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Diversity of ants (*Hymenoptera, Formicidae*) along a heavy metal pollution gradient: Evidence of a hump-shaped effect

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ABSTRACT

Ants, as indicators of ecological changes, are widely used in land management and environmental monitoring. At the same time, effects of industrial pollution are less known compared to many other stressors. We studied variations in habitat variables and ant diversity near the Karabash copper smelter (KCS) in Russia, in 2009 and 2014. Pitfall traps were set up at 10 birch forest sites (3 plots per site and 5 traps per plot) located to the north and south of the smelter within a 1–32-km range, with two samplings per season (June and August). The tree height, stand basal area, species richness of the field and moss layers, and the cover of the field layer decreased towards the smelter. The vegetation almost disappeared at the industrial barren close to KCS. In total, 21 ant species of 7 genera were registered. The highest species richness, diversity, and total occurrence of ants were observed in the transitional zone between moderately polluted forest habitats and industrial barren. Ant diversity and occurrence showed the same pattern in different years. Our results conform to the intermediate disturbance hypothesis predicting the greatest diversity at intermediate frequencies or intensities of disturbance or environmental change. Apparently, contaminant exposure affects ant communities near KCS indirectly, through habitat transformation.

The study emphasizes the need for thorough planning of field studies of pollution effects on ants with examination of the full range of exposures. In addition, the nonlinear pattern of diversity and abundance of the ant community along the pollution gradients suggests caution, when assessing the magnitude of human impact and the state of ecosystems with ants as bioindicators.

1. Introduction

The ants represent a diverse and ubiquitous group with high biomass and importance in the terrestrial ecosystems, which is sensitive to ecological changes. They are known as good indicators of anthropogenic disturbances caused by fires, clear cutting, grazing, industrial pollution, and mine site rehabilitation (Majer, 1983; Stary and Kubiznakova, 1987; Neumann, 1992; Bestelmeyer and Wiens, 1996; Andersen, 1997; Andersen et al., 2003; Underwood and Fisher, 2006). Many authors studied ant responses to the industrial pollution, including accumulation of heavy metals in ants, immune defense, behavior, body size and coloration, colony size, and community structure (Pętal, 1978; Rabitsch, 1995; Eeva et al., 2004; Sorvari et al., 2007; Grześ, 2009a; Sorvari and Eeva, 2010; Skaldina and Sorvari, 2017; Blinova and Dobrydina, 2017; Skaldina et al., 2018; Blinova et al., 2018). It was shown that ants tolerate high concentrations of heavy metals (Folgarait, 1998; Grześ and Okrutniak, 2016) because of social organization, construction of nests with favorable microclimate (Frouz and Jilková, 2008; Farji-Brener and Werenkraut, 2017), and shifting activity to periods with least pollution exposure (Pętal, 1978; Migula and Głowacka, 1996; Grześ, 2010a).

There are significantly less studies of the relationship of ant community structure with heavy metal exposure; moreover, their results are contradictory. The heavy metals and sulfur dioxide emitted by the nonferrous industry pose the strongest effect on the biota compared to other sources of pollution. They can lead to significant degradation of forest habitats up to the formation of vast deforested areas, known as the industrial barrens (Kozlov and Zvereva, 2007). The pollution with heavy metals and sulfur dioxide can decrease the ant abundance and diversity (Pętal, 1978; Hoffman et al., 2000; Zvereva and Kozlov, 2010), or increase the latter (Grześ, 2009a). These authors reported linear changes (increase or decrease) of ant diversity along the pollution gradients. At the same time the hump-shaped pattern of the ant diversity and/or abundance was documented along the gradients of moisture (Fox et al., 1988) and industrial pollution (Kozlov, 1997; Blinova, 2008; Belskaya et al., 2017; Blinova and Dobrydina, 2017),

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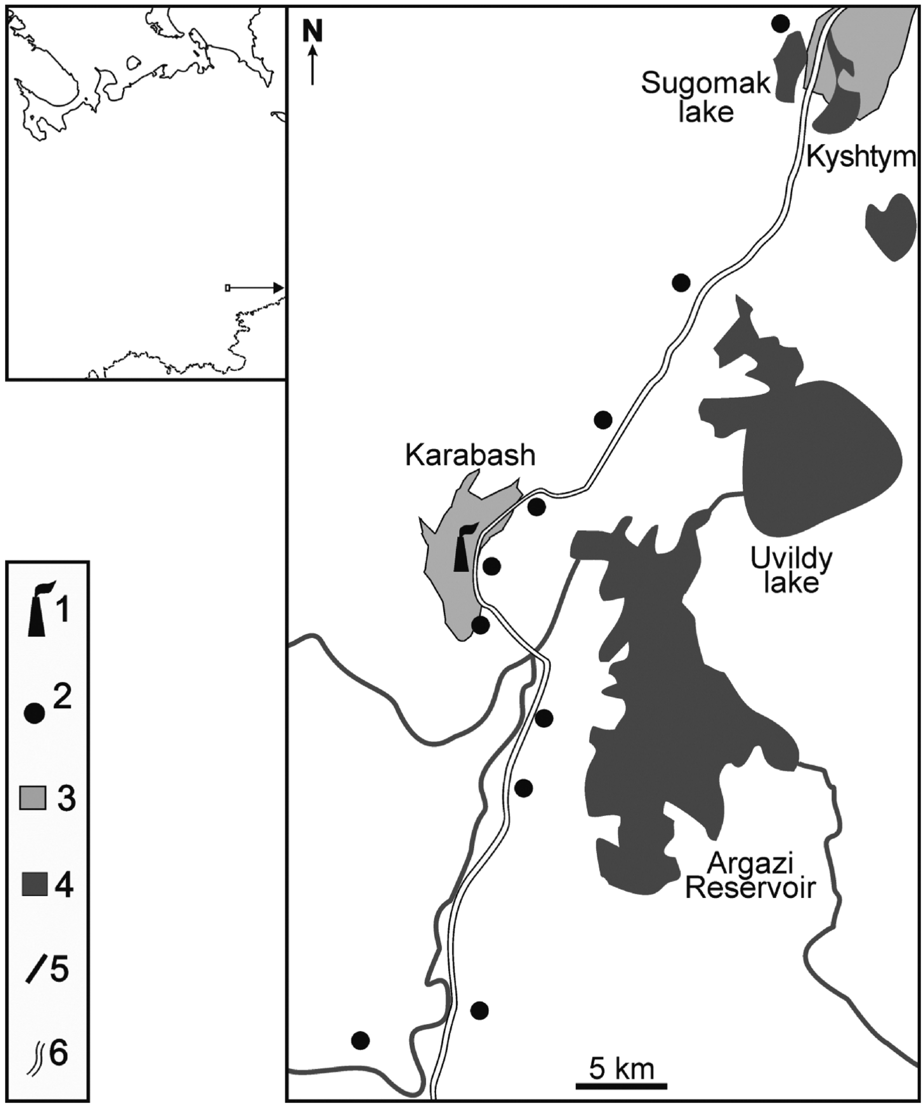

Fig. 1. Locations of study sites along the pollution gradient near the Karabash copper smelter (KCS). Legend: 1: Copper smelter. 2: Study sites. 3: Settlements. 4: Lakes. 5: Rivers. 6: Roads.

and by disturbances caused by military training and fire (Graham et al., 2009). These results conform to the intermediate disturbance hypothesis (Connell, 1978), which predicts the greatest diversity at intermediate frequencies or intensities of disturbance or environmental change. Inconsistency of results determines the need for additional studies of responses of ant communities to pollution. It is unclear whether pollution-related patterns in the ant diversity and occurrence coincide; what factors determine the ant response to pollution: toxic exposure or habitat alteration.

The aim of the current study is to analyze variations in ant communities along a pollution gradient near the Karabash copper smelter. This is one of the most polluted areas in Russia (Kozlov et al., 2009). At the same time, effects of this factory on ground-dwelling invertebrates were not studied. We found only one article on soil invertebrates (Nekrasova, 1993). We focused on ants because of their high abundance and role in terrestrial ecosystems. We tested the intermediate disturbance hypothesis, expecting the hump-shaped diversity and occurrence pattern along the pollution gradient. This hypothesis was confirmed for ant communities near the Middle Ural copper smelter (Belskaya et al., 2017). We aimed to test this hypothesis near another non-ferrous smelter with the same type of emissions (heavy metals and sulfur dioxide), but different environmental conditions. Conducting counts at 10 sites, we intended to study the pollution gradient in more detail, covering different stages of deterioration of the forest habitat typical of the Southern Urals. We studied pollution-related habitat alterations including tree stand, understory, field and moss layers, and soil surface. At the same sites we examined species richness, diversity and occurrence of ants. We tested if their variations along the pollution gradient are nonlinear. By performing investigations over two years, we tested the repeatability of the results. Finally, we tried to link diversity and occurrence of ants with contaminant exposure and habitat characteristics to identify those variables, which determine the responses of the whole ant community.

2. Material and methods

2.1. Pollution source

Karabash (55°27′N, 60°13′E) is an industrial town in Chelyabinsk region, Russia. The Karabash copper smelter (KCS), in operation since 1910, produced considerable amount of copper in Russia (Kozlov et al., 2009). Major pollutants are sulfur, carbon, and nitrogen oxides, and polymetallic dust containing Cu, Pb, Cd, Zn, and As (Kozlov et al., 2009; Smorkalov and Vorobeichik, 2011). Total emissions reached 300–400 thousand tons per year in the 1970s (Kozlov et al., 2009), steadily declined to the beginning of 1990s, when the smelting operations were discontinued, and rose again to 13–16 thousand tons per year during the period of 2009–2012 (Semenov, 2014).

2.2. Study area

Study area is situated in the pre–forest–steppe pine–birch forest subzone at the eastern slope of Southern Urals. It consists of hilly elevations with an altitude of 250–650 m a.s.L. with rivers and lakes lying between them. The mean temperature in January is −16 °C and in July +18 °C; the annual precipitation is 400–600 mm. The frost-free period ranges from 160 to 170 days. The prevailing winds are directed to east. This area is covered with forests formed by Scots pine (*Pinus sylvestris*) and common birch (*Betula pendula*), with well-developed field layer. The soil cover of the area is represented by mountain-forest brown, soddy-podzolic, and gray forest soils (Smorkalov and Vorobeichik, 2011). Initially, the KCS was surrounded by a forest, which declined in the 1940s (Kozlov et al., 2009). The long-term pollution resulted in a vast industrial barren with eroded soil and extremely sparse vegetation around KCS (Kozlov and Zvereva, 2007).

2.3. Site description

The study was conducted in 2009 and 2014 at 10 sites established to the north and south of the smelter within a 1–32-km range (Fig. 1). The sites were chosen to cover the entire pollution gradient and represent different stages of forest habitat deterioration: from background area (i.e., unpolluted) to industrial barren. Study sites are located on the plain or in the lower part of the hillsides. All sites are not isolated forest patches, but parts of a large forest area intersected with glades, cuttroughs, and forest roads. To avoid edge effects we positioned plots at a distance of ≥100 m from forest edges. Special attention was paid to selection of sites with similar tree stand, not subjected to grazing, logging, or fire and with least observable signs of recreation. To avoid pollution from motor vehicles sites were established at distances of 0.5–1.0 km of the asphalt road crossing the study area. There are a few small settlements along the road and two industrial towns apart from Karabash: Kyshtym in the north and Miass in the south. Our most northern site 32N is 3 km away from the west margin of Kyshtym and most southern site 27S is at a distance of 7.5 km to north-west of the border of Miass. Prevailing winds are directed to east and carry atmospheric emissions away from our sites, as evidenced by low litter metal concentrations.

Common birch dominated tree stand at all sites. Proportion of Scots pine and aspen *Populus tremula* equaled on average ~10%. Mean age of the tree stands was 70 years. *Sorbus aucuparia*, *Tilia cordata*, *Chamaecytisus ruthenicus*, and *Rosa acicularis* are the most common species of understory. *Aegopodium podagraria*, *Pteridium aquilinum*, *Calamagrostis arundinacea*, *Rubus saxatilis*, *Lathyrus vernus*, and *Pulmonaria dacica* dominate the field layer at sites with a distance of 26, 27, and 32 km from KCS. *Pteridium aquilinum*, *Calamagrostis arundinacea*, *Rubus saxatilis*, *Brachypodium pinnatum*, *Trifolium medium* dominate the field layer at sites with a distance of 9, 11, 12, and 18 km. *Calamagrostis arundinacea*, *Vaccinium vitis-idaea*, *Orthilia secunda*, and *Pyrola minor* are the most common species at sites with a distance of 3.5 and 5 km. *Calamagrostis epigeios*, *Equisetum arvense*, and *Lactuca tatarica* are represented as single specimens at the industrial barren (1 km from the smelter).

2.4. Measurement of habitat variables

At each of 10 sites, three 25 × 25 m^2 plots were established at a distance of 50–300 m from each other. At each plot, the basic habitat characteristics were measured as follows: Cu, Pb, and Cd concentrations (extracted with 5% HNO_3) in the forest litter (data from Smorkalov and Vorobeichik, 2011); number of trees per 1 ha, height of the top tree canopy and stand basal area (data from Usoltsev et al., 2012); species richness and cover of the understory, field and moss layers; percentages of soil surface covered with woody debris, stones, and bare ground (original data). Cover of the understory, field and moss layers, woody debris, stones, and bare ground were determined visually at 20 subplots, 0.5 × 0.5 m^2 within each plot (altogether 600 subplots). Cover of the field layer is the sum of covers of all species so it can exceed 100%. Habitat variables were measured in 2009. There were no local disturbances and habitats seem not changed after 5 years. Forest ecosystems are very stable and change slowly over time if no catastrophic events (windthrow, fire etc.) occur (Grandin, 2011; Halpern and Lutz, 2013). In polluted areas, the deteriorated plant communities are rather stable as well and show very low recovery even after pollutant deposition reduced strongly or ceased (Isbell et al., 2013; Vorobeichik et al., 2014).

The forest litter samples for chemical analysis were collected in 2010. In addition to the forest litter, common birch leaves were sampled for chemical analysis from 5 trees per site (Belskaya, unpublished). There was no forest litter at the industrial barren, so we calculated metal levels at this site using the regression "metal concentration in birch leaves—metal in the forest litter" (n = 9 sites). The values obtained are consistent with other estimates (Kozlov et al., 2009). The metal concentrations used by us as an exposure index, refer to mobile forms. The mobile metal forms migrate into lower soil horizons over time, but this is a slow process (Tyler, 1978; Vorobeichik and Kaigorodova, 2017). At the same time, ongoing atmospheric deposition of metal particles restores their pool in the topsoil. The mobile metal concentrations in soil depend on a number of factors and show short-term temporal variations (Rieuwerts et al., 1998). Nevertheless, long-living systems, such as ant colonies, average short-term environmental fluctuations and show an integrative response to stressors.

2.5. Sampling

The ant numbers were evaluated over two seasons using pitfall traps (plastic jars 9 cm in diameter, filled with 3% acetic acid). The pitfall trapping is widely used for counts of worker ants outside the nests (Underwood and Fisher, 2006; Tista and Fiedler, 2011); it provides a satisfactory estimate of species richness and abundance of ants foraging on ground and forest litter (Bestelmeyer et al., 2000). The advantage of this method is that it is useful for long-term monitoring allowing repeated counts in the same points and is not laborious. The same sampling technique allows comparison of different years and sites along the pollution gradient under study. At each plot, five traps were placed along a line at a distance of 3 m from each other. The traps were located away from the ant trails, at least 100 m from forest edges, to avoid collecting foraging ants from surrounding habitats; in the same points for all samplings. To reduce the digging-in effect (Greenslade, 1973) we established traps one day before sampling and kept them inverted before and between count sessions. At each sampling event, traps were exposed for five days. The sampling was performed twice a season, at the beginning and at the end of summer, i.e., the 1st decade of June and the 3rd decade of August in 2009 and 2014. By using temporal replications throughout a season, we tried to reduce nondetections of rare species (Dorazio et al., 2006). In further analyses, two sampling sessions were pooled at each plot to yield year-specific values.

Ant species were identified by A.V. Gilev according to Dlussky (1967), Radchenko (1994, 1995, 1996), Seifert (1992) and Czechowski et al. (2002). Because of plenty of intermediate forms between *Lasius niger* and *L. platythorax* in our area, they were treated as *Lasius* cf. *niger*.

2.6. Data processing

To reveal the effects of KCS on habitat characteristics, including tree height and density, species richness and cover of vegetation, we used

Table 1
Characteristics of study sites near the Karabash copper smelter (KCS).

Index	Distance to smelter (km) and direction[a]									
	32 N	27 S	26 S	18 N	12 S	11 N	9 S	5 N	3.5 S	1.2 E
Litter Cu concentration, mg/kg dry mass[b]	37.4 (0.5)	18.6 (1.0)	24.6 (0.7)	89.7 (5.6)	104.5 (3.1)	235.0 (57.9)	421.1 (60.9)	4383.2 (66.3)	3994.6 (333.4)	9600.4 [d]
Litter Pb concentration, mg/kg dry mass[b]	72.0 (2.6)	55.0 (1.5)	75.7 (3.2)	172.9 (6.7)	275.4 (16.6)	401.9 (54.2)	650.0 (34.3)	2260.3 (213.6)	1908.5 (252.8)	3084.2 [d]
Litter Cd concentration, mg/kg dry mass[b]	1.7 (0.1)	1.4 (0.1)	1.6 (0.1)	2.8 (0.1)	4.3 (0.1)	5.7 (0.4)	8.7 (0.3)	27.3 (1.2)	23.2 (1.8)	35.7 [d]
Number of trees per 1 ha[c]	1013.3 (113.5)	901.3 (64.7)	906.7 (227.6)	826.7 (97.8)	1210.7 (228.7)	1368.7 (536.9)	981.3 (161.1)	816 (125)	922.7 (214.9)	0 (0)
Top-canopy height, m[c]	25.7 (0.1)	25.1 (1.2)	25.7 (1.9)	25.4 (0.5)	21.3 (1.9)	22.5 (2.4)	21.2 (0.9)	20.2 (1.8)	19.5 (1.6)	0 (0)
Stand basal area, m^2/ha[c]	35.5 (3.8)	30 (5.5)	31 (1.9)	34 (3.2)	29.2 (4.4)	35 (7.7)	26.7 (6.4)	21.7 (3.7)	17.8 (2.9)	0 (0)
Cover of understory, %	4.7 (3.7)	0.8 (0.2)	0.2 (0.3)	3.7 (2)	5.1 (3.7)	2.5 (1.3)	1.9 (1.5)	0 (0)	0.8 (0.7)	0 (0)
Species richness of understory	4.3 (0.6)	6 (3.6)	4 (1.7)	1.7 (0.6)	4 (1.7)	3 (2)	3.7 (1.5)	1.3 (0.6)	1 (0)	0 (0)
Total cover of the field layer, %	108.1 (8.8)	158.1 (17.7)	127.6 (6.2)	124.5 (18.8)	86.7 (5.8)	87.1 (7.7)	49.8 (8.4)	2.8 (2.7)	1.3 (1.6)	1.4 (1.8)
Species richness of the field layer	64.7 (3.2)	67.3 (5.5)	52 (2.6)	55 (5.3)	61 (2.6)	57.3 (2.3)	44.3 (5.1)	20 (1)	15.3 (4.2)	1 (1)
Cover of the moss layer, %	2.3 (2.2)	0 (0)	1.4 (0.9)	3.5 (3.2)	1.6 (1.1)	3.4 (2.8)	0.4 (0.2)	1.5 (1)	6.9 (4.7)	0 (0)
Species richness of the moss layer	13.3 (1.5)	6 (1)	9.3 (2.1)	8.7 (0.6)	6 (1)	5.7 (0.6)	6.7 (2.3)	1 (0)	1 (0)	0 (0)
Cover of woody debris, %	1.1 (1)	0.6 (1)	3.8 (0.3)	2.3 (2)	0.7 (0.8)	2.2 (1.3)	3 (2.5)	1.1 (1)	0.7 (0.5)	0 (0)
Cover of stones, %	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.3 (0.4)	1.8 (1.8)	0.1 (0.2)	72.6 (2.9)
Cover of bare ground, %	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.4 (0.7)	0.1 (0.2)	26.7 (2)

Note: values represent site means and standard deviation (SD) in parentheses, n = 3 plots per site; [a]direction from KCS: E – east, N – north, S – south; [b]metal concentrations (extraction with 5% HNO_3) from Smorkalov and Vorobeichik (2011); [c]tree stand indices from: Usoltsev et al., 2012; [d]calculated using the regression "metal concentration in birch leaves—metal concentration in the forest litter".

simple linear regression with $\log_{10}$ distance to the smelter as predictor and site as a random effect. Logarithmic distance was used because aerial heavy metal emissions from factories and their effects on biota usually follow logarithmic spatial distribution (Jongman et al., 1987). The analyses were performed with two sets of data: full set including the industrial barren (30 plots) and reduced set without the barren (27 plots). This was done to explore if the effect of pollution is significant without the industrial barren. The false discovery rate (FDR) control for multiple hypothesis testing was performed with Benjamini–Yekutieli procedure. The calculations were performed with JMP 10.0.0 software (SAS Institute Inc., United States, 2012) and Microsoft Excel 2010.

To reduce the number of environmental variables in further analyses of association of ant diversity with habitat characteristics we performed the Principal Component Analysis. For contaminant exposure Cu, Pb, and Cd litter concentrations were used (plot-specific data were kindly provided by E.L. Vorobeichik). The habitat variables were number of trees per 1 ha, top-canopy height, stand basal area, species richness and cover of understory, field and moss layers, and cover of woody debris. Cover of stones and bare ground were excluded because of many zeroes. To meet the normality metal concentrations were $\log_{10}$-transformed and projective covers were arcsine-transformed. To express descriptors in the same units all variables were standardized (x_i − mean)/SD. The PCA was performed with two sets of data: full set including the industrial barren (30 plots) and reduced set without the barren (27 plots). This was done to explore the effect of pollution if the gradient would be shorter and would not include the barren. For the full data set three principal components (PC) were selected with eigenvalues 8.1, 1.7, and 0.99, respectively, which explained together 83.2% of the total variance. The analysis of the reduced data set yielded four PCs with eigenvalues 7.4, 1.7, 1.2, and 1.05, respectively, which explained together 87.2% of the total variance. The calculations were performed with Statistica v10.0 software (StatSoft, Inc.).

For ant communities at each plot and year we determined species richness (S) and number of species occurrences as a measure of abundance (Longino et al., 2002; Masoni et al., 2017). For this, numbers of individuals in each trap were converted to presence/absence data. For each species, the occurrence varied from 0 (never detected in a plot) to 5 (detected in all the traps of a plot). Sum of species occurrences represents the total occurrence of ants at a plot. As a measure of diversity, we used Shannon diversity index (H) using proportions of particular species in the total occurrence at a plot (Chao et al., 2014; Masoni et al., 2017). For plots with no ants H was set to 0. The expected species richness of ants per site (S') was estimated with sample-based rarefaction (Gotelli et al., 2016) using the PAST v.1.92 software (Hammer et al., 2001). For rarefaction we pooled three plots per site with 15 samples (traps) in total. The interannual repeatability of the indices was examined with a dependent *t*-test for paired samples. The normality of the distribution of differences between years was tested with the Kolmogorov-Smirnov test. Since the effect of the year was not significant (see Results), we averaged ant indices at each plot over the two years for further analyses.

Further, we explored associations of ant diversity and occurrence with habitat characteristics, transformed into Principal Components (PC). We performed multiple regression analysis with ant indices as dependent variables and PCs and squared PCs as predictors and site as a random effect. Non-significant terms ($p > 0.05$) were dropped from the models one-by-one. Dropped terms were then introduced back to the reduced model, but in no case they were significant. The calculations were performed with JMP 10.0.0 software (SAS Institute Inc., United States, 2012).

3. Results

3.1. Habitat transformation

The industrial emissions negatively affected forest vegetation. Analysis of the full data set (including industrial barren) showed that tree height, stand basal area, species richness of the understory, field and moss layers, and the cover of the field layer decreased towards the smelter (Tables 1 and 2). Analysis of the reduced data set (without barren) produced almost the same results (except species richness of understory), which indicates that deterioration of forest ecosystems started already at moderate pollution level. The deterioration of forests around Karabash peaked as a vast industrial barren with eroded soil and single specimens of woody (*Betula* spp., *Salix* spp.) and herbaceous species (*Calamagrostis epigeios*, *Equisetum arvense*, *Lactuca tatarica*) growing mainly in ravines.

Table 2
Linear regression of habitat variables on logarithmic ($\log_{10}$) distance to KCS with site as a random effect.

Variables	Estimate	SE	*t*	*FDR-adjusted p*	R^2_{Adj}
Full data set (n = 30, DFDen = 8)					
Number of trees per 1 ha	503.6	214.9	2.34	0.197	0.748
Top-canopy height	15.0	2.7	5.60	0.004	0.972
Stand basal area	21.8	3.3	6.63	0.003	0.868
Cover of understory	2.1	1.3	1.55	0.588	0.512
Species richness of understory	3.4	0.8	4.21	0.014	0.512
Total cover of the field layer	114.7	19.6	5.84	0.004	0.978
Species richness of the field layer	47.8	6.5	7.38	0.003	0.981
Cover of the moss layer	−0.4	1.6	−0.22	1.000	0.481
Species richness of the moss layer	8.1	1.5	5.30	0.004	0.937
Cover of woody debris	1.2	0.8	1.50	0.563	0.458
Reduced data set (industrial barren excluded, n = 27, DFDen = 7)					
Number of trees per 1 ha	6.3	208.3	0.03	1.000	0.311
Top-canopy height	7.2	0.9	8.03	0.003	0.682
Stand basal area	15.2	3.7	4.06	0.028	0.598
Cover of understory	2.0	2.1	0.98	1.000	0.475
Species richness of understory	3.8	1.2	2.99	0.099	0.398
Total cover of the field layer	154.8	22.1	7.01	0.003	0.972
Species richness of the field layer	50.1	10.0	5.03	0.015	0.968
Cover of the moss layer	−3.2	2.0	−1.58	0.662	0.412
Species richness of the moss layer	10.3	2.1	4.94	0.012	0.918
Cover of woody debris	0.8	1.3	0.62	1.000	0.388

The Principal Component Analysis of the full data set (including barren) produced three principal components (PC) with eigenvalues 8.1, 1.7, and 0.99, respectively, which explained together 83.2% of the total variance. Heavy metal concentrations, top-canopy height, stand basal area, species richness of the field and moss layers, and cover of the field layer showed highest correlation with the 1st PC. So, PC1 reflects both contaminant exposure and state of the tree stand and field layer. Cover of mosses contributed to PC2, which likely reflects variations in moisture. Woody debris showed the highest correlation with PC3 (Appendix, Table A.1). The analysis of the reduced data set (without barren) yielded four PCs with eigenvalues 7.4, 1.7, 1.2, and 1.05, respectively, which explained together 87.2% of the total variance. The same variables contributed to PC1 and PC3. The density of trees showed the highest correlation with PC2, indicating variations in insolation. Cover of mosses was the main factor contributing to PC4 (Table A.2).

3.2. Species composition

In total, 21 ant species belonging to 7 genera were registered in the study area (Table 3). The most widespread species were *Lasius* cf. *niger* (registered at all 10 sites, 30 plots), *Formica fusca*, *Myrmica ruginodis*, and *M. rubra* (9 sites; 27, 26, and 23 plots, respectively), *Formica rufa* and *Myrmica lobicornis* (7 sites; 12 and 14 plots), *Leptothorax acervorum* (6 sites; 14 plots), *Camponotus saxatilis*, *Leptothorax muscorum*, and *Myrmica scabrinodis* (5 sites; 11, 10, and 8 plots, respectively), and *Formica sanguinea* (4 sites, 10 plots).

3.3. Community response and differences between sampling years

Indices of ant communities did not depend on the year, which indicated good repeatability of the results. The interannual differences in ant community indices were not significant. The dependent *t*-test for paired samples for species richness equaled t = 1.48, p = 0.15, n = 30 plots; for Shannon diversity t = 0.47, p = 0.64, for total occurrence t = 1.97, p = 0.06.

The species richness (both S and S′), Shannon diversity, and total occurrence of ants increased towards the smelter and were maximal at distances 3.5–5 km, in heavily disturbed forest stands bordering the industrial barren. Further increase of the toxic load and habitat deterioration caused sharp decrease of all indices (Table 3, Fig. 2). Only two species (*Lasius* cf. *niger* and *Tetramorium caespitum*) were registered at the industrial barren. Thus, the diversity and occurrence of ants showed clearly non-linear variations along the pollution gradient.

3.4. Associations of ant diversity and occurrence with habitat variables

The regression analysis showed that indices of ant communities depended only on PC1, i.e. on contaminant exposure and state of the tree stand and field layer (Table 4). The analysis of the full data set showed significant contribution of the second-order term (squared PC) to variations in ant diversity and occurrence. The analysis of the reduced data set showed significant linear pattern of ant indices, which increased with pollution and habitat deterioration (Table 4). This implies that the hump-shaped pattern of ant indices was associated only with the industrial barren.

4. Discussion

The changes in the vegetation near Karabash are in line with known responses of forest communities to industrial pollution. High toxic load caused the decrease of the top-canopy height, stand basal area, cover, and species richness of the field layer, whereas cover of the bare ground increased. The natural vegetation and soil were fully destroyed at the industrial barren around KCS. Therefore, all stages of forest degradation are present along this pollution gradient up to the complete destruction of the ecosystem. The formation of industrial barrens is a typical effect of emissions of large non-ferrous metallurgical enterprises. This is a result of the combined effect of SO_2 and heavy metals, aggravated by a hilly relief (Kozlov and Zvereva, 2007).

Earlier, the intensive ant survey yielded 33 species in this region and more than 70 species in the entire Southern Urals (Gridina, 2003). The species composition of ants in our study corresponds to the published data. *Lasius* cf. *niger*, *Formica fusca*, *Myrmica rubra*, and *M. ruginodis* were the most common. These widespread boreal species are typical of forest and forest-steppe zone of Eurasia. The red wood ants of the genus *Formica*, dominating more northern taiga forests (Dlussky, 1967; Gilev, 2010), were absent at many sites near Karabash and not so abundant than in the more northern area near Revda (Belskaya et al., 2017). This is partly related to the zonal distribution and habitat preferences of this group. We counted ants near Karabash in a deciduous forest inhabited by *F. rufa* and *F. polyctena*, which don't form large colonies in contrast to *F. aquilonia*, preferring coniferous forests and forming big settlements consisting of dozens and hundreds of nests with high abundance of workers (Dlussky, 1967; Gilev, 2010). Our data showed small interannual variability of the ant diversity and occurrence at the same sites. This is because of the residency of ants and longevity of their colonies. The relative stability in time is a good precondition for using ants in ecological monitoring.

The changes in ant diversity and abundance near Karabash are similar to the results of our study near the Middle Ural copper smelter with analogous composition of pollutants (Belskaya et al., 2017). Both study areas showed hump-shaped pattern of diversity of ants along the pollution gradient. This pattern is a result of extremely poor ant assemblages at the industrial barren. In some sense this is the effect of a single point. However, it cannot be ignored. Development of a barren is a logical result of extreme contaminant exposure and exclusion of the barren from the analysis leads to underestimation of the pollution effect. Our findings agree with the results obtained in some industrial regions. Studies near copper-nickel smelter in Monchegorsk (Russia) showed that species richness of ants increased towards the factory, peaked in the transitional forest communities and were minimal at the

Table 3
Number of individuals and species of ants in the birch forest at different distances to the KCS (years 2009 and 2014 combined).

Species	Distance to smelter (km) and direction[a]									
	32 N	27 S	26 S	18 N	12 S	11 N	9 S	5 N	3.5 S	1.2 E
Camponotus herculeanus	0	0	0	0	0	0	0	3	1	0
Camponotus saxatilis	1	0	0	3	0	0	10	25	11	0
Formica exsecta	0	0	0	0	1	0	0	0	0	0
F. fusca	59	29	51	42	121	82	50	15	271	0
F. polyctena	240	0	0	0	0	0	0	0	10,768	0
F. pratensis	0	0	0	0	0	2	0	1	0	0
F. pressilabris	0	0	0	0	1	0	0	0	0	0
F. rufa	82	181	2	3	166	0	9	0	148	0
F. sanguinea	0	0	0	3	0	30	16	477	0	0
F. uralensis	0	0	0	1	0	0	0	0	0	0
Harpagoxenus sublaevis	0	0	0	0	0	0	0	1	0	0
Lasius cf. *niger*	490	127	67	307	161	308	127	188	7	5
Leptothorax acervorum	2	0	1	0	2	11	0	137	45	0
L. muscorum	2	0	0	33	0	7	0	108	9	0
Myrmica lobicornis	8	1	0	0	2	3	2	323	106	0
M. rubra	24	3	17	11	125	148	123	48	6	0
M. ruginodis	36	170	607	331	44	216	10	136	11	0
M. rugulosa	0	0	0	0	0	0	0	0	19	0
M. scabrinodis	0	0	0	1	1	0	3	110	35	0
M. sulcinodis	0	0	0	1	1	0	0	127	118	0
Tetramorium caespitum	0	0	0	0	0	0	0	0	2	1
N	944	511	745	736	625	807	350	1699	11,557	6
S	10	6	6	11	11	9	9	14	15	2

[a] Note: direction from KCS: E – east, N – north, S – south; *N* – total number of individuals; *S* – number of species.

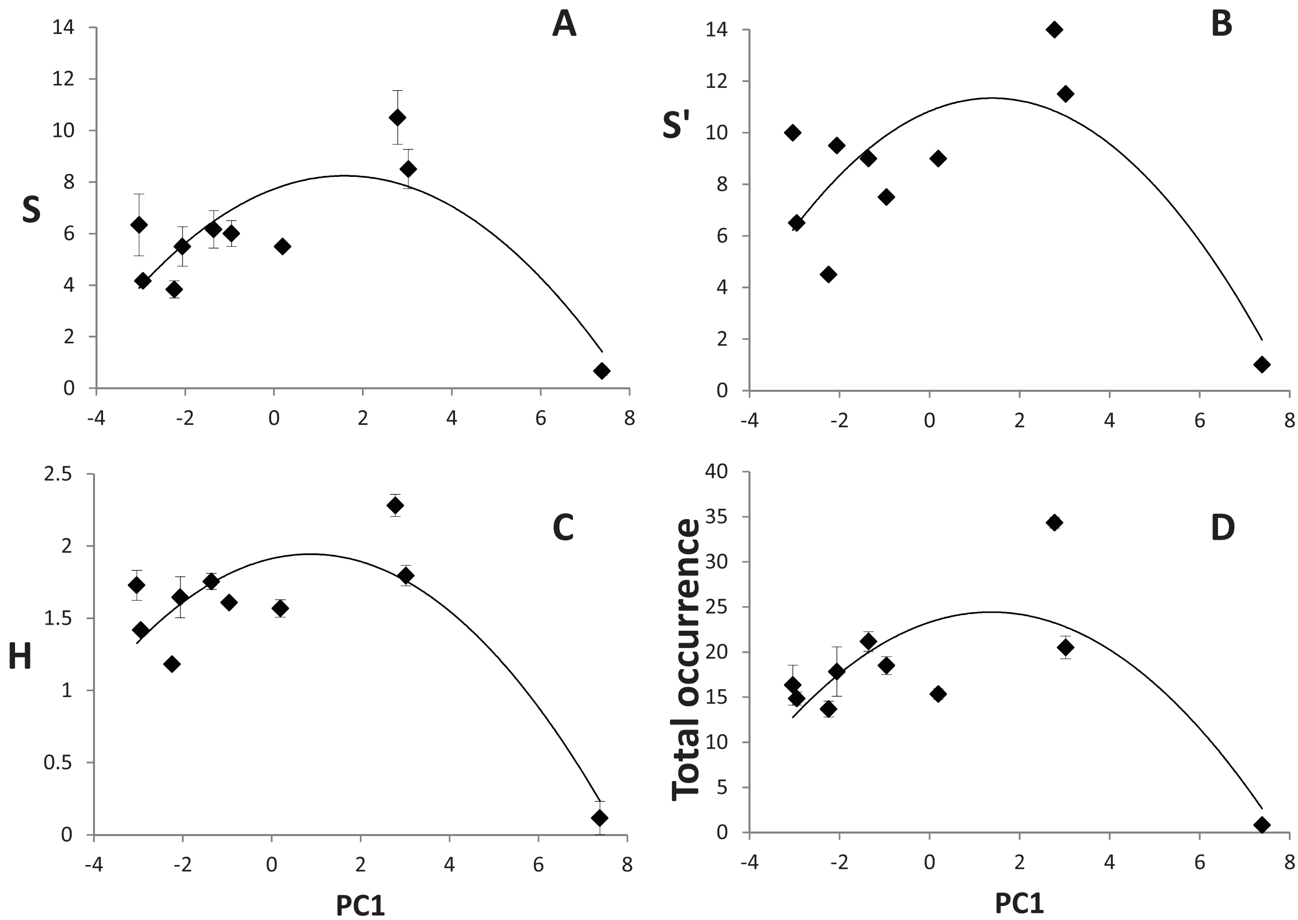

Fig. 2. The relationship of the ant diversity and occurrence (site means ± SE, for S′ only site means) with the 1st principal component (PC1) reflecting pollution level and habitat deterioration near the KCS. Ant indices at each plot (S, H, total occurrence) were averaged over two years and site means were calculated. For S' values of two years were averaged at each site. The curves were plotted using site means as replicates. A) Species richness per plot (S). R^2 = 0.65. p = 0.025; B) expected species richness per site (S'). R^2 = 0.59. p = 0.044; C) Shannon diversity (H). R^2 = 0.75. p = 0.008; D) total occurrence. R^2 = 0.62. p = 0.034.

Table 4
Results of the multiple regression analyses of ant community indices on principal components (PC and squared PC) reflecting pollution level and habitat deterioration with site as a random effect. Only variables included in the final models are shown.

Sites	Index	Variable	Estimate	SE	DFDen	t	p	R^2_{adj}
All	S	PC1	0.601	0.258	9.483	2.33	0.044	0.859
		$(PC1)^2$	−0.199	0.054	9.29	−3.73	0.005	
	Shannon diversity	PC1	0.036	0.046	11.69	0.79	0.448	0.951
		$(PC1)^2$	−0.034	0.010	11.13	−3.56	0.004	
	Total occurrence	PC1	0.258	0.157	13.33	1.64	0.124	0.940
		$(PC1)^2$	−0.108	0.033	12.63	−3.29	0.006	
Barren excluded	S	PC1	0.586	0.149	7.506	3.94	0.005	0.725
	Shannon diversity	PC1	0.063	0.028	9.055	2.20	0.055	0.859
	Total occurrence	PC1	0.283	0.114	9.633	2.48	0.034	0.885

industrial barren (Kozlov, 1997, Fig. 8). The abundance of ants on meadows of Kemerovo region (Russia) subjected to industrial emissions was highest at moderate pollution (Blinova and Dobrydina, 2008; Blinova et al., 2017). At the same time, there are examples of unidirectional patterns. Ant diversity increased towards the emission source and peaked at highest pollution near the copper-nickel smelter in Harjavalta, Finland (Koponen and Niemelä, 1995) and Zn-smelter in Olkusz, Poland (Grześ, 2009a). In contrast, ant diversity decreased towards the copper-lead smelter at Mount Isa, Australia (Hoffmann et al., 2000) and metallurgical, chemical and mining enterprises in Kemerovo region, Russia (Blinova and Dobrydina, 2018).

The pollution-related variations of ant diversity and occurrence near Karabash are consistent with the intermediate disturbance hypothesis, predicting maximum values at intermediate frequencies and/or intensities of disturbances (Connell, 1978). Although this hypothesis seems to be applicable to ant responses to many environmental factors and disturbances (Fox et al., 1988; Samson et al., 1997; Graham et al., 2009; Higgins et al., 2015) some studies failed to confirm it. Testing the hump-shaped diversity/productivity pattern requires a full range of ecological factor or disturbance (Rosenzweig and Abramsky, 1993; Andersen, 1997). The examination of short parts of the gradient can produce different results depending on the factor (disturbance) intensity under study (Jongman et al., 1987). In case of pollution, its effect on ants can be either positive or negative depending on the exposure level: negative effect at extremely high pollution (Hoffmann et al., 2000) or positive effect at lower loads (Koponen and Niemelä, 1995; Grześ, 2009a). The pollution gradient near Karabash is long enough, and as a result of extreme exposure, the forest habitat converts into the industrial barren with almost no vegetation, in contrast to some other studies (Grześ, 2009a), where pollution was not strong enough for full destruction of the forest ecosystem. Near the KCS, species richness and Shannon diversity increased with pollution and peaked in transitional forest communities 3.5–5 km away from the smelter, where pollution caused strong negative effects on the field layer and tree stand. Further increase of the pollution resulted in extremely low diversity and occurrence of ants because of almost full absence of plants and bad food supply at the industrial barren, which looks like a moonscape. Considering the results of this and earlier studies, it can be concluded that hump-shaped response of ants to pollution is quite common in forest ecosystems, if they undergo full destruction terminating with industrial barrens. Additional studies are needed to test generality of this conclusion but it should be taken into account when planning field studies. A potentially nonlinear pattern of ant diversity suggests a detailed examination of pollution gradients, not limiting with two contrast sites. Extreme points within such gradients should differ in the level of pollution (and habitat deterioration) as great as possible (Vorobeichik and Kozlov, 2012). Otherwise, the effect of pollution can be underestimated.

Two main mechanisms can explain the hump-shaped response of the ant diversity to disturbance (Andersen, 1997). The first one is the increased environmental heterogeneity that allows the coexistence of species with different habitat preferences. Along with habitat characteristics, interspecies hierarchy is an important factor in structuring the ant communities. In boreal forests, the territorial red wood ants (*Formica rufa*-group species) dominate the community and affect the abundance of the submissive species (Reznikova, 1980, 1999; Mabelis, 1984; Savolainen et al., 1989; Punttila et al., 1994; Zakharov, 2015). When a habitat changes and the abundance of dominant species decreases, the weakened competition favors the subordinate species. Both mechanisms were shown to act in our earlier study near the Middle Ural copper smelter. In this region, the dominant red wood ants, which were highly abundant in the unpolluted and moderately polluted territory, almost disappeared in the transitional zone between the moderately and heavily disturbed forests, resulting in highest ant diversity (Belskaya et al., 2017). There, increased environmental heterogeneity in the ecotone area combined with the decreased abundance of dominants, and thus weakened the competition. Near Karabash, the red wood ants—*F. polyctena* and *F. rufa*—were not widespread and were unlikely to affect the composition of ant communities. Even high occurrence of *F. polyctena* and *F. rufa*, at the site with a distance of 3.5 km south of KCS, did not hinder the large diversity of the whole community. This may be due to decreased aggressiveness of wood ants in polluted areas (Sorvari and Eeva, 2010). Since competition seems not to affect ant communities in strongly depressed forest stands at distances of 3.5 km south and 5 km north of KCS, it is likely that environmental heterogeneity is the main factor determining ant diversity in transitional forest communities surrounding the barren.

We don't attribute variations of ant diversity solely to pollution. Despite strong association of the indices with the 1st PC, which correlates strongly with metal concentrations, we don't consider it as a cause-effect relationship. The direct toxic exposure is unlikely to explain the absence or presence of individual ant species because of their tolerance to heavy metals (Folgarait, 1998; Grześ, 2009b, 2010a,b; Grześ and Okrutniak, 2016). This suggests the key role of other factors in shaping the ant communities in the polluted areas—first of all the habitat variations. The highest diversity of ants was observed in the transitional zone between the forest habitats and the industrial barren. This ecotone effect was documented for ants in the forested areas not subjected to industrial pollution (Punttila et al., 1994; Zakharov, 2015; Stockan and Robinson, 2016). It is known that most ground-dwelling ant species prefer well-lightened and heated habitats. Near Karabash, the forest deterioration becomes evident at distances of 3.5–5 km from the smelter, where stand basal area, cover of the field layer, and species richness of the field and moss layers decrease. Obviously, better insolation of the soil in these areas contributed to the increased ant diversity. Specifically, the hemixerotherm species preferring drier and warmer areas, such as *C. saxatilis*, *F. sanguinea*, and *T. caespitum*, colonized this territory. Despite vegetation is depressed in forest stands surrounding industrial barren, there is still sufficient food supply and microhabitats suitable for nests.

The ants have both advantages and shortcomings as bioindicators of metal pollution. They are numerous and act as ecological engineers affecting other components of ecosystems because of the high biomass, longevity of their colonies, and complex trophic relationships. They are promising accumulation indicators because of their tolerance to heavy metals and ability to concentrate metals in the body (Stary and Kubiznakova, 1987; Dallinger, 1993; Rabitsch, 1995; Grześ, 2010a; Gramigni et al., 2013). Relatively small feeding area of a colony enables the assessment of contaminant exposure at a local scale. The ants are diverse and populate different habitats. At the same time, this diversity creates some difficulties when using ant communities as ecological indicators. Firstly, species identification requires experienced taxonomists to be involved. To simplify the determination of ant community composition, it was proposed to combine the species into functional groups (Andersen, 1995). The analysis of ecological structure of the ant communities gives good results when species richness is high, in subtropical and tropical regions (Andersen et al., 2003; Bestelmeyer and Wiens, 1996; Gómez et al., 2003; Bernadou et al., 2013; Ottonetti et al., 2010). However, this approach may be less effective in northern latitudes with small species richness of ants. Secondly, different species respond differently to the environmental changes, according to their own ecological preferences. This species-specificity of reactions requires consideration of species composition when using ant communities in environmental monitoring. Finally, the non-linear pattern of diversity along the environmental gradients complicates determination of target indices and values in ant communities, when controlling human impact on ecosystems. The model predictions would be more reliable if based on the linear dose-effect relationships. However, linear model is not always applicable to ant responses to stressors. In addition, geographic position and climatic conditions can modify the pollution effects. The same contaminant exposure can cause different effects in different regions. For example, at Pb litter concentrations of 2800–3000 mg/kg, the ant community was most diverse along the pollution gradient near Revda (Belskaya, 2017) and extremely poor near Karabash, a region with more arid climate. The close association with habitat variables suggests that the ant communities indicate contaminant exposure (or disturbance) indirectly, through the state of habitats.

5. Conclusion

This study showed nonlinear dynamics of ant diversity and occurrence along the pollution gradient near the Karabash copper smelter. The highest species richness, diversity, and total occurrence were observed in the transitional zone between moderately polluted forest habitats and industrial barren. Therefore, our results conform to the intermediate disturbance hypothesis for both diversity and occurrence (measure of productivity) of ants. Importantly, the hump-shaped effect appeared independently on the presence of red wood ants, which dominate boreal communities and are known to affect diversity and abundance of ants. Apparently, contaminant exposure affects ant communities near Karabash indirectly, through habitat transformation.

The study emphasizes the need for thorough planning of field studies of pollution effects on ants. A potentially nonlinear pattern of ant diversity suggests a detailed examination of pollution gradients, including the full range of exposures. The examination of short parts of the gradient can lead to different conclusions depending on the degree of pollution and habitat deterioration. In addition, the nonlinear pattern of diversity and occurrence of ants along the pollution gradients suggests caution, when assessing the magnitude of human impact and the state of ecosystems with ants as bioindicators.

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Declarations of Competing Interest

None.

Appendix

Table A.1
Factor loadings according to Principal Components Analysis with full data set (n = 30 plots).

Variable	Principal Components		
	1	2	3
Log_{10} Cu concentrations in the forest litter	**0.964**	−0.219	−0.003
Log_{10} Pb concentrations in the forest litter	**0.940**	−0.274	−0.001
Log_{10} Cd concentrations in the forest litter	**0.954**	−0.236	−0.002
N trees per 1 ha	−0.617	−0.561	0.064
Top-canopy height	**−0.850**	−0.301	−0.135
Stand basal area. live trees	**−0.887**	−0.263	−0.089
Cover of understory	−0.559	−0.340	0.600
Species richness of understory	−0.698	0.273	0.113
Total cover of the field layer	**−0.896**	0.316	−0.010
Species richness of the field layer	**−0.969**	0.042	0.120
Cover of mosses	−0.088	**−0.810**	0.120
Species richness of mosses	**−0.864**	0.042	−0.050
Cover of woody debris	−0.457	−0.324	**−0.744**

Note: proportion of the total variance explained by PC1 equaled 0.624, PC2 0.131, and PC3 0.076.

Table A.2
Factor loadings according to Principal Components Analysis with reduced data set (industrial barren excluded, n = 27 plots).

Variable	Principal Components			
	1	2	3	4
Log_{10} Cu concentrations in the forest litter	**0.978**	0.032	0.018	0.014
Log_{10} Pb concentrations in the forest litter	**0.972**	0.099	0.047	−0.008
Log_{10} Cd concentrations in the forest litter	**0.983**	0.056	0.031	−0.020
N trees per 1 ha	−0.067	**0.880**	0.210	−0.260
Top-canopy height	**−0.841**	−0.404	−0.069	0.217
Stand basal area. live trees	**−0.757**	0.319	0.246	−0.088
Cover of understory	−0.349	0.678	−0.364	0.299
Species richness of understory	**−0.729**	−0.214	−0.299	−0.178
Total cover of the field layer	**−0.940**	−0.105	−0.034	−0.042
Species richness of the field layer	**−0.934**	0.214	−0.071	−0.074
Cover of mosses	0.375	0.147	−0.298	**0.761**
Species richness of mosses	**−0.825**	0.133	0.184	0.304
Cover of woody debris	−0.152	−0.098	**0.861**	0.363

Note: proportion of the total variance explained by PC1 equaled 0.570, PC2 0.128, PC3 0.093, and PC4 0.081.

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