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Original Research

Development of Microbial Biomass and Enzyme Activities in Mine Soils

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Abstract

This study assessed the development of microbial biomass, basal respiration, and the activities of dehydrogenase, urease, and acid phosphomonoesterase in sandy mine soils reclaimed for forestry and those developing under vegetation from natural succession. The mine soils contained significantly less organic C (C_{org}) and total N (N_t) than the natural forest soils. However, in some of them the microbial biomass and basal respiration attained values typical for the natural forest soils. The content of N_t proved to be the most important control on the microbial biomass, basal respiration, and the activities of dehydrogenase and phosphomonoesterase in the mine soils. All the microbial properties were positively related also to C_{org} content. The activities of dehydrogenase and urease depended strongly on microbial biomass (C_{mic}). Hence, high activities of these enzymes were determined in soils containing high C_{mic}. The acid phosphomonoesterase activity was also positively related to C_{mic}, but its activity was increased in the soils with low P contents.

Keywords: mine soils, microbial biomass, dehydrogenase activity, acid phosphomonoesterase activity, urease activity

Introduction

Afforestation is a common way to reclaim post-mining barrens. One of the most important goals of reclamation for forestry is the reestablishment of stable and productive ecosystems. This goal can be achieved only if soil functionality is restored. Soil microorganisms are involved in several key soil processes such as decomposition and formation of soil organic matter, nutrient cycling, and energy transfer [1]. Therefore, measurement of different soil microbial and biochemical properties has been recommended for soil recovery assessment [2].

Soil enzymes are proteins that catalyze specific reactions required to process complex organic compounds into assimilable subunits such as sugars, amino acids, NH_4^+, PO_4^{3-}, etc. [3, 4]. Determining of activities of enzymes involved in the cycling of nutrients such as N, P, or S may give information on nutrient turnover in soils. Therefore, measurements of various soil enzymes have been widely used for estimation of soil recovery after disturbance and for soil quality assessment [5]. For instance, de Mora et al. [6] used the activities of dehydrogenase, arylsulfatase, and β-glucosidase to assess the effect of various treatments on heavy metal-contaminated soils after the Aznalcollar accident in Spain. Baldrian et al. [7] studied chronosequence of clayey mine soils covered by spontaneously developing vegetation and reported activities of cellobiase, β-xylosidase, acid phosphatase, and arylsulfatase to increase gradually with the site age.

The objective of this study was to assess the development of microbial biomass, basal respiration, and activities

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of soil enzymes involved in N and P cycling in sandy mine soils reclaimed for forestry and those developing under vegetation from natural succession. The natural forest soils were used as a reference.

Material and Methods

Study Area

The study was carried out in Upper Silesia, Poland (19º26' E; 50º16' N), on the grounds of the Szczakowa open-cast sand quarry and its surroundings. The climate is temperate, with mean annual precipitation of ca 700 mm and mean annual temperature of 8ºC. Soils of the study area (mainly Podzols) developed from sands. The sand deposits are fluvioglacial quaternary sediments of a pre-quaternary morphological depression.

Extraction of sands in Szczakowa Quarry created an open cast covering over 2700 ha. Since the late 1950s it has been reclaimed. The standard reclamation procedure included forming and leveling off the surface and adding an organic amendment (approx. 300 $m^3 \cdot ha^{-1}$). The added amendment was a mixture of forest floor (O horizon) and mineral horizons (horizons A_h, E, and partly B), with average organic C content of 0.3-1.0% collected from forest soils in areas to be mined [8]. Then the reclaimed sites were limed (1.5 Mg dolomite ha^{-1}), and lupine (*Lupinus luteus* L.) was cultivated for two years. The lupine cultivations were fertilized with NPK (140 kg $N \cdot ha^{-1}$, 300 kg $P_2O_5 \cdot ha^{-1}$, 180 kg $K_2O \cdot ha^{-1}$). After two years, the lupine biomass was ploughed into the soil as green manure and the sites were afforested with 1-year-old Scots pine seedlings (*Pinus sylvestris*), common birch (*Betula pendula*) and some other deciduous trees. Over the last 25 years, certain modifications were introduced to the reclamation methods; these included cessation of liming, and decreasing the quantity of organic amendment and NPK mineral fertilizers (information from the Szczakowa Sand Quarry). Changes in reclamation procedures caused the reclaimed sites sampled in our study to be differently treated. The oldest site was reclaimed according to the standard procedure as described above. The intermediate site was not limed but received the same amount of organic amendment and mineral fertilizers as the oldest one. At the youngest reclaimed site no liming was applied, NPK fertilizers were applied at lower rates (50 kg $N \cdot ha^{-1}$, 140 kg $P_2O_5 \cdot ha^{-1}$, 120 kg $K_2O \cdot ha^{-1}$), and the organic amendment (amount decreased to approximately 30 $m^3 \cdot ha^{-1}$) was not spread over the entire reclaimed area but applied directly under the seedlings during planting. The reclamation treatments at all the sampled mine soils are summarized in Table 1.

In the 1970s, 1980s, and 1990s some parts of the open cast were abandoned and initial soil formation began there under the vegetation from natural succession. At the abandoned sites, herbaceous communities dominated by gray hair-grass (*Corynephorus canescens* L.) were the first to establish. They were followed by bio-groups of trees with over 50% domination by Scots pine (*Pinus sylvestris* L.) and common birch (*Betula pendula* Roth.), with the occasional trembling poplar (*Populus tremula* L.) [9].

Table 1. Reclamation measures at the sampled mine soils.

Soil	Organic amendment	Liming	Fertilization	Lupine cultivation
MS	-	-	-	-
S7	-	-	-	-
S21	-	-	-	-
S28	-	-	-	-
LUP	-	-	50 kg N ha^{-1}	2 years
			140 kg P_2O_5 ha^{-1}	
			120 kg K_2O ha^{-1}	
R7	30 $m^3 \cdot ha^{-1}$	-	50 kg N ha^{-1}	2 years
			140 kg P_2O_5 ha^{-1}	
			120 kg K_2O ha^{-1}	
R21	300 $m^3 \cdot ha^{-1}$	-	140 kg N ha^{-1}	2 years
			300 kg P_2O_5 ha^{-1}	
			180 kg K_2O ha^{-1}	
R29	300 $m^3 \cdot ha^{-1}$	1.5 Mg dolomite ha^{-1}	140 kg N ha^{-1}	2 years
			300 kg P_2O_5 ha^{-1}	
			180 kg K_2O ha^{-1}	

Soil Sampling

Samples of mineral soil (0-5 cm) were taken in May 2007 at 10 sites (area = 1000 m^2): a degraded site prior to reclamation (MS), a site after two years of lupine cultivation (LUP), reclaimed post-mining sites afforested with Scots pine (7, 21, and 29 years old – R7, R21, and R29, respectively), post-mining sites with spontaneously developing pine forest stands (7, 21, and 28 years old – S7, S21, and S28, respectively), and two natural pine forest stands as reference soils (forest stands under 31 and 109 years old – F31 and F100, respectively). At each site, eight mixed samples consisting of five sub-samples (area of each subsample = 0.16 m^2) were taken. At all the sites (except S28) the soils were classified as sands (sand content 88-99%, silt content 0-9%, clay content 0-2%). At S28 the soil was loamy sand (87% sand, 9% silt, 4% clay) [10].

The samples were sieved (2 mm mesh) and divided into two parts. One part was air-dried and used for chemical analyses, and the other was stored at 4ºC and used for microbial analyses. Prior to microbial analyses the samples were adjusted to 50% of the maximum water holding capacity (WHC) and pre-incubated at 22ºC for 14 days.

Analytical Methods

Maximum water holding capacity (WHC) was determined gravimetrically [11]. The pH of the samples was measured in 1M KCl solution at a 1:10 soil:liquid ratio (w:v) using a digital pH-meter (CP-401, ELMETRON). The content of organic C (C_{org}) and total N (N_t) was determined by dry combustion using a CN analyzer (Vario Max, Elementar Analysensysteme GmbH). The content of extractable P (P_{ext}) was measured after extraction with Mehlich I extractant. Briefly, the soil samples (5 g) were shaken with 25 ml of Mehlich I extractant (0.05 N HCl and 0.025 N H_2SO_4; pH=1.2). Then, the suspensions were filtered through a fine-pore filter and the inorganic P concentration in the filtrates was determined colorimetrically with a continuous flow auto-analyzer (FIAcompact, MLE, Dresden, Germany).

Microbial biomass C (C_{mic}) was determined using substrate-induced respiration method [12]. Basal respiration (RESP) was determined after incubation of the soil samples for 24 h at 22ºC in gas-tight jars. The evolved CO_2 was trapped in 0.2 M NaOH and determined by titration of the excess of hydroxide with 0.1 M HCl in the presence of phenolphthalein as indicator.

Dehydrogenase activity was determined according to von Mersi [13]. 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 40ºC 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 $g^{-1}{\cdot}h^{-1}$.

Acid phosphomonoesterase activity was measured as described by Margesin [14]. 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 37ºC for 1 hour. The p-nitrophenol released by phosphomonoesterase activity was extracted and colored with NaOH and determined photometrically at 400 nm. Acid phosphomonoesterase activity was expressed as μg p-NP $g^{-1}{\cdot}h^{-1}$ and acid phosphomonoesterase efficiency was calculated as acid phosphomonoesterase activity normalized to a per μg of C_{mic} basis.

Urease activity was determined as described by Kandeler [15]. 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 37ºC for 4 hours. 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 $g^{-1}{\cdot}h^{-1}$ and urease efficiency was calculated as urease activity normalized to a per μg of C_{mic} basis.

Calculations and Statistical Data Analyses

All analyses were performed in triplicate and the mean values were used in further calculations. The data presented in the text are means values of eight replicates per site.

Differences in chemical and microbial properties between the forest stands were tested by one-way analysis of variance (ANOVA). The Fisher least significant difference (LSD) test for multiple comparisons was run if significant differences were found ($p<0.05$). Relationships between the measured chemical and microbial properties of the mine soils were studied using Pearson correlations after excluding the samples from natural forest soils (F31 and F100). Comparison of regression lines was performed to check if the relationships between C_{mic} and the RESP and enzyme activities are consistent in the reclaimed and successional soils. Prior to statistical analyses the data were log-transformed or square root-transformed to fulfill the assumption of normality.

The statistical calculations were performed with Statgraphics Plus version 5.1 (Manugistics Inc.).

Results

Chemical Properties of Soils

The lowest contents of C_{org} and N_t were determined in the pre-reclamation mine spoil soils (MS) (0.21 $mg{\cdot}g^{-1}$ and 0.004 $mg{\cdot}g^{-1}$, respectively) and 2-year lupine (LUP) (0.27 $mg{\cdot}g^{-1}$ and 0.011 $mg{\cdot}g^{-1}$, respectively). In afforested mine soils the contents of C_{org} were significantly higher and tended to increase with increasing site age (Fig. 1). However, the increasing trend has not been observed for N_t. The contents of this element in the reclaimed soils were similar (0.096-0.120 $mg{\cdot}g^{-1}$) whereas for the mine soils under natural succession the lowest N_t was determined in soil S21 (0.079 $mg{\cdot}g^{-1}$) and the highest in soil S28 (0.260 $mg{\cdot}g^{-1}$). The natural forest soils contained significantly more C_{org} (14.69-15.71 $mg{\cdot}g^{-1}$) and N_t (0.467-0.635 $mg{\cdot}g^{-1}$) than any of the mine soils (Fig. 1).

The lowest C_{org}-to-N_t ratio (12.3-14.7) was measured in the youngest afforested mine soils (S7 and R7) and the highest in the soil MS (41.5). For the other mine and natural soils the C_{org}-to-N_t ratios were relatively similar and varied from 19.8 to 32.6.

The highest content of P_{ext} was measured in the F31 soil (6.31 $mg{\cdot}kg^{-1}$) and the lowest in MS soil (0.52 $mg{\cdot}kg^{-1}$). Low contents of P_{ext} were measured also in the successional mine soils S7 (1.16 $mg{\cdot}kg^{-1}$) and S21 (1.17 $mg{\cdot}kg^{-1}$). In the other mine soils the P_{ext} contents were higher and similar to the P_{ext} concentration in the natural forest soil F100 (Fig. 1). The highest C_{org}-to-P_{ext} ratios were measured in the soil S28 (4305) and in the two natural forest soils (2899 and 3558 for F31 and F100, respectively). Relatively high C_{org}-to-P_{ext} ratio was determined also in soil S7 (1573).

The highest pH values were measured in the soils MS and LUP (6.1 and 5.8, respectively). The pH values of the afforested mine soils were significantly lower and varied from 4.1 to 4.8 (Fig. 1).

Microbial and Biochemical Properties

The lowest C_{mic} and RESP were measured in the soil MS (10.1 μg·g^{-1} and 8.1 μg C-CO_2 g^{-1}·24h^{-1}, respectively). The LUP soil had similar C_{mic} (20.1 μg·g^{-1}) but significantly higher RESP (16.7 μg C-CO_2 g^{-1}·24h^{-1}) compared with the MS soil (Fig. 2). In the afforested mine soils, C_{mic} was similar and varied from 47.2 μg·g^{-1} to 70.7 μg·g^{-1}. The only exception was soil S28, which contained significantly higher C_{mic} (130.6 μg·g^{-1}). This soil also exhibited the highest RESP among all the studied soils (111.0 μg C-CO_2 g^{-1}·24h^{-1}). Relatively high RESP was measured also in soil R7 (81.0 μg C-CO_2 g^{-1}·24h^{-1}).

The percentage of C_{org} present as C_{mic} (C_{mic}-to-C_{org}) was the highest in the MS and LUP soils, 8.4% and 5.3%, respectively. In the afforested mine soils the C_{mic}-to-C_{org} was lower and decreased with site age. The lowest percentage of C_{org} present as C_{mic} was determined in the natural forest soils (0.7-0.8%).

The highest dehydrogenase activity was determined in soil S28 (20.1 μg INTF g^{-1}·h^{-1}), followed by two natural forest soils (16.7 μg INTF g^{-1}·h^{-1} – 16.9 μg INTF g^{-1}·h^{-1}). In the other mine soils, dehydrogenase activity was lower and the minimum value (1.8 μg INTF g^{-1}·h^{-1}) was measured in soil MS. A similar pattern was observed for urease activity: the highest activity of this enzyme was in soil S28

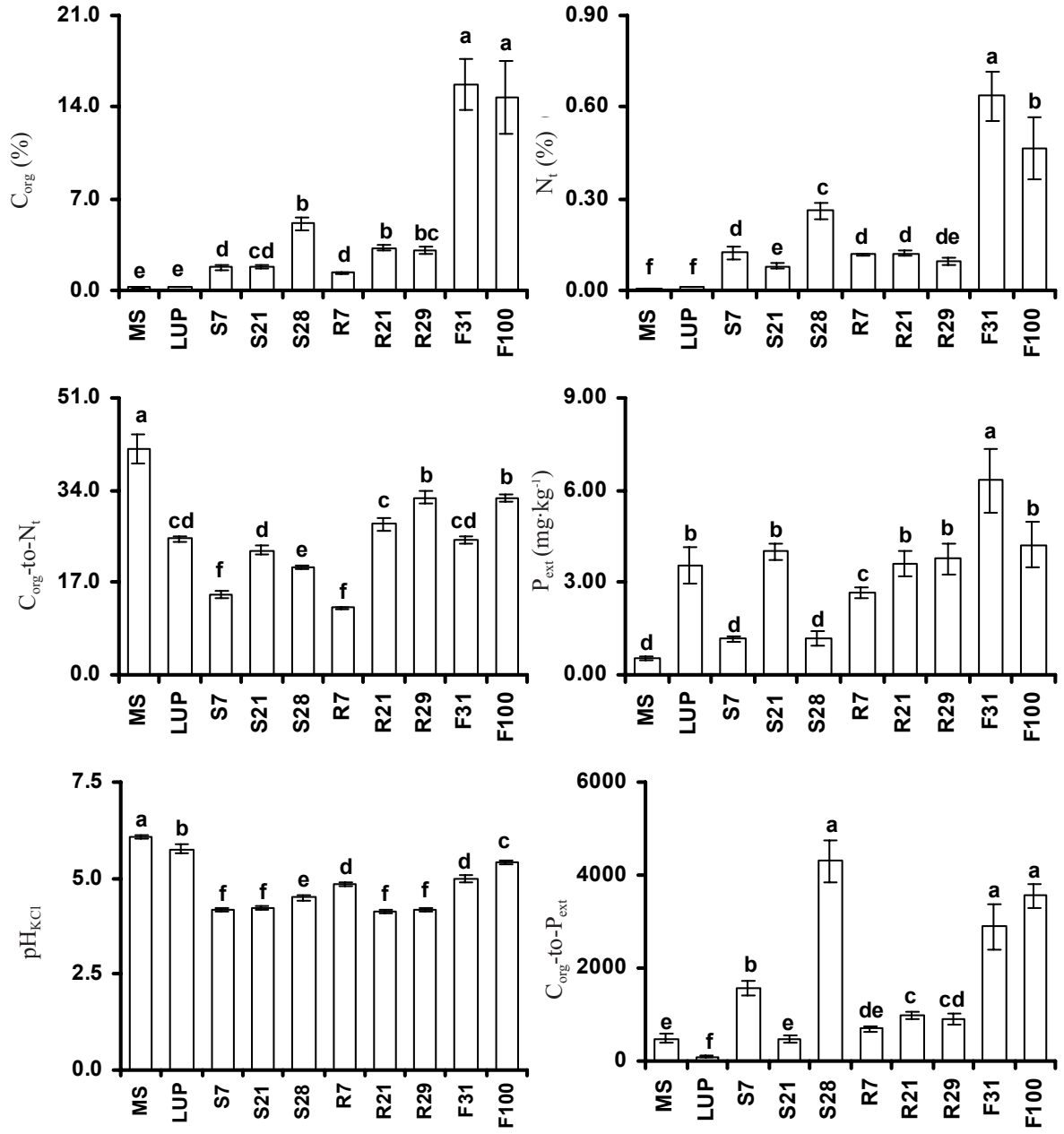

Fig. 1. Mean values (n=8) of the organic C (C_{org}) and total N (N_t) contents, C_{org}-to-N_t ratio, extractable P content (P_{ext}), C_{org}-to-P_{ext} ratio and pH in mine soils (MS, LUP, S7, S21, S28, R7, R21, R29), and natural forest soils (F31 and F100). Error bars indicate standard errors. Columns sharing the same letter do not differ significantly ($p<0.05$).

(2.7 μg N $g^{-1}·h^{-1}$), followed by the soils F31 (1.9 μg N $g^{-1}·h^{-1}$) and F100 (1.6 μg N $g^{-1}·h^{-1}$), and the lowest was in soil MS (0.2 μg N $g^{-1}·h^{-1}$). However, when urease was expressed on microbial biomass basis (urease efficiency), the pattern was quite different with the highest urease efficiency in soil MS (Fig. 2).

For acid phosphomonoesterase activity, the highest values were measured in soils S7 (136.9 μg p-NP $g^{-1}·h^{-1}$) and F31 (108.1 μg p-NP $g^{-1}·h^{-1}$), and the lowest in soils MS (5.6 μg p-NP $g^{-1}·h^{-1}$) and LUP (8.7 μg p-NP $g^{-1}·h^{-1}$). The youngest afforested soils (S7 and R7) exhibited higher acid phosphomonoesterase activities than the older ones. The highest

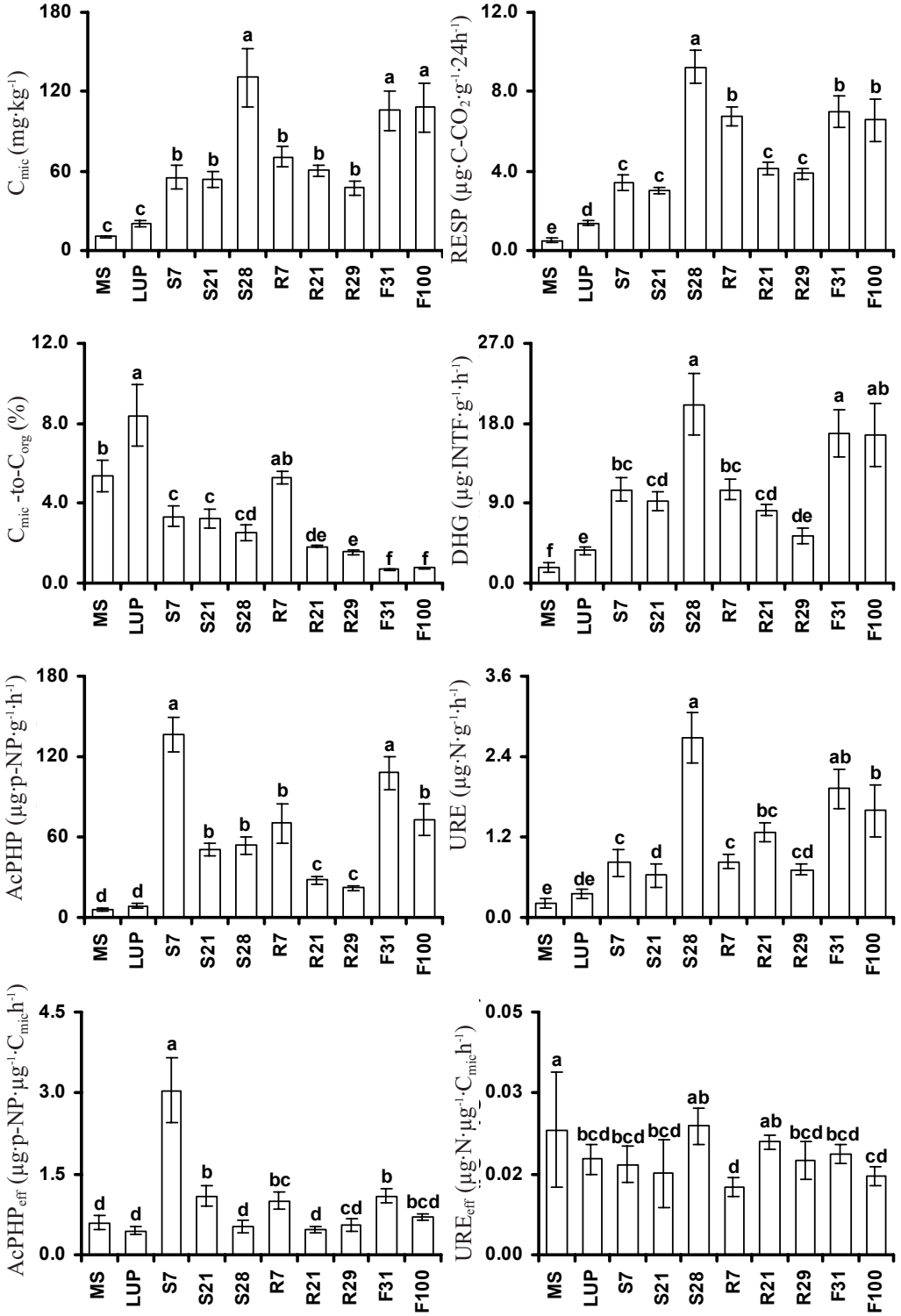

Fig. 2. Mean values (n=8) of microbial biomass (C_{mic}), basal respiration (RESP), C_{mic}-to-C_{org} ratio, activities of dehydrogenase (DHG), urease (URE), acid phosphomonoesterase (AcPHP) and efficiencies of urease (URE_{eff}), acid phosphomonoesterase ($AcPHP_{eff}$) in mine soils (MS, LUP, S7, S21, S28, R7, R21, R29) and natural forest soils (F31 and F100). Error bars indicate standard errors. Columns sharing the same letter do not differ significantly ($p<0.05$).

Table 2. Correlation coefficients between chemical and microbial properties in the mine soils (n=64, p<0.05).

	C_{org}	N_t	C_{org}-to-N_t	P_{ext}	C_{org}-to-P_{ext}	pH	C_{mic}	RESP	DHG	AcPHP	URE	$AcPHP_{eff}$
N_t	0.95											
C_{org}-to-N_t	N.D.	N.D.										
P_{ext}	N.S.	N.S.	N.S.									
C_{org}-to-P_{ext}	0.75	0.81	N.S.	N.D.								
pH	-0.86	-0.80	N.S.	-0.26	-0.57							
C_{mic}	0.77	0.80	-0.49	N.S.	0.64	-0.58						
RESP	0.80	0.85	-0.55	N.S.	0.61	-0.60	0.87					
DHG	0.69	0.75	-0.57	N.S.	0.61	-0.57	0.74	0.78				
AcPHP	0.53	0.63	-0.69	N.S.	0.53	-0.64	0.55	0.55	0.63			
URE	0.74	0.69	N.S.	N.S.	0.71	-0.52	0.70	0.74	0.61	0.39		
$AcPHP_{eff}$	N.S.	N.S.	-0.35	-0.25	N.S.	-0.34	N.S.	N.S.	N.S.	0.73	N.S.	
URE_{eff}	N.S.	N.S.	0.35	-0.25	N.S.	N.S.	-0.29	N.S.	N.S.	N.S.	0.44	N.S.

N.S. – not significant
N.D. – not determined

acid phosphomonoesterase efficiency was measured for the soil S7 (3.1 μg p-NP μg $C_{mic}^{-1} \cdot h^{-1}$). For the other soils, acid phosphomonoesterase efficiency was lower and varied over a relatively narrow range (0.5-1.1 μg p-NP μg $C_{mic}^{-1} \cdot h^{-1}$). There was a trend for decreasing acid phosphomonoesterase efficiency with age in the successional and reclaimed mine soils.

Relationships between Chemical and Microbial Properties in the Mine Soils

The contents of C_{org} and N_t were highly correlated with each other (r=0.95, p<0.0001). However, none of these elements correlated with P_{ext} (Table 2). There was a strong negative relationship between C_{org} and N_t and soil pH (r=-0.86, p<0.0001 and -0.80, p<0.0001, respectively). A weak but significant (p=0.0058) negative relationship also was observed between soil pH and P_{ext} content (r=-0.26).

The microbial biomass and RESP were correlated with each other (r=0.88, p<0.0001) (Table 2). These two microbial properties were strongly positively related to the contents of C_{org} and N_t (r=0.77-0.85, p<0.0001) and moderately, negatively to soil pH (r=-0.58, p<0.0001). The activities of dehydrogenase, urease, and acid phosphomonoesterase correlated positively with RESP (r=0.54-0.80, p<0.0001) and C_{mic} (r=0.55-0.74, p<0.0001). Similarly to C_{mic} and RESP, the enzyme activities depended positively on the contents of C_{org} and N_t (r=0.53-0.75, p<0.0001), and negatively on soil pH (r=-0.57 to -0.64, p<0.0001). None of the soil enzyme activities was related to the content of P_{ext}. However, all properties correlated positively with C_{org}-to-P_{ext} ratio (r=0.53-0.71, p<0.0001). A weak negative relationship was observed between the acid phosphomonoesterase efficiency and the content of P_{ext} (r=-0.25, p=0.0458). The urease efficiency correlated positively with C_{org}-to-N_t ratio (r=0.35, p=0.0082). Comparison of the regression lines revealed that the regression coefficients for the relationships between C_{mic} and RESP, dehydrogenase, acid phosphomonoesterase and urease activities did not differ between the reclaimed and successional soils (Fig. 3). The p-values varied from 0.54 to 0.93, indicating similarity of regression slopes for the reclaimed and successional mine soils.

Discussion

Chemical Properties of Soils

Our results confirmed findings previously reported for this area [10]. The lowest contents of C_{org} and N_t were measured in the pure sand prior to reclamation. Two-years of lupine cultivation did not significantly increase the contents of these elements. Only in the afforested mine soils (reclaimed and successional) were the contents of C_{org} and N_t significantly higher compared with the pure sand, indicating the importance of trees for soil development in post-mining barrens. Among the afforested mine soils, the highest contents of C_{org} and N_t were determined in soil S28, which contained the highest percentage of clay particles among the studied mine soils. Soils with higher clay content can store more C_{org} due to building of organo-mineral complexes resistant to microbial degradation [16-18]. Our results indicate that even a small difference in the clay content may result in significant differences in C_{org} accumulation in the reclaimed mine soils. Similarly, Chodak and Niklińska [19] reported that reclaimed loamy sands under

20-year-old afforestations on the external heap in Bełchatów contained significantly more C_{org} and N_t than the sands.

The C_{org} and N_t contents in all the mine soils were much lower than in the natural forest soils, indicating that build-up of organic matter and accumulation of C_{org} and N_t in reclaimed mine soils is a long-term process [20].

The afforested mine soils had significantly lower pH compared with the MS and LUP soils. This was probably due to accumulation of organic matter, which is often accompanied by a decrease of soil pH [21]. Furthermore, *Pinus sylvestris,* which was the dominating tree species at the experimental sites, is known to cause soil acidification [22, 23].

A relatively high concentration of P_{ext} was measured in the LUP soil and the P_{ext} content in soil R7 was significantly higher than in S7. This indicates the effect of reclamation measures that included P fertilization on the chemical properties of the mine soils. The P_{ext} contents at particular sites did not entirely correspond to previously reported results [10]. However, in our earlier study the P contents were measured with a different method and therefore somewhat different results might have been obtained.

Microbial and Biochemical Properties

The C_{mic} and RESP were strongly related to each other both in the reclaimed and in the successional soils. The lowest values of these properties were measured in the pre-reclamation mine spoil. Two years of lupine cultivation had a relatively small effect on these soil properties and increased only the RESP. In successional mine soils S7 and S21, the C_{mic} and RESP remained constant but peaked in the oldest successional soil, S28. The higher C_{mic} and RESP in soil S28 were probably related not only to gradual development of soil microbial communities, but also resulted from higher content of fine particles (silt and clay) in this soil. Soils with higher content of fine particles usually maintain larger microbial communities than coarsely textured soils, since they provide better protection from faunal predation for microbes and mitigate fluctuations of water availability [16, 17]. For instance, the afforested loamy sands at the reclaimed overburden heap in Bełchatów contained significantly more C_{mic} and exhibited higher RESP than the sands [19].

Among the reclaimed mine soils, the highest C_{mic} and RESP were measured in the youngest soil R7, demonstrating a positive effect of reclamation measures on soil microbial properties. However, this effect proved transient as in the older reclaimed mine soils, R21 and R29, C_{mic} and RESP were lower.

Despite the lower C_{org} and N_t contents, the microbial biomass and basal respiration in soils S28 and R7 reached or even exceeded the values measured in the natural forest soils. We presume that in the natural forest soils a large proportion of C_{org} and N_t was in the recalcitrant form and

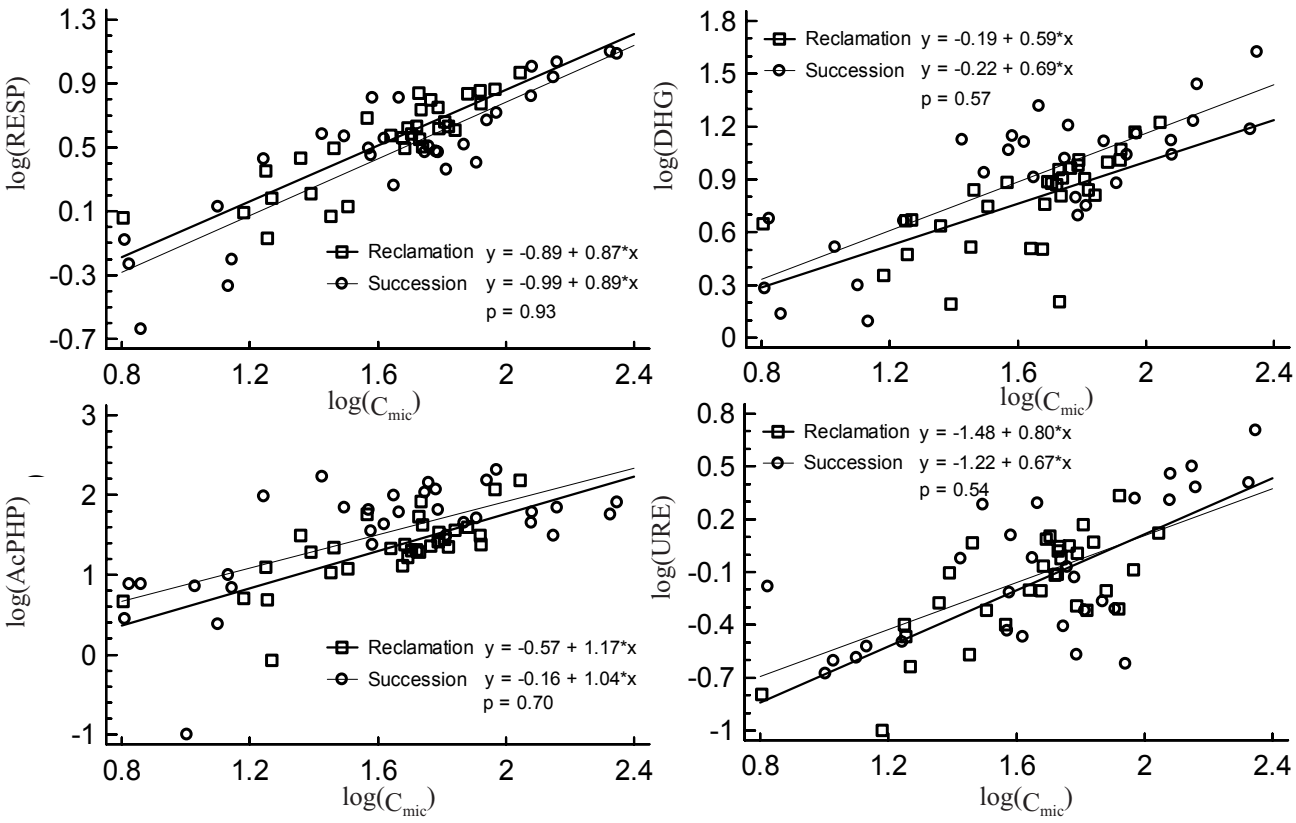

Fig. 3. Relationships between microbial biomass (C_{mic}) and basal respiration (RESP) and activities of dehydrogenase (DHG), urease (URE), acid phosphomonoesterase (AcPHP) in the reclaimed and successional soils. The p-values for the comparison of regression coefficients are also presented.

thus was less available for microbes. Indeed, the percentage of C_{org} present as C_{mic} in the natural forest soils was much lower than in the mine soils, indicating lower availability of C_{org} for soil microorganisms. In the mine soils the percentage of C_{org} present as C_{mic} decreased with age as previously described in several studies [24-26], indicating the progressive accumulation of recalcitrant organic matter [26].

Dehydrogenase activity correlated strongly with C_{mic}, and this relationship was consistent in the reclaimed and successional soils. Consequently, high dehydrogenase activities were determined in the soils with higher microbial biomass. Dehydrogenases are intracellular enzymes that exist only in the living cells; therefore, the measurement of dehydrogenase activity has sometimes been used as a measure of general soil microbial activity [6, 27, 28]. The available assays may underestimate dehydrogenase activity, since the substrates used in the analyses are less effective electron acceptors than oxygen [3]. Our results indicate, however, that in the mine soils the activity of dehydrogenase reflects well their microbial activity.

The highest activities of urease and acid phosphomonoesterase were determined in successional mine soils S28 and S7, respectively. Urease and acid phosphomonoesterase are extracellular enzymes involved in the cycling of N and P, respectively. Urease catalyses the release of NH_4^+ from urea [4] and acid phosphomonoesterase catalyses the hydrolysis of organic P esters to inorganic P [28]. High activity of soil enzymes may indicate nutrient limitation for soil microbes [29]. However, the relationships between extracellular enzyme activities and nutrient contents are not always clear because enzyme activities depend both on nutrient availability and on the microbial biomass [30]. High nutrient contents may suppress synthesis of enzymes but increase microbial biomass. Since the microbial biomass is often a major control on microbiologically mediated processes [31, 32] a decrease in the enzyme synthesis may be compensated by increasing microbial biomass [30]. In our study, the urease and acid phosphomonoesterase activities were positively correlated with microbial biomass and this relationship was consistent in the reclaimed and successional soils. For urease activity the relationship with C_{mic} was relatively strong (r=0.70). This may explain the positive correlation of urease activity with N_t because N_t content had a strong positive effect on the microbial biomass. We think that high urease activity in soil S28 resulted mainly from higher microbial biomass because urease efficiency in this soil did not differ significantly from the other soils.

The negative effect of high contents of N_t on urease activity in our study was evident only when urease activity was expressed per unit of microbial biomass (urease efficiency) and manifested as a positive relationship between urease efficiency and C_{org}-to-N_t ratio. Similarly, Allison et al. [30] observed a negative relationship between N_t content and NAGase activity in the mineral soils in the foreground of Franz Jozef glacier only when the activity of this enzyme was expressed on a microbial biomass basis.

Surprisingly, the acid phosphomonoesterase activity did not correlate with P_{ext} content, although negative correlations between activity of phosphatases and soil P content have been previously reported [30, 33]. In our study the negative relationship between the P_{ext} content and the acid phosphomonoesterase activity was expressed as the positive relationship with the C_{org}-to-P_{ext} ratio and the negative correlation between acid phosphomonoesterase efficiency and P_{ext} content. High acid phosphomonoesterase activity and efficiency in soil S7 may be explained by low content of P_{ext}. Lower P_{ext} content in soil R7 compared with the two older reclaimed mine soils was probably the reason for higher acid phosphomonoesterase activity and efficiency in this soil.

The measured enzyme activities were correlated with each other and depended on microbial biomass and the chemical and physico-chemical soil properties. The correlation analysis indicated that the N_t content was the most limiting factor for the microbial biomass, basal respiration and the activities of dehydrogenase and acid phosphomonoesterase. Only for the urease activity was C_{org} content more important. The N-limitation of microbial growth in soils is a common phenomenon and has been reported for different soils [1].

All the measured microbial properties correlated negatively with soil pH. Soil pH is an important factor affecting soil microbes, but usually positive relationships between pH and microbial properties have been reported [34-36]. In our study the negative relationship of soil microbial properties to soil pH resulted apparently from the fact that the accumulation of organic matter was coupled with decreasing pH. The C_{org} and N_t contents are more important controls on soil microbial properties than soil pH [1].

Conclusions

The mine soils contained significantly less C_{org} and N_t than the natural forest soils. However, in some of the mine soils the microbial biomass and basal respiration attained the values typical for the natural forest soils.

The content of N_t was the most important control on the microbial biomass, basal respiration, and the activities of dehydrogenase and phosphomonoesterase in the mine soils. All the microbial properties were positively related also to C_{org} content.

The activities of dehydrogenase and urease depended strongly on C_{mic}. Therefore, high activities of these enzymes were determined in soils containing high C_{mic}. The acid phosphomonoesterase activity was also positively related to C_{mic}, but its activity was enhanced in the soils with low P contents.

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