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# Development of soil microbial properties in topsoil layer during spontaneous succession in heaps after brown coal mining in relation to humus microstructure development

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#### Abstract

A wide spectrum of soil microbial parameters (microbial biomass, respiration, direct counts of bacteria, fungal mycelium length, cellulose decomposition and FDA activity) was studied in the chronosequence of 1- to 41-year-old plots of spontaneous succession on post-mining sites near the town of Sokolov. At these sites, soil chemical properties and the role of humus micromorphology (driven by soil fauna activity) were recorded, and their relation to changes in microbial properties was evaluated. Growth of herbs and grasses occurred only occasionally on 3- to 14-year-old plots. Shrubs (Salix caprea) covered a plot 15-25 years old, and 26- to 41-year-old plots were forested mainly with Populus tremuloides and Betula spp. Organic matter content increased, while pH decreased during succession. Organic matter accumulation seems to be the main factor affecting the development of the soil microbial community. All investigated parameters, with the exception of mycelium length, correlated positively with soil organic carbon. Most of the parameters measured at 30- to 40-year-old forested sites were of comparable magnitude to undisturbed habitats. Respiration per unit of microbial biomass (metabolic quotient) decreased with increasing succession age. The microbial community was strongly affected by humus form created by the activity of soil invertebrates. A rapid increase in all microbial parameters, again with the exception of mycelium length, was observed when moder developed under shrub cover. A shift from moder, typical of intermediate succession stages, to mull in later succession stages was accompanied by an increase in bacterial numbers and a decrease in microbial biomass, respiration and FDA. These changes correspond to: (i) a decrease in organic carbon content in topsoil layer, caused by earthworm-mediated soil mixing and (ii) a decrease in the availability of soil organic matter.

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# 1. Introduction

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During open-cast coal mining, large amounts of spoil material are excavated and deposited in heaps. This material typically contains low amounts of recent

organic matter (even if the content of fossil geogenic carbon is high in some cases) and display low soil biological activity (Schafer et al., 1979; Frouz et al., 2001). Soil formation in these sites is crucial for restoring ecosystem functions in post-mining landscape. Soil organic matter accumulation and the development of microbial activity play important roles in this process. Transformation of soil organic matter is realized mainly by soil microflora. However, soil fauna may substantially affect the living conditions of microflora and thus indirectly affect microbial activity (Anderson and Ineson, 1984; Lavelle and Martin, 1992; Hendriksen, 1997). In this context, the contribution of soil fauna to the formation of specific topsoil structure (humus form) is particularly important (Lavelle et al., 1997).

At post-mining sites, various reclamation measures are usually applied to accelerate ecosystem development and soil formation. While processes of soil formation in the reclaimed habitats have been widely studied (Dunger, 1968; Schafer et al., 1979; Insam and Domsch, 1988; Frouz et al., 2001), data concerning spontaneous soil formation in post-mining sites is scarce.

The aims of this study are manifold: it describes the development of soil microbial parameters (microbial biomass, respiration, direct counts of bacteria, fungal mycelium length, cellulose decomposition and FDA activity) during spontaneous succession on postmining sites; it compares the values found in spontaneous succession with literature data from both reclaimed post-mining sites and undisturbed habitat; and evaluates the role of soil chemical properties and the role of soil micromorphology—humus form (driven by soil fauna activity) in observed changes in microbial properties.

### 2. Materials and methods

#### 2.1. Study sites

The study considered the chronosequence of 14 post-mining plots 1–41 years old covered by spontaneously developed vegetation located in the Sokolov coal-mining district (Czech Republic). The spoil dumps were formed by tertiary clay material with pH about 8, the prevailing minerals were kaolinite, illite, calcium carbonate and quartz. The surface, because it was not levelled after the heaping process, is characterized by longitudinal depressions and elevations. Thus, two microhabitats (depressions and elevations) were sampled at each site.

Herbs and grasses (*Tusilago farfara* and *Calamagrostis epigeios*) occurred only rarely on 3- to 14year-old plots. Shrubs (*Salix caprea*) occurred on 15- to 25-year-old plots and tree cover (*Populus tremuloides* and *Betula* spp.) on 25- to 41-year-old plots. Shrubs shaded nearly the entire soil surface, resulting in a weak herb and grass cover. When the shrubs develop into forest, dense herb and grass cover appeared, mostly on the elevations.

# 2.2. Methods

Composite soil samples consisting of 5 particular subsamples (each consisting of about 100 g of soil) were taken at each microhabitat (depression and elevation) from each site from top 5 cm below the litter layer in March 2002. Samples were transported to the laboratory, homogenized by sieving through an 8-mm screen and stored at 4 °C until analyzed within 4 weeks.

Bacterial numbers were established as direct counts using DAPI staining and epifluorescent microscopy (Bloem, 1995). To assess microbial respiration, 5 g of fresh soil were hermetically closed in 150 ml vials in three replicates. The dry weight was established in separate samples. CO2 produced in the vials was trapped by 3 ml of 1 M NaOH. Vials were kept at 20  $^{\circ}$ C, and after 10 days the amount of trapped CO<sub>2</sub> was established by HCl titration after the addition of BaCl<sub>2</sub>. Microbial biomass was measured by the chloroform fumigation and extraction method (Vance et al., 1987). FDA (fluorescein diacetate) activity enzymatic breakdown by microbial community was established according to Schnurer and Rosswall (1982). Results were expressed as the difference in absorbance per gram of soil and hour. FDA measurement was carried out at 27 °C. To establish mycelial length, calcofluor stained mycelium were measured by direct epifluorescent microscopy (Bloem, 1995). All above mentioned microbial parameters were expressed per gram of dry soil and per gram of organic C, respectively. Cellulose decomposition was studied using the litterbag field method (Dunger and Fiedler, 1997). Litter bags ( $3 \times 10$  cm; mesh size 1.5

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