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Soil bacterial and fungal communities along a soil chronosequence assessed by fatty acid profiling

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ABSTRACT

Microbial communities are important components of terrestrial ecosystems. The importance of their diversity and functions for natural systems is well recognized. However, a better understanding of successional changes of microbial communities over long time scales is still required. In this work, the size and composition of microbial communities in soils of a deglaciation chronosequence at the Damma glacier forefield were studied by fatty acid profiling. Soil fatty acid concentrations clearly increased with soil age. The abundances of arbuscular mycorrhizal fungi (AMF), bacteria and other soil fungi, however, were more affected by abiotic soil parameters like carbon content and pH than by soil age. Analysis of ratios of the different microbial groups (AMF, fungi, bacteria) along the soil chronosequence indicated that: i) the ratios of AMF to bacteria and AMF to fungi decreased with soil age; and ii) the ratio of fungi to bacteria remained unchanged along the soil chronosequence. These two pieces of evidence suggest that the evolution of this ecosystem proceeds at an uneven pace over time and that the role of AMF is less important in older, more organic and acidified soils than in mineral soils. In contrast to other studies, no successional replacement of bacteria with fungi in more acidified and organic soil was observed.

1. Introduction

Soil is a fundamental component of terrestrial ecosystems. Soil properties develop over time through the interplay of abiotic and biotic processes (Paul, 2007). Several centuries to millennia may need to pass until rock material is transformed into soil, making the process of soil and ecosystem development impossible to observe within human life time. Thus ecologists commonly study areas with a soil developmental gradient resulting from natural processes (e.g., retreat of glaciers, succession on volcanic ashes and islands of uneven ages), thus replacing temporal with spatial gradients (Huggett, 1998; Walker et al., 2010). Chronosequences established on land gradually emerging from underneath of retreating glaciers have formed the basis for numerous studies on soil formation (Dümig et al., 2011; Mavris et al., 2010; Stevens and Walker, 1970) and biological succession (Chapin et al., 1994; Dobas-Miranda et al., 2008; Garibotti et al., 2011; Grzesiak et al., 2009; Sigler et al., 2002). The development of the ecosystem is strongly interlinked with soil microbial activity. Thus, composition, taxonomic and functional diversity of microbial communities on deglaciated lands have been studied intensively (Cázares et al., 2005; Tscherko et al., 2003). This has mostly been accomplished by nucleic acid-based techniques, which offer a deep resolution of identities, but have shortcomings with respect to quantitativeness. Quantitative aspects of microbial communities are best assessed using biochemical markers such as fatty acids (Kaštovská et al., 2007; Tscherko et al., 2005).

The establishment of microbial communities on newly deglaciated areas is a complex and multifaceted process. The gradient of environmental conditions along the soil chronosequence has been postulated as the main factor governing the structure (composition and diversity) and activity of the microbial communities (Lazzaro et al., 2009, 2010; Noll and Wellinger, 2008). Although differences between recently and earlier deglaciated soils in temperature (lower in the vicinity of ice), rates of weathering and accumulation of soil organic matter (SOM) seem to be obvious, their impact on microbial communities is still poorly understood. Therefore, in this study we attempted to disentangle the effects of different soil properties (i.e., nutrients, soil age and pH) along a natural gradient of soil development on the biomass of the main groups of the microbial community.

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The size of a nutrient pool such as phosphorus and nitrogen in the topsoil usually increases with increasing soil age, and this is mostly accompanied by an increase of microbial and plant biomass with soil age. However, nutrients typically limit plant and microbial growth differentially at various stages of soil development. Moreover, microbial groups differ in nutrient acquisition strategies and trophic requirements, which should be reflected in correlations between the biomass of different soil organisms and the levels of soil nutrients in different soil developmental stages. Soil fungi are active participants but also main beneficiaries of SOM decomposition. Thus, their biomass is expected to correlate with soil age as well as soil carbon (C) content. In case of arbuscular mycorrhizal fungi (AMF) and bacteria obtaining C from other sources (AMF: photoassimilates from the host plant and bacteria: autotrophic C fixation) a weaker correlation with soil age and a dependency on elements other than C can be presumed. Phosphorus (P) could be a potentially important agent, lowering the biomass of AMF community as shown in other environments (Jansa et al., 2009), whereas the availability of nitrogen (N) and other biogenic elements preferentially affects bacterial communities (Madigan and Martinko, 2006).

Acidification is an integral process of soil development which has been found to be important in shaping the structure of microbial communities (Noll and Wellinger, 2008; Rousk et al., 2010). However, to the best of our knowledge, the influence of pH on the biomass of different microbial groups in recently deglaciated areas as it is presented in our work has not been elucidated. The Damma glacier forefield exhibits a gradient of soil pH ranging from 4.4 to 3.5, which is expected to shift the microbial community from dominance of bacteria to that of fungi (Aliasgharzad et al., 2010). Moreover, based on experience from other ecosystems such as forests and an experimental agroecosystem (Coughlan et al., 2000; Wang et al., 1993) a shift in AMF biomass along the soil pH gradient can be hypothesized, i.e., a positive correlation between pH and AMF biomass.

In the present work we aimed to estimate the microbial biomass and to identify the abiotic determinants of the size and coarse diversity of microbial communities in a soil chronosequence along the Damma glacier forefield, distinguishing AMF, other fungi, and bacterial communities. In particular, the dominance of fungi over bacteria in older soils and the successional replacement of AMF with other symbiotic associations (i.e., ericoid and ectomycorrhizal) were tested. To ensure the selection of the lipid fraction with the highest informative power for the investigated ecosystem, different lipid fractions were analyzed separately by fatty acid profiling.

2. Materials and methods

2.1. Sampling sites

This study was conducted at the forefield of the retreating Damma glacier, situated in the Western Alps in the Canton of Uri, Switzerland (N46°38.117′, E8°27.677′). The glacier forefield is situated in front of an ice block, which is a remnant of a glacier tongue and currently detached from the main glacier body. The forefield is oriented south-west to north-east and measures approximately 1000 m in length and 600 m in width. Average inclination of the forefield area from the ice block to the bottom of the forefield valley is about 21%. Two main recent advances of the glacier resulted in terminal moraines dated back to 1992 and 1927. Side moraines date back to 1850.

Twenty three experimental sites along the forefield chronosequence were selected in July 2007. These sites are identical to the so called “common sampling design” of the BigLink project (Bernasconi et al., 2011). Of these, 21 sites were distributed along a recent soil chronosequence (Fig. S1) at the valley bottom, thus minimizing effects of the side slopes. The approximate ages of soil (i.e., years after the last deglaciation) at each experimental site were calculated based on historic glaciological records available from the Swiss Glacier Monitoring Network (glaciology.ethz.ch/messnetz/glaciers/damma, accessed 11th March 2012). The soil age at the forefield ranged between 7 and 137 years. The two remaining sites, at a distance of about 500–800 m from the forefield (Fig. S1), were deglaciated much earlier (probably more than 3000 years ago) and served as reference sites. An approximate age of 3000 years was assigned to the reference sites for statistical analyses. Soils at the experimental and reference sites were classified as Hyperskeletic Leptosol and Haplic Cambisol, respectively (Bernasconi et al., 2011).

The vegetation is very patchy in the young part of the chronosequence and dominated by individual herb species. As succession continues, plant cover is increasing up to 100% coverage. The initial dominance by herbs and grasses (*Agrostis gigantea*, *Poa alpina*, *Leucanthemopsis alpina*) is gradually replaced by dominance of woody plants (*Salix helvetica*, *Salix herbacea*) and shrubs (*Rhododendron ferrugineum*, *Vaccinium* sp., *Calluna vulgaris*, *Loiseleuria procumbens*) in the middle and old parts of the chronosequence, respectively. The reference sites are dominated by grasses (*A. gigantea*, *P. alpina*, *Festuca rubra*).

2.2. Soil sampling and processing

Soil samples were collected from the 23 sites between 10th and 12th September 2007 (see Bernasconi et al., 2011 for further details). Soil from each site was collected from three subplots (0.5 m × 0.5 m each) within a 2 m × 2 m quadrant, with separate sampling from two depths (0–5 cm and 5–10 cm). Soil from each depth was passed through an 8 mm sieve directly in the field, homogenized by mixing, preserved in plastic bags, transported to the laboratory and stored at −20 °C until analyses. Subsequently, soil samples were freeze-dried, passed through a 2 mm sieve, ground to a fine powder with mortar and pestle at room temperature and weighed directly into 50 ml glass centrifuge tubes for lipid extraction. Fifteen, 10 and 5 g dry soil was processed for young (deglaciated approx. 7–14 years ago), middle-aged (deglaciated approx. 59–79 years ago), and old soils (deglaciated more than 100 years ago), respectively.

2.3. Lipid analyses

Lipids were extracted from all samples following the procedure of Frostegård et al. (1991) with slight modifications. Briefly, 15 ml of a chloroform: methanol: citrate buffer (0.15 M, pH 4.0) mixture (1:2:0.8, v:v:v) was added to the samples in the centrifuge tubes. Each sample was spiked with 900 μg of the first internal standard (Fig. S2), nonadecanoic fatty acid (19:0, Sigma–Aldrich, Buchs, Switzerland) dissolved in hexane. Samples were incubated at room temperature for 2 h with occasional stirring. Phase separation was attained by addition of 6.2 ml of chloroform: citrate buffer (1:1, v:v) and centrifugation at 750*g* at 16 °C for 15 min. The lower organic phase was collected with a 10 ml Hamilton syringe, filtered through syringe-driven hydrophobic filters (Millex-FR, Millipore, Zug, Switzerland), transferred to a new 50 ml glass tube and evaporated to dryness under mild vacuum (water-driven pump). Total lipids in this fraction were redissolved in 200 μl of chloroform and divided in two parts. One half was evaporated to dryness under vacuum and stored at −20 °C for analysis of whole cell fatty acids (WCFA). The other half was used for lipid fractionation using pre-packed silica columns (Bond Elut LRC-Si 100 mg, Varian, Darmstadt, Germany). Elution volumes for each fraction were adjusted experimentally to assure complete elution, with 5.2 ml of chloroform, 7.8 ml of acetone and 5.2 ml of methanol eluting neutral lipids (NL),