



# The effects of tree species and substrate on carbon sequestration and chemical and biological properties in reforested post-mining soils



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## ABSTRACT

The effects of tree species, parent material (substrate), and faunal bioturbation on carbon sequestration and chemical and microbial properties in soil were studied at three post-mining sites (Piaseczno, Szczakowa, and Bełchatów) in Poland that were reforested with pine, birch, oak, and alder. The samples were taken from the 0–5-cm mineral soil horizons beneath the litter layer. The samples were analyzed for pH, organic C and total N content, texture and based exchange cations. Microbial analyses included determination of ergosterol and phospholipid fatty acid (PLFA) profiles. Soil thin section was done to described faunal activity by the percentage of the soil volume occupied by earthworm casts or macrofaunal excrements. Substrate quality varied considerably among the sites; soil pH ranged from 4.0 to 6.4, and clay content ranged from 1 to 16%. The C:N ratio and other soil properties depended more on the specific combination of tree species and substrate than on tree species or substrate alone.

Carbon content and C stock were positively correlated with bioturbation caused by soil macrofauna. Bioturbation was positively correlated with soil pH and the content of basic cations. In contrast, C stock was negatively correlated with the fungal to bacterial ratio (F:B ratio). Bioturbation was also positively correlated with total microbial biomass and negatively correlated with F:B ratio. Microbial biomass was positively correlated with N content and pH, both of which were negatively correlated F:B ratio.

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## 1. Introduction

Reclaimed mine soils are usually characterized by a lack of recent soil organic matter, poor air and water properties, compaction, extreme acidity or alkalinity, and excessive salinity (Brevik and Lazari, 2014; Roberts et al., 1988). Mine soils may also be deficient in some nutrients and in some regions, they are characterized by an excessive concentration of heavy metals and sulphur (Frouz et al., 2005; Sołek-Podwika et al., 2016). A characteristic feature of mine soil at post-mining sites is a large spatial variability in soil properties (Pietrzykowski et al., 2014). At the same time, post-mining sites provide a unique opportunity to study the early development of ecosystems and soils (Frouz et al., 2013).

Active reclamation is often used to accelerate the recovery of ecosystem functions (Bradshaw, 1983; Frouz and Nováková, 2005; Zipper et al., 2011). In the case of post-mining sites, reforestation is frequently used to restore environmental sustainability (Macdonald et al., 2015; Pietrzykowski, 2014; Zipper et al., 2011). One important factor affecting

reforestation success and soil development at post-mining sites is the selection of tree species (Chodak and Niklińska, 2010; Šourková et al., 2005; Woś and Pietrzykowski, 2015). Deciduous species, especially nitrogen-fixing species like alder, have litter with a lower carbon to nitrogen ratio (C:N ratio) than coniferous species like pine (Cools et al., 2014; Frouz et al., 2013). Many deciduous species (e.g., beech and locust tree) cause C to accumulate in the top- and subsoil, but coniferous species cause C to accumulate mainly in the topsoil (litter layer and in the uppermost mineral horizons) (De Marco et al., 2013; Galka et al., 2014). As a consequence, the development of biological soil properties in reclaimed soils is better with deciduous than with coniferous trees (Frouz et al., 2013; Józefowska et al., 2016). Prescott and Grayston (2013) emphasized that tree effect on mineral soil could be less visible compared to litter (forest floor). According to mentioned authors and Binkley (1994) the tree effect on soil properties is slow or very limited. In both natural and in post-mining soils, the substrate (parent material) from which soil is developing had influence on soil properties including a biological one (Józefowska et al., 2016; Reich et al., 2005). That is the reason why vegetation (e.g. tree species), soil biological and physico-chemical properties should be viewed as co-developing components (Lovett et al., 2002; Reich et al., 2005)

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**Table 1**  
Characteristics of the post-mining sites that were studied in Poland.

Study site	Mean annual temperature (°C)	Mean annual precipitation (mm year <sup>-1</sup> )	Tree species	Tree-substrate abbreviation <sup>a</sup>	Age of trees (years)	Substrate	Reclamation treatments
Piaseczno external slope N 50 33.622; 21 34.185	7.0	650	Scots pine	P-QsNc	40	Mixture of quaternary sands and neogene Krakowiec Beds formation clays (QsNc)	Sowing a mixture of grasses and legumes, NPK fertilisation NPK(80, 50, 60 kg K ha <sup>-1</sup> ), 2-year papilionaceous (Papilionaceae) plants and grasses cultivation, planting of tree
			Common birch	B-QsNc	40		
			Pedunculate oak	Q-QsNc	40		
Szcakowa sand quarry 50 14.394; 19 25.140	8.0	700	Scots pine	P-Qls	25	Fluvioglacial quaternary loamy sands and loams (Qls)	2-Year cycle of fertilisation NPK (total amount 140, 130, 150 kg ha <sup>-1</sup> ), 2-year lupine cultivation, planting of tree
			Common birch	B-Qls	25		
			Red oak	Q-Qls	25		
			Black alder	A-Qls	33		
Bełchatów external slope N 51 13.196; 19 25.659	7.6	580–600	Scots pine	P-Ns	19	Neogene sands with loam and clay, carbonated and sulphurised (Ns)	Neutralisation with bog lime, NPK (60, 70, 60 kg ha <sup>-1</sup> ), one year cultivation of grasses and leguminous plants, planting of tree
			Common birch	B-Ns	19		
			Black alder	A-Ns	19		
			Red oak	Q-Ns	19		

<sup>a</sup> The letter before the hyphen indicates the tree species and the letter after the hyphen indicates the substrate, P = pine, B = birch, Q = oak, and A = alder, QsNc = mixed quaternary sands and neogene clays, Qls = quaternary loamy sands and loams, Ns = neogene acidic sands.

The biotic and the abiotic factors are connected and determine the stabilization of C in soil. In natural habitats, soils with low C:N ratios and with high pH values (what is connected with Ca content) tend to have high faunal activity and a microbial community dominated by bacteria rather than fungi (Prescott and Grayston, 2013; Reich et al., 2005). Such conditions favor the stabilization of C in organo-mineral aggregates (Ponge, 2003). Mentioned condition can be altered in post-mining soils because they are often planted with trees that are not adapted to the site, as noted earlier.

Vesterdal et al. (2013) emphasized that the mechanisms of C sequestration are complex and that the influences of litter inputs, root inputs, and macrofauna activity in different tree stands require additional study. Prescott and Grayston (2013) noted that connection between soil organic matter and tree species, soil fauna and microbial community should be investigated. Vindušková and Frouz (2013) also emphasized that additional research is needed to understand C accumulation in post-mining sites. New post-mining ecosystems have a significant potential for C sequestration (Amichev et al., 2008; Shrestha and Lal, 2006). Ladegaard-Pedersen et al. (2005) reported that tree species influence C sequestration and that it is possible to manage C sequestration during afforestation by selecting the proper tree species if soil properties (or substrate properties in the case of post-mining sites) is considered.

It is well known that different tree species produce litter with different properties and that litter properties affect soil formation (Cools et al., 2014; Frouz et al., 2013). In addition to litter properties, soil characteristics (e.g., texture and pH) are also important during early soil formation (Chodak and Niklińska, 2010; Józefowska et al., 2016). A recent study (Frouz et al., 2013) also showed that the effect of trees can be greatly modified by soil organisms. The aim of the current paper was to determine the effect of tree species, parent material (substrate), and faunal activity on C and N accumulation in soil and on other soil properties.

## 2. Materials and methods

### 2.1. Study sites

This research was conducted at reforested post-mining sites in southern and central Poland. The sites included a spoil heap produced by a sulphur mine in Piaseczno, a sand mine pit in Szcakowa, and an external slope heap produced by a lignite open-pit mine in Bełchatów. Basic information about the study sites is provided in Table 1. Sampling was performed in pure stands of pine (*Pinus sylvestris* L.), birch (*Betula pendula*), oak (*Quercus robur* L. or *Quercus rubra* L.), and black alder (*Alnus glutinosa*) growing on the following substrates: mixed

**Table 2**  
Soil chemical and physical properties as affected by 11 combinations of tree species and substrates at post-mining sites in Poland. F and p values refer to one-way ANOVAs. Within each column, statistically homogeneous groups are marked by the same letter (Tukey post hoc test  $p < 0.05$ ).

Tree-substrate	pH <sub>KCl</sub>	Ca <sup>2+</sup> cmol(+)·kg <sup>-1</sup>	K <sup>+</sup>	Base saturation	C %	N	C:N ratio	Sand %	Clay
P-QsNc	6 ± 0.5 <sup>bcd</sup>	6.2 ± 1.5 <sup>ab</sup>	0.1 ± 0.0 <sup>a</sup>	68.4 ± 8.7 <sup>a</sup>	1.68 ± 0.18 <sup>c</sup>	0.09 ± 0.01 <sup>ce</sup>	18.2 ± 1.4 <sup>ab</sup>	74 ± 3 <sup>ab</sup>	3 ± 0 <sup>a</sup>
B-QsNc	6.4 ± 0.3 <sup>e</sup>	6.8 ± 2.8 <sup>ab</sup>	0.1 ± 0.0 <sup>a</sup>	78.5 ± 9.8 <sup>a</sup>	1.47 ± 0.32 <sup>bc</sup>	0.09 ± 0.02 <sup>ce</sup>	16.2 ± 0.6 <sup>ab</sup>	74 ± 9 <sup>ab</sup>	3 ± 1 <sup>a</sup>
Q-QsNc	6.1 ± 0.2 <sup>cde</sup>	5.3 ± 2.2 <sup>ab</sup>	0.2 ± 0.1 <sup>a</sup>	71.4 ± 2.6 <sup>a</sup>	1.17 ± 0.47 <sup>abc</sup>	0.08 ± 0.04 <sup>bcd</sup>	17.2 ± 5.5 <sup>ab</sup>	69 ± 16 <sup>ab</sup>	3 ± 1 <sup>a</sup>
P-Qls	4.2 ± 0.3 <sup>a</sup>	0.7 ± 2.8 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	20.1 ± 9.8 <sup>b</sup>	0.87 ± 0.32 <sup>ab</sup>	0.03 ± 0.02 <sup>ab</sup>	31.8 ± 0.6 <sup>ab</sup>	82 ± 9 <sup>ab</sup>	2 ± 1 <sup>a</sup>
B-Qls	4.6 ± 0.4 <sup>abc</sup>	1.8 ± 1.4 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	43.1 ± 19.5 <sup>ab</sup>	1.01 ± 0.24 <sup>abc</sup>	0.04 ± 0.02 <sup>abcd</sup>	24.1 ± 6.1 <sup>a</sup>	81 ± 13 <sup>ab</sup>	2 ± 1 <sup>a</sup>
Q-Qls	5.0 ± 0.2 <sup>abcde</sup>	0.4 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	19.2 ± 3.1 <sup>b</sup>	0.69 ± 0.14 <sup>a</sup>	0.03 ± 0 <sup>ab</sup>	26.1 ± 3.7 <sup>ab</sup>	87 ± 8 <sup>b</sup>	2 ± 2 <sup>a</sup>
A-Qls	4.0 ± 0.2 <sup>a</sup>	1.1 ± 0.4 <sup>a</sup>	0.1 ± 0.0 <sup>a</sup>	18.7 ± 6.3 <sup>b</sup>	1.69 ± 0.21 <sup>c</sup>	0.12 ± 0.02 <sup>e</sup>	14 ± 0.4 <sup>b</sup>	76 ± 5 <sup>ab</sup>	1 ± 0 <sup>a</sup>
P-Ns	4.8 ± 0.1 <sup>abcd</sup>	4.3 ± 0.2 <sup>ab</sup>	0.1 ± 0.0 <sup>a</sup>	54.7 ± 3 <sup>ab</sup>	0.78 ± 0.2 <sup>ab</sup>	0.04 ± 0 <sup>abd</sup>	21.1 ± 4.4 <sup>ab</sup>	54 ± 5 <sup>a</sup>	10 ± 2 <sup>b</sup>
B-Ns	5.1 ± 0.6 <sup>abcde</sup>	3.9 ± 0.2 <sup>ab</sup>	0.1 ± 0.1 <sup>a</sup>	60.8 ± 16.8 <sup>ab</sup>	0.48 ± 0.13 <sup>a</sup>	0.02 ± 0.01 <sup>a</sup>	26.6 ± 10.6 <sup>ab</sup>	57 ± 11 <sup>a</sup>	10 ± 1 <sup>b</sup>
A-Ns	6.2 ± 1.4 <sup>de</sup>	10.3 ± 7.9 <sup>b</sup>	0.2 ± 0.1 <sup>a</sup>	72.8 ± 35.9 <sup>a</sup>	1.15 ± 0.15 <sup>abc</sup>	0.04 ± 0.02 <sup>abcd</sup>	30.5 ± 10.3 <sup>a</sup>	65 ± 17 <sup>ab</sup>	8 ± 3 <sup>b</sup>
Q-Ns	4.4 ± 0.2 <sup>ab</sup>	5.7 ± 0.7 <sup>ab</sup>	0.3 ± 0.0 <sup>b</sup>	40.8 ± 6.9 <sup>ab</sup>	2.4 ± 0.25 <sup>d</sup>	0.08 ± 0.01 <sup>cde</sup>	29.1 ± 2.6 <sup>ab</sup>	56 ± 2 <sup>a</sup>	7 ± 0 <sup>b</sup>
F	6.30	3.83	7.37	7.50	15.81	10.52	3.71	3.84	17.90
p	0.000	0.004	0.000	0.000	0.000	0.000	0.005	0.004	0.000

P = pine, B = birch, Q = oak, and A = alder, QsNc = mixed quaternary sands and neogene clays, Qls = quaternary loamy sands and loams, Ns = neogene acidic sands.

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