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Particulate organic carbon at reclaimed and unreclaimed post-mining soils and its microbial community composition

Martin Bartuška ^{a,b}, Mark Pawlett ^c, Jan Frouz ^{a,b,*}

^a Institute for Environmental Studies, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 01 Prague 2, Czech Republic

^b Institute of Soil Biology, Biology Centre of the Academy of Sciences of the Czech Republic, Na Sádkách 7, CZ-370 05 České Budějovice, Czech Republic

^c School of Applied Science, Cranfield University, Cranfield, Bedfordshire MK43 0AL, UK

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Recovery of soil organic matter and associated microbial biomass is a fundamental precondition for successful restoration of post-mining soils. The aim is to compare the dynamics of soil C fractions and of microbial communities associated with these fractions in two chronosequences of post-mining sites with different plant communities. Soil carbon, pH, bulk density and the light fraction of particulate organic carbon (POC), free or bound in soil aggregates, were studied along two chronosequences, both covering successional ages from 10 to 50 years. One chronosequence had been reclaimed by planting of alder, while the other had been vegetated by natural regrowth (Salix caprea, Populus tremula and Betula pendula). In intermediate and late successional stages, microbial community in bulk soil and POC fractions were studied using phospholipid fatty acid analysis. Soil C content increased and pH decreased with plot age, these trends being more pronounced at reclaimed sites. The light and bound POC fractions increased with age, higher values and a larger increase being found at reclaimed sites. In both chronosequences, the light fraction was an order of magnitude higher than the bound fraction. C content in both fractions increased with successional age, with higher C content at reclaimed sites. Microbial communities were more affected by the POC fraction than plot age. The bulk soil of reclaimed sites was more similar to bound POC, while the bulk soil of unreclaimed soils was similar to the light POC fraction. Observed differences correspond with a higher level of bioturbation at the reclaimed sites, which promotes faster accumulation of bound POC and drives bulk soil microbial communities closer to those of bound POC.

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1. Introduction

Soil formation and the reestablishment of soil biological functions are among the fundamental preconditions for ecosystem development during primary succession on new surfaces or in places where soil has been destroyed [\(Bradshaw, 1997\)](#page--1-0). Soil microorganisms are essential for ecosystem processes such as organic matter decomposition and mineralization ([Coleman et al., 2004](#page--1-0)). The quantity and activity of soil microorganisms are sensitive indicators of soil quality [\(Powlson et al.,](#page--1-0) [1987\)](#page--1-0). Consequently, many studies have explored the development of soil microbial communities during soil development ([Frouz and](#page--1-0) [Novakova, 2005; Helingerova et al., 2010; Sourkova et al., 2005](#page--1-0)). Most of these studies, however, investigated only bulk soil, even though soil is very heterogeneous. Some studies took this heterogeneity into account by exploring differences between individual soil horizons or between the rhizosphere and bulk soil ([Baldrian et al., 2008; Elhottova](#page--1-0) [et al., 2009](#page--1-0)), but variation among individual microhabitats within soil

E-mail address: frouz@natur.cuni.cz (J. Frouz).

has seldom been considered [\(Frouz et al., 2011; Mummey and Stahl,](#page--1-0) [2004; Mummey et al., 2006\)](#page--1-0).

In this study, we explored how microbial communities differ between two different soil microhabitats, how the occurrence of microbial microhabitats changes during the course of succession and how this variation corresponds with the microbial community in bulk soil. To define individual microbial microhabitats, we used individual fractions of soil organic matter (SOM). SOM was divided into fractions with a rapid and slow turnover rate [\(Molina et al., 1983; Parton et al., 1987;](#page--1-0) [Van Veen and Pauli, 1981; Van Veen et al., 1984; Verberne et al.,](#page--1-0) [1990\)](#page--1-0). To describe C changes in SOM quality, we physically fractionated SOM based on density [\(Golchin et al., 1994\)](#page--1-0). Physical fractionation by density yields a light and a heavy SOM fraction. The light fraction consists largely of non- or partially decomposed plant residues that are not associated with soil minerals ([Sollins et al., 1994](#page--1-0)) and that have a rapid turnover rate; the C pool in the light fraction is assumed to play a dominant role in soil nutrient dynamics. The heavy fraction is more closely associated with minerals and represents a more recalcitrant and stable part of SOM [\(Spycher et al., 1983](#page--1-0)).

Studies of post-mining soils are not only relevant to their restoration but also because post-mining soils are well-suited for chronosequence analyses. New sites are repeatedly created by mining operations over

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[⁎] Corresponding author at: Institute for Environmental Studies, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 01 Prague 2, Czech Republic.

long time periods. These sites are usually formed of the same substrate and with the same technology, so they can be easily dated. The present study was conducted in one of the major open-cast coal mining areas in the Czech Republic. A considerable amount of information about soil development and microbial communities in the area has already been amassed [\(Baldrian et al., 2008; Frouz and Novakova, 2005; Helingerova](#page--1-0) [et al., 2010; Sourkova et al., 2005](#page--1-0)). In particular, a remarkable difference was found between unreclaimed sites revegetated by natural regrowth resulting in litter with a high C/N ratio and sites reclaimed by planting of alder trees that produce litter with a low C/N ratio. Bioturbation, mainly due to earthworm activity, is more intensive at reclaimed sites than at unreclaimed ones [\(Frouz et al., 2007\)](#page--1-0). Development of humus forms ([Ponge,](#page--1-0) [2003\)](#page--1-0) differs between these chronosequences; moder or moder-mull develops at reclaimed sites, while moor dominates at young and mediumaged unreclaimed sites. At older unreclaimed sites, the humus form is also shifted towards moder or moder-mull [\(Frouz et al., 2007\)](#page--1-0).

The aim of this study was to test the following hypotheses: i) More intensive bioturbation at reclaimed sites changes distribution of carbon sources between individual soil microhabitats described here as soil C fractions; ii) Microbial communities differ between individual C fractions; iii) Differences in the proportions of individual fractions between reclaimed and unreclaimed sites and differences in microbial community in these fractions may explain differences in microbial communities among sites. To do so, we studied the composition of individual soil fractions at reclaimed and unreclaimed soils and the composition of the microbial community in individual fractions as well as in bulk soil using phospholipid fatty acid (PLFA) analysis.

2. Methods

2.1. Study sites

The study was conducted at post-mining sites near the town of Sokolov in the Czech Republic (50°14′29″N, 12°40′14″E). The sites have an elevation of 500–600 m a.s.l. (Kronstadt), a mean annual precipitation of 650 mm and a mean annual temperature of 6.5 °С [\(Frouz](#page--1-0) [et al., 2001](#page--1-0)). The spoil heaps in the Sokolov region (Fig. 1) originated from open-cast brown-coal mining and consist mainly of tertiary clays with a high content of mineral nutrients ([Sourkova et al., 2005; Stys,](#page--1-0) [1981\)](#page--1-0). The spoil material also contains significant amounts of fossil

organic matter, which can account for 5–6% of soil C. This organic matter is not related to coal, but is mostly represented by type II Kerogen derived from dead algae; the algae had fallen to the bottom of a tertiary lake that deposited sediments over the coal layers in the geological past [\(Kribek et al., 1998\)](#page--1-0). Although the spoil material is similar, some minor differences exist.

Two chronosequences, both covering successional ages from 0 to 50 years, were chosen. All sites were selected on tertiary clays described above on a gentle, south-facing slope of one large post-mining heap. One chronosequence consisted of eight sites reclaimed by planting of a mixture of alder species (Alnus glutinosa and Alnus incana) directly into graded overburden. The other chronosequence consisted of six unreclaimed sites spontaneously colonized by vegetation (dominated by Salix caprea, Betula pendula and Populus tremula). The litter C/N ratio for reclaimed sites was 23.7 \pm 7.2 and for unreclaimed sites 31.3 \pm 6.1 [\(Frouz et al., 2013\)](#page--1-0). Although these differences in the litter C/N ratio were not significant, differences in soil C/N are ([Table 1\)](#page--1-0). Understorey vegetation in both chronosequences was scarce at initial sites. A dense understorey occurs at intermediate reclaimed sites whereas older reclaimed sites had almost no understorey. By contrast, intermediate unreclaimed sites had almost no understory, and a dense understory was found at older succession sites. In both chronosequences, Calamagrostis epigejos was the dominant understorey species. Site ages and soil characteristics are provided in [Table 1](#page--1-0). For details about soil development and the microclimate see [Kuraz et al. \(2012\),](#page--1-0) and for details about understorey vegetation see [Mudrak et al. \(2012\)](#page--1-0). In agreement with previous studies [\(Frouz and Novakova, 2005; Frouz et al., 2008](#page--1-0)), unreclaimed sites dominated by herbs were classified as early stages (U9, U19 and U21), sites dominated by shrubs as intermediate stages (U24 and U28) and sites dominated by forest as late stages (U50). Correspondingly, we used the same categories for reclaimed sites of similar age (R10-21 as early, R25-34 as intermediate and R49 as late).

2.2. Sampling

For each site, three composite samples for each of the two layers (0–5 and 5–10 cm below the litter layer) were prepared by mixing two samples per composite obtained using a cylindrical corer (5.1 cm diameter, 5 cm long). The samples were transported to the laboratory, weighed, homogenized and passed through a 2-mm sieve.

Fig. 1. Map of investigated area with position of sampling points, open circles denote reclaimed sites, and full circles denote unreclaimed sites. The position of municipalities (black), postmining heaps (dark grey) and the mining pit (light grey) is shown. Inserted picture shows the position of the study area in the Czech Republic.

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