Enzymatic activities in the rhizosphere of different plants at a glacier forefield

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Abstract
Availabilities of nitrogen (N) and phosphorus (P) are important determinants of primary ecosystem succession. One of the plant strategies to acquire sufficient amounts of P and N in young ecosystems like glacier forefields is to establish mycorrhizal symbiosis. Mycorrhizal fungi have developed different mechanisms to acquire nutrients from soils such as extensive mycelium growth, exudation of organic acids and/or lytic enzymes. Large amounts of such enzymes are produced by many ericoid (ER) and some ectomycorrhizal (ECM) fungi, thus providing access to organic forms of the nutrients. In contrast, arbuscular mycorrhizas (AM) are rather functioning as efficient pumps of soluble N and P forms from the soil solution, with limited access to the recalcitrant forms of nutrients. To quantify differences in enzymatic activities between ER, ECM and AM types, rhizosphere soils from four different plants species were collected at different soil developmental stages in a glacier forefield in Switzerland. Activities of different enzymes in the soil samples were assessed using fluorogenic substrates. Elevated chitinase and protease activities were associated with ECM and ER types, respectively, whereas no clear trends were observed for acid phosphatase. Soil developmental stage was an important factor of the background enzymatic activity levels.

Key Words
Mycorrhiza, nitrogen cycle, phosphorus cycle, soil enzymes, soil formation gradient.

Introduction

Development of soils and patterns of ecosystem succession on glacier forefields have been studied over the last years from different points of view. Among them, issues such as availability, turnover and cycling of nitrogen (N) and phosphorus (P) received particular attention of botanists, mycologists and microbiologists. It has been shown that the size of N and P pools as well as their forms vary strongly with soil developmental stages, which has consequences for nutrient availabilities for plants and soil microorganisms. On areas that have only recently been deglaciated, inorganic forms of N and P predominate, which originate either from parent rock weathering and/or deposition. Concentrations of both N and P are usually very low in those young soils. In more developed soils, significant accumulation of N and P is frequently observed and those nutrients are usually present in organic polymers (such as DNA, proteins, chitin etc.). To cover their nutritional demands, plants colonizing glacier forefields employ various mechanisms to acquire N and P from the different soil pools. Among them, formation of mycorrhizal symbioses is of major importance. About 90% of all terrestrial vascular plant species form mycorrhizas (Brundrett 2002), of which the arbuscular mycorrhizas (AM), ectomycorrhizas (ECM) and ericoid mycorrhizas (ER) are the most common types (Smith and Read 2008). Mycorrhizas are known to enhance N and P acquisition of their host plants. However, the mechanisms behind these processes are not the same for all mycorrhizal types. For example, AM fungi can efficiently gather soluble inorganic N and P from the soil solution, whereas their direct access to recalcitrant nutrient sources is very limited. In contrast, some ECM and ER fungi were shown to exude large amounts of exoenzymes and organic acids, which facilitate N and P uptake from recalcitrant organic and inorganic pools such as proteins and apatites (Smith and Read 2008).
Enzymatic activities of different mycorrhizal fungi have been recently studied in pure cultures (Bajwa and Read 1986; Joner *et al.* 2000; Read and Perez-Moreno 2003; Jayakumar and Tan 2005), which may be of limited relevance to natural ecosystems. For that reason, the main aim of this study was to investigate enzymatic activity of rhizosphere samples taken directly from natural conditions. We expected to find different enzymatic activities in different mycorrhizal types formed by various plant species along a soil formation gradient. This study was embedded in the interdisciplinary BigLink project (Bernasconi *et al.* 2008).

Methods

Study area

This study was carried out at the forefield of the Damma glacier, situated in Urner Alps, Switzerland (N 46°38', E 08°27') (Figure 1A). In July 2009, eight experimental sites were selected for enzymatic activity

investigations. These sites have been deglaciated for different periods of time, ranging from 7 to 110 years (Figure 1B). Approximate ages of soil in each of the experimental sites were calculated based on historic glaciological records available from 1921 through the Swiss Glacier Monitoring Network.

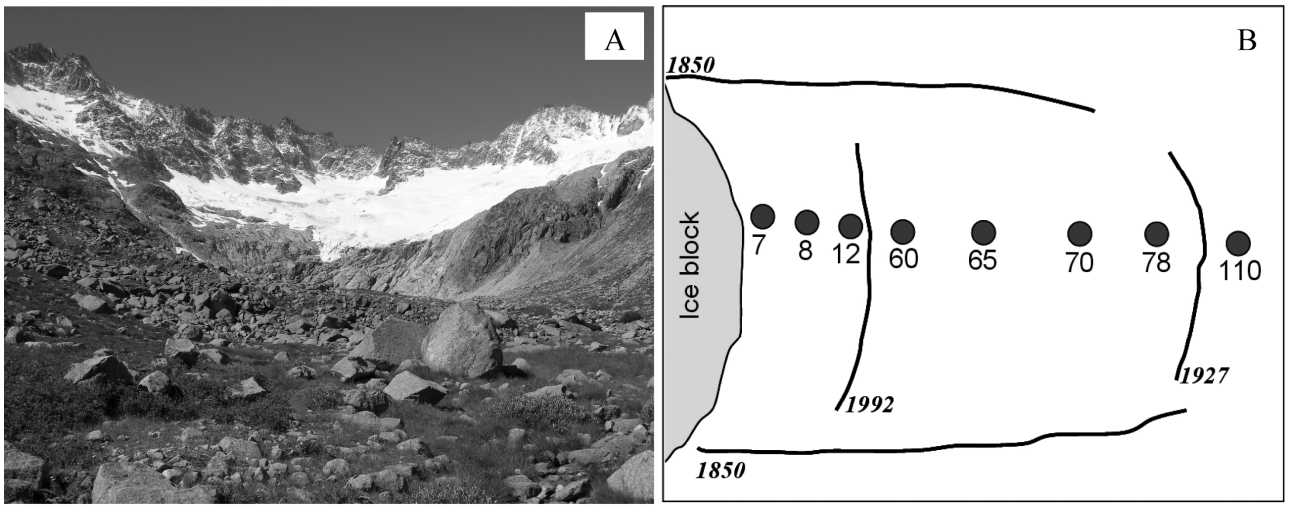

Figure 1. The Damma glacier forefield in the Swiss Alps (A), and schematic representation of the forefield (B). Points indicate individual experimental sites. Numbers below the points indicate approximate soil ages (times after deglaciation). Lines with dates indicate positions of side (1850) and end (1927 and 1992) moraines.

Soil sampling

Samples (~200 g each) were collected from each experimental site in the close vicinity of roots of four model plants, representing different mycorrhizal types: *Salix helvetica* (predominantly ECM plant), *Rhododendron ferrugineum* (ER plant), *Leucanthemopsis alpina* and *Agrostis gigantea* (both AM plants). From each site, an additional sample was collected from a spot not covered by vegetation. The soils were passed through a 2 mm sieve, aliquoted for subsamples of about 5 g each, and stored at -80°C. Soil carbon (C), N and P concentrations were assessed by standard methods (Table 1).

Table 1. Selected soil chemical properties

Site	Soil $pH_{(CaCl2)}$	Total C [A] (g/kg)	Total N [A] (g/kg)	Extractable P [B] (mg/kg)
1	4.37 (±0.36)	0.79 (±0.17)	0.03 (±0.03)	10.88 (±2.74)
2	4.49 (±0.28)	0.95 (±0.35)	0.05 (±0.05)	10.44 (±0.86)
3	4.17 (±0.07)	7.42 (±4.38)	0.44 (±0.16)	28.60 (±8.72)
4	4.13 (±0.09)	4.07 (±3.80)	0.22 (±0.20)	21.33 (±4.58)
5	4.10 (±0.07)	5.78 (±2.91)	0.36 (±0.17)	26.28 (±2.59)
6	4.03 (±0.18)	3.63 (±1.53)	0.22 (±0.11)	21.91 (±6.37)
7	3.91 (±0.16)	8.53 (±3.04)	0.42 (±0.17)	13.55 (±5.30)
8	3.72 (±0.16)	17.49 (±10.96)	0.95 (±0.63)	15.87 (±3.25)

Values are means of five analytical replicates (± standard deviation), [A] Dry combustion method, [B] Ammonium acetate-EDTA extraction followed by colorimetric quantification with malachite green as a color agent.

Enzyme assays and measurements

Enzymatic activities were assessed in soil suspensions. Approximately 1 g of soil was suspended in 40 mL of sterile distilled water, sonicated for 2 min (35 kHz) and shaken horizontally (2.3 Hz) for 1.5 h at room temperature. The suspensions were allowed to sediment for 15 min, after which the liquid samples were distributed into black 96-well microplates containing the relevant buffers. Acid phosphatase (EC 3.1.3.2) and chitinase (EC 3.2.1.14) assays were buffered with MES (Stemmer 2002), whereas the protease (leucine aminopeptidase, E 3.4.11.1) assay required modified universal buffer (Stemmer 2004). Standards and fluorogenic substrates used for the assays followed the description by Stemmer (2002). Enzymatic activities were quantified using a Biotek FLx800 microplate fluorometer (excitation at 360 nm and emission at 460 nm). Measurements were done at the following incubation times: 0, 30, 60, 90, 120, and 180 minutes.

Results

For all soils, enzymes activities were strongly affected by plant species that differ in their mycorrhizal status, but varied also greatly between the sites. High variability in soil properties (Table 1) was observed across the sites, with a general increase in C and N contents, and decreasing pH with soil age. Phosphatase activity increased with soil age, but no clear differences could be observed between the different plant species (data not shown). The highest chitinase activity was observed in a site deglaciated 12 years ago (Figure 2A). High activities were recorded in the rhizospheres of *S. helvetica* and *R. ferrugineum*, which were ECM and ER plant, respectively. Protease activity followed similar pattern to chitinase, but the values were several fold lower than those of chitinase (Figure 2B). Elevated protease activity (as compared to unvegetated soil) was sometimes associated with *S. helvetica* and *R. ferrugineum*.

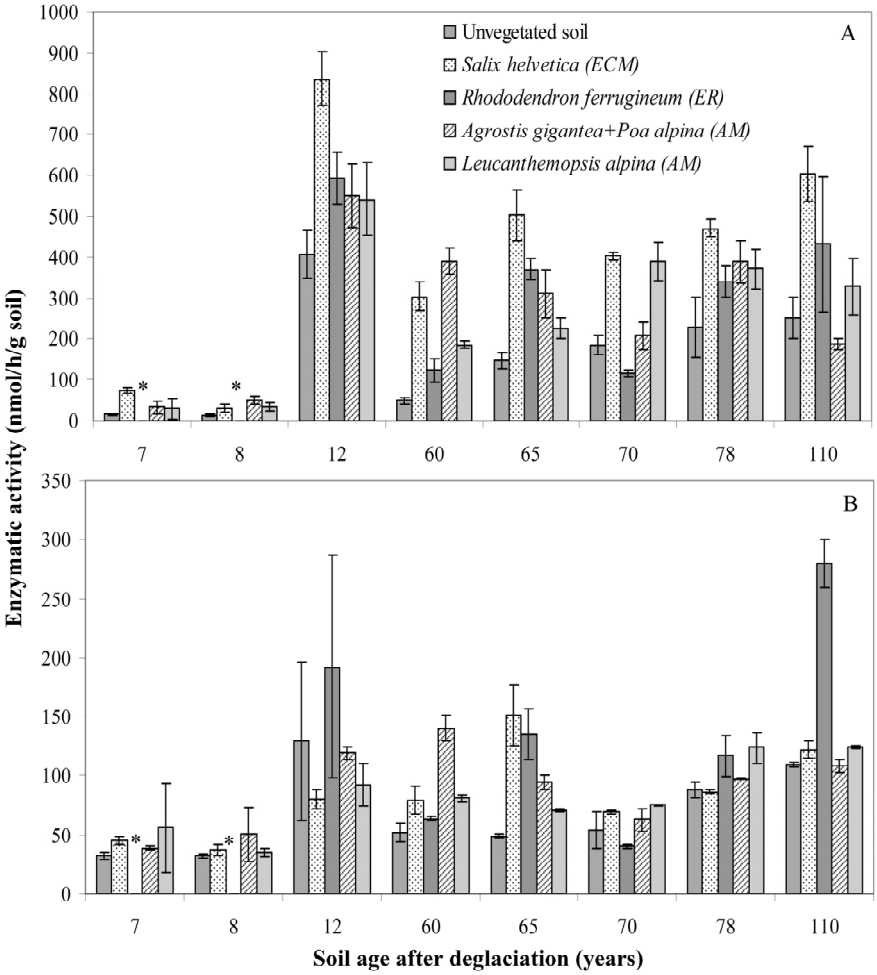

Figure 2. Chitinase (A) and protease (B) activities in rhizospheres of different plants growing in soils of different ages. Values are means of analytical replicates (n=3, ± standard deviation). Asterisks indicate absence of the plant species on a particular site.

Conclusion

The results of the present study showed that different mycorrhizal types, formed by various plant species along a soil formation gradient, differentially influenced enzymatic activities of their rhizospheres. While phosphatase is produced by many different organisms and this enzyme has its optimum pH at acidic conditions, increased acid phosphatase activity is intuitive along the soil formation gradient, where soil organic matter content dramatically increases with soil age (Table 1). High chitinase and protease activities were frequently associated with *Salix helvetica* and *Rhododendron ferrugineum*. This can be explained by the presence of ECM and ER mycorrhizal fungi associated with roots of these plants, with their capacity to exude proteases (Bajwa and Read 1986; Leake and Read 1991; Hodge *et al.* 1995). However, interpretation of enzymatic activities in the soil samples should also consider other ecosystem properties such as soil chemistry, plant cover composition and density, ecological optimum of the individual plant species as well as the levels of the mycorrhizal colonizations in their roots. These factors should receive appropriate attention in the future. Moreover, in order to better understand the origin and consequences of soil enzymatic

activities, further measurements of the enzymatic activities at the surface of roots are planned. Parallel measurements, scrutinizing enzymatic activities of the plant's roots collected in the field and cultivated in the growth chamber will be conducted. Plants inoculated or not with different mycorrhizal fungi will be compared for their root- and rhizosphere-bound enzymatic activities in the future, and quantification of fungal biomass by quantitative PCR is planned.

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