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Soil physicochemical properties and vegetation as factors affecting soil microorganisms in areas contaminated with heavy metals

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Soil microorganisms, mainly bacteria and fungi, play many important roles in ecosystems. They decompose dead organic matter of plant and animal origin into inorganic compounds that can be taken up and used by plants. Some microorganisms are able to fix atmospheric nitrogen and enrich the soil with it. Microorganisms interact with plants, supporting or limiting their growth and development (mycorrhizal fungi, rhizobacteria, pathogens). Soil microbes improve soil structure and some of them can degrade organic pollutants.

Unfavourable environmental factors such as heavy metal pollution may decrease the activity, biomass and diversity of soil microbial communities, and may alter their structure (Kandeler *et al.* 1996; Ge and Zhang 2011; Pan and Yu 2011). Any disturbance to the structure or functioning of microbial communities may upset the functioning of the whole ecosystem. Pioneering studies of the effects of heavy metals on soil processes in forests near a zinc smelter in Palmerton, Pennsylvania, showed that Zn, Pb and Cd pollution decreases the rate of organic matter decomposition while increasing the rate of its accumulation (Strojan 1978). It has been found that heavy-metal contamination of soil considerably reduces the activity of soil enzymes such as urease, acid phosphatase, dehydrogenase, arylsulphatase, protease, ß-glucosidase and endocellulase (Kandeler *et al.* 1996; Kuperman and Carreiro 1997; Pan and Yu 2011). In view of the important functions played by microorganisms and other soil organisms in ecosystems, the priority tasks of reclamation should include enhancement of the diversity and activity of microorganisms as well as micro-, meso- and macrofauna, and also restoration of the biological functions of degraded post-industrial soils (Gómez-Sagasti *et al.* 2012). Among the important indicators of soil biological quality which should be included in biomonitoring are soil microbial parameters such as respiration, biomass, taxonomic structure and diversity, the activity of C, N, S or P cycling-related enzymes exuded by microorganisms, microbial utilisation of different organic compounds, and level of root

mycorrhization (Filip 2002; Avidano *et al.* 2005; Gómez-Sagasti *et al.* 2012).

Assessing the effects of pollution on soil microorganisms in the field (e.g. in the vicinity of smelters) is not an easy task. Soil microbiological quality is affected by metal pollution but by other biotic and abiotic factors as well, such as temperature, soil moisture, soil structure, nutrient content, pH, the presence or lack of plants, and the species richness and composition of plant communities (Stephan *et al.* 2000; Marschner *et al.* 2004; Niklińska *et al.* 2005; Balogh *et al.* 2011; Chodak *et al.* 2013). The C/N ratio and soil pH have been shown to be more important for soil microorganisms than heavy metals even at highly polluted sites (Niklińska *et al.* 2005). Those biotic and abiotic factors affect microbes in polluted soils both directly and indirectly, by modifying the behaviour of toxicants: their mobility, bioavailability and toxicity (Babich and Stotzky 1983; Hinsinger *et al.* 2006; Gao *et al.* 2012). For example, high content of the organic matter and clay fraction in soil improves trophic conditions but also promotes immobilisation of metals, lowering their bioavailability (Bååth 1989). Soil pH affects soil microbial community structure (e.g. fungi/bacteria ratio; Blagodatskaya and Anderson 1998) but also affects metal sorption by organic matter (Kabata-Pendias and Pendias 1993).

Plants influence soil microorganisms through deposition of litter and production of root exudates of varying amounts and chemical composition. Dead organic matter is a source of nutrients for saprotrophic soil organisms; it modifies the soil environment, influencing physicochemical characteristics such as soil nutrient content and pH. Reich *et al.* (2005) found significant differences in the quantity and quality of litter produced by 14 tree species growing in experimental monocultures. Ca content ranged from 3.7 mg g^{-1} in *Pinus nigra* needles to 22.4 mg g^{-1} in *Tilia cordata* leaves. More importantly, Ca content in tree assimilation organs was significantly correlated with the Ca content and pH of the soil beneath the plants (Reich *et al.* 2005). Herbaceous species may considerably modify soil physicochemical properties as well. Four grass species (*Agrostis capillaris*, *Festuca ovina*, *Lolium perenne*, *Nardus stricta*) were found to differently affect soil moisture, pH and inorganic N content (Markham *et al.* 2009).

Plants produce numerous compounds that are exuded into the soil (rhizodeposition). These substances include organic compounds (carbohydrates, amino acids, amides, fatty acids, sterols, vitamins, growth regulators), gases (ethylene, carbon dioxide) and hydrocyanic acid (Grayston *et al.* 1996). Root exudates may be specific to a plant species or even a variety. Differences in the quantity and quality of root exudates and organic matter are one of the main causes of differences in the activity and diversity of soil microorganisms in both the rhizosphere and bulk soil. The diversity and/or activity of soil microbial communities increase as plant diversity increases (Stephan *et al.* 2000; Loranger-Merciris *et al.* 2006; Eisenhauer *et al.* 2011). Some species, for example legumes, may particularly benefit soil microbes (Stephan *et al.* 2000).

This study, part of the project "Vegetation of calamine soils and its importance for biodiversity and landscape conservation in post-mining areas" (EEA FM PL0265), examined the microbiological quality of soils in areas affected by mining and processing of metal ores. The study had four component tasks: (1) to analyse microbial activity and biomass as well as the ability of soil bacteria and fungi to utilise organic compounds in two soil layers; (2) to compare the effects of heavy metals on soil microorganisms between forest and

non-forest (grassland, fallows) ecosystems; (3) to compare soil microbiological parameters between habitat types dominating the studied post-mining area (forest and grassland on sand and on mining waste, and fallows); and (4) to assess the effects of soil physicochemical properties and of the diversity and species composition of herbaceous vegetation on soil microorganisms.

Materials and methods

As in other research in this project, 49 sites representing six dominant habitat categories (GW, MW, FW, GS, FS, P; Kapusta and Godzik – Chapter 6, this volume) were studied. Microbial parameters were measured in two soil layers: upper (humus A, Ap or AE, depending on the site) and lower (B). Soil samples were taken as described in Chapters 6 (Kapusta and Godzik) and 13 (Kapusta *et al.*) of this volume, and sieved (2 mm mesh). Soil moisture and maximum water holding capacity (WHC) were measured before the microbiological measurements. Soil microbial activity was measured as soil basal respiration. Soil samples were adjusted to 50% of maximum WHC and incubated in gas-tight jars at 22°C. Evolved CO_2 was absorbed in 0.2 M NaOH, and the excess of NaOH was titrated with 0.1 M HCl after the addition of $BaCl_2$ and phenolphthalein as indicator. The duration of incubation ranged from 24 to 120 h depending on soil activity. The amount of evolved CO_2 was calculated based on the amount of HCl used for titration. After basal respiration measurements the soil was amended with glucose (10 mg g_{DW}^{-1} soil) in order to assess substrate-induced respiration. After 4 h incubation at 22°C, respiration was measured as described above. Microbial biomass carbon (C_{mic}) in soil was calculated according to the formula: C_{mic} (μg g^{-1}) = 40.04x + 0.37, where x is the respiration rate given in μl CO_2 h^{-1} g^{-1} (Anderson and Domsch 1978). Soil basal respiration and microbial biomass were expressed both per dry weight and per soil organic matter unit. The metabolic quotient qCO_2 was calculated as the soil respiration/biomass ratio; qCO_2 is helpful in assessing the effects of stress factors such as heavy metal pollution on soil microbial communities.

The ability of soil microorganisms to degrade organic compounds was assessed with 96-well Biolog plates: GN2 for bacteria and SFN2 for fungi (Preston-Mafham *et al.* 2002; Stefanowicz 2006). The plates contain 95 carbon compounds including carbohydrates, amino acids and carboxylic acids, which are nutrients for heterotrophic microorganisms. The GN2 plates also contain a dye indicator of bacterial activity.

Soil samples were shaken in physiological saline and the extracts were diluted 10-fold with physiological saline (GN2 plates) or with solution of agar, detergent (polyoxyethylene sorbitan monooleate – Tween 80), streptomycin and chlortetracycline (SFN2 plates). The addition of agar and detergent allows uniform dispersion of fungal spores in a solution, and the antibiotics prevent bacterial growth (Dobranic and Zak 1999; Buyer *et al.* 2001; Kraus *et al.* 2004). The extracts were inoculated onto the plates and the plates were further incubated at 22°C for ca. 115 h (GN2) or 216 h (SFN2). During incubation, absorbance, reflecting microbial activity on the plates, was measured twice a day at 590 nm (GN2 – colour development due to reduction of the dye by bacteria) or once a day at 650 nm (SFN2 – turbidity development due to hyphal growth).

Microbial activity on each carbon substrate was expressed as area under curve (incubation time vs. absorbance). Average bacterial and fungal activity in each soil was

expressed as average area under curve (calculated across all substrates). The number of substrates metabolised by microorganisms was recorded; the number of compounds utilised reflects the functional richness of the microbial community.

Factor analysis was performed to obtain non-correlated factors describing habitat properties (Kapusta *et al.* – Chapter 13, this volume). This analysis included 30 variables: content of sand and clay fractions, organic C, total Ca, Cd, Fe, K, Mg, Mn, N, P, Pb, S and Zn, $BaCl_2$-extractable Ca, Cd, K, Mg and Zn, water-soluble Cd, Pb and Zn, available P, pH, number of herbaceous plant species, plant species composition represented by DCA1 and DCA2 axes (Kapusta *et al.* – Chapter 13, this volume), total plant cover, and cover of two plant functional groups: graminoids and forbs. Legume cover was not included in the factor analysis as this variable did not meet the assumptions of the analysis. The obtained factors were further used in multiple regressions as independent variables in order to determine the effects of habitat properties, represented by the factors, on the microbial parameters of the upper soil layer (N = 49).

The nonparametric Mann-Whitney U test was used to compare microbial activity between the two soil layers in 34 of the 49 study sites for which the two soil layers were distinguishable (N = 34). Data from sites where one of the two soil layers was not detected or where only the transition layer AB was present were removed from the analysis.

To assess the effect of metals on microorganisms of the upper soil layer, correlation analyses were done separately for forest (N = 21) and non-forest (grasslands and fallows; N = 28) habitats. One-way ANOVA followed by Tukey's test for unequal N were performed to compare microbial parameters between the six habitat categories.

Results

Detailed data on soil respiration, microbial biomass and microbial activity on the Biolog plates are presented in Table 1. Microbial community activity and biomass were significantly higher in the upper (A, Ap or AE, depending on the site) than in the lower (B) soil layer, probably due to better trophic conditions of the upper (humus) layer. Microbial parameters varied greatly between the study sites for both soil layers (Table 1). Microbial biomass and respiration of the upper soil layer showed the highest variability (CV = 123% and CV = 97% respectively); fungal functional richness (number of Biolog substrates metabolised) showed the lowest variability (CV = 31%). The opposite was observed for the lower soil layer: the variation coefficients for 5 of 7 microbial variables exceeded 100% and were highest for fungal activity (CV = 154%) and richness (CV = 127%), and for qCO_2 (CV = 127%). Microbial biomass showed the lowest variability (CV = 47%).

The results of correlation analysis for total Zn, Pb and Cd and the microbial parameters are presented in Table 2. For soil from the non-forest sites, the concentration of total heavy metals was significantly and negatively correlated with microbial biomass (calculated per organic matter unit) and the activity and functional richness of the bacterial communities. Non-forest sites also showed significant positive relationships between total Cd, Pb and Zn in soil and the metabolic quotient qCO_2. Metals did not influence fungal activity and functional richness. None of the microbial parameters were negatively affected by metal pollution in forest soils. Soil microbial properties differed significantly between the six site categories (GW, MW, FW, GS, FS, P; Kapusta and Godzik – Chapter 6, this volume; Fig. 1). Respiration, biomass, and bacterial and fungal

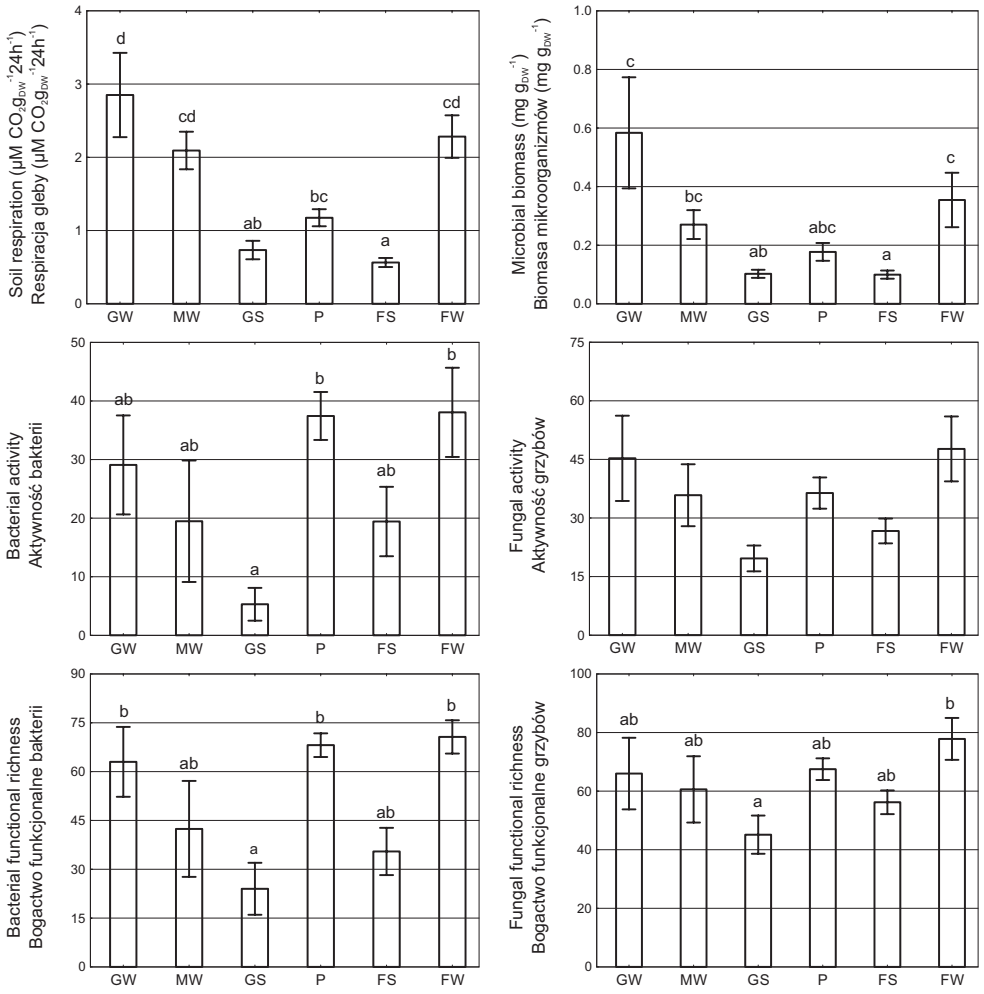

Fig. 1. Soil basal respiration, microbial biomass, activity and functional richness of the bacterial and fungal communities for six habitat categories (means and standard errors)

Ryc. 1. Respiracja bazowa gleby, biomasa mikroorganizmów oraz aktywność i bogactwo funkcjonalne zespołów bakterii i grzybów glebowych dla sześć kategorii siedlisk (średnie i błędy standardowe)

Values bearing different letters differ significantly between habitat types by Tukey's test for unequal N ($p < 0.05$). Activity – measured with the use of Biolog plates. Functional richness – number of substrates used on Biolog plates. GW – grassland on mining waste (N = 7), MW – moist grassland dominated by *Molinia caerulea* on mining waste (N = 5; one site was discarded due to an admixture of sand in the subsoil), GS – grassland on sand (N = 7), P – grassland on abandoned and relatively fertile arable fields (fallows; N = 8), FS – pine forest on sand (N = 15), FW – pine forest on mining waste (N = 6). $_{DW}$ – soil dry weight.

Istotne różnice między typami siedlisk oznaczono różnymi literami (test Tukeya dla nierównych N, $p < 0.05$). Aktywność – oznaczona z wykorzystaniem płytek Biolog. Bogactwo funkcjonalne – liczba zużytych substratów na płytkach Biolog. GW – murawy na odpadzie dolomitowym (N = 7), MW – łąki z dominacją *Molinia caerulea* na odpadzie (N = 5; jedno stanowisko odrzucono ze względu na domieszkę piasku w podłożu), GS – murawy na podłożu piaszczystym (N = 7), P – murawy na porzuconych, stosunkowo żyznych polach uprawnych (N = 8), FS – lasy sosnowe na podłożu piaszczystym (N = 15), FW – lasy sosnowe na odpadzie (N = 6). $_{DW}$ – sucha masa gleby.

Table 1. Soil microbial parameters of two soil layers (N = 34 for each layer)

Tabela 1. Wartości parametrów mikrobiologicznych dla dwóch poziomów gleby (N = 34 dla każdego z poziomów)

Variable Zmienna	Layer Poziom	Minimum Minimum	Lower quartile Dolny kwartyl	Mean (SD) Średnia (SD)	Upper quartile Górny kwartyl	Maximum Maksimum
Soil respiration Respiracja gleby ($\mu M\ CO_2\ g_{DW}^{-1}\ 24h^{-1}$)	upper górny	0.19	0.52	1.22 (1.19)	1.36	5.27
	lower dolny	0.03	0.07	0.62 (0.71)	1.14	2.13
Microbial biomass Biomasa mikrobów ($mg\ g_{DW}^{-1}$)	upper górny	0.04	0.09	0.25 (0.30)	0.33	1.57
	lower dolny	0.04	0.06	0.11 (0.05)	0.14	0.27
qCO_2 ($mg\ C\text{-}CO_2\ mg^{-1}\ C_{mic}\ h^{-1}$)	upper górny	0.001	0.002	0.003 (0.001)	0.004	0.007
	lower dolny	0.0002	0.0006	0.003 (0.004)	0.005	0.020
Bacterial activity Aktywność bakterii	upper górny	0.1	7.7	26.6 (21.6)	43.4	80.0
	lower dolny	0.0	0.9	10.4 (12.2)	17.8	60.0
Fungal activity Aktywność grzybów	upper górny	7.3	20.4	33.8 (18.1)	47.7	74.9
	lower dolny	0.0	0.7	5.2 (7.9)	4.9	27.4
Bacterial functional richness Bogactwo funkcjonalne bakterii	upper górny	1	17	50 (29)	74	87
	lower dolny	0	4	28 (25)	54	79
Fungal functional richness Bogactwo funkcjonalne grzybów	upper górny	20	51	62 (19)	77	91
	lower dolny	0	3	17 (22)	21	75

Upper soil layer – A, Ap or AE, depending on site; lower layer – B. Microbial biomass – the amount of carbon in biomass (C_{mic}). Metabolic quotient qCO_2 – basal respiration/microbial biomass ratio. Activity – measured with the use of Biolog plates. Functional richness – number of substrates used on Biolog plates. $_{DW}$ – soil dry weight, SD – standard deviation.

Górny poziom gleby – w zależności od stanowiska, A, Ap lub AE; dolny poziom – B. Biomasa mikrobów – ilość węgla w biomasie (C_{mic}). Współczynnik metaboliczny qCO_2 – stosunek respiracji bazowej do biomasy mikroorganizmów. Aktywność – oznaczona z wykorzystaniem płytek Biolog. Bogactwo funkcjonalne – liczba zużytych substratów na płytkach Biolog. $_{DW}$ – sucha masa gleby, SD – odchylenie standardowe.

activity on the Biolog plates were lowest in the soil samples from grassland and forest developed on sand. Respiration and microbial biomass were highest in soil samples from mining waste; Biolog-derived parameters were high in soil from fallows and mining waste.

Factor analysis reduced the original physicochemical and plant variables to 5

Table 2. Correlations between total heavy metal concentrations in soil and microbial parameters

Tabela 2. Korelacje między ogólną zawartością metali ciężkich w glebie a parametrami mikrobiologicznymi

		Grasslands and fallows (N = 28) Murawy i odłogi			Forests (N = 21) Lasy		
		Zn	Pb	Cd	Zn	Pb	Cd
Basal respiration $_{DW}$ Respiracja bazowa $_{DW}$		0.61***	0.48**	0.61***	0.82***	0.76***	0.82***
Basal respiration $_{OM}$ Respiracja bazowa $_{OM}$		0.19	0.06	0.15	0.21	0.32	0.19
Biomass $_{DW}$ Biomasa $_{DW}$		0.22	0.14	0.24	0.66**	0.58**	0.68***
Biomass $_{OM}$ Biomasa $_{OM}$		–0.46*	–0.53**	–0.47*	–0.25	–0.23	–0.23
qCO_2		0.47*	0.41*	0.44*	0.30	0.39	0.28
Activity Aktywność	bacteria bakterie	–0.42*	–0.50**	–0.44*	0.26	0.22	0.29
	fungi grzyby	0.08	–0.01	0.12	0.51*	0.47*	0.54*
Functional richness Bogactwo funkcjonalne	bacteria bakterie	–0.27	–0.39*	–0.28	0.43	0.38	0.46*
	fungi grzyby	0.05	–0.03	0.07	0.56**	0.51*	0.60**

Statistically significant correlations are asterisked (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Metabolic quotient qCO_2 – basal respiration/microbial biomass ratio. Activity – measured with the use of Biolog plates. Functional richness – number of substrates used on Biolog plates. $_{DW}$ – variables calculated per soil dry weight unit; $_{OM}$ – variables calculated per organic matter unit.

Korelacje statystycznie istotne oznaczono gwiazdką (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Współczynnik metaboliczny qCO_2 – stosunek respiracji bazowej do biomasy mikroorganizmów. Aktywność – oznaczona z wykorzystaniem płytek Biolog. Bogactwo funkcjonalne – liczba zużytych substratów na płytkach Biolog. $_{DW}$ – zmienne przeliczone na jednostkę suchej masy gleby; $_{OM}$ – zmienne przeliczone na jednostkę materii organicznej.

non-correlated factors, interpreted as subsoil type (mining waste vs. sand), soil fertility, plant species richness, water-extractable metals and P availability (Table 3). The factors were further used as independent variables in multiple regression analysis (Table 4), which showed that the soil microbial communities were shaped mainly by 3 factors: subsoil type, soil fertility and plant species richness. Subsoil type, reflecting the amount of contaminated mining waste in the subsoil, affected soil basal respiration and microbial biomass. Soil fertility, related predominantly to exchangeable forms of Mg, Ca, K as well as organic C and total N, correlated positively with all measured microbial parameters. Plant species richness and composition were particularly important for soil bacteria, barely influencing soil fungi. Water-soluble metals correlated negatively with soil basal respiration and positively with fungal activity and functional richness. P availability did not affect the microorganisms.

Discussion

In this study the influence of heavy metals on soil microbial communities was assessed. For both grassland and fallow soils it was found

Table 3. Results of factor analysis performed on 30 physicochemical and vegetation variables (N = 49)
Tabela 3. Wyniki analizy czynnikowej wykonanej na 30 zmiennych fizykochemicznych i roślinnych (N = 49)

Factor Czynniki	Variance explained Wyjaśniana zmienność (%)	Variables with the highest (> 0.6) factor loadings Zmienne o najwyższych (> 0.6) wartościach ładunków czynnikowych	Interpretation Interpretacja
1	25.8	Zn_T (0.93), Cd_T (0.92), Pb_T (0.89), Fe_T (0.88), Mn_T (0.77), Ca_T (0.67), Mg_T (0.66), pH (0.65), K_T (0.65), S (0.64)	Subsoil type Rodzaj podłoża
2	16.0	Mg_{EX} (0.86), Ca_{EX} (0.83), K_{EX} (0.76), C_{ORG} (0.68), N (0.65), Cd_{EX} (0.64)	Soil fertility Żyzność gleby
3	13.4	S'_P (0.91), Cov_F (0.90), DCA1 (–0.72)	Plant species richness Bogactwo gatunkowe roślin
4	7.9	Pb_W (0.76), Cd_W (0.75), Zn_W (0.67)	Water-soluble metals Zawartość metali rozpuszczalnych w wodzie
5	7.0	P_{AV} (0.81)	Phosphorus availability Dostępność fosforu

$_{AV}$ – available (Olsen method), $_{EX}$ – extracted with $BaCl_2$ (exchangeable), $_T$ – total, $_{ORG}$ – organic, $_W$ – water-soluble, Cov – herbaceous plant cover, Cov_F – forb cover, S'_P – plant species richness (number of species), DCA1 – first DCA axis (see Kapusta *et al.* – Chapter 13, this volume), representing herbaceous plant species composition.

$_{AV}$ – dostępny (metoda Olsena), $_{EX}$ – ekstrahowany $BaCl_2$ (wymienny), $_T$ – ogólny, $_{ORG}$ – organiczny, $_W$ – rozpuszczalny w wodzie, Cov – pokrycie roślin zielnych, Cov_F – pokrycie roślin zielnych z wyłączeniem traw, turzyc i motylkowatych, S'_P – bogactwo gatunkowe roślin (liczba gatunków), DCA1 – pierwsza oś z analizy DCA (por. Kapusta i in. – Rozdział 13, niniejszy tom), reprezentująca skład gatunkowy roślin zielnych.

that the metals negatively affected microbial biomass, bacterial activity and bacterial ability to utilise organic compounds. Toxic effects of heavy metals on microorganisms have been reported by other authors (Wang *et al.* 2007; Niemeyer *et al.* 2012; Chodak *et al.* 2013). Niemeyer *et al.* (2012) found negative correlations between contamination level and respiration, microbial biomass, phosphatase activity and asparaginase activity in soils polluted with Pb, Zn, Cd and Cu from a Pb smelter. Wang *et al.* (2007) reported reduction of microbial biomass, bacterial diversity and phosphatase activity, and altered bacterial community structure, in soils contaminated with Zn and Cu near a Cu smelter.

The adverse effect of Zn, Pb and Cd on microorganisms of grassland and fallow soils was confirmed by a positive relationship between soil heavy metal pollution and the metabolic quotient qCO_2. Changes in qCO_2 may indicate altered microbial community structure or altered use of resources by the community (Wardle and Ghani 1995; Insam *et al.* 1996). An increase of qCO_2 in response to contamination may suggest that the microorganisms are using more resources for maintenance, including detoxification of heavy metals, instead of for biomass production.

No toxic effect of metals on fungal activity and functional richness was detected in this study. This suggests that the studied fungi are less sensitive to metal pollution than the bacteria. The negative relationship between total metal concentration and microbial biomass presumably resulted, therefore, from the effect

Table 4. Relationships between habitat properties and microbial variables (N = 49)

Tabela 4. Zależności miedzy właściwościami siedliska a zmiennymi mikrobiologicznymi (N = 49)

	Regression summary Podsumowanie regresji		Subsoil type Rodzaj podłoża	Soil fertility Żyzność gleby	Plant species richness Bogactwo gatunkowe roślin	Water-soluble metals Zawartość metali rozpuszczalnych w wodzie	Phosphorus availability Dostępność fosforu
	Adjusted Poprawione R_2	p					
Basal respiration $_{DW}$ Respiracja bazowa $_{DW}$	0.79	< 0.0001	0.64***	0.46***	0.38***	0.21**	–0.13
Basal respiration $_{OM}$ Respiracja bazowa $_{OM}$	0.37	< 0.001	0.29*	–0.17	0.47***	–0.33**	–0.02
Biomass $_{DW}$ Biomasa $_{DW}$	0.60	< 0.0001	0.35***	0.60***	0.28**	0.30**	–0.03
Biomass $_{OM}$ Biomasa $_{OM}$	0.27	< 0.01	–0.44***	0.24	0.28*	–0.02	0.12
Bacterial activity Aktywność bakterii	0.43	< 0.0001	–0.23*	0.38**	0.53***	0.11	–0.05
Fungal activity Aktywność grzybów	0.42	< 0.0001	0.05	0.54***	0.16	0.40***	–0.07
Bacterial functional richness Bogactwo funkcjonalne bakterii	0.43	< 0.0001	–0.06	0.48***	0.48***	0.16	–0.02
Fungal functional richness Bogactwo funkcjonalne grzybów	0.42	< 0.0001	0.05	0.47***	0.26*	0.43***	–0.07

Habitat properties are represented by the factors obtained in factor analysis and presented in Table 3. Statistically significant correlations are asterisked (*p < 0.05, **p < 0.01, ***p < 0.001). Activity – measured with the use of Biolog plates. Functional richness – number of substrates used on Biolog plates. $_{DW}$ – variables calculated per soil dry weight unit, $_{OM}$ – variables calculated per organic matter unit.

Właściwości siedliska reprezentowane są przez czynniki otrzymane w analizie czynnikowej i zestawione w Tabeli 3. Korelacje statystycznie istotne oznaczono gwiazdką (*p < 0.05, **p < 0.01, ***p < 0.001). Aktywność – oznaczona z wykorzystaniem płytek Biolog. Bogactwo funkcjonalne – liczba zużytych substratów na płytkach Biolog. $_{DW}$ – zmienne mikrobiologiczne przeliczone na jednostkę suchej masy gleby, $_{OM}$ – zmienne przeliczone na jednostkę materii organicznej.

of the metals on the bacterial component of the microbial community. These results are consistent with other authors' findings of greater susceptibility to heavy metals in bacteria than in fungi (Bååth 1989; Rajapaksha *et al.* 2004; Stefanowicz *et al.* 2008, Wang *et al.* 2010). Both bacteria and fungi have evolved mechanisms protecting them to some extent against the adverse effect of metals, but bacterial cells are microscopic and have a high surface/volume ratio; these features make them highly susceptible to environmental effects. With their widely expanding mycelium, fungi are able to penetrate a wider space and apparently are better than bacteria at avoiding harmful factors such as contamination (Baldrian 2010). It should be noted that the content of water-soluble forms of metals (factor 4 obtained in

factor analysis) correlated positively with the activity and functional richness of the fungi. The increase of fungal activity with the increase of factor 4 could have two explanations: elimination of metal-sensitive bacteria that normally compete with fungi, resulting in better functioning of the fungi in polluted environments; or the positive impact of organic C on the fungi, in the absence of adverse effects of the metals (the loading of organic C in factor 4 was relatively high, 0.47; data not shown in Table 3). Higher content of organic C (organic matter) in the soil results in higher availability of nutrients for saprotrophic microorganisms and, consequently, an increase of their activity and biomass.

No negative impact of metals on forest soil microorganisms was found in this study. This may be due to the lower metal concentrations in forest soils as compared with the soils of grasslands and fallows; the tree canopy and the soil organic layer protect the humus and mineral layers of forest soils, to some extent, against deposition of pollutants from the atmosphere (Kapusta and Godzik – Chapter 6, this volume). The studied forest soils also showed lower variability of metal concentrations between sites; this could hinder detection of the effects of metals on the microbes in these ecosystems.

Heavy metals were not a major factor influencing the soil microbial communities. The most important factors for the microorganisms were subsoil type and soil fertility (factors 1 and 2). Subsoil type, related to the mining waste content of the subsoil, strongly influenced microbial biomass and respiration. Soil fertility, correlated mainly with the content of exchangeable Mg, Ca and K as well as organic C and total N, significantly influenced all the examined microbial parameters. The study sites differed in terms of mining waste and sand content. The mining waste, consisting mostly of dolomite and calcite, was heavily contaminated with metals but also contained large amounts of macronutrients (Ca, Mg, K) and was alkaline (Kapusta *et al.* – Chapter 13, this volume); those last two characteristics could benefit the microorganisms. The negative impact of the metals in the waste on microbial biomass was disclosed when the latter was calculated per unit of organic matter. Calculating microbiological parameters per organic matter unit partially corrects for differences in organic matter content between study sites, allowing the effects of other factors to be detected, in this case the negative impact of metals (Soler-Rovira *et al.* 2013). The situation was similar for factor 4, representing water-soluble metal concentrations. This factor correlated negatively with soil respiration calculated per unit of organic matter.

A number of studies of the relationships between physicochemical and microbiological characteristics of soil in areas contaminated with heavy metals have shown that the nutrient content and pH of soil are more important to microbes than the presence of metals (Niklińska *et al.* 2005; Chodak *et al.* 2013; Soler-Rovira *et al.* 2013). Niklińska *et al.* (2005) found that the respiration and microbial biomass of humus were influenced mainly by the soil's S content and C/N ratio. In their study the effect of pH and heavy metals was minor but statistically significant. Soil pH had a large effect on bacterial activity and physiological profiles in the Biolog plate tests. Chodak *et al.* (2013) reported similar results. In their study, respiration and microbial biomass depended mainly on the content of organic C and N, while the community structure and diversity depended on soil pH. The effect of metals was observed for respiration level and bacterial community diversity, and was lower than the effects of other physicochemical factors. Soler-Rovira *et al.* (2013) showed that the

respiration of vineyard soils contaminated with Cu from fungicides depended mainly on pH and organic C content, and not on the Cu contamination level.

The third factor strongly affecting soil microbial parameters was plant species richness, that is, the number of species of herbaceous plants found on the research plots. The number of species correlated positively with soil respiration, biomass and, particularly strongly, with soil bacteria activity and functional richness. The plant species composition can also be important to soil microorganisms. This parameter was strongly correlated with plant species richness (both variables had high loadings on factor 3). Plants can affect microbial communities both directly and indirectly. Direct effects are associated with deposition of litter of different chemical composition and with exudation of a number of compounds from plant roots, stimulating soil microbial activity (Grayston *et al.* 1996; Spehn *et al.* 2000; Zak *et al.* 2003; Reich *et al.* 2005). Higher plant species diversity is generally associated with increased diversity of plant-derived substances that serve as nutrients for heterotrophic microorganisms. The diversity of these compounds can influence the diversity and activity of microorganisms (Eisenhauer *et al.* 2010). The higher the diversity of plant communities, the more likely the occurrence of plant species beneficial to soil microbes. Such species include legumes, which fix nitrogen from the atmosphere (Spehn *et al.* 2000; Milcu *et al.* 2008). Stephan *et al.* (2000) showed that the presence of *Trifolium repens* led to increased activity and functional diversity of microorganisms; in soil from *T. repens* monoculture those parameters were as high as in soil from plant communities of high species diversity.

In contaminated areas, plants affect microorganisms indirectly by affecting the amount and mobility of heavy metals in soil. Plants can alter the pH of soil solution and the content of dissolved organic C content, especially in the rhizosphere, which may lead to an increase or decrease of metal mobility (Kim *et al.* 2010). Plants having unique features such as the ability to accumulate large amounts of metals in their tissues exert a particularly pronounced effect on the soil. Gremion *et al.* (2004) found that the presence of the Zn-Cd hyperaccumulator *Thlaspi caerulescens* not only reduced the amounts of Zn and Cd in contaminated soil due to metal uptake and accumulation in plant tissues, but also stimulated the metabolic activity of soil bacteria. Gao *et al.* (2010) reported beneficial effects of plants on microorganisms in contaminated soil. The growth of *Solanum nigrum* in soil experimentally contaminated with Cd and Pb increased the populations of actinomycetes, other bacteria and fungi, and the effect of the presence of both *Solanum nigrum* and *Zea mays* on those organisms was even stronger. Plant growth in contaminated soil also boosted respiration, the activity of phosphatase, urease and dehydrogenase, and the genetic diversity of microbial communities (Gao *et al.* 2010).

This study showed that although high metal concentrations may adversely affect microbial activity, biomass or functional diversity, the high nutrient content of contaminated soils and the presence of highly diverse plant communities can significantly mitigate those effects. Further research should address the impact of different levels of plant species diversity on microorganisms of degraded soils. Few authors have investigated this problem, and studies on the subject are limited to assemblages of only several plant species (up to 2–4 species) (Yang *et al.* 2007; Gao *et al.* 2010; Gao *et al.* 2012). Field studies of soil microorganisms from under plant communities developing through natural succession in post-mining areas need to be done.

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