negative relationship between SF OTU number and litter thickness in the impact zone and a similar but less pronounced pattern in the control area (Table 3). According to the regression coefficients for a 1-cm increase in litter thickness, the

**Table 2** SF and AMF diversity parameters in the control and impact zones

| Parameter | SF | | AMF | |
|---|---|---|---|---|
| | Control zone | Impact zone | Control zone | Impact zone |
| Total number of OTUs[a] | 200 (382) | 166 (251) | 75 (166) | 61 (151) |
| Number of OTUs per sample[b] | 53.05±1.25 | 37.27±2.41 | 23.33±1.22 | 18.8±0.81 |
| Shannon index[b] | 2.92±0.07 | 2.29±0.11 | 2.01±0.08 | 1.79±0.10 |
| Pielou's evenness[b] | 0.73±0.01 | 0.63±0.02 | 0.64±0.02 | 0.61±0.03 |

*OTU* operational taxonomic unit

[a] Number of OTUs including rare OTUs is shown in parentheses

[b] Mean±standard error

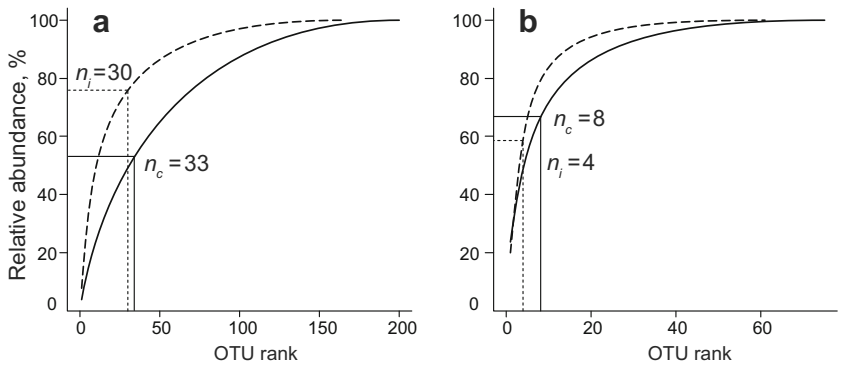


**Fig. 2** Cumulative rank abundance curves of SF (**a**) and AMF (**b**) in the control (*solid lines*) and impact (*dashed lines*) zones. Projections correspond to the inflection points of the curves; $n_c$ and $n_i$, number of dominating OTUs in the control and impact zones, respectively

number of SF OTUs would decrease by two. The litter water content was positively related with SF OTU richness only in the control area. A significant negative relationship between litter toxicity and SF OTU number was registered in the impact territory.

For AMF communities, the only significant positive relationship with metal concentrations was noted in the control sites, which apparently demonstrates the stimulating effect of a slight increase of biogenic metal concentrations in substrates. We did not find other AMF diversity determinants among the studied environmental parameters.

### 3.4 Beta Diversity and Spatial Structure

According to the within-zone values of *BC*, beta diversity was similar between the control and impact areas for both SF [$BC_c$=0.90 (range 0.55–0.97), $BC_i$=0.90 (0.54–0.99) with $F(1; 34)$=0.10, $p$=0.75] and AMF [$BC_c$=0.71 (0.21–0.97), $BC_i$=0.71 (0.28–0.94) with $F(1; 34)$=0.07, $p$=0.79].

The spatial organization degree of SF and AMF communities differed between the zones. In the control area, a significant positive autocorrelation of SF composition was revealed in the distance class of 30 m (Fig. 4a). However, in the impact zone, this positive relationship persisted only in the first distance class (up to 10 m), whereas a negative autocorrelation was registered for the second distance class (20 m).

No spatial organization was revealed for AMF communities in the control area (Fig. 4b). We registered a significant negative autocorrelation between the AMF compositions of sample plots from the first distance class, which indicated patchiness in the 10-m scale.

The water content in litter can be considered the only possible factor of the observed spatial structure of SF communities in the control area (Table 4). The heavy metal content in litter, its water content, and herb species composition determined the spatial distribution of AMF in the control sites. No spatial structure determinants of either SF or AMF were revealed in the impact zone.

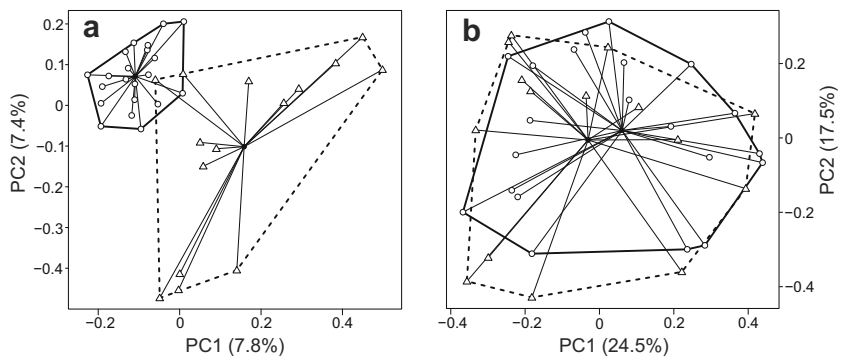


**Fig. 3** Differentiation of SF (**a**) and AMF (**b**) communities' composition between the control (*circles)* and impact (*triangles*) zones. Samples are shown in projection of the first principal coordinates (PC) built on *BC* matrices. The percentage of explained variance for each PC is shown in parentheses

**Table 3** Relationship of SF and AMF OTU numbers with environmental parameters

| Environmental parameters | SF | | | | | | AMF | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control zone ($n$=21) | | | Impact zone ($n$=15) | | | Control zone ($n$=21) | | | Impact zone ($n$=15) | | |
| | $B$ | $SE_B$ | $R^2$ | $B$ | $SE_B$ | $R^2$ | $B$ | $SE_B$ | $R^2$ | $B$ | $SE_B$ | $R^2$ |
| Litter thickness | **−1.55*** | **0.91** | **0.13** | **−2.09**** | **0.83** | **0.33** | 0.16 | 0.96 | 0 | −0.18 | 0.34 | 0.02 |
| Litter toxicity | −2.55 | 3.09 | 0.03 | **−0.35**** | **0.15** | **0.30** | **5.68*** | **2.79** | **0.18** | 0.01 | 0.06 | 0 |
| Litter water content | **8.66**** | **3.85** | **0.21** | −8.44 | 7.70 | 0.08 | −4.29 | 4.13 | 0.05 | −0.59 | 2.71 | 0 |
| Herb coverage | 0 | 0.05 | 0 | 0.08 | 0.27 | 0.01 | 0.06 | 0.05 | 0.06 | −0.05 | 0.09 | 0.02 |
| Moss coverage | 0.03 | 0.04 | 0.03 | −0.17 | 0.07 | 0.32 | −0.05 | 0.03 | 0.08 | −0.01 | 0.03 | 0.01 |

Linear regression coefficient ($B$), its error ($SE_B$), and determination coefficient ($R^2$) are shown

*$B$ significant at $p \leq 0.10$; **$B$ significant at $p \leq 0.05$

Therefore, litter humidity is considered the common parameter governing the spatial distribution of fungal communities in the control area. The effect of litter humidity on the spatial structure of the herb layer in the control forest was also more pronounced than those of other environmental

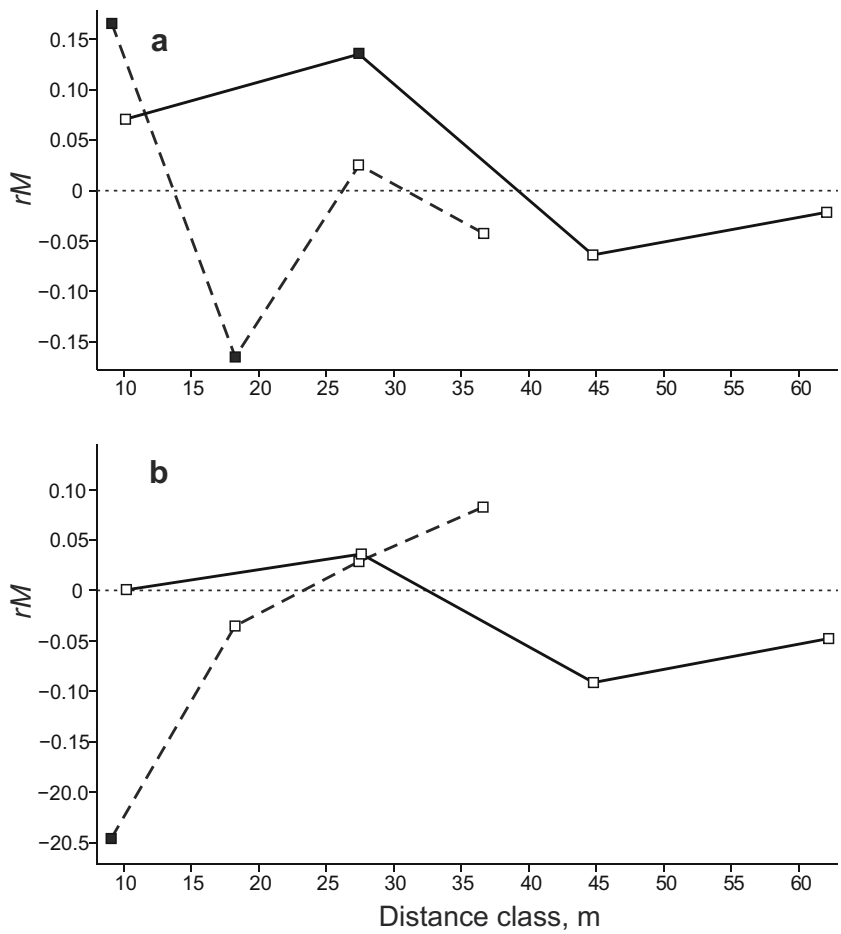


**Fig. 4** Correlograms of SF (**a**) and AMF (**b**) communities' similarity in the control (*solid line*) and impact zones (*dashed line*). A significant Mantel's correlation coefficient (*rM*, *filled marker*) indicates that for the given distance class, the multivariate similarity among sites is higher (for positive *rM*) or lower (for negative *rM*) than expected by chance (i.e., the mean within-class similarity is different from the mean among-class similarity)

**Table 4** Relationships between SF and AMF community spatial structure and environmental parameters

| Environmental parameters | SF | | | | AMF | | | |
|---|---|---|---|---|---|---|---|---|
| | Control zone | | Impact zone | | Control zone | | Impact zone | |
| | *E* | *p* | *E* | *p* | *E* | *p* | *E* | *P* |
| Litter toxicity[a] | 0 | 0.49 | −0.07 | 0.73 | **0.16** | **0.10** | −0.19 | 0.96 |
| Spatial structure of herbal layer[a] | −0.05 | 0.74 | −0.04 | 0.62 | **0.15** | **0.07** | 0 | 0.49 |
| Litter water content[a] | **−0.10** | **0.06** | 0.11 | 0.19 | **0.18** | **0.02** | 0.04 | 0.37 |
| Sample plot position relative to canopy density[b] | 1.02 | 0.45 | 0.99 | 0.44 | 1.11 | 0.32 | 0.65 | 0.72 |

Effect size (*E*) and corresponding *p* values are shown

[a] *E* denotes *rM* for continuous variables

[b] *E* denotes pseudo-*F* ratio for nominal variables

parameters included in the analysis (Table S4, Supplementary information).

## 4 Discussion

### 4.1 SF Communities

The diversity and evenness of SF communities decrease under the copper smelter emission effects, which supports our first hypothesis and is in accordance with the results of studies of the fungal community diversities exposed to a long-term excess of heavy metals (Bååth et al. 2005; Giller et al. 1998; Hui et al. 2012; Pečiulytė and Dirginčiutė-Volodkienė 2010). Although the linearity of the relationship between diversity and function is not obvious, especially for soil ecosystems (Loreau 2000; Ramsey et al. 2005), the reduction of biota functioning in polluted areas is thought to result from diversity loss. Therefore, the decline of fungal cellulolytic and xylotrophic activity in the impact area shown in previous studies of the territory around the smelter (Vorobeichik 2007; Vorobeichik and Pishchulin 2011; Stavishenko and Kshnyasev 2013) implies a decrease in the diversity of primarily saprotrophic species in SF group. More stable functioning was shown for ectomycorrhizal and phytopathogenic fungi (which are also included in SF), since their abundance does not change or slightly increases under exposure to pollution (Veselkin 2005; Stavishenko and Kshnyasev 2013). This, however, does not necessarily characterize communities of ectomycorrhizal and phytopathogenic fungi in the polluted area as identical to control communities in terms of diversity or structure.

Only a few OTUs were unique to the impact zones. OTUs dominating in impact communities were common and abundant in the control zones. Despite this, our second hypothesis, which suggested that the OTU structure of SF communities differed between the control and impact areas, was also confirmed. This difference primarily resulted from the elimination of pollution-sensitive OTUs, and it is hardly attributable to the replacement of certain OTUs by new ones. A similar shift of OTU or species composition of fungal communities in territories polluted with heavy metals was demonstrated by other researchers (Bååth et al. 2005; Frostegård et al. 1996; Hui et al. 2012). The exact mechanisms of this process remain obscure. It is commonly believed that the fungal communities in industrially polluted areas emerge from a pool of innate metal-tolerant species with little possibilities for genetic adaptation within species (Bååth et al. 2005). However, there is some evidence of fungal species adaptation to an excess of heavy metals in serpentine soils (Southworth et al. 2013), although the operation period of industrial enterprises is negligibly small on the time scale of existence of serpentine areas.

The spatial structure of SF communities differed between the studied zones. A gradual shift in community composition with increasing distance between localities was typical for the control territory, whereas distinctly delimited ca. 10-m patches with different SF compositions were noted in the impact zone. This is in corroboration with our third hypothesis because the distance of autocorrelation of SF communities is lower in polluted areas. Nevertheless, the autocorrelation degree (absolute *rM* values) increased in the impact zone.

We found no spatial structure determinants of SF excluding litter humidity in the control area. In the impact zone, litter toxicity weakly determined the SF OTU number and had no effect on their spatial structure. This is surprising because the impact sites are substantially heterogeneous regarding plant abundance and particularly litter toxicity. Therefore, we suppose that communities in more toxic localities formed at random from the OTU common pool of the polluted area. Therefore, at least in the actual diapason of heavy metal concentrations, SF species could possess similar metal tolerance degrees in the polluted area, which is usually not the case for higher plants (Antonovics et al. 1971). However, it is doubtless that OTU identification to the species or genus level and the use of phylogenetic or ecological distances between them are required for the ultimate verification of this suggestion.

In a previous research of the studied territory, we revealed that the complete inhibition of cellulose decomposition was found only in localities with an extremely high heavy metal content. Possibly, the diversity dependence on litter toxicity also has its threshold, which was not reached in our sample.

It is also possible that diversity estimated using total SF DNA is unrelated to heavy metal concentrations (Van der Linde and Haller 2013). Specifically, the composition of active SF communities can be masked by dead mycelium remnants, dormant spores, and DNA absorbed onto soil particles (Pietramellara et al. 2009), which could reflect the SF community diversities that previously existed under lower levels of pollution. The active SF diversity, which can be assessed through RNA analysis, possibly depends on the substrate toxicity. For instance, in parallel analysis of fungal DNA and RNA in a coniferous forest litter (Baldrian et al. 2012), it was found that the community compositions of total and active SF were significantly different despite the similarity of their diversity levels, i.e., several highly active taxa exhibited low abundance or even absence in the DNA pool. However, some studies reported contradictory results demonstrating similarity between the DNA- and RNA-derived profiles of SF communities (Anderson et al. 2008; Korkama-Rajala et al. 2008). Unfortunately, there is a lack of research on the relationship between active and total diversity in microbial communities in polluted areas.

Large trees are efficient "ecosystem engineers" that substantially affect the environmental parameters around them (Jones et al. 1994). Therefore, we expected that trees would be powerful determinants of the spatial structure of SF communities. However, we did not find a sample plot position effect relative to canopy density on the structure or richness of SF communities, possibly resulting from the intersection of individual areas of the influence of different trees in the forest (Table 1). This is in line with our previous study, in which the cellulose-decomposing activity of litter microorganisms in the studied territory was not affected by a test point position relative to a tree stem (Vorobeichik and Pishchulin 2011).

The need in the analysis of the effect of other environmental parameters (e.g., litter pH, concentrations of nutrient elements, organic compounds, litter structure, and light intensity) on SF composition is doubtless. However, the spatial structure of SF communities is affected by both environmental parameters ("environmental filtering") and distribution potential ("dispersal limitations"; Kadowaki et al. 2013). In our opinion, the contribution of the latter constituent of the SF spatial structure formation mechanism could be the same as, if not larger than, the former. Namely, in previous studies, dispersal limitations were considered to cause the phenomenon of "foci" in the spatial structure of cellulose decomposition intensity in the impact zone (Vorobeichik 2002, 2007). Fungal dispersal occurs via the vegetative spread of mycelium, and propagule dispersal occurs via animal carriers or through the aerial spore distribution from fruit bodies. Chemical stress inhibits the colonization potential of most fungi because the growth rate of active mycelium and consequently its amount and spore production decreases (Bååth et al. 2005). In addition, a negative copper-dependent chemotropism of SF is well known (Fomina et al. 2000).

Moreover, according to research on the soil fauna in the studied territory, the abundance of primary spore carriers, i.e., soil animals, is much lower in the impact zone than that in the control area. Particularly, the abundance of epigeic carabids (Zolotarev and Belskaya 2012), springtails (Kuznetsova 2009), saprotrophic millipedes, and enchytraeids (Vorobeichik et al. 2012) were lower in the impact zone. The low abundance of rodents and shrews in the polluted zone (Mukhacheva 2007) is particularly important considering their contribution to fungal spore distribution (Kataržytė and Kutorga 2011). In a study of earthworm abundance, we demonstrated that the 50–80-km$^2$ area around the smelter comprises the "lumbricid desert" (Vorobeichik 1998). A similar "mole desert" extends over a greater distance from the smelter (Nesterkova 2014). The absence of burrowing

activity resulted in a lack of mechanical mixing of forest litter in the impact zone and drastically decreased the dispersal potential of SF, resulting in decreases of their spatial autocorrelation distance. The supposed primary importance of dispersal limitation for SF community spatial structure on the local scale is opposite to the leading role of environmental filtering for the SF distribution revealed on the regional scale (Kivlin et al. 2014).

### 4.2 AMF Communities

Similar to the findings for SF, the diversity and evenness of AMF communities decreased under industrial pollution effects, which supports our first hypothesis and corresponds with other studies (Zarei et al. 2010). However, we have expected a more pronounced decrease of diversity together with between-zone differentiation of AMF communities. The observed between-zone similarity of AMF communities was surprising because the herb layer of the impact territory is drastically different from that of the control area in composition, alpha diversity, and abundance (Table 1), and it is believed that AMF diversity and composition should be closely related to plant community parameters (Johnson 2010). Also AMF are considered among the most sensitive fungi to a heavy metal excess (Baldrian 2010), which is also confirmed with our estimates of AMF root colonization frequency.

The observed similarity of impact AMF communities to control can be explained considering that a vast number of AMF are generalists toward host plants. For example, in primeval unmanaged boreonemoral forest, from 1 to 32 AMF OTUs were recorded in individual plants of six herb species (Saks et al. 2013). Thus, the total intraradical diversity of AMF comprised 76 OTUs, 32 of which were associated either with *C. arundinacea* or five other species. Such nonspecificity admits that few plant species found in the impact area may maintain observed levels of AMF diversity. However, we registered from 15 to 24 AMF OTUs in six bare sample plots, which is surprising, because of a limited dispersal ability of AMF (Smith and Read 2008) and scarce spore carriers (see above). It is very likely that OTUs found in the bare plots as well as the revealed between-zone similarity of AMF communities resulted from burials of AMF spores in the litter's thick layer in the polluted territory. This phenomenon of "buried spores" also elucidates the lack of a link between AMF and herb layer parameters together with litter toxicity in the impact zone, in contrast to the control sites, in which the dependence of OTU richness on herb abundance and litter chemistry was registered (see Table 4).

In our estimation, the revealed spatial structure and composition of AMF communities represented a "footprint" of weakly disturbed stages of the forest herb layer, which were characteristic for the impact zone before or at the beginning of operation of the smelter. This spore bank in analogy with a seed bank presents the pool for the root colonization of plants in polluted sites during revegetation after a decrease of emissions or soil mechanical disturbance (Trubina 2009). Therefore, our results support the idea that the low contribution of arbuscular mycorrhizal plant species to the early succession of polluted community should not be attributed to the low availability of AMF spores at elevated metal concentrations (Ruotsalainen and Kozlov 2006). AMF spores can successfully germinate after 6–8 years of storage (Plenchette and Strullu 2003), providing a reservoir of inoculum which persists for many years. Considering that there are no data on the long-term viability (dozens of years) of AMF propagules in the field and that analyzed DNA in the litter samples can be derived from dead propagules, further verification of the hypothesis is needed.

## 5 Conclusion

The obtained results clarify the directions of further investigations and have a methodological outcome. It was demonstrated that severe industrial pollution affects the taxonomic structure, diversity, and spatial structure of soil fungal communities. Among the studied environmental parameters, we did not reveal any specific spatial structure determinant of fungal communities in the polluted zone. This outlines the need to estimate the relative contribution of environmental parameters and dispersal limitation into the spatial structure of microbial communities. In this direction, the most promising tool appears to be the combination of spatial eigenfunction analysis, proposed to create spatial predictors that can be easily incorporated into explorative models together with environmental predictors (Dray et al. 2006) and variation partitioning (Legendre et al. 2012). However, to realize this approach, much larger samples are required.

The spatial autocorrelation scales revealed in the study agree with the results of other research, which

reported pronounced spatial autocorrelation of fungal community composition in different soil horizons at distances of 3–50 m (Kadowaki et al. 2013; Lilleskov et al. 2004). Based on these results, we can suggest that for appropriate inventory of fungi in a temperate forest biotope, an appropriate distance between sampling plots is ca. 20–30 m. Conversely, in polluted areas, samples can be collected closer to each other. The best alternative is undoubtedly the preliminary estimation of the spatial dependence scale to apply it in sampling design for diversity analysis.

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