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Development of microbial properties in a chronosequence of sandy mine soils

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ABSTRACT

Soil microbial communities are of crucial importance for the functioning of ecosystems developing in post-mining areas. The objective of this study was to compare the properties of microbial communities in mine soils reclaimed for forestry and in mine soils developing under vegetation from natural succession. Soil samples were taken from a degraded site prior to reclamation (MS), a site after 2 years of lupine cultivation (LUP), reclaimed post-mining sites afforested with Scots pine (R6, R20, R28), post-mining sites with spontaneously developing pine forest stands (S6, S20, S27) and two natural pine forest stands (F30, F100). The examined microbial properties included basal respiration (RESP), microbial biomass (C_{mic}), C_{mic}-to-C_{org} ratio, potential nitrogen mineralization rate (Min_N) and community-level physiological profiles (CLPPs) studied using Biolog® Ecoplates. The lowest C_{mic}, RESP and Min_N values were determined in soil MS (C_{mic} = 26.5 mg kg^{-1}, RESP = 1.06 μg C-CO_2 g^{-1} 24 h^{-1}, Min_N = 5.2 μg N kg^{-1} 24 h^{-1}). Among the reclaimed mine soils the most biologically active was R6 (C_{mic} = 89.2 mg kg^{-1}, RESP = 7.28 μg C-CO_2 g^{-1} 24 h^{-1}, Min_N = 213.2 μg N kg^{-1} 24 h^{-1}). C_{mic}, RESP and Min_N gradually increased with soil age in S6, S20 and S27. Cluster analysis of CLPPs revealed a distinct difference between the natural forest soils and the mine soils. Among the mine soils, the least physiologically diverse was MS. In the successional mine soils, the metabolic abilities developed gradually with soil age. The CLPPs of the youngest successional mine soils resembled more the profiles of soil MS than those of natural forest soils whereas for the youngest reclaimed mine soils the opposite was the case. The results indicate that reclamation measures not only boost the gross microbial properties but also promote rapid development of metabolic abilities characteristic of natural forest soil microbial communities.

1. Introduction

Areas degraded by open-cast mining are often reclaimed for forestry. The ultimate goal of this kind of reclamation is to restore a stable (i.e. able to withstand a disturbance) and productive forest ecosystem. This goal can be achieved only if soil functionality is restored. Soil microbial communities are crucial to the functioning of soils. This is because soil microbes are responsible for establishing biogeochemical cycles and for energy transfer, and are involved in forming soil structure (Diaz-Raviña et al., 1993; Bauhus and Khanna, 1999; Preston et al., 2001). Several gross microbial properties such as the amount of microbial biomass, soil respiration rate and metabolic quotient (Graham and Haynes, 2004; Frouz and

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Novakova, 2005; Šourková et al., 2005) have been used to assess soil development in reclaimed post-mining lands. For numerous chronosequences of reclaimed mine soils a gradual increase of organic C and microbial biomass has been reported (Ruzek et al., 2001; Graham and Haynes, 2004; Šourková et al., 2005). Increasing contents of C_{org} and microbial biomass may result in increased functional diversity of soil microbial communities (Yan et al., 2000) and thus increased functionality and stability of soil ecosystems (Degens et al., 2001; Lynch et al., 2004).

In recent years, different analytical methods of assessing soil microbial diversity have been proposed as measures of ecosystem restoration (Mummey et al., 2002a,b; Graham and Haynes, 2004; Machulla et al., 2005). Measurement of the functional diversity is considered to provide information relevant to the functioning of soils (Nannipieri et al., 2003; Graham and Haynes, 2004). One of the methods that can be applied to measure functional diversity of microbial communities is the Biolog® test. The Biolog® test, a method of analyzing the metabolic abilities of microbial communities, uses a number of sole carbon substrates. The carbon substrate utilization pattern (referred to as the community-level physiological profile – CLPP) can potentially provide information on the functional abilities of the microbial community. CLPPs have been used to assess the influence of plants on soil microbes (Grayston et al., 1998), the effects of acid precipitation and pollutants on soil microbial communities (Fritze et al., 2000; Pennanen, 2001) and the effects of forest management techniques on soil microbes (Pietikäinen et al., 2000). However, the Biolog® assay has several limitations. The method is sensitive to inoculum density (Insam, 1997) and selects for culturable microorganisms capable of growing under experimental conditions (Garland and Mills, 1991). Thus, Biolog® CLPPs reflect the functional abilities not of the entire microbial community but of limited subset of microbial genera (Ros et al., 2008). Furthermore, the method favors fast-growing bacteria and structural changes may occur during the incubation (Smalla et al., 1998). Despite these drawbacks, Biolog® test could be a valuable tool for evaluating mine soil rehabilitation processes as it is a rapid and convenient analytical technique.

During afforestation of post-mining barrens seedlings of selected tree species are planted in appropriately prepared and fertilized spoil material, and the growing plants transform the raw parent material into soil. Sometimes vegetation from primary succession on the abandoned mine land is used in reclamation (Jochimsen, 1996), since the succession of plants may lead to spontaneous soil formation. Frouz and Novakova (2005) reported that gross microbial properties (FDA activity, respiration, microbial biomass) in mine soils spontaneously developing on brown coal mining heaps were similar to or higher than the levels in reclaimed mine soils. However, little is known about the functional diversity and metabolic abilities of microbial communities in spontaneously developing mine soils.

The objective of this study was to compare the temporal development of microbial biomass, soil respiration, potential N mineralization rate and the physiological profiles of microbial communities in mine soils reclaimed for forestry and in mine soils developing under vegetation from natural succession. Microbial properties measured in the natural forest soils were used as a reference.

2. Materials and methods

2.1. Study site

The study was carried out in Upper Silesia, Poland (19°26′E; 50°16′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 8 °C. Soils of the study area (mainly Podzols) developed from sands. The sand deposits are fluvioglacial Quaternary sediments of a pre-Quaternary morphological depression. The dominating tree species in the forests surrounding the sand quarry is Scots pine (*Pinus sylvestris*), which constitutes 72.9% of the forest stands, followed by common birch (*Betula pendula*) constituting 16.2% of the stands (data from the Chrzanów Forest District administration).

The Szczakowa open-cast quarry has been extracting sand since 1954. Mining created an open cast 5–25 m deep, covering over 2700 ha. Since the late 1950s it has been reclaimed and reforested. The standard reclamation procedure included forming and leveling off the surface and adding an organic amendment (approx. 300 m^3 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 (Strzyszcz, 2004). Then the reclaimed sites were limed (1.5 Mg dolomite ha^{-1}), and lupine (*Lupinus luteus* L.) was cultivated for 2 years. The lupine cultivations were fertilized with NPK (140 kg N ha^{-1}, 300 kg P_2O_5 ha^{-1}, 180 kg K_2O ha^{-1}). After 2 years, the lupine biomass was ploughed into the soil as green manure and the sites were afforested with 1-year-old seedlings of Scots pine (*P. 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, 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 were to be treated differently. 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 ha^{-1}, 140 kg P_2O_5 ha^{-1}, 120 kg K_2O ha^{-1}) and the organic amendment (amount decreased to approx. 30 m^3 ha^{-1}) was not spread over the entire reclaimed area but applied directly under the seedlings during planting.

In the 1970s and 1980s, some parts of the open cast were abandoned, only to be mined again some 10–20 years later. They were eventually abandoned due to falling demand for sand. Vegetation appeared there spontaneously, and initial soil formation began by natural primary succession. At the abandoned sites, herbaceous communities dominated by gray hair-grass (*Corynephorus canescens* L.) were the first to establish. They were followed by groups of trees with over 50%