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Effects of pioneering plants on microbial structures and functions in a glacier forefield

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Abstract This study investigates the small-scale spatial impact of the pioneering plant *Leucanthemopsis alpina* (L.) Heywood (*L. alpina*) on biological and chemical–physical parameters in an early successional stage of a glacier forefield. Considering the frequent occurrence of isolated patches of this pioneer plant in the forefield of the Dammaglacier (Switzerland), we hypothesized that the impact of the plant would establish gradients in nutrients, and microbial community structure and activity that may be of importance for the successional processes occurring in the forefield. Our results indicated that, in young successional soils, the rhizosphere effect of *L. alpina* plant patches can influence bacterial cell numbers and activities not only within the root zone, but even at 20 cm distance from the plant. Microbial cell counts, active cells, and saccharase, glucosidase, and acid phosphatase activities revealed

significant distance effects, decreasing from soil directly underneath the plant to soils at 20 and 40 cm distance. Soil chemical and physical parameters did not exhibit significant trends. Fingerprinting analysis of amplified 16S rDNA fragments was used to characterize the microbial community. A selective effect of the plant on the microbial community could not be shown because the bacterial communities were similar regardless of distance to the plant.

Keywords Glacier forefield · Pioneering plants · Plant–bacteria interaction · Soil bacteria · Soil development · Enzyme activity · Active biomass

Introduction

The majority of Swiss glaciers are currently receding as a consequence of global warming (Haeberli and Beniston 1998). Since 1850, around the end of the little Ice Age, European glaciers lost about 50% of their original glaciated area (Zemp et al. 2006). The forefield of receding glaciers is initially vegetation-free, with low nutrient content (Sigler et al. 2002; Darmody et al. 2005). In a continuous glacier retreat, greater distance from the termini corresponds to longer periods of ice-free exposure, resulting in a successional chronosequence (Matthews 1992).

Whereas the succession of flora and fauna has been extensively studied in this system (Matthews 1992; Chapin et al. 1994; Kaufmann 2001; Caccianiga and Andreis 2004; Hodkinson et al. 2004), few studies addressed bacterial processes in glacier forefields (Ohtonen et al. 1999; Sigler and Zeyer 2002; Bardgett and Walker 2004; Tschérko et al. 2004). Sigler and Zeyer (2002), using molecular methods, reported that community structures and metabolic functions change along the chronosequence of a glacier forefield. Tschérko et al. (2003) demonstrated that microbial functional diversity increased along a glacier forefield chronosequence.

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As plant-cover is one of the most obvious gradients in a glacier forefield, microbial activity is likely to vary with vegetation. Plants translocate between 5 and 60% of their photosynthetically fixed carbon into the rhizosphere, which may stimulate microbial communities and their associated enzymatic activities (Marschner 1995; Walker et al. 2003). However, along a glacier forefield chronosequence, the interaction between microorganisms and vegetation might differ at the various successional stages. Tscherko et al. (2004) investigated microbial community structure and enzymatic activities in the rhizosphere and bulk soil of *Poa alpina* plants at various successional stages of a glacier forefield. In an additional study, Tscherko et al. (2005) investigated the interrelationship of different successional plant communities with microbial diversity and enzymatic activity patterns, providing evidence that microbial community structure and enzymatic activity patterns are strongly conditioned by the successional stage, as well as the carbon and nitrogen content of the forefield soils. The relationship between chronosequence and microbial community structure in bulk and rhizospheric soils of the pioneering plant *L. alpina* was investigated by Edwards et al. (2006). A major focus of this study was the changing patterns of rhizodeposits (in the form of organic acids and sugars) in the *L. alpina* rhizosphere and how they can condition the structure of microbial communities, as well as their ecological strategies.

Patchy vegetation is a common observation in early successional stages. A question not addressed by the previous literature is the spatial extent of plant microbial interaction in these environments. We hypothesized that pioneer plants provide hot spots of microbial activity that extend into the surrounding bulk soil, leading to gradients in soil chemistry, microbial activity, and microbial community structure. To address this question we chose *L. alpina* because it is one of the earliest and most abundant vascular plant pioneers in the young Damaglacier forefield soils and because there are previous studies on the plant–microbial interactions for this plant (Edwards et al. 2006). The current work therefore focuses on resolving the spatial effect of *L. alpina* on microbial cell counts, community structure, and enzyme activities in an early successional soil. We applied chemical–physical measurements (e.g., pH, dissolved carbon, and nitrogen parameters) to describe the conditions for the microbial community. Microbiological (microscopic counts of total and active cells) and molecular [fingerprinting of bacterial 16S rDNA genes using restriction fragment length polymorphisms (RFLP) and denaturing gradient gel electrophoresis (DGGE)] methods describe the size and structure of the microbial community, respectively, and enzymatic activity assays (saccharase, glucosidase, phosphatase, and urease) were selected to reflect major microbial processes related to

nutrient cycling and energy metabolism. In combination, these data provide detailed information about chemical and biological gradients around isolated patches of *L. alpina* plants.

Materials and methods

Field site and sample collection

The research site at the terminus of the Damaglacier is located in the Central Alps, in the canton of Uri, Switzerland (N 46°38.177' E 008°27.677'), about 2,100 m above sea level. The climate in this area is characterized by a short vegetation period, and about 2,400 mm precipitation per year (Sigler and Zeyer 2002). Between July and October 2003, we noted very large day–night temperature fluctuations, the observed maximum and minimum soil surface temperatures ranged between 38 and 0°C. At the research plot, soil conditions varied between fine fluvial sands and coarse sandy areas.

Sampling took place shortly after snow melt in May 2003. Plants were collected from three isolated patches of *L. alpina* that were situated about 60 m distant from the glacier terminus, corresponding to a deglaciation time of 5 to 10 years. *L. alpina* occurred at a frequency of about 1,000 individuals per hectare at this location. The whole plants including roots were collected for determination of root biomass. The soil attached to the roots together with soil from within 10 cm of the plants was collected and arbitrarily considered as plant center soil. In addition, samples were taken along two transects from two further distance classes, at 20 cm and at 40 cm distance from the *L. alpina* plants. For each distance class, up to four samples were collected from the 0–5 cm soil layer; larger rocks prevented sampling in some cases. The samples were sieved (2 mm) and homogenized, and roots were manually separated from soil samples. Subsamples were dried at 105°C for 24 h and subsequently weighed for dry mass determination of root material and soil.

Analysis

Soil chemical properties were analyzed by extracting field moist soil samples with 0.01 M CaCl₂ (ratio 1:5 of soil/extractant). Samples were shaken for 1 h in an overhead shaker. Nitrate, phosphate, total sugars, and organic acids were determined in CaCl₂ extracts. Ion chromatography with suppressed conductivity detection (DX-100, Dionex, Sunnyvale, CA, USA) was used to measure nitrate, phosphate, lactate, acetate, propionate, formate, butyrate, pyruvate, oxalate, and citrate in their anionic forms (Edwards et al. 2006). Total soluble sugars were determined by acid hydrolysis as glucose equivalents (Chabrierie et al. 2003). Available NH₄⁺ was measured colorimetrically by

extracting soil samples with 2 M KCl (ratio 1:5 of soil/extractant) shaken for 1 h at room temperature (Mulvaney 1996). Soil pH was measured (MP 225, Mettler-Toledo, Greifensee, Switzerland) by diluting 5 g of air-dried soil in 15 ml 0.01 M CaCl₂ solution. Total organic C (TOC) and total N were determined by combustion of finely ground air-dried soil samples using a LECO 932 CHNS device (Leco, Krefeld, Germany).

Bacterial cells were fixed in field moist soil samples (1 g) with 4% paraformaldehyde in phosphate-buffered saline solution (pH 7). Total bacterial cell numbers were determined after 4',6'-diamidino-2-phenylindole (DAPI) staining using an epifluorescence microscope (Zarda et al. 1997). Active bacterial cells were counted after fluorescein diacetate (FDA) hydrolysis by bacterial cells based on the method cited by Alef (1995) with minor changes. Briefly, field moist soil samples (5 g) were diluted in 5 ml of 60 mM phosphate buffer (pH 7.6) and shaken with 1.2 g of sterile glass beads (0.5 mm) for 2 h. This suspension was further diluted (1:10) with sterile distilled water, and FDA was added to a final concentration of 10 µg ml⁻¹. The diluted and FDA-stained samples were shaken (2 min) and centrifuged (2 min; 10,000×g). The supernatant was removed and excess FDA was washed off by flushing with 60 mM phosphate buffer. Cells were resuspended in phosphate buffer (1 ml), and 10 µl of the supernatant was dried on microscopic slides for 10 min at 37°C. Active fluorescent bacterial cells were determined microscopically by counting 10 fields per slide and sample.

DNA was extracted from triplicate field moist soil samples (0.7 g each), using the bead-beating method previously described by Sigler et al. (2002) and pooling triplicate extracts for subsequent PCR amplification. For analysis of RFLP, 16S rRNA gene fragments were amplified using oligonucleotides targeting bacterial sequences using the EUB 338 (5'-ACT CCT ACG GGA GGC AGC-3')/uni-b-rev (5'-GAC GGG CGG TGT GTR CAA-3') primers (Amann et al. 1995). PCR products were purified with equal volumes of chloroform and reprecipitated with isopropanol. Subsequently, the PCR products (2 µl) were digested with *Hae*III (2U; Promega, Madison, WI, USA) during overnight incubation at 37°C. RFLP gels (12% Bis-/acrylamide) were loaded with 3 µl of the digested PCR products that were separated for 3.5 h at 200 V. For RFLP analysis, the DCode Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA) was used, the running buffer was 1×TAE buffer (0.04 M Tris base, 0.02 M of sodium acetate, and 1.0 mM EDTA, pH 7.4) at 35°C. The gel was stained for 30 min with GelStar® (Cambrex Bioscience, Baltimore, MA, USA), 1:10,000 in 1×TAE buffer. The restriction patterns were photographed under UV light using the GelDoc 2000 system and Quantity One® software (Bio-Rad). After using a rolling disk algorithm

for background correction, band intensities and relative band position were determined. Digital image data were exported to the statistical software SPSS 11.0. Hierarchical cluster analysis was performed using the Ward's method in SPSS.

We obtained PCR products for DGGE using the primers F 968-GC (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC-3') and R-1401 (5'-CGG TGT GTA CAA GAC CC-3') as described by Nübel et al. (1996). Each PCR reaction contained 1×PCR buffer, 2 mM MgCl₂, 0.2 mM of each deoxyribonucleotide triphosphate, 0.5 µM of each primer, 0.2 mg ml⁻¹ BSA, 1 U of *Taq* polymerase (Invitrogen, Carlsbad, CA, USA), and 1 ng of template DNA. PCR products were screened on a 1% agarose gel. Equal amounts of PCR products were electrophoresed on a 8% polyacrylamide denaturing gel (35–60% denaturant gradient consisting of urea and formamide as defined by Muyzer et al. (1993). Gels were run in 1×TAE at 60°C for 5 h at 200 V using the DCode System, stained, and analyzed as described for RFLP.

Shannon diversity index was calculated from DGGE band intensity data according to

$$H = -\sum_{j=1}^x p_i \ln p_i$$

with p_i being the proportional intensity of band i to total band intensity. Richness is the number of identified phylotypes (identifiable DGGE bands per lane).

We measured five different extracellular enzymatic activities involved in carbon, nitrogen, and phosphorus cycling. All measurements were made in triplicates. Saccharase activity was determined according to the method by Schinner and von Mersi (1990), which was adapted for glacier forefield soils. Briefly, field moist soils (5 g) were mixed with 5 ml of sucrose solution (1.2%) and 5 ml of acetate buffer (2 M, pH 5.5). After incubation for 3 h at 50°C, the released reduced sugars were determined photometrically at 690 nm. β-Glucosidase activity was quantified according to the method described by Tabatabai (1994). Samples were measured by colorimetric determination of *p*-nitrophenol released by β-glucosidase after incubation in a *p*-nitrophenyl-β-D-glucoside solution. We quantified urease activity according to Kandeler and Gerber (1988) by colorimetric determination of the ammonia released after incubation of field moist soil samples (5 g) with 2.5 ml urea solution (Kandeler and Gerber 1988). Estimations of alkaline and acid phosphatase activity were performed according to the method described by Tabatabai (1994). For both measurements, field moist soil (1 g) was mixed with 4 ml of alkaline (pH 11) or acid (pH 6.5) modified universal buffer accordingly, together with toluene (0.25 ml) and 1 ml of *p*-nitrophenyl phosphate solution

(15 mM). All samples were incubated for 1 h at 37°C in the dark. The reaction was stopped by adding CaCl_2 at a final concentration of 0.05 M, and NaOH at 0.2 M. The phosphatase activity, assayed by *p*-nitrophenol release, was measured photometrically.

Statistical analysis

Statistical analysis was carried out using the SPSS 11.0 software package. Mean values are given ± 1 standard error of the mean. The significance threshold was set to 0.05 for the *p* value.

To test for the distance effects on chemical and physical parameters and on microbial abundance, we applied one-way ANOVA on each parameter set ($n=25$), with distance classes as fixed factors [0 cm ($n=3$), 20 cm ($n=11$), 40 cm ($n=11$)]. To test for pairwise differences between distance classes, we applied unpaired sample *t* tests to analyze the differences of means.

We applied hierarchical clustering to investigate microbial community fingerprints using the Ward's linkage method. Distances were recorded as squared Euclidian distances.

Results

The measured chemical and physical soil properties showed no significant effect with distance from the plant (Table 1), although all measures of soil carbon were highest in plant center soil. The measurements revealed the acidic character of the soil, with a pH of 5.03 of the plant center soil of *L. alpina*. TOC values were generally low, ranging from $285.89 \mu\text{g C (g dry soil)}^{-1}$ at 0 cm distance to $127.77 \mu\text{g C (g dry soil)}^{-1}$ at 40 cm distance from the plants. Extractable organic carbon was dominated by organic acids [240 to $270 \mu\text{g (g dry soil)}^{-1}$], with lactate and formate being the most abundant acids in all samples. Total sugar values were more than a magnitude lower [10 to $13 \mu\text{g glucose (g dry soil)}^{-1}$]. $\text{NH}_4^+\text{-N}$ values fluctuated between 5.06 and $2.87 \mu\text{g (g dry soil)}^{-1}$. $\text{NO}_3^-\text{-N}$ values ranged between 18.06 and $18.69 \mu\text{g (g dry soil)}^{-1}$. Soluble phosphate was around $100 \mu\text{g (g dry soil)}^{-1}$ in plant center soil and below detection at further distance from the plant.

In contrast to chemical data, cell count data showed significant trends with distance from the plant (Table 2). Root biomass significantly decreased with distance from the plant (one-way ANOVA, $p<0.01$), but 20 and 40 cm were not significantly different (*t* test) (Table 2). DAPI counts were highest in the plant center soils [7.88×10^8 cells (g dry soil) $^{-1}$] and significantly decreased to about 50% at 40 cm. Active bacteria counts (as measured by FDA) also decreased significantly with distance (one-way ANOVA, $p=0.01$). On average, about 30% of the total bacterial cells

were detected as active, with no significant effect of distance from the plant. Only DAPI (*t* test, $p<0.01$) and active bacteria (*t* test, $p<0.05$) showed significant differences between 20 and 40 cm samples.

RFLP analysis revealed complex restriction patterns (Fig. 1). Cluster analysis revealed no clear distance effects; overall, all samples showed a high degree of similarity. Closely related patterns tended to originate from the same plant, but the plant or site effect was likewise marginal (Fig. 1).

Fingerprinting by DGGE confirmed the diverse microbial community structure and lack of spatial effects (data not shown). However, in comparison to RFLP, the information obtained from DGGE profiling is more directly related to the microbial species level because, theoretically, each DGGE band could be related to a distinct bacterial phylotype (Muyzer et al. 1993). We therefore used DGGE banding information to investigate two different diversity parameters: richness (number of different phylotypes) and Shannon index (Table 3), which is a general diversity parameter.

Species richness, as indicated from DGGE band numbers, was highest in samples from the rhizosphere and decreased with distance (Table 3). Shannon diversity values near the plants were slightly higher than those obtained from profiles at 40 cm distance ($p=0.06$; unpaired *t* test). Overall, the decrease was only marginally significant (Pearson correlation -0.352 , significance 0.07).

Like cell counts, several enzyme activities were significantly related to distance (Table 2). Saccharase activity decreased significantly from the plant center soil of *L. alpina* [$119.46 \text{ nmol glucose (g dry soil} \cdot \text{h)}^{-1}$], (ANOVA, $p<0.01$) to $29.25 \text{ nmol glucose (g dry soil} \cdot \text{h)}^{-1}$ at 40 cm distance (Table 2). The ratio of saccharase activity divided by the number of FDA stained bacterial cells also decreased with distance (Table 2), but the trend was not significant ($p=0.155$). β -Glucosidase activity was significantly higher in the plant center soil than in the soil sampled at 40 cm (ANOVA, $p<0.001$). The ratio of β -glucosidase activity divided by the number of active (FDA stained) bacterial cells followed the same trend, which was also statistically significant, $p<0.001$ (Table 2). The sugar-related enzyme activities showed no significant differences between 20 and 40 cm (*t* test). Alkaline phosphatase activity was highest in the plant center soil [$57.80 \text{ nmol phenol (g dry soil} \cdot \text{h)}^{-1}$] and decreased with distance to $34.63 \text{ nmol phenol (g dry soil} \cdot \text{h)}^{-1}$ (Table 2). Acid phosphatase activity was about an order of magnitude higher [$480 \text{ nmol phenol (g dry soil} \cdot \text{h)}^{-1}$ in plant center soil] and decreased significantly with distance (ANOVA, $p<0.01$). Differences between 20 and 40 cm were again not significant. Urease activity fluctuated between 90 and $120 \text{ nmol N (g dry soil} \cdot \text{h)}^{-1}$ with no significant differences between distance classes (Table 2).

Table 1 Chemical and physical properties across the investigated distance classes of central *L. alpina* plants in the Damaglacier forefield

	Distance from plant			ANOVA	
	0 cm (<i>n</i> =3)	20 cm (<i>n</i> =11)	40 cm (<i>n</i> =10)	<i>F</i> _{1,24} ratio	<i>p</i> value ^a
Soil water content (%)	8.01±2.03	8.32±1.37	9.58±1.75	0.22	0.81
pH	5.03±0.1	4.91±0.03	4.92±0.02	1.67	0.21
Carbon parameters					
TOC [μg C (g dry soil) ⁻¹]	285.89±165.06	221.11±66.67	127.77±38.53	0.46	0.64
Total sugar [μg (g dry soil) ⁻¹] ^b	13.08±3.58	10.94±1.37	10.42±1.32	0.32	0.73
Total organic acids [μg (g dry soil) ⁻¹] ^b	344.62±23.11	283.91±17.81	313.56±29.35	0.86	0.44
Lactate [μg (g dry soil) ⁻¹] ^b	268.03±9.62	240.43±10.81	248.00±14.82	0.53	0.60
Formate [μg (g dry soil) ⁻¹] ^b	76.59±13.62	43.48±8.10	65.56±16.16	1.18	0.33
Nitrogen parameters					
Total nitrogen [μg N (g dry soil) ⁻¹]	<100 ^c	<100 ^c	<100 ^c	–	–
Nitrate [μg N (g dry soil) ⁻¹] ^b	18.12±0.79	18.69±0.85	18.06±0.86	0.16	0.86
Ammonium [μg N (g dry soil) ⁻¹]	4.70±2.66	2.87±0.53	5.06±1.84	0.69	0.52
Phosphate [μg (g dry soil) ⁻¹] ^b	108.32±62.54	<24 ^d	<24 ^d	–	–

Values are given ± 1 standard error of the mean. The distance effect was tested with ANOVA

^a Significance threshold 0.05

^b Measurements were done in 0.01 M CaCl₂ extracts

^c Below detection limit [100 μg N (g dry soil)⁻¹]

^d Below smallest standard concentration [24 μg (g dry soil)⁻¹]

Discussion

L. alpina occurs together with other early pioneering plants such as *Agrostis*, *Cerastium*, or *Poa* species. One of the earliest and most abundant vascular plant species that we investigated at our field site is the perennial plant *L. alpina*. In general we observed single plants or small clusters of

plants in the study area, with individual plants reaching up to 10 cm diameter and making a vigorous appearance, in contrast to the sampling of Edwards et al. (2006), when individual plants were very small, probably representing seedlings from the year. We therefore assume that the plants sampled in this study had established the previous year or earlier.

Table 2 Biological properties across three investigated distance classes of central *L. alpina* plants

	Distance from plant			ANOVA	
	0 cm (<i>n</i> =3)	20 cm (<i>n</i> =11)	40 cm (<i>n</i> =10)	<i>F</i> _{1,24} ratio	<i>p</i> value ^a
Biomass					
Root biomass [mg (g dry soil) ⁻¹]	1.35±0.95	0.05±0.03	0.03±0.01	8.937	0.001
DAPI [10 ⁸ cells (g dry soil) ⁻¹]	7.88±0.58	5.54±0.45	3.87±0.34	12.383	<0.001
Bacterial activity					
FDA [10 ⁸ cells (g dry soil) ⁻¹]	2.02±0.15	1.56±0.12	1.23±0.11	5.753	0.010
FDA/DAPI [%]	25.70±1.01	29.18±2.66	33.36±3.72	0.850	0.441
Enzyme activities					
Saccharase activity [nmol glucose (g dry soil • h) ⁻¹]	119.46±47.32	40.68±8.66	29.25±7.91	7.534	0.003
Glucosidase activity [nmol phenol (g dry soil • h) ⁻¹]	677.53±231.62	152.60±27.54	101.60±17.63	20.375	<0.001
Urease activity [nmol N (g dry soil • h) ⁻¹]	101.59±21.3	127.37±24.39	99.09±21.57	0.438	0.651
Alkaline phosphatase activity [nmol phenol (g dry soil • h) ⁻¹]	57.80±6.94	31.64±6.01	34.63±5.86	2.265	0.127
Acid phosphatase activity [nmol phenol (g dry soil • h) ⁻¹]	482.02±124.75	262.16±28.82	264.93±22.20	5.801	0.009
Enzyme activities per FDA stained cells					
Saccharase activity [fmol glucose (cell • h) ⁻¹]	0.57±0.19	0.29±0.08	0.24±0.07	2.029	0.155
Glucosidase activity [fmol phenol (cell • h) ⁻¹]	3.25±1.04	1.00±0.17	0.80±0.11	14.873	<0.001
Urease activity [fmol N (cell • h) ⁻¹]	0.50±0.08	0.89±0.19	0.79±0.14	0.636	0.539
Alkaline phosphatase activity [fmol phenol (cell • h) ⁻¹]	0.28±0.02	0.20±0.03	0.30±0.05	1.626	0.219
Acid phosphatase activity [fmol phenol (cell • h) ⁻¹]	2.32±0.49	1.79±0.24	2.32±0.25	1.332	0.285

Values are given ± 1 standard error of the mean. The effect of distance was tested by ANOVA

^a Significance threshold 0.05

Fig. 1 RFLP fingerprinting patterns of 16S rDNA fragments, originating from different distance classes (*top panels*) obtained from two transects (**a** and **b**) across *L. alpina* plants together with corresponding results obtained by hierarchical clustering using Ward's method (*lower panels*)

We found significantly increased total and active cell numbers and enzymatic activities and elevated soluble organic carbon concentrations in plant center soils, as would be expected based on numerous studies on the rhizosphere effect (e.g., (Youssef and Chino 1987; Badalucco et al. 1996; de Neergaard and Magid 2001; Corgie et al. 2004). The root biomass measurements showed that the root zone of *L. alpina* did not extend beyond the 10 cm diameter sampled as plant center soil, indicating that the 40 cm soil was not in obvious contact with the *L. alpina* root system. The effect on the microbial biomass, however, clearly extended to the 20-cm samples, with both total and active cell counts significantly increased relative to the 40-cm samples. It is noteworthy that previous studies in similar environments have not observed a significant difference in total cell counts or bacterial biomass between interspace and rhizosphere in young soils (Tscherko et al. 2004; Edwards et al. 2006). This may be

related to the species and age of the sampled plants, but may also indicate that the sampled interspace/bulk soils could have originated from within the zone of influence of the plant. In the case of Edwards et al. (2006), interspace soil was taken from within 15 cm of the plant.

Similar to total and active cell counts, several enzymatic activities showed a significant decrease with distance, although differences between the 20 and 40 cm soil were no longer significant. For saccharase and glucosidase, this result may be related to easily available sugars that can readily be metabolized in the rhizosphere, but it may also reflect the ability of young mineral soil substrates to preserve organic matter and enzymes due to sorption processes on mineral surfaces (Burns and Dick 2002; Guggenberger and Kaiser 2003). Acid phosphatase, which also showed a significant trend with distance, may originate from the plant, as well as microbes (Dick et al. 1983). The

Table 3 Summary of diversity parameters calculated from DGGE banding patterns for samples from three distance classes

	Distance from plant			PC	sig	p value ^a
	0 cm	20 cm	40 cm			
Shannon index	2.66±0.07	2.51±0.08	2.39±0.11	−0.352	0.07	0.06
Richness	18.67±1.20	17.55±1.55	16.20±1.69	−0.202	0.31	0.25

Values are given ±1 standard error of the mean ($n=27$)

PC Pearson correlation, sig two-tailed significance

^a Unpaired sample *t* test comparing 20-cm with 40-cm samples, significance threshold 0.05

soil we studied has been shown to become both P- and N-limited in the presence of sufficient carbon (Bleikolm and Bürgmann, unpublished data); however, we observed no effect on urease activity, possibly because urea may not be a significant source of nitrogen in this soil, where soluble nitrogen is mostly inorganic (Edwards et al. 2006). Unlike our results, Tscherko et al. (2004) did not observe enhanced enzymatic activities in the rhizosphere of the alpine grass *P. alpina* in pioneering successional stages, while such differences were occasionally observed in more developed soils. These contrasting results might reflect different underlying environmental parameters such as nutrient availability, organic matter content, and pH at the research sites, or the different plant species and associated root exudation patterns.

Enzyme assays measure both intracellular and extracellular activities (Nannipieri et al. 2003). In this study, we have applied a new approach that relates enzyme activities to the number of active (FDA-stained) bacteria. This ratio is an alternative to previously used ratios of enzymatic activity to total bacterial biomass measurements, which include dead and dormant cells (Landi et al. 2000). This provides the possibility to qualitatively link the observed enzyme activities to microbial processes. Our results showed that per-cell enzymatic activity generally (and for glucosidase significantly) decreased with distance (Table 2); e.g., the saccharase and glucosidase activities per FDA-stained cell in 40 cm soil were only 42 and 24% of values of plant center soil, respectively. This would indicate that the bacterial populations show lower levels in the microbial biomass and are increasingly nutrient- or energy-limited with distance from the plant and show lower levels of microbial activity. The overall decrease in activity is therefore due both to the decreasing trend in the biomass and decreasing levels of activity of individual cells.

How can the increased bacterial biomass and activity beyond the actual root zone be explained? It is possible that fine roots, which may not be detected by our root biomass measurements, may provide exudates to the 20 cm soil. Alternatively, in these young soils, low biomass and a predominantly mineral character (low organic matter and clay content) may allow diffusion or advection of dissolved organic carbon to a much greater extent than has been determined in developed soils (Karthikeyan and Kulakow 2003; Poll et al. 2006). The occurrence of isolated plant patches in young soils could thus promote the formation of strong gradients and lead to a spatially extended impact of plants on young soils. However, the lack of a clear trend in the extractable organic carbon and other chemical parameters rather supports the involvement of fine roots. Finally, the presence of isolated vegetation may have indirect effects that change the living conditions for the microbial biomass independent of the root zone, through effects such as increased aeolic sedimenta-

tion rates, decomposition of aboveground biomass, attraction of animals, shading, etc. (Matthews 1992). These effects would have to be studied in more detail in future research.

We performed RFLP and DGGE analysis to characterize the soil microbial community structure at different distances from *L. alpina* plants. At the observed scale, the differences in microbial activities were not related to an obvious change of the microbial community structure.

Previous research suggests that rhizospheres can have a selective effect on soil bacteria (Marilley et al. 1998). Specific compounds in the root exudates might even selectively stimulate certain beneficial bacterial groups, which have mechanisms to potentially improve plant growth (Griffiths et al. 1999; Dobbelaere et al. 2003; Bürgmann et al. 2005). Edwards et al. (2006) observed different microbial communities in rhizosphere of *L. alpina* and interspace soil also sampled in this study using DGGE. The different findings may be explained by the different sampling procedures because Edwards et al. (2006) sampled rhizosphere soil directly within the root zone. However, Tscherko et al. (2005) studied the microbial community directly in *P. alpina* rhizosphere (root-adhering soil) using phospholipid fatty acid analysis and found similar microbial community structure in bulk soil and rhizosphere in young soils. Our results support the conclusion of Tscherko et al. (2005) that in young glacial soils the rhizosphere community is mostly recruited from the bulk soil community. However, they also observed no significant differences in enzymatic activities and microbial biomass, unlike our results. Here we observed that despite a significant increase in numbers and activity of the microbial community in the vicinity of *L. alpina*, the community was not subject to selective pressure that affected the community composition at the coarse resolution that could be studied using RFLP and DGGE (Nocker et al. 2007). In conclusion, the currently available data remain inconclusive as to under which conditions shifts in microbial community structure in the rhizosphere of pioneer plants occur. This may depend on specific spatial, temporal, or plant species-related effects. The variation of rhizodeposition patterns might be a general mechanism regulating the growth of root-associated bacteria, depending on the environmental conditions and nutrient requirements. Recently, Edwards et al. (2006) reported that the rhizosphere of *L. alpina* at different successional stages exhibited distinct root-exudation patterns.

In conclusion, we have shown, in accordance to our hypothesis, that vegetational patches occurring in a glacial forefield affected microbial biomass and activity, and that this effect extended to 20 cm distance from the plant. This creation of relatively large zones of microbial activity may create islands with improved conditions for further biological colonization, e.g., by plants and animals. Similar scenarios have been reported for semiarid and desert zones

where isolated patches of shrub canopies represent areas of enhanced nutrient availability (“resource islands”) (Herman et al. 1995; Su et al. 2004). In contrast to our original hypothesis, the microbial community composition and soil chemistry did not reveal obvious gradients on the studied scale. The dominant members of the bacterial community remained the same despite a doubling of the bacterial cell count, indicating little selective pressure.

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