

## Microbial Diversity and Activity along the Forefields of Two Receding Glaciers

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### ABSTRACT

Forefields of two receding glaciers were sampled along either a 150 or 200 m long transect at identical spatial intervals for assessment of soil microbial activity and community diversity trends. The forefields belonged to the Dammaglacier (forefield area is 157 ha, 2000 m above sea level) and Rotfirnglacier (100 ha, 2200 m) and at the time of sampling were receding at an estimated rate of 8 and 10 m yr<sup>-1</sup> over the past 5 years, respectively. Direct counting of bacteria (DAPI staining), assessment of dehydrogenase activity (DH), and fluorescein diacetate hydrolysis activity (FDA) were performed to estimate bacteria number and soil microbial activity. Along the Dammaglacier forefield (from youngest to oldest soil), bacteria number ( $8.21 \times 10^7$  to  $1.49 \times 10^9$  cells g<sup>-1</sup> soil), DH activity (0 to 61 µg TTC reduced g<sup>-1</sup> soil h<sup>-1</sup>), and FDA activity (0 to 100 µg fluorescein produced g<sup>-1</sup> soil h<sup>-1</sup>) increased, suggesting the development of microbial populations increasing in number and activity. The Rotfirn forefield exhibited similar trends per gram of soil in bacteria number ( $1.13 \times 10^8$  to  $5.93 \times 10^9$  cells), DH activity (0 to 36 µg TTC reduced), and FDA activity (2 to 70 µg fluorescein produced), but with more variability among samples than the Damma forefield samples. Molecular assessment of bacterial diversity included denaturing gradient gel electrophoresis (DGGE) and ribosomal intergenic spacer analysis (RISA) of soil DNA. DGGE and RISA revealed that the composition and succession of bacterial populations were different in both forefields. Comparison of Shannon diversity index values indicated that all populations sampled from the Damma forefield were significantly different ( $p < 0.05$ ). Conversely, similar populations existed in the Rotfirn forefield succession. Overall, the results indicate that diverse bacterial assemblages increasing in number and activity characterize these glacier forefield soils with both forefield successions exhibiting differing modes of bacterial community establishment.

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## Introduction

Forefields that result from receding glaciers represent unique environments of newly exposed terrain free of glacial ice in which microbial communities may be studied. Although the glacial environment has received little attention with respect to microbial ecology study, the importance of these sites is becoming apparent. A recent report by the Swiss Federal Office for Environment, Forest, and Landscape (BUWAL) has completed a geomorphological and biological inventory of Switzerland's alluvial zones [18]. Of 197 glacier forefields designated as potentially important sites, 78 forefields were eventually determined to be of national significance and are predicted to be subject to protection and scientific research.

Although much of our current knowledge of glacial ecosystems has evolved from studying the succession of forefield plant communities, bacteria are most likely the primary pioneers of these sites [40]. Despite this, little research has been initiated to characterize the microbial populations present in the developing soils of this environment. Because of the limited data that exist describing microbial communities native to glacial forefields, one must rely on studies of similar environments such as the arctic, alpine, and other succession environments in order to make preliminary assumptions about glacial forefield ecology. As a succession environment, the glacier forefield contains young soils as well as mature soils of varying physical composition [26]. Furthermore, studies indicate that organism distribution is not random but is heavily dependent on the presence of "safe sites" such as cracks in rocks, concave surfaces and depressions that allow protection from wind and temperature extremes [22, 27]. Examples of the importance of bacteria populations in cold climate environments in terms of soil development, plant growth, and biogeochemical cycling are abundant [4, 6, 9, 30, 42, 43, 46, 47, 49]. Thus, in light of the diverse functional roles of bacteria in cold environments, it is likely that bacteria will represent important colonizers in the developing glacial forefield community. Moreover, given the examples of plant-growth-promoting bacteria and their impact on plant establishment [7] it is likely that the development of certain pioneer bacteria species may greatly affect the subsequent succession of other organisms.

Assessing the diversity of natural environments using culture-based methods has proven limited since less than 1% of organisms are truly culturable [45] and analyzing bacterial populations through traditional methods can re-

sult in the misidentification of organisms [2] and biases in diversity assessment [12]. However, throughout the past decade, the assessment of microbial community diversity has benefited from the use of molecular methods, which have become commonplace for studying microbial diversity in environmental samples [34]. The direct extraction of DNA from soil and the subsequent PCR amplification of 16S rRNA genes conserved within the domain Bacteria has greatly facilitated ecological studies and several methods of community fingerprinting share this strategy as a starting point. Specifically, the method of denaturing gradient gel electrophoresis (DGGE) has been frequently used to assess the diversity of dominant microbial communities through the separation of PCR-amplified partial 16S rRNA genes (for a review see Muyzer and Smalla, [29]). More recently, ribosomal intergenic spacer analysis (RISA) has become popular as a molecular fingerprinting technique. RISA is based on the length heterogeneity of the 16S-23S rRNA gene spacer in bacteria and has been effective in assessing the bacterial community structure in soils [3, 36, 37].

Although effective for assessing microbial community structure, DNA-based methods suffer from difficulty in their usefulness in revealing the activity of microbial communities. Despite this, soil microbial activity can be assessed through the measurement of soil enzymes where the enzyme reaction rate indicates the amount of enzyme present [16] and thus an estimation of the soil microbial activity. Moreover, soil enzyme activities have been sensitive indicators of changes in soil properties, especially in succession sites [11]. Thus, soil enzyme activity measurements provide a useful activity-based complement to molecular assays.

In this work, we have investigated the microbial communities of two glacial forefield successions. Our principal objectives were to characterize the glacier forefield succession environment with respect to bacteria abundance, overall microbial activity, and diversity of the bacterial community. These objectives were accomplished through the use of traditional enzymatic activity assays and molecular tools, a combination that assessed the forefield environment from both a structural and functional perspective.

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## Methods

### *Field Site Description*

Two glacial forefields were selected for investigation in this study. The forefields of the Dammaglacier (forefield area is 157 ha, 2000

m above sea level at the glacier terminus, 25% slope), and Rotfirnglacier (100 ha, 2200 m, 7% slope) are located in roughly perpendicular valleys in Göschenalp, Switzerland are separated by approximately 2.5 km (Fig. 1). Since 1850, the Dammaglaciar and Rotfirnglacier have experienced a decrease in length of 22% and 34%, respectively, which has resulted in the liberation of 157 ha and 100 ha of terrain in the Damma and Rotfirn forefields, respectively. Over the past 5 years, the recession rate of the Dammaglaciar and the Rotfirnglacier has been 10 m yr<sup>-1</sup> and 8 m yr<sup>-1</sup>, respectively ([19]; Andres Bauder, personal communication). The sites are further characterized by a mean annual temperature of between 0 and 5°C, and approximately 2400 mm of precipitation per year [41]. Because of the NW to SE aspect of the Rotfirn forefield, it receives more direct sunlight per day than the Damma forefield. Despite this, there is little vegetation present at the Rotfirn forefield site with the exception of sporadic grasses growing at 200 m from the glacier terminus and beyond. On the other hand, intermittent grasses at 60 m from the glacier characterize the Damma forefield vegetation pattern. Additionally, extensive vegetation including grasses (*Poa* spp.), and broadleaf perennials are present beginning at 100 m from the glacier. The subsurface of both glacier forefields is composed of granite as the parent material.

In this study, the term “soil” is difficult to define as a variety of “soils” or “soil precursors” are found along the two forefields. Thus, for the sake of simplicity, all materials analyzed will be referred to as “soil.” The pH of the soils at these two sites ranges from 4.8 to 6.0 but shows no clear correlation with distance from

the glacier terminus and organic carbon contents were below 1%. Soil moisture contents (w/w) in the Damma forefield increased from 5% near the glacier to 22% at 150 m distant from the glacier. Rotfirn forefield soil moisture contents were between 7% and 12% but varied along the forefield showing no clear correlation with soil age.

### Soil Sampling and Handling

Soil samples were harvested from each forefield at predetermined spatial intervals beginning at the terminus of each glacier. Multiple samples (four or more) of approximately 500 g each were harvested with a small spade from along 25-m-long transects approximately 0 (debris from glacial ice), 25, 50, 100, and 150 m distant from the terminus of the two glaciers. An additional sampling was performed at 200 m from the Rotfirnglacier. To avoid the interference of vertical heterogeneity in soil samples resulting from the formation of distinct soil horizons, we sampled only surface soils (2 cm depth) free of distinct horizon development. Samples were placed into sterile plastic bags and transported to the laboratory. Samples from common transects were pooled, sieved (2.0 mm), and assessed for moisture content and pH. Soils used for enzyme assays and cell counts were processed immediately and samples to be used for DNA extractions were frozen at -80°C but were analyzed within 3 days. Forefield samples dedicated to bacteria counting (approximately 500 mg each) were fixed in triplicate with 4% paraformaldehyde solution (pH 7) in 1.5 ml microcentrifuge tubes and placed on ice for 4 h. Tubes were centrifuged for 10 min at 13,000 rpm, the supernatant was discarded, and 1 mL of phosphate-buffered saline (130 mM NaCl, 10 mM NaPO<sub>4</sub>, pH 7.2) was added. The tubes were mixed well and centrifuged again as described above. The supernatant was discarded and the soil sample resuspended in 1 mL phosphate-buffered saline:ethanol (1:1) and stored at -20°C until analyzed.

### Total Counts of Soil Bacteria as Determined by DAPI Staining

DAPI (4',6-diamidino-2-phenylindole) staining was used to estimate the total numbers of soil bacteria in each sample according to the method of Zarda et al. [51].

### Assays of Microbial Activity

Assays of dehydrogenase (DH) activity, and fluorescein diacetate hydrolysis (FDA) activity were performed on freshly harvested forefield soils. Both assays were completed twice, in triplicate, according to the methods of Alef [1]. In specific, the DH assay was performed by incubating 2 g field moist soil with 2 mL of triphenyltetrazolium chloride (TTC) solution (0.1 g TTC 100 mL<sup>-1</sup>, 100 mM Tris buffer, pH 7.6) in sterile glass test tubes at 30°C for 24 h. Acetone (20 mL) was added to each sample followed by further incubation for 2 h at room temperature in

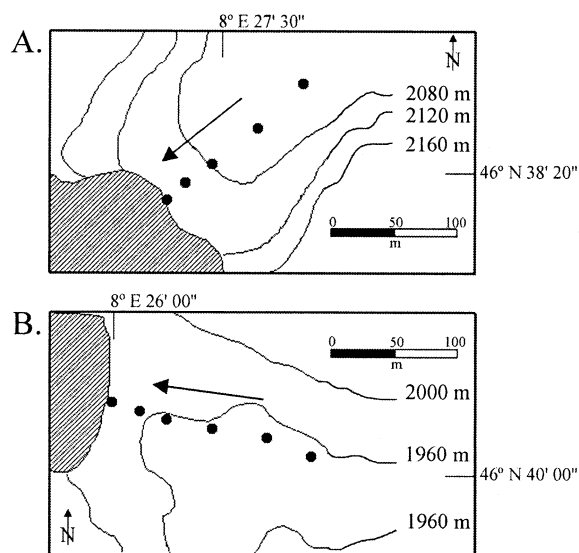


Fig. 1. Soil collection sites for samples analyzed in this study. Sampling positions within the (A) Damma and (B) Rotfirn forefields are denoted by the closed circles. The arrow indicates the direction of glacial movement. The shaded area indicates the glacier terminus. Adapted from *Landeskarte der Schweiz 1231, Urseren*, Bundesamt für Landestopographie, Switzerland.

the dark. Each soil suspension was filtered and measured for absorbance at 546 nm. Absorbance results were compared against a standard series of triphenyl formazan solutions, the end product of TTC reduction. The DH activity was calculated for each sample and expressed as  $\mu\text{g TTC reduced g}^{-1}$  dry soil. The hydrolysis of FDA was performed by incubating 2 g of moist soil with 25 mL 60 mM  $\text{PO}_4$  buffer (pH 7.6) and fluorescein diacetate at a final concentration of  $10 \mu\text{g ml}^{-1}$  in sterile Falcon tubes. The tubes were shaken for 1.5 h at  $24^\circ\text{C}$  followed by the addition of an equal volume of acetone to stop the reaction. The supernatant was clarified by centrifugation, and absorbance was measured at 490 nm and compared against a standard series of fluorescein solutions. The hydrolysis activity was calculated for each sample and expressed as  $\mu\text{g fluorescein produced g}^{-1}$  dry soil. Both assays included two negative controls per triplicate soil sample tested: an uninoculated soil and a no-soil blank.

### Soil DNA Extraction and Genetic Fingerprinting

Approximately 0.6 g of each pooled forefield soil was sampled in at least triplicate for DNA extraction. Samples were placed into 2 ml microcentrifuge tubes along with 1.25 ml of extraction buffer (50 mM NaCl, 50 mM EDTA, 50 mM Tris, and 5% sodium dodecyl sulfate, pH 8) and 0.5 g glass beads (0.10–0.11 mm). Tubes were shaken with a Fast-prep instrument (BIO 101, La Jolla, CA) at  $5.5 \text{ m s}^{-1}$  for 30 s and centrifuged for 4 min at  $10,000 \times g$ . Nucleic acids were isolated from the supernatant with phenol/chloroform extraction and isopropanol precipitation [38]. The concentration of the extracted DNA was determined by measuring absorbance at 260 nm and purity was estimated from the ratio of  $A_{260}:A_{280}$ . Because of the low concentration of nucleic acids extracted from several of the soils harvested near the glacier terminus, multiple extractions were performed for each triplicate DNA sample and the samples pooled and concentrated prior to further analysis. Partial bacterial 16S rRNA gene fragments were amplified for use in DGGE analysis with primers U968-GC (5'-GC clamp-AAC GCG AAG AAC CTT AC-3') [24] and L1401 (5'-CGG TGT GTA CAA GAC CC-3') according to the method of Felske et al. [14]. PCR reactions were performed in a MJ Research PTC-200 thermalcycler (MJ Research, Inc., Watertown MS) using approximately  $1 \text{ ng DNA } \mu\text{l}^{-1}$  PCR reaction. For RISA, bacterial 16S-23S rRNA intergenic fragments were amplified with primers 1406-f (5'-TGY ACA CAC CGC CCG T-3'; [24]) and 23S-r (5'-GGG TTB CCC CAT TCR G-3'; [3]) according to the method of Yin et al. [50]. DGGE was performed in  $16 \text{ cm} \times 16 \text{ cm} \times 1 \text{ mm}$  gels made of 6% acrylamide in a gradient of denaturant ranging from 30 to 55%. Denaturant was added in the form of urea and formamide and increased linearly in concentration in the direction of electrophoresis. A 100% denaturing solution is defined as 40% vol/vol formamide and 42% wt/vol (7.0 M) urea. Gels were run in  $1 \times$  TAE buffer (0.04 M Tris base, 0.02 M sodium acetate, and 1.0 mM EDTA, pH 7.4) at  $60^\circ\text{C}$  and 170 V for 6 h using the DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA). RISA amplification products were elec-

trophoresed in 2% Metaphor agarose (FMC, Rockland, ME) at 80 V for 3 h. Staining of both types of gels was performed following electrophoresis by gently agitating the gel for 15 min in 50 ml of  $1 \times$  TAE containing a 1:10,000 dilution of SYBR Green dye (Molecular Probes Inc., Eugene, OR).

### Image Analysis

The resulting banding patterns from both fingerprinting techniques were visualized with UV transillumination and photographed using the Gel Doc 2000 gel documentation system (Bio-Rad Laboratories, Hercules, CA). DGGE and RISA gels are composed of several lanes containing multiple bands of DNA with varying intensities. Band patterns and average intensities were analyzed using Quantity One image analysis software version 4.0 (Bio-Rad Laboratories). After applying a rolling disc background subtraction (setting 9), the software performed an analysis of each lane, acquiring information concerning each band's position and average intensity relative to the cumulative intensity value of all bands in the given lane. A band of DNA was detected if it accounted for greater than 0.2% of the total lane intensity. The same software settings were used in the analysis of all fingerprints. Identification of bands occupying common positions in multiple lanes was also performed. A matrix was constructed using this information and used to calculate numerical values describing the diversity of the bacterial communities. The Shannon index ( $H' = -\sum (n_i/N) \ln (n_i/N)$ ), and the Simpson index ( $D = \sum n_i^2$ , expressed as  $1/D$ ) where  $n_i$  is the area of each peak and  $N$  is the sum of all peak areas [25], were used as numerical descriptors of bacterial community diversity.

### Statistics

The results of the analyses are reported as averages over the replications described above. Comparisons of Shannon diversity indices were performed first by estimating the variance in  $H'$  of each forefield sample:

$$\text{Var}H' = \frac{\sum \rho_i (\ln \rho_i)^2 - (\sum \rho_i \ln \rho_i)^2}{N} - \left( S - \frac{1}{2} N^2 \right)$$

where  $p_i$  is the proportional abundance of the  $i$ th species (band),  $N$  is the total number of species (bands), and  $S$  is the total number of individuals detected (summed band intensities). A  $t$ -test allowed the comparison of two fingerprint lanes by

$$t = \frac{H'_1 - H'_2}{(\text{Var}H'_1 + \text{Var}H'_2)^{1/2}}$$

where  $H'_1$  is the diversity of sample 1 and  $\text{Var} H'_1$  is its variance. The associated degrees of freedom were calculated by

$$df = \frac{(\text{Var}H'_1 + \text{Var}H'_2)^2}{\frac{(\text{Var}H'_1)^2}{N_1} + \frac{(\text{Var}H'_2)^2}{N_2}}$$

## Results

### Estimation of Bacterial Biomass as Determined by DAPI Staining and DNA Extraction

In the Damma forefield (from youngest to oldest soil), bacteria counts increased from  $8.21 \times 10^7$  to  $1.49 \times 10^9$  cells  $g^{-1}$  dry soil (Fig. 2A). Bacteria numbers also increased with soil age in the forefield of the Rotfirnglacier; however, the increase was not as consistent as the trend revealed by the Damma forefield samples. Cell numbers increased from  $1.13 \times 10^8$  near the glacier to  $1.9 \times 10^9$  cells  $g^{-1}$  dry soil at the 150 m sample, then decreased to  $5.93 \times 10^8$  cells at 200 m from the glacial front (Fig. 2B). Damma forefield soil DNA concentrations ranged from  $1.61 \mu g g^{-1}$  dry soil in samples harvested from debris on the glacial ice (0 m) to  $13.52 \mu g g^{-1}$  dry soil at 150 m from the glacier terminus (Fig. 2A). Overall, Rotfirn forefield soil samples yielded similar quantities of DNA, from  $0.40 \mu g g^{-1}$  dry soil at the glacier terminus to  $15.39 \mu g g^{-1}$  dry soil in the 150 m sampling (Fig. 2B). Furthermore, soil DNA concentrations were found to strongly correlate with cell number estimates (Rotfirn  $r^2 = 0.948$ ; Damma  $r^2 = 0.981$ ), suggesting that the amount of DNA recovered from the forefield soils was a reasonable predictor of bacterial biomass.

### Assays of Microbial Activity

As determined by both the DH and FDA assays, overall metabolic activity in each glacier forefield increased as soil age increased. The two glacier forefields displayed different trends in soil DH activity. While the 0 m sample from both forefields was mostly inactive ( $2.00 \mu g$  TTC reduced  $g^{-1}$  soil  $h^{-1}$ ), the DH activity of the Damma forefield rose

steadily across all samples to a maximum of  $60.8 \mu g$  TTC reduced  $g^{-1}$  soil  $h^{-1}$  in the 150 m sample (Fig. 2A). Conversely, the 0, 25, 50, and 100 m samples from the Rotfirn forefield showed little DH activity. The 150 and 200 m samples exhibited values of  $30.1$  and  $36.3 \mu g$  TTC reduced  $g^{-1}$  soil  $h^{-1}$ , respectively (Fig. 2B).

The results of the FDA assay were similar to those of the DH assay as the activities in soils from both glacier forefields exhibited low activity in the 0 m samples and then increased to higher levels as distance from the glacier terminus increased. Specifically, Damma forefield soils increased in FDA activity from  $0.8$  to  $97.5 \mu g$  fluorescein produced  $g^{-1}$  dry soil  $h^{-1}$  from 0 to 150 m, respectively (Fig. 2A). Likewise, the soils of the Rotfirn forefield revealed an increase in activity from  $1.8$  to  $70.0 \mu g$  fluorescein produced  $g^{-1}$  dry soil  $h^{-1}$ . However, in a similar manner to the DH trend, these values did not increase with the consistency of the Damma forefield samples as the maximum fluorescein produced was observed in the 150 m sample ( $100.9 \mu g$  fluorescein produced  $g^{-1}$  dry soil  $h^{-1}$ ; Fig. 2B). Furthermore, both measures of microbial activity were found to positively correlate with each other (Fig. 3C, F) and with cell numbers (Figs. 3A, B, D, E) indicating the significant role of bacteria in the microbial activity of this environment.

### Glacial Forefield Community Diversity as Determined by DGGE

Forty-seven different bands were detected in the five Damma forefield DGGE profiles according to the detection parameters outlined in Methods (Fig. 4A) while 57 different bands were detected in the six Rotfirn forefield profiles (Fig. 4B). Both Rotfirn and Damma forefield DGGE profiles exhibited bands shared by at least two lanes

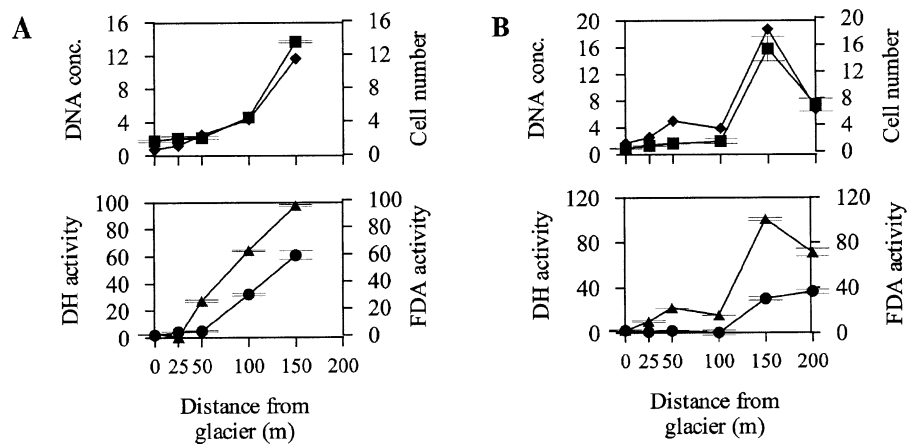


Fig. 2. Trends in soil enzyme activity and estimation of biomass investigated for the (A) Damma and (B) Rotfirn forefields. Legend: dehydrogenase activity ( $\mu g$  TTC reduced  $g^{-1}$  soil  $h^{-1}$ , ●), fluorescein diacetate hydrolysis activity ( $\mu g$  fluorescein produced  $g^{-1}$  soil  $h^{-1}$ , ▲), cell number ( $\times 10^7 g^{-1}$  soil, ◆), and DNA concentration ( $\mu g g^{-1}$  soil, ■). Error bars represent the standard error measurements of three replications.

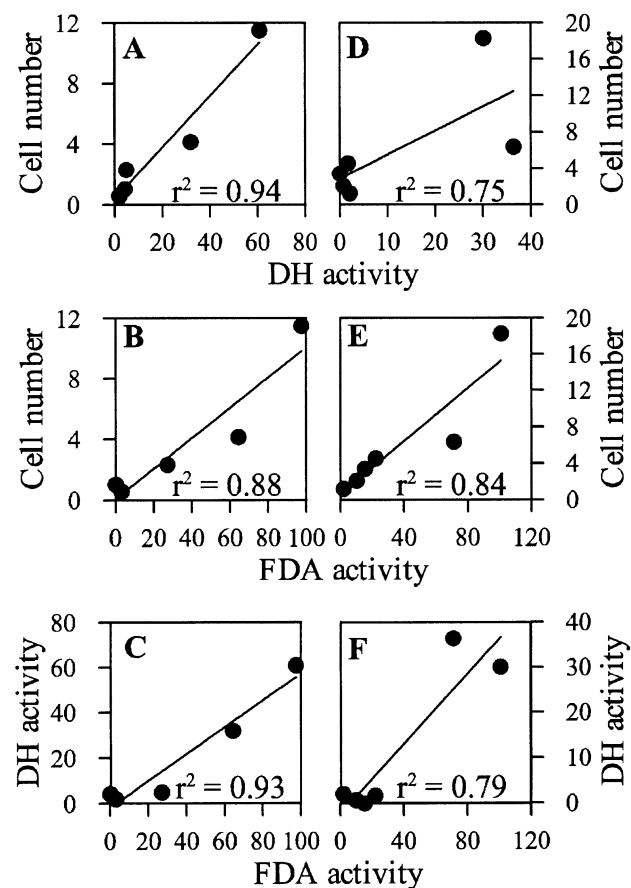


Fig. 3. Correlation of mean cell number ( $\times 10^{-7}$  soil  $\phi$ ), DH activity ( $\mu\text{g}$  TTC reduced  $\text{g}^{-1}$  soil  $\text{h}^{-1}$ ), and FDA activity ( $\mu\text{g}$  fluorescein produced  $\text{g}^{-1}$  soil  $\text{h}^{-1}$ ), within Damma (A, B, and C) and Rotfirn (D, E, and F) forefield soil samples.

as well as bands unique to individual lanes. Sixteen bands were common to at least two lanes in the Damma forefield profiles while 22 bands were shared in the Rotfirn forefield profiles.

The number of bands detected in each sample within a forefield also varied greatly, but did indicate a definite trend in the Rotfirn forefield with respect to age of the deglaciated soil. As distance from the glacier increased, the number of detected bands decreased or remained the same in each of the successive Rotfirn forefield samples. These numbers ranged from 25 bands detected at the glacier terminus to 11 bands in samples harvested from 200 m distant from the glacial ice (Fig. 4B). This overall trend was evident in samples from the Damma forefield (from 15 bands at the terminus to 9 bands at 150 m); however a constant decrease in band number associated with increased distance from the glacier was not observed (Fig. 4A.).

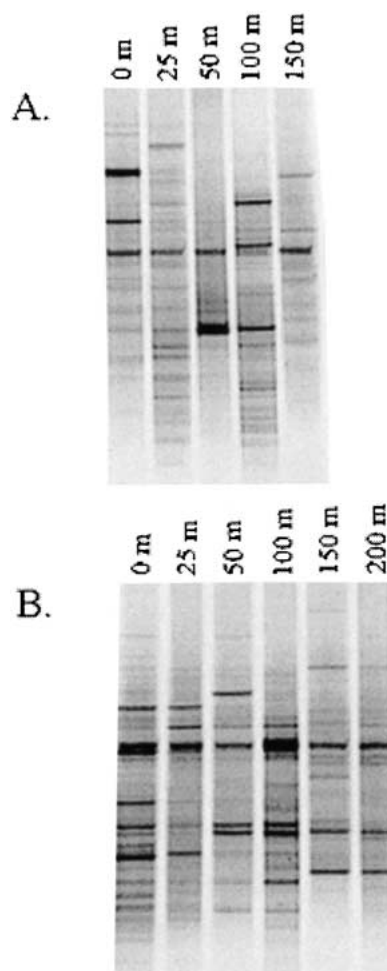


Fig. 4. DGGE analysis of (A) Damma and (B) Rotfirn forefield soil bacterial populations after DNA amplification with Bacteria primers. Lane labels represent sampling distance in meters from the glacier terminus.

In order to further compare the bacterial communities in the forefield soils, numerical indicators of microbial diversity were calculated from values of DGGE band number and intensity in the form of Shannon ( $H'$ ) and Simpson ( $1/D$ ) indices. Values of  $H'$  decreased from 2.31 in most recently deglaciated terrain to 1.97 (Fig. 5A) in the oldest samples harvested from the Damma forefield, and from 2.83 to 2.25 in Rotfirn forefield samples (Fig. 5C). Simpson index values showed a similar trend ranging from 12.99 to 8.64, and 7.39 to 6.04 in Rotfirn and Damma forefield samples, respectively. While a gradual decrease in  $H'$  and  $1/D$  was apparent in the Rotfirn forefield, the trend in diversity along the Damma forefield exhibited higher variability. This is evidenced by higher standard deviations of both diversity indices when compared to samples from the Rotfirn forefield (Rotfirn forefield SD = 0.22 for

$H'$ , 1.82 for 1/D; Damma forefield SD = 0.62 for  $H'$ , 5.28 for 1/D). Student's  $t$ -test statistics indicated that all Damma forefield samples harbored significantly different bacterial populations ( $p < 0.05$ ). Conversely, similar populations were found to inhabit the soils of the Rotfirn forefield ( $p < 0.05$ ). For example, populations from 25, 50, and 100 m were determined to be similar as well as populations at 100 and 150 m, and at 150 and 200 m from the terminus.

#### Glacial Forefield Community Diversity as Determined by RISA

RISA analysis resulted in an average of 7 bands detected in each lane of the Damma forefield profile (Fig. 6A) and 8 detected in Rotfirn forefield lanes (Fig. 6B). The total number of bands detected in the Rotfirn forefield samples decreased sequentially from 9 at 0 m to 5 at 200 m (SD = 1.38; Fig. 5D). Conversely, the number of bands detected in Damma forefield profiles varied, decreasing overall from 6 at 0 m to 4 at 150 m (SD = 2.51); however, as many as 9 bands were detected in intermediate forefield samples, confirming the variability in band number observed in DGGE profiles (Fig. 5B). Sixteen total bands were detected in the Rotfirn forefield RISA profiles (11 shared among two or more lanes) while 15 bands were detected in Damma forefield profiles (9 shared). Calculated Student's  $t$ -statistics indicated that all population fingerprints generated from the Damma forefield samples were significantly different ( $p < 0.05$ ). As seen with DGGE, Rotfirn forefield RISA community fingerprints were found to define more than one distinct population: samples harvested from 0, 25, 50, and 100 m vs those harvested from 150 and 200 m.

## Discussion

In order to develop an understanding of microbial population development along a primary succession, soils from two glacier forefields were sampled with a focus on microbial population development and activity. Parameters such as soil biomass and metabolic efficiency have been previously investigated in other forefields [21, 33], but our study was different as we aimed to investigate the additional perspective of molecular diversity of the forefield bacterial community. Of particular importance in the glacial environment is the concept of a forefield being viewed as a physical timeline with the youngest soils (or

soil precursors) found at the glacier terminus and the oldest ones found distant from the glacier. The two glacier forefields chosen for this study were in close proximity to one another and were derived from the same parent material [41], so variables such as geologic history were similar for each site. On the other hand, variables such as the southern exposure, slope, valley width, and vegetation pattern were different for each forefield, which could play a key role in sunlight exposure, water relations, debris transport, and overall soil development. The historical position of the glacier itself is also of importance at these sites. Many Swiss glaciers have experienced periods of both advancement and retreat since the end of the Little Ice Age in 1850 [19]. Because of complications involved with accurate sample dating, we have intentionally avoided associating the forefield sample positions with a given

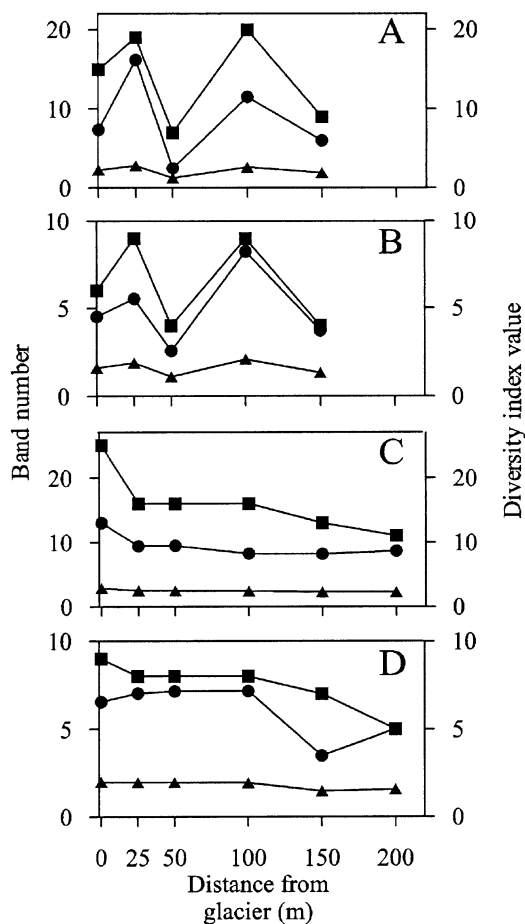


Fig. 5. Band number and diversity index values for glacier forefield soils harvested from the Damma (A and B) and Rotfirn (C and D) forefields. Panels A and C represent DGGE values, panels B and D represent RISA values. Legend: band number, (■); 1/D, (●); and  $H'$ , (▲).

deglaciation date. We have simplified the complexities of sample dating by assuming that samples distant from the glacier terminus have been deglaciated for a longer period of time than those closer to the glacier. It is obvious that glacier forefields are heterogeneous environments from which accurate sampling can be difficult. To address this problem, our sampling scheme was designed to minimize as much heterogeneity-derived error as possible by generating representative composites of each transect through subsampling and pooling of the individual samples.

The two glacial forefields chosen for this initial investigation were different in each parameter tested: biomass estimation, activity assays, and population diversity assessment. As revealed by DH and FDA assays, metabolic activity increased along the Rotfirn and Damma forefields. Increased DH and FDA activity with soil age has previously been shown to result from increased biomass and the establishment of metabolically active soil organism populations [20] and the overall trend of increased activity has also been observed in other forefield environments [21, 33]. The positive correlation of cell number with metabolic activity along the forefields in the current study further confirms this finding (Figs. 3A, B, D, E). Decreased DH activity in soils has been attributed to acidic conditions [1] and could be a factor in the low DH activity observed in the Rotfirn forefield since five of the six soil samples harvested from this forefield were acidic. Although DH and FDA hydrolysis are accepted indicators of general microbiological activity, we cannot attribute the observed microbial activity entirely to bacteria as the assays fail to specifically separate the activity of bacteria from the activity of other groups of organisms. Despite this, the correlation of the results of both assays with cell numbers leads us to conclude that the bacteria population is an important contributor to the observed metabolic trends in both forefield environments. Although the DH and FDA hydrolysis assays are commonly used to assess microbial activity, TTC, fluorescein, and FDA can adsorb to soil organic matter and remain undetected during spectroscopy; thus care must be taken when interpreting results.

Forefield community fingerprints indicated the presence of several dominant populations in the soils of both forefields regardless of the fingerprinting method used. Given the low metabolic status and cell number in the youngest forefield soils, it was expected that only a few phylotypes would dominate those samples. Surprisingly, diverse communities inhabited soil samples harvested

from the glacial ice as detected by DGGE, indicating that the youngest soils in the forefield sequence are capable of supporting a variety of bacteria. For example, the 0 m sample (Fig. 4B), harvested from within the ice of the Rotfirnglacier, contained the highest number of dominant bands (25) of any sample tested and a higher diversity of dominant organisms ( $H' = 2.83$ ,  $1/D = 12.99$ ) than any other sample in the forefield. We postulate that decreased competition for space and resources in the young forefield soils allowed for the establishment of a greater number of pioneer bacteria species than in older soils. The isolation of diverse assemblages of bacteria from a mixture of rock and glacial ice has been reported previously [10] and, combined with our observations, reinforces our characterization of glacial forefields as microbially diverse environments.

Trends in RISA band number and diversity confirmed the findings of the DGGE analysis. Each forefield displayed similar  $H'$  trends regardless of which analysis technique was used. Diversity trends as measured by  $1/D$  were also consistent between the two analysis techniques with the exception of inconsistencies in the comparison with  $H'$  in some Rotfirn forefield samples (0, 25, and 50 m samples). Diversity index values as determined by RISA were consistently lower than DGGE-derived values. This overall decrease can be explained by the lower number of detected bands associated with the RISA fingerprints. Nevertheless, a comparison of the DGGE and RISA fingerprints revealed that the number of detected bands was positively correlated for each set of forefield samples (Rotfirn  $r^2 = 0.6932$ ; Damma  $r^2 = 0.948$ ).

Molecular fingerprinting revealed differences in the development of microbial populations in the two forefields. A visual inspection of Damma forefield fingerprints showed that several bands appear in only one lane, indicating that a high degree of phylotype replacement had occurred along the succession gradient. Pairwise  $t$ -test results also revealed that regardless of fingerprinting method used, all Damma forefield populations were significantly different (as determined by comparison of  $H'$  values). Conversely, the numerous shared bands as well as  $t$ -test results indicated that less phylotype replacement was taking place along the Rotfirn forefield. We can partially attribute these forefield differences to the differential weathering, vegetation cover, topography, and exposure to wind and/or sunlight at the two sites. These factors have been found to affect other environmental gradients as well [50]. However, it is likely that inconsistencies in the

movements of the respective glaciers throughout history have played a role in the apparent development of microbial populations as well.

In this study, soil was harvested from the glacier forefields and processed for either enzyme assays or DNA extraction. There are limitations and biases involved with this approach that must be addressed. The efficiency and reproducibility of DNA extractions from soil has been found to be variable depending on extraction buffer, energy, and purification method [5, 15]. In the present study, much care was taken during DNA extraction to handle all samples identically to render any inherent biases systematic in nature, thus, enabling sample-to-sample comparisons. Calculating diversity indices based partially on the intensity of bands in DGGE and RISA fingerprints relies on the assumption that band intensity is related to initial DNA template amount in the extracted sample and, thus, original organism number. Although biases are often associated with PCR amplification of mixtures of DNA templates (reviewed by [44, 48]), band intensity will often relate to initial cell numbers [17] and has been frequently related to relative phylotype abundance [13, 23, 28, 31, 35, 39]. DGGE and RISA can also present limitations when analyzing complex samples following 16S rRNA gene amplification. Multiple *rrn* operons and/or comigration of bands of different sequence but similar melting behavior can over- or underestimate community diversity following PCR and DGGE [8, 32]. A similar problem is associated with RISA, although comigration is due to DNA size similarities of spacer regions. Nevertheless, an inspection of our DGGE and RISA fingerprints reveals similar profiles even though differing regions of the rRNA gene were used in the two methods (compare Figs. 4A and B with Figs. 6A and B).

To our knowledge, this is the first study to develop numerical descriptors of the microbial diversity of a glacier forefield using molecular fingerprinting methods. By comparing community fingerprints with metabolic assays, we have shown that although values of general microbial metabolic activity increased with soil age, activity was not strongly coupled to trends in the diversity of dominant bacterial species. However, cell number, DH and FDA activity, and community fingerprint results all reflected the differences in microbial population development in the two sites. Our results underscore the idea that both metabolic and structural diversity within and between forefields is an important characteristic in this environment. The biological heterogeneity observed in the two forefields

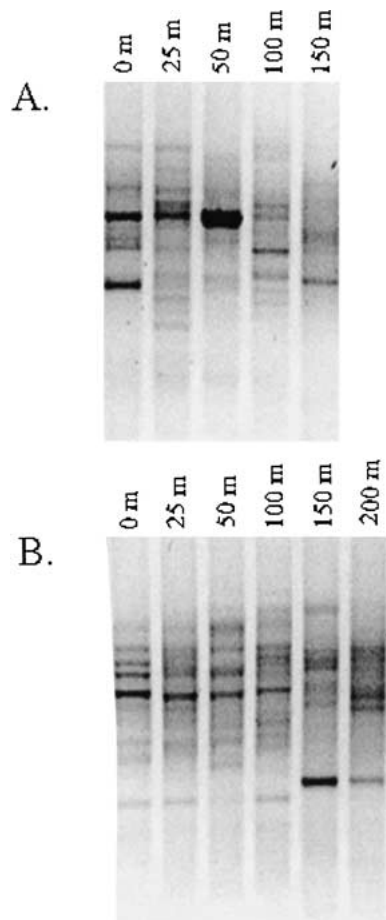


Fig. 6. RISA analysis of (A) Damma and (B) Rotfirn forefield soil bacterial populations after DNA amplification with 16S-23S rRNA gene intergenic spacer primers. Lane labels represent sampling distance in meters from the glacier terminus.

reflects the differences in current site characteristics but is probably also a result of differences in glacial history. Thus, future efforts should be directed toward connecting the microbial ecology of the forefield site with glacial position and soil age. Nevertheless, the diverse bacterial populations detected in the two forefields should alert ecologists to the potential of this environment to facilitate basic ecology research as well as investigation of specific issues such as microorganism–plant community relationships and microbial population evolution.

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