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Effect of texture and tree species on microbial properties of mine soils

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ABSTRACT

Reestablishment of soil microbial communities is a prerequisite for successful reclamation of post-mining barrens. The objective of this study was to assess the effect of texture of soil substrate and the planted tree species on microbial properties of mine soils reclaimed for forestry. Soil samples were taken from loamy sands and sands afforested with Scots pine and silver birch either in monocultures or in the mixed stands. The samples were measured for the contents of organic C (C_{org}), total N (N_t) and pH. The examined microbial properties included basal respiration (RESP), microbial biomass (C_{mic}), C_{mic}-to-C_{org} ratio, activities of dehydrogenase, acid phosphomonoesterase and urease and community level physiological profiles (CLPPs) studied using Biolog® Ecoplates. The loamy sands had higher pH, contained more C_{org}, N_t and C_{mic} and exhibited higher basal respiration and enzyme activities than the sands. However, their C_{mic}-to-C_{org} ratio was lower indicating less availability of C_{org} for soil microbes compared with the sands. The CLPPs in the loamy sands differed from those in the sands although there was no difference in microbial diversity (expressed as Shannon's diversity index) and activity on the Biolog® plates between the two textural classes. Tree species did not affect C_{org}, N_t and C_{org}-to-N_t ratio and had only a weak effect on CLPPs. However, the values of C_{mic}, RESP, C_{mic}-to-C_{org} ratio, dehydrogenase and urease activities were significantly lower under pine compared with the birch and mixed stands. The obtained results suggest that the texture of soil substrate is of higher importance for microbial properties of the studied mine soils than the planted vegetation.

1. Introduction

Soil microorganisms play a crucial role in energy transfer and nutrient cycling (Bauhus and Khanna, 1999; Nannipieri et al., 2003). Therefore, reestablishment of soil microbial communities is a prerequisite for successful reclamation of post-mining barrens. In natural soils microbial communities are affected by the composition of vegetation and the soil texture (Bauhus et al., 1998; Bauhus and Khanna, 1999; Müller and Höper, 2004). These factors are likely to affect also microbial communities in the reclaimed mine soils. However, the reclaimed mine soils constitute a specific environment for microbes. They are often built of excavated materials that do not contain organic matter, have disadvantageous structure and exhibit extremely low microbial activity (Baldrian et al., 2008). Therefore, the relationships between vegetation, texture and the microbial properties in the reclaimed mine soils may differ from those known from the natural forest soils. The studies assessing the effect of vegetation and soil texture on soil microbial properties are scarce. Šourková et al. (2005) studied afforested mine soils in eastern Germany and Czech Republic and suggested that the vegetation type was of higher importance for the microbial properties than the substrate quality. However, this study included only determination of microbial C, microbial P and basal respiration.

The status of soil microbial communities in reclaimed soils may be assessed using a variety of methods (Gil-Sotres et al., 2005; Harris et al., 2005). Commonly studied properties include microbial biomass, basal respiration (Insam and Domsch, 1988; Šourková et al., 2005) and activities of different soil enzymes (Izquierdo et al., 2005; Baldrian et al., 2008). Measurements of microbial diversity may give additional information on microbial communities in reclaimed mine soils (Mummey et al., 2002; Graham and Haynes, 2004; Machulla et al., 2005; Chodak et al., 2009). In particular, assessment of the functional abilities of soil microbial populations may provide information relevant to the functioning of soils (Nannipieri et al., 2003; Graham and Haynes, 2004).

The Biolog® test is a method of analyzing the physiological profiles of microbial communities, based on the measurement of the use of a set of sole carbon substrates. The method has several limitations – it is sensitive to inoculum density (Insam, 1997), selects for culturable microorganisms capable of growing under experimental conditions (Garland and Mills, 1991) and reflects the functional abilities of a limited subset of microbial genera present in the soil (Ros et al., 2008). Thus, the Biolog® test cannot be applied as stand alone technique and for thorough description

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of soil microbial communities should be accompanied by other complementary methods such as PLFA or molecular techniques. Nevertheless, Biolog® assay may be a valuable tool for rapid and convenient screening of functional abilities in a large number of soil samples. The method is sensitive and reproducible (Gomez et al., 2004), yields information on important functional attributes of microbial communities and its results have been described to match with the results of culture-independent PLFA method (Grayston et al., 2004).

The objective of this study was to assess the effect of soil texture and tree species on microbial properties of mine soils reclaimed for forestry. We have chosen the mine soils that were reclaimed with the same methods and afforested with two tree species – Scots pine (*Pinus sylvestris*) and silver birch (*Betula pendula*) in monocultures and in a mixed stand. The chosen tree species are commonly used in reclamation of post-mining barrens and are known to differently affect several chemical and microbial properties of natural forest soils (Priha and Smolander, 1999; Priha et al., 2001; Kiikkilä et al., 2006).

2. Materials and methods

2.1. Study site

The study was carried out in Central Poland at the external heap of Lignite open-pit mine Bełchatów (19°25′ E; 51°13′ N). The climate in the study area is temperate with *ca.* 580 mm mean annual precipitation and mean annual temperature of 7.6 °C.

The external spoil heap of the Lignite Mine Bełchatów was built in the years 1977–1993. It has an area of approximately 14.8 km^2 and a height of 195 m. The heap was built of Quaternary loamy sands, loams, clays as well as Tertiary sands with loam and clay admixture, containing lignite and sulphides. The overburden material was dumped unselectively creating a mosaic of soils with different chemical and physical properties. However, the largest part of the heap surface is built of sands and loamy sands. From the late 1970s to the mid 1990s the heap was reclaimed and afforested. The standard reclamation procedure included forming and leveling of the surface and NPK fertilization (60 kg N ha^{-1}, 70 kg P ha^{-1}, 60 kg K ha^{-1}). Subsequently, grasses and leguminous plants were sown (60 kg seeds ha^{-1}) and cultivated for 1-year. The final step of reclamation was planting of different tree species (*P. sylvestris*, *B. pendula*, *Larix decidua*, *Alnus incana*, etc.). The tree seedlings have been planted both in monocultures and in mixed stands.

2.2. Soil sampling

Samples of the mineral soil (0–5 cm) were taken in October 2008 from two areas built of two kinds of Quaternary strata: sands and loamy sands. The experimental areas were chosen based on the lithological information obtained from the State Forests managing the reclaimed spoil heap. Within each area sampled were three forest stands – pure Scots pine (*P. sylvestris*), silver birch (*B. pendula*) and mixed pine – birch forest stand (area of each sampled forest stand = 5000 m^2). The sampled forest stands were 23–24 years old. At each forest stand, six mixed samples were taken. The mixed samples consisted of five subsamples (area of each subsample = 0.16 m^2) located at the corners and in the middle of a 3 m × 3 m square. The subsamples were pooled together, sieved (2 mm mesh) and divided into two parts. One part was air-dried and used for physical, physico-chemical and chemical analyses, and the other one was stored field-moist at 4 °C and used for microbial analyses. Prior to microbial analyses, the samples were adjusted to 50% of maximum water holding capacity (WHC) and pre-incubated at 22 °C for 6 days. WHC was determined gravimetrically according to Schlichting and Blume (1966).

2.3. Physical, physico-chemical and chemical analyses

The pH of the samples was measured in 1 M KCl solution (soil:liquid ratio 1:2.5, w/v) with a digital pH-meter (CP-401, ELMETRON). Content of organic C (C_{org}) and total N (N_t) was determined by dry combustion with a CN analyser (Vario Max, Elementar Analysensysteme GmbH). The soil texture of the samples was determined hydrometrically (Mocek et al., 2000). Available P (P_{avl}) was measured according to the Egner-Riehm method (Lityński et al., 1976). Briefly, the soil samples (2 g) were shaken with 100 ml of 0.04 M calcium lactate at pH = 3.6. Then, the suspensions were filtered through a fine-pore filter. The P_{avl} concentration in the filtrates was determined colorimetrically.

2.4. Determination of microbial biomass and basal respiration

To measure basal respiration (RESP) and microbial biomass C (C_{mic}), samples (50 g d.w.) unamended for RESP measurements and amended with 200 mg glucose monohydrate for C_{mic} measurements were incubated at 22 °C in gas-tight jars. The incubation time was 24 h for determination of RESP and 4 h for C_{mic}. The jars contained small beakers with 5 ml 0.2 M NaOH to trap the evolved CO_2. After the jars were opened, 2 ml 0.5 M $BaCl_2$ was added to the NaOH; the excess of hydroxide was titrated with 0.1 M HCl in the presence of phenolphthalein as indicator. C_{mic} was calculated from the substrate-induced respiration rate according to the equation given by Anderson and Domsch (1978): C_{mic} [mg g^{-1}] = 40.04y + 0.37, where y is ml CO_2 h^{-1} g^{-1}.

2.5. Soil enzyme activities

Dehydrogenase activity was determined according to von Mersi (1996). The soil samples (1 g d.w.) were mixed with 1.5 ml Tris buffer (pH 7) and 2 ml 0.5% INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride) solution, and incubated at 40 °C for 2 h. The reduced iodonitrotetrazolium formazan (INTF) was extracted with 10 ml dimethyloformamid/ethanol (1:1) and measured photometrically at 464 nm. Dehydrogenase activity was expressed as μg INTF g^{-1} h^{-1}.

Acid phosphomonoesterase activity was measured as described by Margesin (1996). The soil samples (1 g d.w.) were mixed with 1 ml disodium p-nitrophenyl phosphate solution (115 mM) and 4 ml buffer solution (pH 6.5) and incubated at 37 °C for 1 h. The p-nitrophenol released by phosphatase activity was extracted and colored with NaOH and determined photometrically at 400 nm. Acid phosphomonoesterase activity was expressed as μg p-NP g^{-1} h^{-1}.

Urease activity was determined as described by Kandeler (1996). The soil samples (5 g d.w.) were mixed with 2.5 ml urea (720 mM) and 20 ml borate buffer (pH 10) and incubated at 37 °C for 4 h. The released ammonium was extracted with acidified potassium chloride solution, coloured in the modified Berthelot reaction and measured photometrically at 690 nm. Urease activity was expressed as μg N g^{-1} h^{-1}.

2.6. Community level physiological profiles (CLPP)

The physiological profiles of the microbial communities were analysed using Biolog® Ecoplates (Insam, 1997). Samples (10 g d.w.) were shaken for 60 min in 20 ml of a 10 mM Bis–Tris solution (pH 7) and allowed to settle for 30 min. Then the extracts containing microbes were decanted, and subsamples of the extracts (2 ml) were immediately frozen in liquid nitrogen and stored at −70 °C

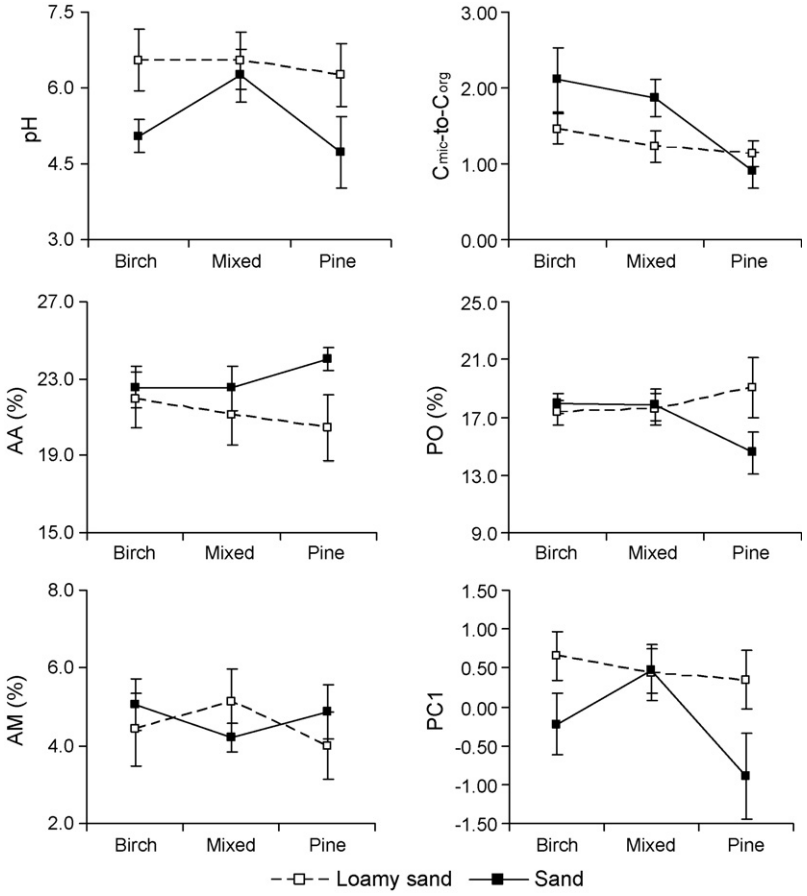

Fig. 1. Influence of soil texture (loamy sands or sands) on soil pH, C_{mic}-to-C_{org} ratio, the relative use of amino acids (AA), polymers (PO) and amines and amides (AM) on the Biolog® plates and the first principal component based on Biolog® data affected by different composition of forest stand (pine, birch and mixed pine-birch).

until analysis. Freezing at −70° C was reported not to affect community level physiological profiles measured with Biolog® assay (Boivin, 2005). In order to ensure similar inoculum density (Insam and Goberna, 2004), prior to analysis the thawed extracts were diluted with Bis–Tris solution to obtain 0.5 μg C_{mic} in 1 ml solution. The solutions were inoculated on microplates (100 μl per well) and incubated at 22 °C. Substrate utilization was monitored by measuring light absorbance at 590 nm. The first measurement was made immediately after inoculation, and the subsequent ones at 12 h intervals for 6 days. The readings for individual substrates were corrected for background absorbance by subtracting the absorbance of the first reading. The corrected absorbance values were used to calculate the area under the absorbance curve (AUC). The calculated AUC values were standardized by dividing them by the average area under the curve (AAUC) and used in statistical analyses (Garland, 1997; Insam and Goberna, 2004). AAUC was used to express overall microbial activity on the plates.

2.7. Calculations and data analyses

Principal component analysis (PCA) performed on covariance matrix was used to investigate Biolog® data (Statgraphics Plus 5.1 software, Statistical Graphics Corporation). PCA reduces the number of independent variables to a smaller number of new variables called principal components (PCs). Interpretation of the PCs was based on significant factor loading of the individual substrate on each of the PCs. Biolog® data were further used to compare metabolic preferences of the bacterial communities under the studied forest stands. The carbon substrates were grouped into six guilds – carbohydrates (CH), carboxylic acids (CA), amino acids (AA) polymers (PO), amines and amides (AM) and miscellaneous (Misc) (Zak et al., 1994). For each guild the AUC values of the substrates were summarized and expressed as a percentage of total AUC value of the plate. The functional diversity of microbial communities was calculated using the Shannon's index: $H' = \Sigma_{i=1\ldots n}\, p_i\, (\ln p_i)$, where

Table 1
Effects of soil texture and tree species on chemical, physico-chemical and physical properties of the analysed mine soils (mean ± standard deviation) by two-way ANOVA (*$p < 0.05$; **$p < 0.01$). Different letters indicate significant ($p < 0.05$) differences between the studied forest stands.

Factor	Level	Sand (%)	Silt (%)	Clay (%)	C_{org} (mg g^{-1})	N_t (mg g^{-1})	C_{org}-to-N_t	P_{avl} (mg kg^{-1})	pH
Texture (Tx)		**	**	**	**	**	N.S.	**	**
	Loamy sand	82 ± 3.8	14 ± 2.9	4 ± 1.5	35.4 ± 10.9	2.07 ± 0.8	18 ± 2.3	40.0 ± 13.6	6.4 ± 0.6
	Sand	91 ± 5.0	7 ± 4.5	2 ± 1.0	17.4 ± 4.8	0.94 ± 0.3	19 ± 1.5	15.9 ± 9.5	5.3 ± 0.9
Tree (Tr)		**	**	N.S.	N.S.	N.S.	N.S.	N.S.	**
	Birch	89 ± 6.0a	8 ± 4.7b	3 ± 1.7	26.4 ± 14.1	1.52 ± 1.0	18 ± 1.8	25.2 ± 14.8	5.8 ± 0.9ab
	Mixed	83 ± 5.3b	14 ± 3.7a	3 ± 2.0	28.3 ± 14.1	1.67 ± 1.0	18 ± 2.0	34.5 ± 16.4	6.4 ± 0.6a
	Pine	88 ± 5.7a	9 ± 4.6b	3 ± 1.2	24.5 ± 7.4	1.31 ± 0.5	19 ± 2.2	24.1 ± 17.1	5.5 ± 1.0b
Interaction	Tx × Tr	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*

Table 2
Effects of soil texture and tree species on basal respiration (RESP), microbial biomass (C_{mic}), C_{mic}-to-C_{org} ratio and activities of dehydrogenase (DHG), acid phosphomonoesterase (AcPHP) and urease (URE) in the analysed mine soils (mean ± standard deviation) by two-way ANOVA (*$p < 0.05$; **$p < 0.01$). Different letters indicate significant ($p < 0.05$) differences between the studied forest stands.

Factor	Level	RESP (μg C-CO_2 g^{-1} 24 h^{-1})	C_{mic} (μg g^{-1})	C_{mic}-to-C_{org}	DHG (μg INTF g^{-1} h^{-1})	AcPHP (μg p-NP g^{-1} h^{-1})	URE (μg N g^{-1} h^{-1})
Texture		**	**	**	**	**	**
(Tx)	Loamy sands	28.6 ± 6.1	442 ± 131	1.27 ± 0.24	72.7 ± 26.9	372 ± 165	21.2 ± 10.6
	Sands	17.0 ± 6.6	270 ± 91	1.63 ± 0.61	52.2 ± 11.0	234 ± 86	9.3 ± 3.2
Tree		**	**	**	**	N.S.	**
(Tr)	Birch	26.2 ± 6.4a	419 ± 142a	1.78 ± 0.47a	77.3 ± 26.8a	323 ± 167	17.3 ± 10.7a
	Mixed	24.5 ± 8.9a	390 ± 118a	1.54 ± 0.39a	61.4 ± 15.4a	302 ± 157	18.2 ± 10.5a
	Pine	17.7 ± 7.8b	258 ± 107b	1.02 ± 0.23b	48.7 ± 14.8b	284 ± 114	10.2 ± 5.2b
Interaction	Tx × Tr	N.S.	N.S.	*	N.S.	N.S.	N.S.

n is the number of wells and p_i is the use of the ith substrate (AUC value) as a proportion of the sum of the use of all substrates of a plate.

Multifactor analysis of variance (MANOVA) was used to test the effect of the soil texture and tree species on soil chemical and microbial properties. The Tukey's honestly significant differences (HSD) test for multiple comparisons was run if significant differences were found ($p < 0.05$). The right-skewed data (C_{org}, N_t, P_{avl}, dehydrogenase, acid phosphomonoesterase and urease activities) were log-transformed to fulfill the assumption of normality.

All analyses were performed with Statgraphics Plus 5.1 software (Statistical Graphics Corporation).

3. Results

3.1. Physical, chemical and microbial characteristics of the sample population

There was an obvious difference in contents of sand, silt and clay between the two studied textural classes (Table 1). However, differences in clay and sand content were evident also between the studied tree species with the mixed stand containing significantly more silt and less sand particles compared with the other stands (Table 1).

The loamy sands contained more C_{org}, N_t and P_{avl} and had higher pH compared with the sands. There was no significant difference in C_{org}, N_t and P_{avl} between the studied tree species but the mixed stands exhibited higher pH values than the pine stands (Table 1). The C_{org}-to-N_t ratio did not differ either between the textural classes or between the tree species. Significant interaction between soil texture and tree species was found for soil pH; under the mixed stand, the loamy sands and the sands did not differ in soil pH (Fig. 1).

3.2. The effect of texture and tree species on microbial biomass, basal respiration and soil enzyme activities

Significantly higher values of C_{mic}, RESP, dehydrogenase, acid phosphomonoesterase and urease activities were determined under the loamy sands compared with the sands (Table 2). In turn, the sands exhibited higher C_{mic}-to-C_{org} ratio. There was a significant interaction between the texture and the tree species for this soil property; under the pine stand the difference between the loamy sands and the sands was not significant (Fig. 1).

Among the tree species the lowest values of C_{mic}, RESP, C_{mic}-to-C_{org} ratio, dehydrogenase and urease activity were determined under pine (Table 2). Differences between the birch and the mixed stand were statistically not significant. Activity of acid phosphomonoesterase was not affected by the tree species.

3.3. Analysis of community level physiological profiles

The first two PCs explained 40.3% of variance in Biolog® data (PC1: 26.2%, PC2: 14.1%) (Fig. 2). The largest loadings on PC1 were from γ-hydroxybutyric acid (0.75), L-serine (−0.34) and glycogen (0.27) and on PC2 from β-methyl-D-glucoside (0.60), α-D-lactose (0.40) and D-galacturonic acid (−0.27). The PC1 depended on the tree species, the texture and interaction of these factors (Table 3, Fig. 2). Lower values of PC1 were calculated for the sandy soils compared with the loamy sands and for the pine stand compared to the mixed stand. The PC2 did not separate neither the forest stands nor the textural classes. Analysis of guilds indicated significant effect of soil texture on the relative use of amino acids (higher in the sands), carboxylic acids, polymers and CH-to-AA ratio (higher in loamy sands). Tree species affected the use of carbohydrates as there was a difference between the mixed stand and the pine stand (Table 3). Neither soil texture nor the tree species had an influence on Shannon's diversity index and AAUC (Table 3).

3.4. Relationships between the measured properties

Microbial biomass, RESP and the activities of dehydrogenase, acid phosphomonoesterase and urease correlated positively ($r = 0.39–0.91$) with C_{org}, N_t and P_{avl} (Table 4). The enzyme activities were strongly correlated with C_{mic} ($r = 0.80–0.90$).

Shannon diversity index (H') and AAUC correlated only with each other and with PC2 ($r = 0.68–0.73$). The first PC depended strongly on pH ($r = 0.85$) and positively correlated also with C_{org}, P_{avl}, RESP, C_{mic} and ($r = 0.33–0.50$).

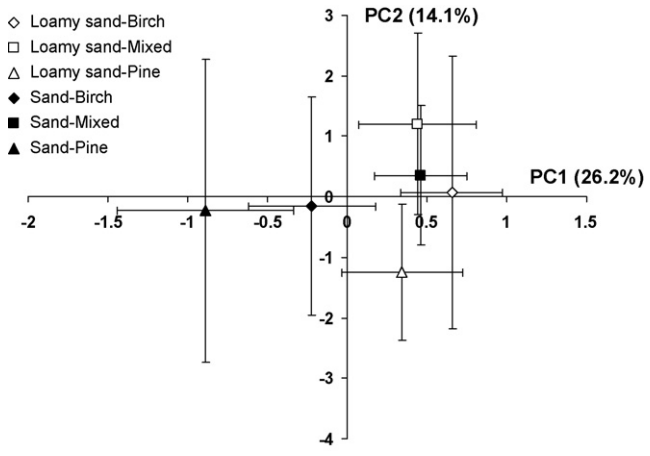

Fig. 2. Mean values ($n = 6$) of principal components (PC1 and PC2) for the loamy sands and the sands under the birch, the pine and the pine-birch stands. The PCs are based on Biolog® data. Whiskers indicate standard deviations. Percent of explained variance in parentheses.

Table 3
Effects of soil texture and tree species on functional diversity index (Shannon H'), microbial activity on Biolog® plates (AAUC), principal components based on Biolog® data, the relative use of carbohydrates (CH), amino acids (AA), carboxylic acids (CA), polymers (PO), amines and amides (AM) and miscellaneous substrates (Misc) and the ratio of relative use of carbohydrates to amino acids (CH-to-AA) in the analysed mine soils (mean ± standard deviation) by two-way ANOVA (*$p < 0.05$; **$p < 0.01$). Different letters indicate significant ($p < 0.05$) differences between the studied forest stands.

Factor	Level	Shannon H'	AAUC	PC1	PC2	CH (%)	AA (%)	CA (%)	PO (%)	AM (%)	Misc (%)	CH-to-AA
Texture (Tx)		N.S.	N.S.	**	N.S.	N.S.	**	*	*	N.S.	N.S.	*
	Loamy sands	3.24 ± 0.03	108 ± 17	0.50 ± 0.39	0.01 ± 2.03	30.0 ± 1.8	21.1 ± 1.7	20.5 ± 1.8	18.0 ± 1.6	4.5 ± 1.0	5.8 ± 1.1	1.8 ± 0.2
	Sands	3.25 ± 0.04	112 ± 14	−0.17 ± 0.72	−0.01 ± 1.97	29.9 ± 1.9	23.0 ± 1.2	19.1 ± .18	16.8 ± 2.0	4.7 ± 0.7	6.5 ± 0.7	1.7 ± 0.1
Tree species (Tr)		N.S.	N.S.	**	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Birch	3.25 ± 0.04	109 ± 14	0.22 ± 0.59ab	−0.04 ± 2.13	29.9 ± 1.5	22.2 ± 1.3	19.2 ± 2.1	17.6 ± 0.8	4.7 ± 0.9	6.3 ± 1.0	1.7 ± 0.1
	Mixed	3.25 ± 0.03	114 ± 8	0.45 ± 0.34a	0.78 ± 1.47	29.0 ± 1.4	21.8 ± 1.5	20.8 ± 1.1	17.7 ± 1.1	4.7 ± 0.8	6.0 ± 1.1	1.7 ± 0.2
	Pine	3.24 ± 0.04	106 ± 21	−0.27 ± 0.81b	−0.74 ± 2.10	30.9 ± 2.1	22.2 ± 2.2	19.5 ± 2.0	16.8 ± 2.9	4.4 ± 0.9	6.1 ± 0.7	1.8 ± 0.2
Interaction	Tx × Tr	N.S.	N.S.	**	N.S.	N.S.	*	N.S.	**	*	N.S.	N.S.

4. Discussion

4.1. Experimental design

The experimental design used in our study includes pseudoreplication because all the sampling sites were located within one reclaimed object (Hurlbert, 1984). Such data should be interpreted assuming that there are no other factors affecting the studied properties stronger than the considered ones (Menyailo et al., 2002). Since climate, age of vegetation and reclamation measures may have an important effect on soil chemical and microbial properties we took care to keep these factors constant at the experimental sites. Therefore, we assume that the major differences in the studied properties can be attributed to the effect of texture of parent material and tree species.

4.2. Physical and chemical properties of the mine soils

The texture and planted vegetation were primary differences between the studied soils. The reclaimed mine soils at the studied heap developed from Quaternary materials excavated from large depths. These materials initially contained no organic matter and the planted vegetation is the only source of soil C and N (Baldrian et al., 2008). Despite a short time of soil development the contents of C_{org} and N_t were significantly higher in the loamy sands indicating that a small difference in clay content in the dumped material distinctly affects its ability to store C_{org} and N_t. Soils with higher clay content may contain more C_{org} because the presence of fine particles supports organic matter protection owing to building of organo-mineral complexes resistant to microbial degradation (Franzluebbers et al., 1996; Müller and Höper, 2004).

Mine soils may sometimes contain certain amounts of geogenic C that origins from lignite particles present in the dumped overburden (Rumpel et al., 1998). However, we presume that this source of C_{org} was negligible in our studied soils because lignite particles occur in Tertiary strata and the studied soils developed from dumped Quaternary materials. Moreover, the lignite extracted in Bełchatów contains only a small amount of N and its C-to-N ratio is 71 (Chodak et al., 2007). In our studied soils, C_{org}-to-N_t ratio was much narrower and averaged 17–18 indicating that even if there was some geogenic C present, its contribution to C_{org} was low.

The loamy sands had higher pH than the sands. This was probably due to their higher buffering capacity. Accumulation of organic matter may lead to soil acidification (Bolan et al., 1991) and decreases of soil pH have been observed in chronosequences of afforested mine soils (Graham and Haynes, 2004; Chodak et al., 2009). We presume that higher buffering capacity of the loamy sands counteracted the acidification processes and hence their higher pH compared with the sands. Differences in texture may explain also the higher soil pH under the mixed stand and the observed significant interaction between the tree species and soil texture. The content of silt under the mixed stands was higher than under the other stands. We presume that this was the reason for higher pH under these stands because the silt fraction may have a significant contribution to soil cation exchange capacity and buffering capacity (Curtin and Rostad, 1997; Stewart and Hossner, 2001).

The tree species did not affect C_{org}, N_t and C_{org}-to-N_t ratio of the studied soils although silver birch and Scots pine are known to produce litter of different quality (Kiikkilä et al., 2006; Adamczyk et al., 2008; Kanerva et al., 2008). We presume that longer time is needed for trees to cause significant changes in chemical properties of soils. Similarly to our results, Priha and Smolander (1997) reported no difference in C_{org}, N_t, C_{org}-to-N_t ratio and pH in mineral soils under 23–24 years old birch and pine forest stands in Finland.

Table 4
Coefficients of correlation between the microbial and chemical properties of the mine soils. Presented values are significant at $p < 0.05$.

	DHG	AcPHP	URE	C_{mic}	RESP	H'	AAUC	PC1	PC2	C_{org}	N_t	C_{org}-to-N_t	P_{avl}
AcPHP	0.68												
URE	0.74	0.86											
C_{mic}	0.83	0.80	0.90										
RESP	0.59	0.66	0.77	0.85									
H'	N.S.	N.S.	N.S.	N.S.	N.S.								
AAUC	N.S.	N.S.	N.S.	N.S.	N.S.	0.68							
PC1	N.S.	N.S.	N.S.	0.44	0.48	N.S.	N.S.						
PC2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.73	N.S.					
C_{org}	0.63	0.84	0.88	0.81	0.78	N.S.	N.S.	0.33	N.S.				
N_t	0.65	0.86	0.91	0.81	0.76	N.S.	N.S.	N.S.	N.S.	0.99			
C_{org}-to-N_t	−0.48	−0.68	−0.64	−0.55	−0.48	N.S.	N.S.	N.S.	N.S.	−0.63	−0.73		
P_{avl}	0.39	0.50	0.66	0.66	0.75	N.S.	N.S.	0.50	N.S.	0.75	0.74	−0.47	
pH	N.S.	N.S.	N.S.	N.S.	0.41	N.S.	N.S.	0.85	N.S.	N.S.	N.S.	N.S.	0.38

DHG – dehydrogenase activity, AcPHP – acid phosphomonoesterase activity, URE – urease activity, C_{mic} –microbial biomass, RESP – basal respiration, H' – Shannon index, AAUC – average area under curve on Biolog® plates, PC1 and PC2 – the first and second principal components based on Biolog® data, P_{avl} – available phosphorus, N.S. – not significant.

4.3. Microbial biomass, basal respiration and enzyme activities

Differences in physical and chemical properties of the studied soils were reflected in their microbial properties. The loamy sands exhibited higher values of RESP, C_{mic} and soil enzyme activities compared to the sands. Soils containing more clay particles may maintain larger microbial communities than those with lower clay content due to higher C_{org} content in these soils, better protection from faunal predation and less fluctuation of water availability (Kaiser et al., 1992; Franzluebbers et al., 1996; Müller and Höper, 2004). Larger and more active microbial biomass in the loamy sands might have been also due to their higher pH (Bauhus and Khanna, 1999). Despite the higher content of microbial biomass in the loamy sands, the proportion of C_{mic} in C_{org} in these soils was lower than in the sands. This indicates rapid accumulation of recalcitrant organic matter and lower availability of C_{org} accumulated in these soils (Graham and Haynes, 2004).

Although tree species did not affect the contents of C_{org}, N_t and C_{org}-to-N_t ratio, the soils under the pine stands exhibited lower values of C_{mic}, RESP, C_{mic}-to-C_{org} ratio and dehydrogenase and urease activities than the soils under the other stands. The observed tree species dependent differences resulted probably form different quality of organic matter under the studied forest stands. The pine litter contains less tannins and easily degradable compounds that support growth of soil microorganisms compared with the birch litter (Kiikkilä et al., 2006; Adamczyk et al., 2008) and this may explain lower values of C_{mic}, RESP, C_{mic}-to-C_{org} and enzyme activities under the pine stands.

Differences in soil enzymes between particular soil types or tree species resulted from differences in microbial biomass. Higher enzyme activities were found in soils with higher microbial biomass. Strong dependence between microbial biomass and dehydrogenase activity was expected because dehydrogenases are enzymes that exist only in living cells (Nannipieri et al., 2002). Dehydrogenase activity has often been used as a measure of general soil microbial activity (Gil-Sotres et al., 2005; Tan et al., 2008) although it is known that the existing assays may underestimate the activity of this enzyme (Nannipieri et al., 2002). Urease and acid phosphomonoesterase are enzymes involved in cycling of N and P, respectively (Caldwell, 2005; Tan et al., 2008). High activity of soil enzymes may indicate insufficient nutrient supply for microbes (Sinsabaugh et al., 1993). However, clear relationships between nutrient availability and enzyme activity are difficult to establish because enzyme activity depends on specific activity (enzyme activity per microbial biomass) and on total microbial biomass (Allison et al., 2007). In our study, urease and acid phosphomonoesterase activities were positively related to C_{mic}, C_{org}, N_t and P_{avl}. This suggests that the microbial biomass and the organic matter content are major determinants of urease and acid phosphomonoesterase activities in the studied mine soils. Similar dependencies were found in other studies on microbial properties of the mine soils (Baldrian et al., 2008; Chodak and Niklińska, 2010).

4.4. Community level physiological profiles

Despite significant differences in microbial biomass, neither the tree species nor the soil texture affected the microbial activity and the physiological diversity on the Biolog® plates. Larger microbial communities may be functionally more diverse (Lynch et al., 2004). However, functional diversity increases with increasing microbial biomass only to a certain threshold value (Lynch et al., 2004). Yan et al. (2000) used data given by Sharma et al. (1997) and found that the functional diversity measured with Biolog® method increased with increasing C_{mic} up to a threshold of 105.6 $\mu g\, g^{-1}$ and remained constant above this value. Chodak et al. (2009) reported even lower threshold value of C_{mic} (35 $\mu g\, g^{-1}$) for reclaimed mine soils afforested with pine. In our studied soils microbial biomass was much larger and this may explain the absence of differences in functional diversity between the studied textural classes and tree species.

The MANOVA performed on the PCs based on Biolog® data revealed the effects of soil texture, tree species and their interaction on CLPPs. Differences in CLPPs between the two textural classes resulted probably from different soil pH. Loamy sands had significantly higher pH than the sands and soil pH is an important factor affecting physiological profiles of microbial communities (Niklińska et al., 2005). In the study of Priha et al. (2001) CLPPs of microbial communities from mineral soils did not distinguish the two sampling sites that differed in texture but did not differ in pH.

The observed effect of tree species on CLPPs was probably an artifact resulting from differences in the silt content between the analysed forest stands that affected soil pH. In our study PC1 correlated with soil pH and the difference in PC1 was significant only between the mixed and the pine stands that differed also in soil pH. For the pine and the birch stands the values of both PCs did not differ indicating similarity of CLPPs. Similarly to our results, Priha et al. (2001) reported that CLPPs of microbial communities under birch and pine forests in Finland did not differ from each other.

Soil texture affected the relative use of amino acids, carboxylic acids and polymers. The CH-to-AA ratios were higher in the loamy sands. Sharma et al. (1998) suggested that higher CH-to-AA ratios indicate higher content of easily degradable carbon compounds in the soils. However, in our study such an explanation of higher CH-

to-AA ratios under the loamy sands is doubtful considering that C_{mic}-to-C_{org} ratio was significantly lower in the loamy sands suggesting higher share of stabile C in these soils. Biolog® test is a culture-based method that analyses only a limited subset of microbial genera (Ros et al., 2008). Therefore, the results of Biolog® should be interpreted with caution. Our results indicate that higher CH-to-AA ratios do not necessarily indicate higher amount of easily degradable compounds and that some other factors may affect this microbial property. Indeed, even in the study of Sharma et al. (1998) decreasing trend of CH-to-AA values was evident in two luvisols from Denmark and Germany but not in the andosol from Italy.

5. Conclusions

The texture of soil substrate was of higher importance for microbial properties of the studied young mine soils than the composition of the planted vegetation. The afforested loamy sands contained significantly more C_{org} and N_t and had higher pH than the sands. Consequently, they maintained larger and more active microbial biomass.

Soil afforested with pine exhibited lower C_{mic}, RESP and enzyme activities indicating significance of vegetation for these soil properties.

The functional diversity measured with Biolog® assay did not depend neither on soil texture nor the tree species, but the community level physiological profiles depended on the soil texture.

The contents of C_{org} and N_t were the major determinants of microbial biomass, basal respiration and enzyme activities, whereas the physiological profiles of soil microbial communities depended mainly on soil pH.

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