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# Effect of black alder (*Alnus glutinosa*) admixture to Scots pine (*Pinus sylvestris*) plantations on chemical and microbial properties of sandy mine soils

Katarzyna Sroka[a,*], Marcin Chodak[a], Beata Klimek[b], Marcin Pietrzykowski[c]

[a] *AGH University of Science and Technology, Department of Environmental Management and Protection, al. Mickiewicza 30, 30-059 Kraków, Poland*
[b] *Jagiellonian University, Institute of Environmental Sciences, Faculty of Biology and Earth Sciences, ul. Gronostajowa 7, 30-387 Kraków, Poland*
[c] *University of Agriculture in Kraków, Department of Forest Ecology and Reclamation, Institute of Ecology and Silviculture, Faculty of Forestry, al. 29 Listopada, 31-425 Kraków, Poland*

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## ABSTRACT

Phytomelioration and tree species selection on poor mine soils are important for reclamation and afforestation success. Black alder (*Alnus glutinosa*), as N-fixing species, is often planted on afforested post-mining barrens as an admixture to improve soil properties and enhance growth of target tree species. Objective of this study was to assess the effect of black alder admixture on chemical, physical and microbial properties of sandy mine soils afforested with Scots pine (*Pinus sylvestris*). The topsoil samples (0–5 cm) were taken in areas afforested with Scots pine with black alder admixture at 1 m, 2 m, 4 m and 8 m from the black alder rows and measured for texture, maximum water holding capacity (WHC), pH, $C_{org}$ and N contents. The measured soil microbial properties included microbial biomass ($C_{mic}$), basal respiration rate (RESP), N mineralization rate and urease activity (URE). Community level physiological profiles (CLPP) of soil microbial communities were determined with Biolog® Ecoplates. The highest $C_{org}$, N, $C_{mic}$, RESP, N mineralization rate and URE values were measured at 1 m from the alders row and then decreased rapidly. The influence of alders ranged to ca 2 m–3 m from the alders row depending on the soil property. Soil microbial communities close to the alders row were functionally more diverse than those in soils from larger distances. There were distinct differences in CLPPs between microbial communities from soil at 1 m and 8 m from the alders row wherein those close to the alders were more efficient in degradation of carbohydrates and those at 8 m in degradation of polymers. Results of the study clearly indicate positive influence of black alder on several soil properties crucial for their biological activity and fertility.

## 1. Introduction

Afforestation is a common way of reclamation of post-mining barrens such as overburden heaps or open pits left after mining extraction. However, such objects are often built of infertile materials characterized by disadvantageous chemical and physical properties and exhibiting extremely low biological activity (Hüttl and Weber, 2001; Baldrian et al., 2008; Pietrzykowski, 2014). Therefore, only some tree species are able to grow on these materials. In central Europe Scots pine (*Pinus sylvestris*) is commonly used for afforestation of infertile post-mining barrens since this tree species has low nutritional requirements and is able to survive under harsh environmental conditions (Kelly and Conolly, 2000; Pietrzykowski et al., 2013). However, extremely low N content in post-mining barrens may limit growth of planted Scots pine trees. Mineral N fertilization of such barrens may not always be sufficient, especially in case of coarse textured materials as the applied fertilizers are relatively quickly leached from the soils. However, long term N supply of barren materials poor in this element may be ensured by planting N-fixing plant species (Krzaklewski et al., 2012).

Alder species (*Alnus* sp.) are well known for their symbiosis with nitrogen-fixing actinomycetes of genus *Frankia* and enriching soils in N (Malcolm et al., 1985; Hibbs and Cromack, 1990; Binkley, 1994; Hart et al., 1997). Admixture of different alder species to forest plantations on poor soils has been described to increase their N and organic matter content (Cromack et al., 1999; Seltmans et al., 2005; Chiti et al., 2007; Chodak and Niklińska, 2010a). In Central Europe black alder (*Alnus glutinosa*) and grey alder (*Alnus incana*) have often been used for reclamation of various barrens, including quaternary and neogene sands,

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[*] Corresponding author.
*E-mail address:* sroka@agh.edu.pl (K. Sroka).

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gravel-sands and clays or even fly ashes and combustion wastes (Frouz et al., 2001; Šourková et al., 2005; Krzaklewski et al., 2012; Józefowska et al., 2017) and proved to have a positive effect on several chemical and microbial properties of soils developing in the reclaimed barrens. Soils under pure alder stands had higher organic matter and N content compared with those under other tree species (Šourková et al., 2005; Chodak and Niklińska, 2010) or under natural succession (Helingerová et al., 2010). In consequence they were also more biologically active–harbored larger microbial biomass and exhibited higher respiration and enzymatic activities compared to soils under natural succession or other tree species (Šourková et al., 2005; Helingerová et al., 2010; Šnajdr et al., 2013). Despite positive influence on several soil properties black alder is rarely introduced in pure plantations on coarse textured mine soils. This is because under harsh conditions of post-mining barrens planted black alder is low competitive to other target tree species and can survive in tree stands up to only ca. 15–20 (Pietrzykowski, 2015). Therefore on such areas black alder is often planted as an admixture and introduced in rows that are to improve soil properties and support growth of target tree species in their vicinity. Although this method of alders planting has been used in reclamation practice little is known about the distance at which alder rows affect soil properties. Therefore, in this study we addressed the question what is the range of influence of black alders admixed row-wise into the Scots pine plantations on various chemical and microbial properties of sandy mine soils.

## 2. Material and methods

### 2.1. Study site

The study was carried out in Upper Silesia, Poland (19°26′ E; 50°16′ N) at Szczakowa sand mine pit. The climate in the study area is temperate, with ca. 700 mm mean annual precipitation and 8 °C mean annual temperature. The sand deposits extracted in the quarry are fluvioglacial Quaternary sediments of a pre-Quaternary depression.

The Szczakowa quarry has been extracting sands since 1954, with mining creating an open cast 5–25 m deep, covering over 3000 ha. The sampling area has been reclaimed for forestry in years 1999–2000. The reclamation procedure included forming and levelling the surface and lupin (*Lupinus luteus L.*) cultivation for two years. The lupin cultivations were fertilized with NPK (140 kg N $\mathrm{ha}^{-1}$, 300 kg $P_2O_5$ $\mathrm{ha}^{-1}$, 180 kg $K_2O$ $\mathrm{ha}^{-1}$) (Pietrzykowski et al., 2017). After two years the lupin biomass was ploughed into the soil as green manure and the sites were afforested with 1-year-old seedlings of Scots pine (*Pinus sylvestris*) and common alder (*Alnus glutinosa*) wherein the pine saplings were planted area-wise while black alder saplings row-wise.

### 2.2. Soil sampling

Samples of mineral soil (0–5 cm) were taken in October 2016 from six sites (20 m × 16 m). Organic horizons were omitted as they were thin, discontinuous and present only at few locations close to the alder rows. In order to avoid pseudoreplication problem the sampling sites were located as far as possible from each other and the distance between the nearest sites was ca. 300 m. Each site consisted of 20 m long row of black alders (*Alnus glutinosa*) surrounded by pure Scots pine (*Pinus sylvestris*) stands. To assess the range of black alder influence on soil chemical and microbial properties the mixed soil samples were taken at distances of 1 m, 2 m, 4 m and 8 m from the alders row. Each sample consisted of four sub-samples (area of each sub-sample = 0.16 $\mathrm{m}^2$) taken at both sides of the alders row. All the sampled forest stands were 16 years old.

The samples were 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 and biochemical analyses. Prior to microbial analyses the samples were adjusted to 50% of maximum water holding capacity (WHC) and pre-incubated at 22 °C for six days.

### 2.3. Chemical and microbial analyses

The WHC was determined gravimetrically according to Schlichting and Blume (1966). The soil texture of the samples was determined hydrometrically. The content of organic C ($C_{\mathrm{org}}$) was measured by dry combustion using (CS Eltra 500) and soil N content was according to Kjeldahl method (Büchi, Kjelflex K-360).

The pH of the samples was measured in water and in 1 M KCl solution (soil:liquid ratio 1:5, w:v) with a digital pH-meter (Elemtron CPC-401).

### 2.4. Determination of microbial biomass, basal respiration and urease activity

To measure basal respiration (RESP) and microbial biomass ($C_{\mathrm{mic}}$), samples (50 g d.w.) unamended for RESP measurements and amended with 8 mg glucose monohydrate for $C_{\mathrm{mic}}$ measurements were incubated at 22 °C in gas-tight jars. The incubation time was 24 h for determination of RESP and 4 h for $C_{\mathrm{mic}}$. 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 0.9 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. $C_{\mathrm{mic}}$ was calculated from the substrate-induced respiration rate according to the equation given by Anderson and Domsch (1978): $C_{\mathrm{mic}}$ [mg $\mathrm{g}^{-1}$] = 40.04 y + 0.37, where y is ml $CO_2$ $\mathrm{h}^{-1}$ $\mathrm{g}^{-1}$. Urease activity (URE) 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 2 h. The released ammonium was extracted with acidified potassium chloride solution, colored in the modified Berthelot reaction and measured photometrically at 690 nm. Urease activity was expressed as µg N $\mathrm{g}^{-1}$ $\mathrm{h}^{-1}$ and urease efficiency ($\mathrm{URE}_{\mathrm{eff}}$) was calculated as urease activity normalized to a per µg of $C_{\mathrm{mic}}$ basis (Allison et al., 2007).

### 2.5. Community level physiological profiles (CLPP)

The physiological profiles of the microbial communities were analyzed using Biolog® Ecoplates (Insam, 1997). Samples (9 g d.w.) were shaken for 60 min in 30 ml of a 0.9% NaCl solution and allowed to settle for 30 min. Then the extracts containing microbes were decanted and diluted ($10^{-2}$) with 0.9% NaCl solution. The solutions were inoculated on microplates (125 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 24 h intervals for six days. The readings for individual substrates were corrected for background absorbance by subtracting the absorbance of the control (water) well and 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 summed for each soil sample and the proportions of individual substrates from this total area were expressed as area%. The area% values for all substrates were then used for characterization of community-level physiological profiles and statistical analyses. The Biolog® data were further analyzed to compare metabolic preferences of the microbial communities at different distances from the alders row. The carbon substrates were grouped into seven guilds–carbohydrates (CH), carboxylic acids (CA), amino acids (AA), polymers (PO), phosphates (Phos), amines and amides (AM), phenolics (PH) 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. Average area under curve (AAUC) was used to express overall microbial activity on the plates.

### 2.6. Statistical analysis

Simple regression analysis was used to determine relationship between soil chemical and microbial properties and the distance from the black alders row. Among several regression models tested the reciprocal-X model ($y = a + b/\text{Dist}$; where y is the soil property, Dist is distance from the alders row in m and a and b are equation parameters) proved to be the best fitting one for most of the studied properties and was used in the study. Only for $\text{URE}_{\text{eff}}$ linear regression model was applied.

To assess the effects of soil chemical properties on microbial parameters ($C_{mic}$, RESP, N mineralization rate and URE) multiple regression analysis was performed. In this analysis, we used the $C_{org}$, $N_t$ and $C_{org}$-to-Nt ratio as variables representing nutrient availability for the microbes, $pH_{H2O}$ was used as a variable representing soil acidity and sand content as variable representing soil texture. The right-skewed data ($C_{mic}$, RESP, N mineralization rate and URE, $C_{org}$, and $N_t$) were log-transformed to fulfill the assumption of normality. In order to directly assess the effects of significant variables all the independent variables were standardized prior to analysis to have a mean value of 0 and variance of 1. The percentage of total variance explained by the models was reported as the $R^2$ value adjusted for degrees of freedom.

Principal component analysis (PCA) performed on covariance matrix was used to investigate Biolog® data (Statgraphics Plus 5.1 software, Statistical Graphics Corporation). In this analysis we used area% values for 7 substrate guilds (CH, CA, AA, PO, PH, Phos, AM, Misc). Interpretation of the PCs was based on significant factor loading of the substrate guilds on each of the PCs. 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 n is the number of wells and $p_i$ is the use of the *i*th substrate (AUC value) as a proportion of the sum of the use of all substrates of a plate.

All analyses were performed with Statgraphics Centurion XVI software (Statistical Graphics Corporation).

## 3. Results

### 3.1. Physical, chemical and microbial properties of mine soils at different distances from the alders row

All studied soils were coarse textured and were classified as sands (USDA classification) (Fig. 1). Only in one sample taken at the distance of 8 m from the row of alders the shares of silt and clay particles were higher (33% and 6%, respectively) and the sample was classified as sandy loam (Fig. 1).

The highest contents of $C_{org}$ (8.15 mg $g^{-1}$–11.05 mg $g^{-1}$) and N (0.52 mg $g^{-1}$–0.90 mg $g^{-1}$) were measured at 1 m from the alders row and then rapidly decreased at 2 m ($C_{org}$ to 3.02 mg $g^{-1}$–6.92 mg $g^{-1}$ and N to 0.25 mg $g^{-1}$–0.46 mg $g^{-1}$) and at larger distances (Fig. 2). The only exception was the sandy loam sample taken at the distance of 8 m for which relatively high $C_{org}$ and N contents were measured (Fig. 2).

The maximum WHC values followed the same trend as $C_{org}$ and N with the highest values (30%–38%) at 1 m and the lowest (22%–25%) at 8 m from the row of alders. The influence of alders was evident also for pH and manifested by decreasing pH values with decreasing distance to the alders row (Fig. 2). Most of samples had acid pH with values around 5.8–6.0 at 8 m and 5.2–5.5 at 1 m from the row of alders. Only at one site the pH values were distinctly higher but also at this site the trend for lower pH at shorter distance from the alders row was apparent (Fig. 2).

The highest $C_{mic}$ (229 μg $g^{-1}$–391 μg $g^{-1}$) and RESP values (19.6 μg C-$CO_2$ $g^{-1}$ 24 $h^{-1}$–29.5 μg C-$CO_2$ $g^{-1}$ 24 $h^{-1}$) were measured at 1 m from the alders row and then decreased rapidly at 2 m and at larger distances (Fig. 3). Similar trend was observed for N mineralization rate and URE, however for URE the decrease at the 2 m distance was large and the measured values were at similar level as at 4 m and 8 m distance. The $\text{URE}_{\text{eff}}$ exhibited a different trend–the lowest values were determined at 1 m from the alders row and then gradually increased to reach the highest level at 8 m (Fig. 3).

Relatively high values of URE, RESP and $C_{mic}$ were measured at 8 m from the alders row in the sandy loam sample.

The change of the measured soil properties with the distance from the alders row was well described by reciprocal-X model, with $r^2$ values ranging from 0.23 to 0.84 ($p = 0.0000$–$0.0007$). The models indicated that the effect of alders on the studied soil properties disappeared relatively rapidly and ranged from ca. 1.5 m (URE, N mineralization rate, pH) to ca. 2.5 m–3 m ($C_{org}$, N, $C_{mic}$ and RESP) or even ca. 4 m for WHC (Fig. 2 and 3).

### 3.2. Relationship between chemical and microbial properties of mine soils

Multiple regression models for $C_{mic}$, RESP, N mineralization rate and URE were highly significant ($p < 0.0001$) and explained from 54.8% (URE) to 93.0% ($C_{mic}$) of the variance in these properties (Table 1). The $C_{org}$ content was the most important variable, positively related to $C_{mic}$ and RESP (regression coefficient (β) = 0.59 and 0.68 for $C_{mic}$ and RESP respectively). Both $C_{mic}$ and RESP were affected also by pH, however for $C_{mic}$ the effect of pH was positive while for RESP negative (Table 1). Urease activity was positively related to N content and $C_{org}$-to-N ratio, but the N mineralization rate depended only on WHC (Table 1).

### 3.3. Analysis of the BIOLOG® data

The AAUC and H' values were highest at 1 m from the alders row where ranged from 31.3 to 71.6 and from 2.84 to 3.14, respectively and then decreased gradually with increasing distance from the alders row (Fig. 4). This decrease was well described by reciprocal-X model ($p = 0.0002$–$0.0061$; $r^2 = 0.29$–$0.48$).

The first PC explained 45.4% and was positively related to the use of CA (0.72) and negatively to the use of AA (−0.60) but did not separated soils from any distances (Fig. 5). However, the second PC that explained 36.4% of the variance in Biolog® data separated soils taken at 8 m from the alders row from those at 1 m. The largest loadings on PC2 were from the use of PO (0.78) and CH (−0.50). High PC2 values for

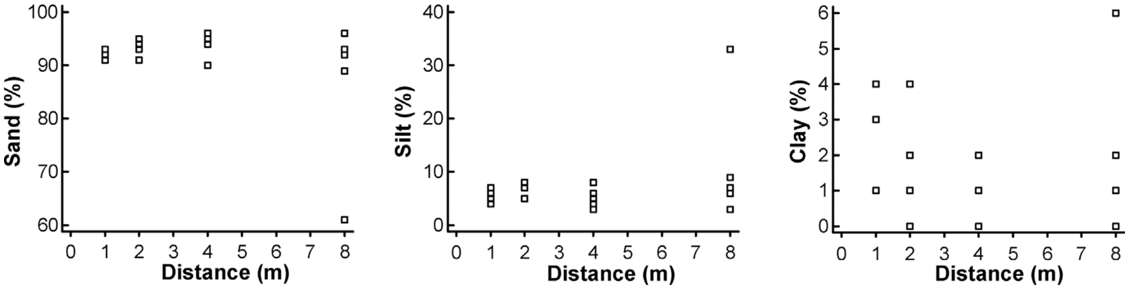

**Fig 1.** Shares of sand (2 mm–0.05 mm), silt (0.05 mm–0.002 mm) and clay (< 0.002) particles in soils at different distances from the black alders row.

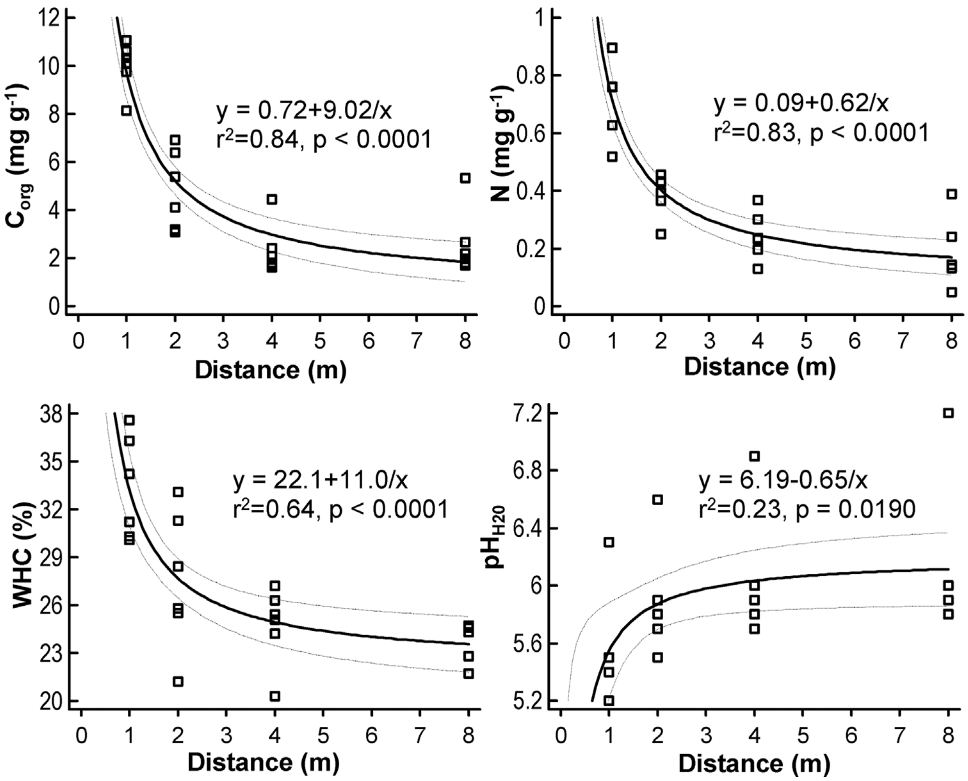

**Fig. 2.** Contents of organic C ($C_{org}$) and nitrogen (N), pH and maximum water holding capacity (WHC) in soils at different distances from the black alders row. Thick lines indicate modelled values and thin lines confidence intervals (95%).

the soils at 8 m distance indicated therefore preferable use of PO and less efficient use of CH while for the soils at 1 m distance the opposite was the case.

## 4. Discussion

### 4.1. The effect of alders on chemical and physical soil properties

Admixture of black alder to Scots pine stands planted on sandy barrens had a significant effect on several chemical and microbial properties of mine soils. As expected soil N contents were the highest in the vicinity of black alder rows and decreased with distance. Similar effect of black alder was observed also for $C_{org}$. Elevated $C_{org}$ and N contents were up to ca. 3.5 m from the alders row. The obvious reason for the observed higher soil N contents was N-fixation by alders owing to their symbiosis with *Frankia* actinomycetes (Hibbs and Cromack, 1990). Higher $C_{org}$ contents were probably due to larger biomass production by alders and Scots pines growing in their vicinity. The studied sandy mine soils consisted of excavated materials that initially contained extremely low amounts of N, hence the growth of planted Scots pine could be limited. Planted black alders alleviated soil N deficiency in their vicinity and allowed better growth of Scots pines leading to larger litter production resulting in greater C inputs to the soil and in consequence higher $C_{org}$ accumulation (Resh et al., 2002).The higher soil $C_{org}$ contents close to the alders row might have been also due to slower decomposition rate of humified organic matter since high N contents in the litter inhibit decomposition of humified soil C (Berg, 2000). This is because the low molecular weight N compounds may be incorporated into phenolics or lignin molecules creating larger molecules resistant to microbial decomposition (Camiré et al., 1991). High N contents may also suppress fungal lignolytic enzymes responsible for decomposition of recalcitrant C compounds (Carreiro et al., 2000). In line with our results, Chodak and Niklińska (2010a) reported significantly higher $C_{org}$ content in mineral soils under mixed Scots pine–black alder stands compared with pure Scots pine stands. Similarly, Chiti et al. (2007) found higher amounts of $C_{org}$ in mine soils afforested with mixed oak-alder (*Quercus robur* L., *Alnus cordata* Loisel.) than in soils under pure oak stands.

Organic carbon contents were lower at larger distances from the alders row, except the site where soils with higher clay and silt fraction content occurred. High $C_{org}$ (and N) content in this soil was probably due to its higher silt and clay content. Soils with higher share of fine particles may contain more $C_{org}$ because the presence of these particles supports organic matter protection owing to building of organo-mineral complexes resistant to microbial degradation (Franzluebbers et al., 1996; Müller and Höper, 2004). Indeed, Chodak and Niklińska (2010b) reported that loamy sands afforested under 23–24 years old pine and birch stands contained more $C_{org}$ than sands and concluded that even small change in clay content in coarse textured mine soils may lead to large differences in organic matter accumulation.

The WHC of studied soils was highly variable, but higher values were found to ca. 3 m–4 m from the alders row. Improved water retention ability in soils close to the alders row resulted apparently from their higher organic matter content. The studied soils had sandy texture and in coarse soils increase of $C_{org}$ content is known to improve water retention in sandy soils (Rawls et al., 2003).

The effect of alders on soil properties manifested also in lower soil pH determined to ca. 2 m from the alders row. This was probably because the process of N fixation by plants is often connected with the release of $H^+$ ions into the rhizosphere (Bolan et al., 1991). Increased leaching of $NO_3^-$ might have also contributed to decrease of soil pH in the vicinity of black alder rows (Verburg et al., 2001; Rothe et al., 2002). Contrary to our results, Chodak and Niklińska (2010a) reported that soil pH under mixed pine-alder stand was not lower than under the pure pine stand. However, the share of alders in their study approximated only ca. 25%. In our study, decrease of soil pH was the most evident at 1 m from the alders row where the relative share of alders and in consequence their effect on soil properties was much higher. reported a decrease of pH from 8.3 to 6.1 in mine soils under 30-years old pure *Alnus glutinosa* and *Alnus incana* stands.

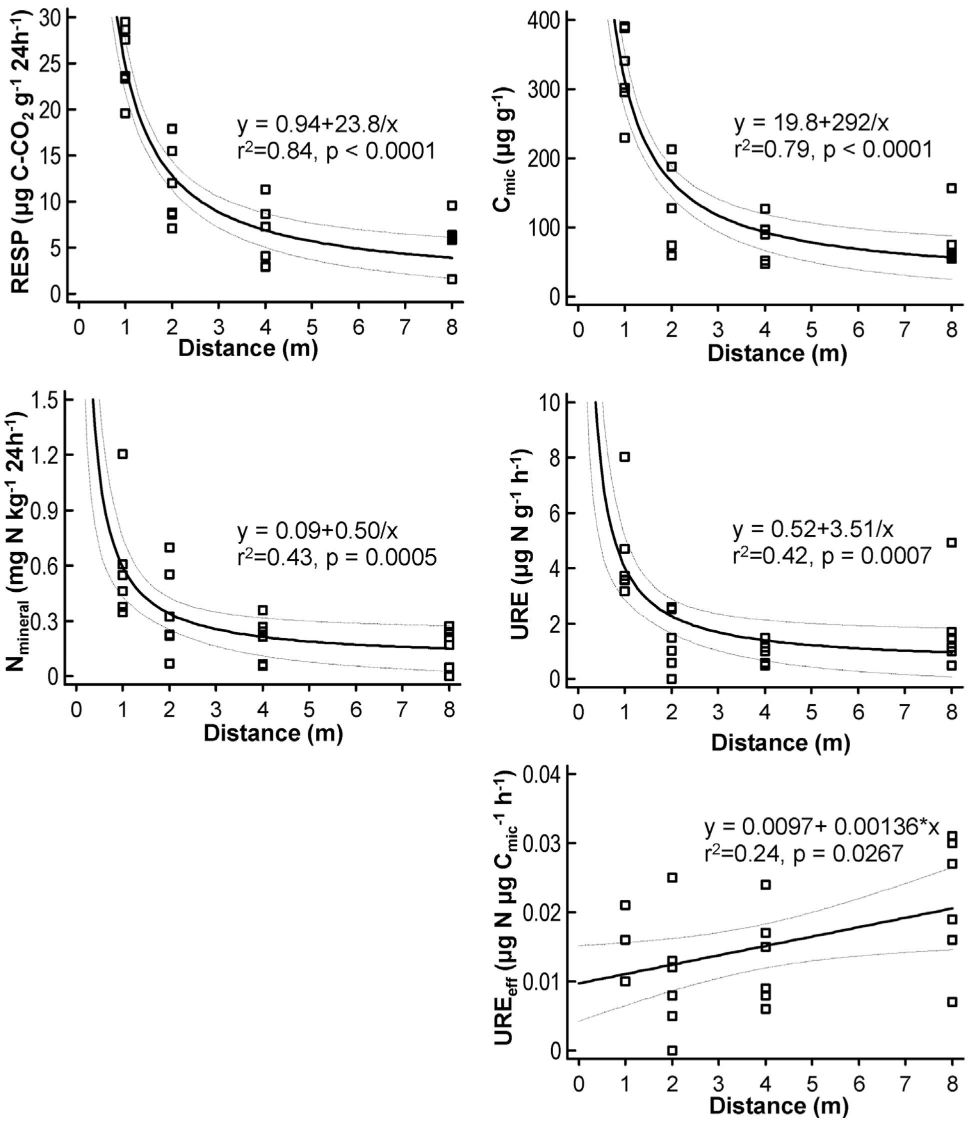

**Fig. 3.** Microbial biomass ($C_{mic}$), basal respiration rate (RESP), N mineralization rate ($N_{mineral}$), urease activity (URE) and urease efficiency ($URE_{eff}$) in soils at different distances from the black alders row. Thick lines indicate modelled values and thin lines confidence intervals (95%).

**Table 1**
Relationships between microbial biomass ($C_{mic}$), basal respiration (RESP), N mineralization rate ($N_{mineral}$) and urease activity (URE) and soil physical and chemical properties. Results of multiple regression with backward selection, only significant parameter are presented.

| Microbial property | $R_{adj}^2$ | $p_{mod}$ | Equation parameter | β | p |
|---|---|---|---|---|---|
| RESP | 90.6 | < 0.0001 | Intercept | 2.19 | < 0.0001 |
| | | | $C_{org}$ | 0.68 | < 0.0001 |
| | | | $C_{org}$-to-N | −0.16 | 0.0108 |
| | | | $pH_{H2O}$ | −0.20 | 0.0055 |
| $C_{mic}$ | 93.0 | < 0.0001 | Intercept | 4.82 | < 0.0001 |
| | | | $C_{org}$ | 0.59 | < 0.0001 |
| | | | WHC | 0.18 | 0.0340 |
| | | | $pH_{H2O}$ | 0.12 | 0.0206 |
| $N_{mineral}$ | 71.2 | < 0.0001 | Intercept | −1.40 | < 0.0001 |
| | | | WHC | 0.53 | 0.0002 |
| URE | 54.8 | < 0.0001 | Intercept | 1.00 | < 0.0001 |
| | | | N | 0.45 | < 0.0001 |
| | | | $C_{org}$-to-N | 0.29 | 0.0026 |

### *4.2. The effect of alders on soil microbial properties*

The planted alders had positive effect on gross microbial properties as higher values of $C_{mic}$, RESP, URE and N mineralization rate were found up to ca. 3 m–4 m from the alders row. Higher accumulation of $C_{org}$ close to the alders row was the most important reason for higher $C_{mic}$ and RESP values (Table 1). Indeed, the $C_{org}$ content has often been described as the main factor limiting microbial growth (Demoling et al., 2007; Spohn and Kuzyakov, 2013). However, positive effect of alders on soil microbial properties was also due to improved water retention and N accumulation (Table 1). Water availability and N content are also important factors influencing growth and activity of soil microorganisms (Demoling et al., 2007).

The effect of alders on microbial properties related to N cycling had somewhat shorter range. Higher urease activity was measured only at 1 m from the alders row then decreased and was at similar level at 2 m, 4 m and 8 m from the alders row. Urease is an extracellular enzyme that catalyses the release of $NH_4^+$ from urea (Caldwell, 2005). Its activity in soil is controlled both by N content and microbial biomass (Allison et al., 2007). High N contents may suppress synthesis of urease but decrease in the urease synthesis may be compensated by increasing microbial biomass (Allison et al., 2007). In our study, urease activity correlated positively with microbial biomass ($r = 0.79$, $p < 0.0001$; data not shown). We think therefore that high urease activity in soils at

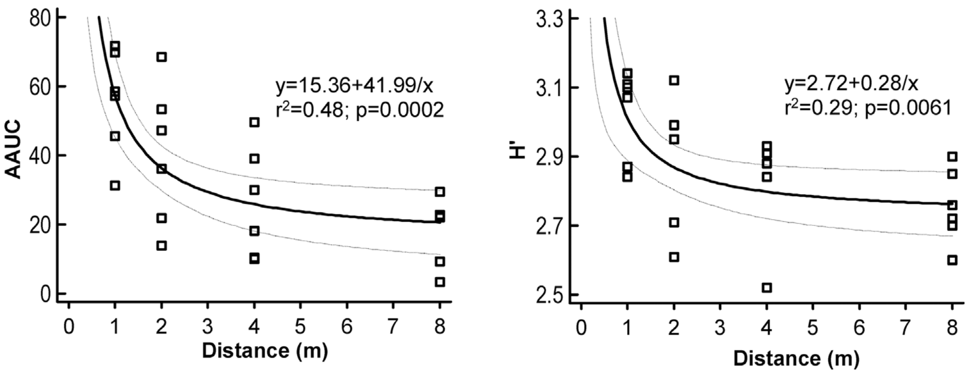

**Fig. 4.** Average area under curve (AAUC) and Shannon's diversity index values in soils at different distances from the black alders row. Thick lines indicate modelled values and thin lines confidence intervals (95%).

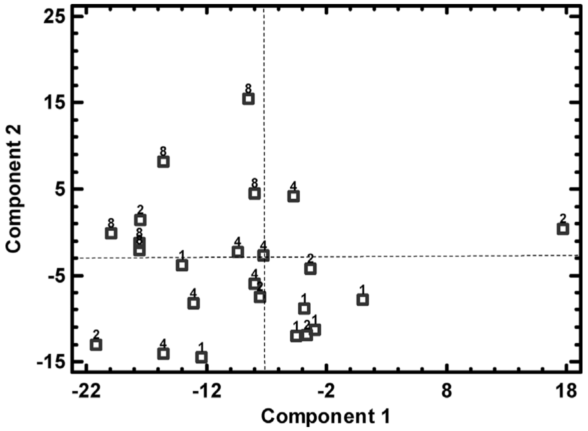

**Fig. 5.** Principal components (PC1 and PC2) calculated for microbial communities from soils at different distances from the alders row (1m, 2m, 4 m and 8m) based on Biolog® data. Percent of explained variance in parentheses.

1 m from the alders row resulted mainly from much higher microbial biomass there. At larger distances $C_{mic}$ diminished but urease synthesis increased due to lower N content as indicated by increased $URE_{eff}$ values. In consequence urease activity at 4 m and 8 m was at similar level as at 2 m from the alders row. The only exception was high urease activity in the sandy loam sample, 8 m from the alders row. High urease activity in this soil was probably due to high microbial biomass there. However, urease adsorbed on fine particles might have additionally contributed to higher URE determined in this soil (Nannipieri et al., 2002).

The N mineralization rate was the highest at 1 m from the alders row. However, in contrast to soil respiration rate the increase in N mineralization rate in soil at 1 m from the alders row compared to soils at larger distances was less pronounced. The measured N mineralization rate was a net value and resulted from two counterflowing processes–gross N mineralization and N immobilization in soil samples during their incubation. In forest soils microbial immobilization of mineral N may be high (Hart et al., 1994; Shi et al., 2006) leading in consequence to lower net N mineralization. Relatively weak increase of N mineralization rate close to the alders row might have been also caused by reduced nitrification rate due to lower pH there (Hart et al., 1994; Shi et al., 2006).

### 4.3. The effect of alders on functional properties of soil microbial communities

The alders influenced not only the gross microbial properties but affected also functional properties of soil microbial communities. The H' values close to the alder rows were higher than at larger distance suggesting higher functional diversity of microbial communities in the vicinity of alders. Soil microbial communities at 1 m and 8 m from the alders row exhibited different CLPPs indicating different C substrate preferences. The microbial communities at 1 m from the alders row were more efficient in using carbohydrates while those at 8 m used more effectively polymers. Quality of organic matter is an important factor affecting the physiological abilities of soil microbial communities since the ability of a microbial community to decompose specific C substrates is likely to depend on the type, abundance and bioavailability of C substrates present in the soil organic matter pool (Degens et al., 2000; Orwin et al., 2006; Banning et al., 2012). Therefore, the observed differences may be attributed to different soil organic matter quality at the two distances. At 8 m from the alders row the pine litter was the major source of soil organic matter. Since the Scots pine litter contains large proportion of recalcitrant organic compounds (Johansson, 1995; Kanerva et al., 2008) it favoured development of soil microbial communities well adapted to degradation of polymers but less efficient in decomposing easily decomposable compounds such as carbohydrates. At 1 m distance the share of black alder litter in soil organic matter pool was large and the litter of alders contains more N, less lignin and is more easily degradable than the pine litter (Niemi et al., 2007). In consequence soil microbial communities close to the black alders row were better adapted to degradation of easily degradable compounds (carbohydrates) but less efficient in decomposing polymers. The CLPPs determined with Biolog® test may biased by different cell numbers (or microbial biomass) inoculated into the wells (Preston-Mafham et al., 2002). However, we used standardized results (area%) and therefore, we suppose that the differences in CLPPs resulted mainly from differences in intrinsic properties of the studied microbial communities (Garland, 1997). However, we cannot rule out that different pattern of the substrate use in soils at 1 m and 8 m distance from the alders row might have partly resulted also from lower inoculum densities of the soil samples taken at 8m. One should also bear in mind that Biolog® Ecoplates measure the activity of only the selected groups of bacteria capable of growing in the plates (Ros et al., 2008). Hence, the differences demonstrated in our study refer to only small component of the soil microbial communities. Nevertheless, we think that the information on functional diversity obtained with Biolog® Ecoplates was useful for assessing the effects of alders admixture on the microbial properties of the studied mine soils.

## 5. Conclusions

Black alder planted in rows among Scots pine stands had a distinct, positive effect on several chemical and microbial properties of the reclaimed sandy mine soils. Introduction of alders alleviated N deficiency and increased $C_{org}$ accumulation in these soils and in consequence increased their microbiological activity. Thus planting of black alders proved to be a useful alternative for the use of artificial fertilizers. The range of alders influence on soil properties in the studied 16 years old forest stands varied from ca. 2 m to ca. 3 m for different soil properties. These values should be considered in preparation of afforestation and species composition design (ie. percentage of alder saplings and their spatial arrangement within target species plantations) for reclaimed sandy mine soils.

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