

Bacterial Succession in Glacial Forefield Soils Characterized by Community Structure, Activity and Opportunistic Growth Dynamics

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ABSTRACT

The succession of bacterial communities inhabiting the forefield of the Dammaglacier (Switzerland) was investigated in soils ranging in successional age from 0 to 100 years since deglaciation. Overall activity per bacterial cell was estimated by the amount of fluorescein diacetate (FDA) hydrolyzed per DAPI-stained cell, and an index of “opportunism” was determined from the ratio of culturable to total cells (C:T ratio). Ribosomal intergenic spacer analysis (RISA) was used to estimate the richness of dominant phylotypes and to construct rank-abundance plots of the dominant populations. We observed a biphasic trend in specific cellular activity, which exhibited minima in the 0- and 100-year-old soils while a maximum activity per cell was reached in the 70-y soil. On average, the C:T ratio showed the same trend as the specific activity, although we observed some differences between the two sampling transects. RISA revealed a decrease in dominant phylotype richness as successional age increased, and rank-abundance plots indicated that the evenness of the dominant bacterial phylotypes significantly decreased with successional age. The combination of specific cellular activity and C:T ratio results suggested the presence of an r-K continuum of bacteria while RISA showed that richness and evenness of dominant phylotypes decreased with successional age. We conclude that bacterial succession in the glacier forefield was a dynamic process with adaptation to the differing stages of succession occurring on both the individual and community levels.

Introduction

The forefield of a receding glacier offers the opportunity to investigate several decades of microbial succession over the distance of a few hundred meters. The appeal of this

environment to ecologists is evident in the numerous studies that have focused on plant succession [6, 12, 16]. It is understood that the physical, chemical, and biological gradients characteristic of the forefield soils will have a great impact on the growth strategy and community structure of its inhabitants. One often-observed manifestation of these gradients involves the change in growth

strategy of succession organisms, the r-K continuum [17]. Likewise, it is widely accepted that community structure and organization will change as succession progresses, reflecting the response of the populations to the shifting environmental conditions [34]. Despite the fact that bacteria are the pioneer organisms on these sites [44, 45, 46], few studies have taken advantage of the forefield environment to study the primary succession and population distribution of bacteria.

The use of bacterial populations to study primary succession appears to have several advantages over the use of macroorganisms. For example, the large diversity of bacteria found in soils [54] might exhibit patterns of diversity and activity that are useful for characterizing successional processes. Additionally, sampling is simplified, as many samples of glacial forefield soil can be easily collected, transported, and processed in a relatively short period of time. From each soil sample, a large number of bacteria may be subject to analysis, which in combination with an adequate sampling scheme increases the confidence in achieving a representative sample of the real bacterial population. Analysis of the dominant soil bacterial community is facilitated by the use of molecular fingerprinting methods that have become routine. Moreover, classical methods such as bacteria isolation and enzyme assays are also easily applied and are often necessary to provide a valuable metabolic complement to the largely structure-based molecular approach [24]. In previous studies, we demonstrated that the combination of molecular and traditional methods was useful for characterizing bacterial populations in two Swiss glacier forefields [44, 45]. Using genetic fingerprinting, enzyme assays, and direct bacterial counts, our study showed that the forefield succession was a dynamic but orderly process that combined replacement as well as maintenance of dominant phylotypes.

The characterization of organism metabolic status is an important parameter that helps to define the various stages of primary succession [38]. It has been predicted that early succession communities will be characterized by a life strategy focused on high reproductive capacity of the species (r-selection), leading to unstable populations of low metabolic efficiency. On the other hand, organisms associated with late succession will invest few resources into reproduction and more into maintenance-related activities (K-selection), resulting in a metabolically efficient population [17, 29]. The theory of r- vs K-selection as an indicator of microbial succession has been applied by assessing bacterial culturability as a surrogate for oppor-

tunism, an attribute of r-strategists [18]. The authors concluded that the ratio of opportunistic cells (those able to grow on a nonselective medium) to total cells (detected by microscopic cell counts) was higher in early than in late succession environments, indicating a propensity for cellular reproduction (r) over maintenance (K). This observation reflects the theory of Tilman [52], who suggested that those organisms able to reproduce and grow fastest would have a competitive advantage over slower-growing organisms, but conclusive evidence of bacterial growth strategy in the glacier forefield succession is, as far as we know, unavailable.

In addition to metabolic status, bacterial community structure is also affected by successional stage [9, 14, 36]. Indeed, for plant [41, 56] and mycorrhizae [22] communities, it has been shown that species evenness will increase with succession age. The authors attributed the shift to the rapid colonization of open niches by opportunistic species and to the lack of organic inputs in early succession. Also of particular interest is the biofilm succession study of Jackson et al. [25], which revealed that early succession habitats supported an unorganized community with low species evenness and richness. Late succession was defined by increased habitat variation and resource availability, which consequently led to increased bacterial community evenness and richness, a pattern often observed in forefield plant succession [30].

It is apparent that both opportunistic growth and community structure can be useful indicators of the successional state of a bacterial population. Despite this, a combination of these parameters has remained unapplied in the natural environment. In the present study, we were interested in showing how microbial succession in the glacier forefield environment could serve as a model for the study of evolving bacterial assemblages. Specifically, we tested two hypotheses: first, that the bacterial populations would exhibit a shift from r- to K-strategy as successional age increased; and second, that the species evenness of the most abundant bacteria would follow a trend similar to forefield plant populations, and increase as succession age increased.

Materials and Methods

Site and Soil Description

Soils were harvested from the forefield of the Dammaglacier (8° E 27' 30", 46° N 38' 00") in the Canton of Uri, Switzerland (Fig. 1).

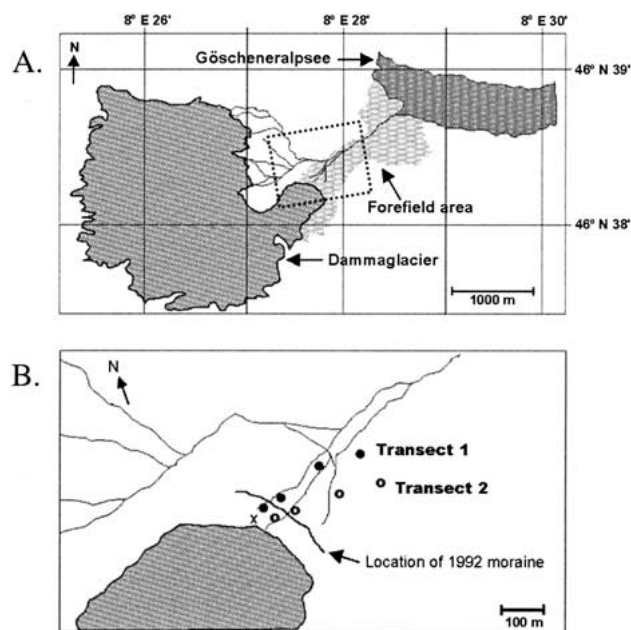


Fig. 1. Maps of the Dammaglaciers region in Göschenalp, Switzerland. (A) General overview of the Dammaglaciers and forefield with the sampling area denoted by the dotted rectangle. (B) Close-up of the sampling transect positions with respect to the glacier terminus. Legend; ×, 0-year soil location; ●, Transect-1 sites; ○, Transect-2 sites.

Two-500 m sampling transects that ran parallel to the forefield were established beginning at the glacier terminus. Approximately 2-kg of soil was collected from each of five sites ([45], Table 1) along each transect by pooling at least six subsamples harvested from a roughly 10 m diameter circle surrounding the given sampling point. To avoid the vertical heterogeneity imparted by soil horizon development, only surface soils (to approximately 5 cm deep) were harvested. The soils were sieved on

site (<2 mm) and processed immediately upon return to the laboratory.

For pH measurement, approximately 5 g of fresh soil was suspended in 15 mL of 0.01 M CaCl₂, stirred for 20 min, and then measured for pH. Total carbon and nitrogen content (%) of the soil was assessed with a LECO 932 CHNS analyzer according to the manufacturer's instructions (LECO, Krefeld, Germany) and total phosphorus (%) was determined with an X-lab 2000 X-ray fluorescence analyzer (Spectro AG, Kleve, Germany, Table 1).

For each soil, the time since deglaciation was inferred from data compiled by the Laboratory of Hydraulics, Hydrology and Glaciology at the Swiss Federal Institute of Technology (Zurich, Switzerland). The elevation of the individual harvest sites and distance from the glacier terminus were determined by barometric pressure (error unknown) and global positioning (± 3 m), respectively.

Estimation of Microbial Number and Activity

Subsamples of soil from each harvest site were treated in triplicate with 4% paraformaldehyde solution in phosphate-buffered saline (pH 7.0) to fix bacterial cells. The total number of bacteria in each soil was estimated following DAPI (4',6-diamidino-2-phenylindole) staining and microscopic counting [57]. Bacterial activity was estimated in triplicate soil samples by monitoring the hydrolysis of fluorescein diacetate (FDA) according to the spectrophotometric method of Adam and Duncan [1] and expressed as μg fluorescein produced g^{-1} dry soil and also cell^{-1} . For each soil assayed, a non-FDA-inoculated soil sample was analyzed in order to account for background coloration as well as a no-soil negative control.

Enumeration of Cultured Cells

The number of cells culturable from each of the forefield soils was estimated by suspending 0.5 g of soil in 5 mL of sterile

Table 1. Site and soil characteristics of the Dammaglaciers forefield

Site	Distance from glacier (m)	Time since deglaciation (y)	Elevation (m.a.m.s.l.) ^b	Soil texture	%C (SE) ^c	%N (SE)	%P	pH
Transect-1								
1 ^a	0	0–1	2053	Coarse sand	0.017 (0.001)	ND ^d	0.052	6.1 (0.06)
2	60	10	2052	Sand	0.047 (0.002)	0.004 (0.004)	0.040	5.4 (0.03)
3	100	46	2058	Fine sand	0.375 (0.009)	ND	0.071	4.2 (0.46)
4	350	70	1985	Fine sand	0.457 (0.001)	0.048 (0.023)	0.078	4.6 (0.01)
5	500	100	1979	Coarse sand	0.558 (0.041)	ND	0.048	4.6 (0.16)
Transect-2								
1 ^a	0	0–1	2053	Coarse sand	0.017 (0.001)	ND	0.052	6.1 (0.06)
2	60	10	2050	Sand	0.082 (0.001)	0.006 (0.001)	0.058	4.9 (0.02)
3	100	46	2052	Sand	0.376 (0.002)	0.036 (0.001)	0.034	4.9 (0.42)
4	350	70	1985	Sand	0.799 (0.009)	0.059 (0.001)	0.048	4.3 (0.01)
5	500	100	1978	Loamy sand	1.384 (0.044)	0.109 (0.002)	0.090	4.5 (0.18)

^a Site 1 is the origin of both transects.

^b (m.a.m.s.l.), Meters above mean sea level.

^c (SE), Standard error of three replications.

^d ND, None detected.

distilled water, vortexing for 1 min, then diluting the soil suspension to 10^{-4} and plating 50 μL of each dilution in triplicate. The media contained, per liter, 1 g carbon (10% from casein hydrolysate, 90% from cellulose), 0.5 g $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.5 g KNO_3 , 1.0 g K_2HPO_4 , 0.06 g $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$, 11.0 g agar, and 100 ppm cycloheximide to prevent fungal growth [23]. Plates containing between 30 and 300 colonies of bacteria were counted following 7 days of growth in the dark at room temperature.

Soil DNA Extraction and Genetic Fingerprinting of Forefield Communities

Approximately 0.6 g of each pooled soil was extracted for DNA in at least triplicate as described previously [44]. The concentration of the extracted DNA was determined by measuring absorbance at 260 nm and purity was estimated from the ratio of $A_{260}:A_{280}$. For ribosomal intergenic spacer analysis (RISA), the spacer region between the small- and large-subunit rRNA and large-subunit rRNA genes was amplified and equally loaded (according to densitometric screening of PCR products on agarose gels) and separated on 5% acrylamide gels according to the method of Borneman and Triplett [5]. Following electrophoresis, staining was performed by gently agitating the gel for 15 min in 50 mL of $1 \times \text{TAE}$ containing a 1:10,000 dilution of SYBR Green dye (Molecular Probes Inc., Eugene, OR). RISA banding patterns were visualized with UV transillumination and photographed using the Gel Doc 2000 gel documentation system (Bio-Rad Laboratories, Hercules, CA).

Bacterial Community Analysis

RISA banding patterns were used to visually distinguish the amplified spacer region sequences. A single band of DNA served as an indicator for a specific spacer region sequence and thus, a specific soil bacterial population (phylotype). Likewise, the band's relative intensity provided information concerning the relative abundance of the particular sequence [20, 33]. For each lane, the banding pattern and average band intensity were analyzed using Quantity One image analysis software (version 4.0, Bio-Rad Laboratories). The average intensity of each band was calculated and compared to the cumulative intensity of all detected bands in the given lane to give an estimate of the relative abundance of the detected sequence types. Evidence of bacterial community changes during the differing stages of succession was visualized by comparing the presence and/or absence of bands between different samples. The maintenance of phylotypes was detected as bands common to two or more adjacent lanes (i.e., soils). Likewise, phylotype replacement was identified by the presence of a band followed by its disappearance from the lane representing the next older soil. The genetic fingerprints of each soil were compared for similarity by calculating a band-based Dice coefficient (S_D),

$$S_D = \frac{2N_C}{(N_Q + N_T)}$$

where N_Q represented the number of bands detected in the query soil, N_T represented the number of bands detected in the test soil, and N_C represented the number of bands common to both soils. Following S_D calculation, fingerprints were clustered with the Quantity One software using the unweighted pair group method with average linkages (UPGMA) [47].

Rank-abundance plots were constructed by plotting, in decreasing order of rank (from the most abundant organism to the least abundant), the \log_{10} of the relative abundance (intensity) of each detected phylotype (band). Each plot was fitted with a linear model so that relative changes in plot slope with succession age could be determined.

Statistics

The results of direct cell counts, culturable cell enumeration, and FDA hydrolysis assays were reported as averages and standard errors over the replications described above. For the rank-abundance plots, the significance in correlation ($p < 0.001$) was calculated and the plots within each transect were tested for significant differences ($p < 0.05$) by applying an analysis of covariance (ANCOVA) to the resultant slopes.

Results

Estimation of Bacterial Number and Activity

Although soils were collected in two transects that were spatially separated by approximately 50 m, soils of similar age were comparable in terms of overall microbial number and activity. Microscopic counts of bacteria revealed that the cell number g^{-1} dry soil increased from a minimum of 3.69×10^7 ($\pm 7.95 \times 10^6$) in 0-y soil to 1.78×10^9 ($\pm 1.00 \times 10^8$) and 2.94×10^9 ($\pm 1.49 \times 10^8$) in the 100-y soils of Transect-1 and -2, respectively (Fig. 2A).

Patterns of FDA hydrolysis showed that in general, microbial activity increased with succession age (Fig. 2B). Specifically, 1.21 (± 0.17) μg fluorescein was produced g^{-1} dry soil in the 0-y soil, while 100-y samples yielded 135.00 (± 7.31) and 201.00 (± 5.15) μg fluorescein g^{-1} dry soil for Transects-1 and -2, respectively. In both transects, activity increased consistently until the 70-y sample, but stabilized (Transect-2) or even decreased (Transect-1) after 70 y.

When calculated on a per-cell basis, we observed an increase in cellular activity from 3.28×10^{-8} in the 0-y soil to 1.31×10^{-7} and 2.15×10^{-7} μg fluor cell^{-1} in the 70-y soils of Transects-1 and -2, respectively. Following this increase, a distinct decrease in specific activity occurred between the 70- and 100-y soils (Fig. 2C), dropping to approximately 7.2×10^{-8} μg fluor cell^{-1} (average of both transects).

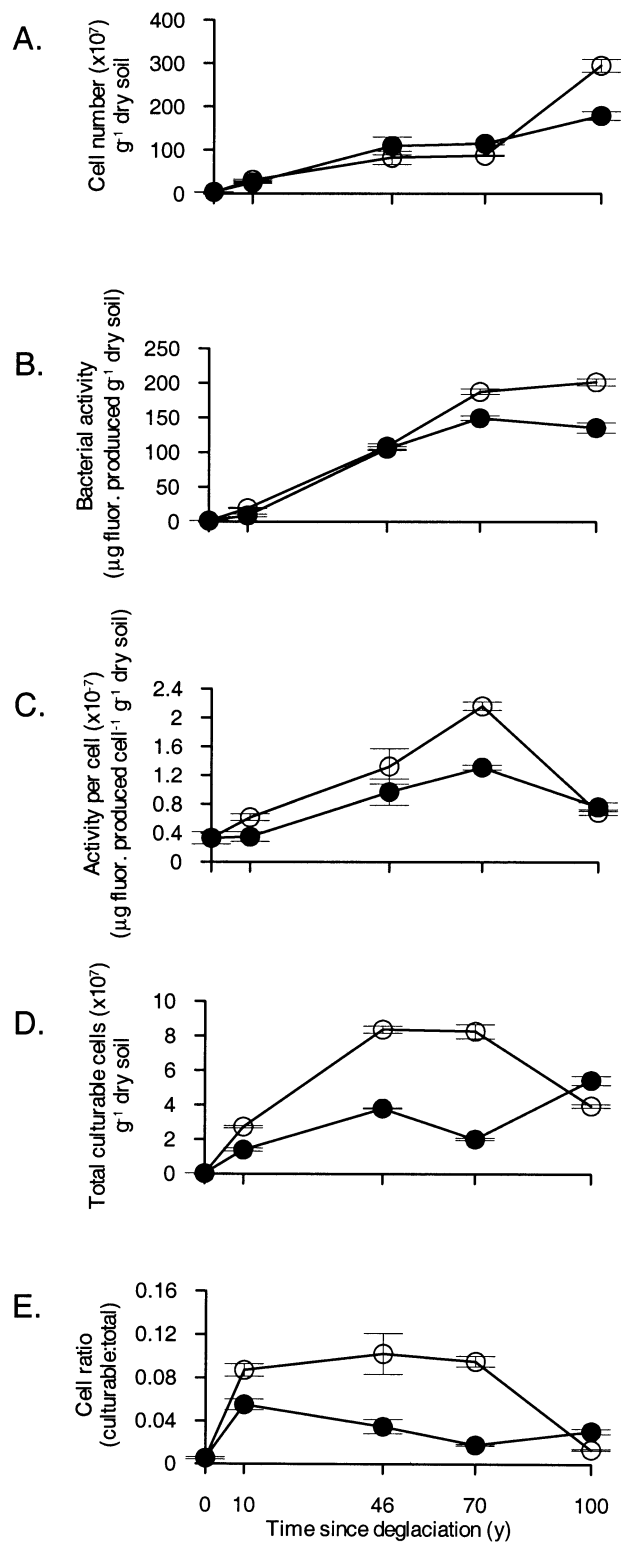


Fig. 2. Trends in (A) bacterial cell number; (B) overall microbial activity; (C) activity per bacterial cell; (D) total culturable bacteria number; and (E) ratio of culturable to total bacteria number, along the two sampling transects in the Dammaglacier forefield. Legend: ●, Transect-1; ○, Transect-2. Error bars represent the standard error over the replications described in Materials and Methods.

Enumeration of Cultured Cells

In both transects, culturable cell numbers increased steadily from 1.89×10^5 CFU g⁻¹ dry soil in the 0-y soil to 3.78×10^7 (Transect-1) and 8.35×10^7 (Transect-2) in the 46-y soil (Fig. 2D). Beyond this site, in Transect-1, CFUs decreased in the 70-y soil to 2.00×10^7 CFU g⁻¹ dry soil, then increased again in the 100-y soil to 5.41×10^7 CFU g⁻¹ dry soil. In Transect-2, the number of CFUs decreased slightly between 46- and 70-y soils (from 8.35×10^7 to 8.24×10^7 CFU g⁻¹ dry soil), then decreased further in the 100-y soil to a final value of 3.93×10^7 CFUs g⁻¹ dry soil.

With the exception of the 0-y soil, the ratio of culturable to total cells (C:T) was relatively high in the younger soils but decreased as successional age increased (Fig. 2E). Specifically, within the first 10 years following deglaciation, the C:T ratio increased from 0.005 (± 0.001) in the 0-y soil to 0.055 (± 0.005) in Transect-1, and 0.087 (± 0.006) in Transect-2. Transect-1 exhibited a maximum C:T ratio in the 10-y soil ($0.055 \pm .005$), while the 46-y soil yielded the highest C:T ratio in Transect-2 (0.097 ± 0.019). As successional age increased beyond the ages of maximum C:T ratio, the ratios in both transects decreased to 0.017 (± 0.001) (Transect-1, 70-y soil) and 0.013 (± 0.001) (Transect-2, 100-y soil), although the C:T ratio between the 70- and 100-y soil in Transect-1 increased slightly to 0.030 (± 0.002).

Bacterial Community Similarity

RISA fingerprinting indicated that several dominant bacterial phylotypes were present in each of the Dammaglacier forefield soils (Fig. 3) and that the community structure changed as successional age increased. The similarity of banding patterns of the forefield soils as determined by S_D showed that the 0-y soil was most similar to the 10-y soil (44% and 45% similar for Transects-1 and -2, respectively), while the similarity to the 0-y soil of the successively older soils tended to decrease with successional age to a minimum of 20% similarity between 100-y and 0-y soil (Transect-1) and 26% for Transect-2 (Fig. 4). The relationship describing the similarity between adjacent soils was consistent for both transects.

Phylotype richness (S) was estimated by the number of detected bands in a given lane and used as an additional parameter to quantify the differences in genetic finger-

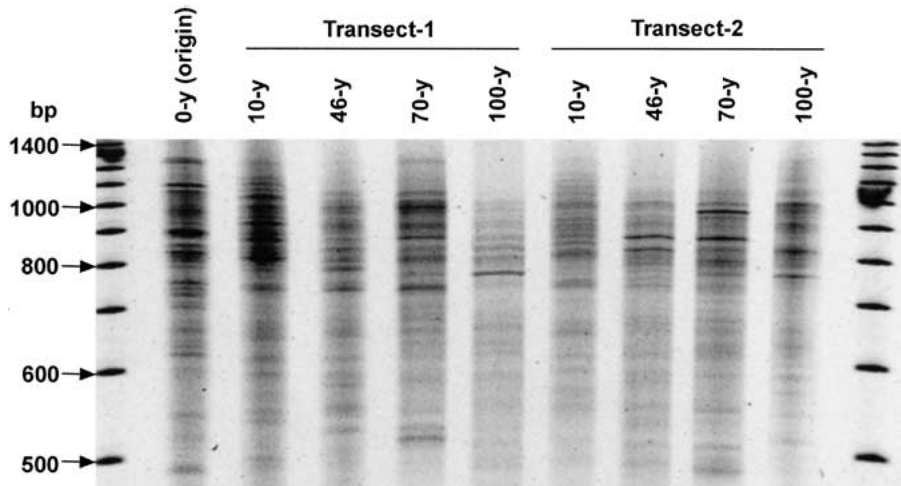


Fig. 3. Gel image of ribosomal intergenic spacer analysis of DNA isolated from the soils of the Dammaglacier forefield. The lanes are labeled with the soil's successional age (e.g., "10-y" represents the soil harvested from 10-year-old soil) and arranged according to the transect from which they were harvested. The outside lanes contain DNA size markers.

prints. Overall, *S* followed a decreasing trend in both transects as successional age increased (Table 2).

Bacterial Community Evenness

Slopes and coefficients of determination (r^2) of the rank-abundance plots showed that early succession communities (0-y soil) demonstrated significantly higher evenness ($P < 0.05$) than late succession communities (70-y and 100-y soils), although the trend in Transect-2 was not as consistent as in Transect-1 (Table 2). Specifically, in the soils of Transect-1, the slopes of the plots increased from a minimum of 0.006 (0-y soil) to 0.014 (100-y soil) (Fig. 5A). The rank-abundance slopes of Transect-2 soils increased

overall to 0.012 in the 100-y soil but exhibited some variation with a maximum of 0.019 in the 46-y soil (Fig. 5B).

Discussion

Bacterial Growth Strategy in the Forefield Succession

Based on the relationship between environmental conditions and bacterial growth strategy, ecologists have divided organisms into two broad categories: r-strategists, and K-strategists. Although defining a single organism in terms of r- or K-strategy is impossible [17], organisms can be compared on a relative basis. De Leij et al. [10] proposed that by assessing the differing growth characteristics of cells on solid media, a relative categorization could be assigned. An application of this methodology was pro-

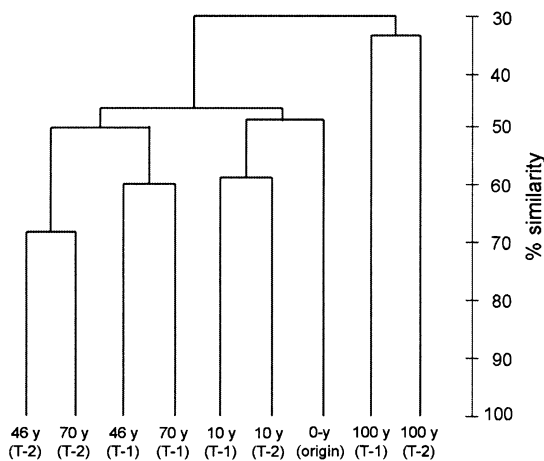


Fig. 4. Cluster analysis of soil bacterial communities as determined by RISA band-based Dice coefficient and unweighted pair group method with average linkages (UPGMA) from Transect-1 (T-1), and Transect-2 (T-2). The successional age of the soil is labeled as in Fig. 3.

Table 2. Rank-abundance plot statistics and phylotype richness (*S*) of bacteria in the Dammaglacier forefield

Site	Slope ^b	r ²	S
Transect-1			
1 ^a	0.006 ^a	0.98	18
2	0.006 ^a	0.99	17
3	0.009 ^b	0.96	13
4	0.011 ^c	0.92	14
5	0.014 ^c	0.82	12
Transect-2			
1 ^a	0.006 ^a	0.98	18
2	0.009 ^b	0.83	13
3	0.019 ^c	0.82	11
4	0.008 ^{bd}	0.82	16
5	0.012 ^{bc}	0.93	13

^a Site 1 is the origin of both transects.

^b Values superscripted with the same letter are not significantly different ($p < 0.05$) as determined by analysis of covariance. All curves were fitted with a linear model with highly significant correlations ($p < 0.001$).

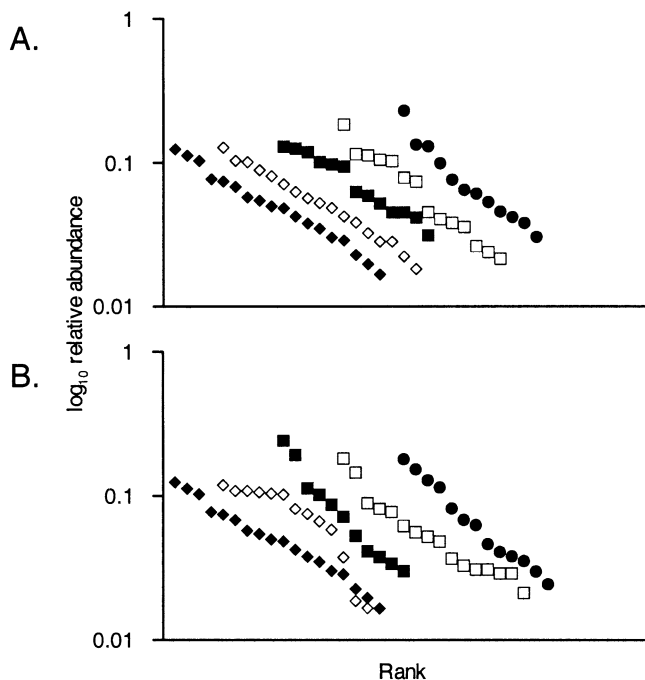


Fig. 5. Rank-abundance plots of estimated phylotype abundance data generated from RISA gels for soils of (A) Transect-1 and (B) Transect-2 of the Dammaglacier forefield. The plots have been artificially separated along the x-axis for clarity. Legend; \blacklozenge , 0-year soil; \diamond , 10-year soil; \blacksquare , 46-year soil; \square , 70-year soil; \bullet , 100-year soil.

vided by Garland et al. [18], who suggested that the ratio of culturable cells to total cells (C:T ratio) described the differing stages of bacterial succession in a bioreactor and in the wheat rhizosphere. The general theory behind this type of approach is that the ability of a bacterium to grow on a “nonselective” medium represents an opportunistic growth strategy. Based on the definitions outlined above, r-strategists inhabiting the glacier forefield would be highly culturable. Conversely, K-strategists, cells adapted to high-density sites where high reproductive potential is not a priority, would be more difficult to culture.

In the current study, the ratio of culturable cells to total DAPI-stained cells (C:T) along the Dammaglacier forefield displayed a trend indicative of a r-K continuum (Fig. 2E). After an initial increase in C:T ratio during the first 10 years following deglaciation, the ratio in both transects decreased as successional age increased, indicating an apparent shift from r- to K-strategy. Within the first 10 years after deglaciation, it appeared that microbial populations experienced rapid increases in opportunistic character. These cells were able to reproduce in a relatively extreme environment (in terms of temperature, moisture, and nu-

trients) resulting in an increase in total cells (Fig. 2A) and culturable cells relative to total cells. Following this initial period of r-strategy, stabilization (Transect-2) or even a decrease (Transect-1) of C:T ratio occurred, as populations appeared to shift to K-type growth strategy. It is likely that the observed shift was the result of predictable changes in the environment that were paralleled by a shift in the population’s opportunistic attributes. One explanation for this observation might be that succession selected for organisms that allocated a greater proportion of resources into maintenance relative to growth [34], resulting from the onset of species competition for food and space [17].

Shifts in Bacterial Population Activity

Based on the amount of FDA hydrolysis per cell as an indicator of general activity, our results showed that the specific activity of the forefield bacterial community increased during 70 years of succession, then decreased in the 100-y soil (Fig. 2C), indicating a shift in activity of the community as a whole. FDA is hydrolyzed by a variety of enzymes including lipases, esterases, and proteases [21, 39]. The increase in FDA activity over the first 70 years could have resulted from an increase in the amount or variety of enzymes released by the bacterial community in response to an increased diversity of plant species (carbon sources) in the forefield [48].

Although the relationship between growth strategy and overall activity was difficult to determine, in both transects it was apparent that cells were involved in activities beyond those required for opportunistic growth. For example, overall activity continued to increase for 70 years following deglaciation while the C:T ratio was relatively stable between 10- and 70-y soils (Transect-2) or decreased (Transect-1), suggesting that the shift in growth strategy was, in part, independent of overall activity. A shift in overall activity could result from conditions greatly differing from those impacting a shift in growth characteristics (r- vs K-strategy), as those actions directly concentrated toward resource allocation and growth strategy comprise a fraction of the total number of metabolic activities possible.

We are aware of the diversity of organisms capable of performing FDA hydrolysis, especially fungi [19], which could compromise our use of the parameter as a bacterial activity measurement. However, we are confident that primarily bacteria accounted for the activity in our assay, as following both visible-light microscopic examination as

well as direct FDA staining of soil microorganisms [49], no fungal hyphae were observed. Furthermore, using fungal-specific primers in PCR assays of the forefield soil DNA samples (in addition to positive controls of mixed fungal assemblage DNA and appropriate negative controls), no evidence of fungi was detected (data not shown).

Molecular Assessment of Bacterial Communities

Succession implies a directional change in the organization of community members [34]. Thus, changes in the molecular fingerprint banding patterns were expected to reflect the succession of the most competitively fit bacterial communities. Analysis of the between-site similarity was provided by the Dice coefficient (S_D), which showed not only that the communities of adjacent soils were relatively similar, but also that soils of similar age, but in differing transects, were similar. This suggests that although the bacterial population distribution in the Damaglacier forefield has been previously characterized as heterogeneous [44, 45], succession was progressing in a somewhat orderly manner. In the investigated transects, a total of 31 bands (Transect-1) and 25 bands (Transect-2) were in common among the soils from adjacent sites, indicating the survival of several phylotypes from one succession period to the next. The replacement of phylotypes was apparent as well, totaling 31 occurrences in Transect-1, and in Transect-2. Thus, the observation of RISA bands not only served as a convenient measure of phylotype maintenance and replacement, it also showed, through the calculation of S_D , that forefield bacterial succession of dominant types was an ordered process.

It is important to remember that DNA extraction and molecular fingerprinting methods are generally biased toward the most abundant organisms in an ecosystem [28, 32]. Thus, descriptive parameters such as visual analysis of band patterns, similarity coefficients, and species richness and evenness must not be viewed as absolute descriptors of a bacterial population, but as trends describing dominant members of the community. RISA analyzes the length heterogeneity of the 16S-23S ribosomal intergenic spacer, and the separation of the resulting PCR products can provide a useful discrimination of soil bacterial phylotypes [5]. Although biases are often associated with PCR amplification of mixtures of DNA templates [50, 55], band intensity will often relate to initial population numbers [20] and has been frequently related to relative phylotype abundance [11, 27, 31, 33, 44]. Pianka [37] proposed that

the early succession environment was an “ecologic vacuum” in contrast to the organism-“saturated” late succession environment. In both situations, the number of bacterial species in minor abundance will be in great excess over the number of dominant ones [2]. Nevertheless, it has also been shown that the numerically dominant bacteria species will often be the most active [13] and thus might direct many of the biological processes that drive succession.

Bacterial Evenness Trends in the Forefield Succession

Rank-abundance plots have been used previously to describe a variety of organism/environment combinations including slime molds in cold deserts [42], plants in soil [56], and invertebrates in freshwater streams [48] and rice fields [43]. The slope of the plot gives an indication of the evenness of the detected community. A horizontal plot (slope = 0) suggests that all organisms are detected in similar abundance, and thus perfect community evenness. Conversely, a steeply sloping plot results from a community exhibiting high, moderate, and low organism abundance, and thus indicates lower relative evenness. The use of rank-abundance plots to describe bacterial communities has remained rare, probably because of the complexities involving analysis. A notable exception is the work of Jackson et al. [25], who generated rank-abundance plots based on banding patterns of DNA fingerprints of biofilm succession. They showed, overall, that the evenness of the bacterial community became greater as succession progressed. Our observations of forefield succession revealed the opposite trend. In both transects, the slope of the rank-abundance plots significantly increased from the 0-y to 100-y soil ($p < 0.05$) (Fig. 5, Table 2). These data, combined with the band-based estimation of species richness, suggested that not only did the number of dominant organism types decrease with succession, so did the community evenness. Our previous work in this forefield also indicated decreased diversity of dominant phylotypes with succession age [44], but the current study improved on the former by extending the sampling to soils of greater successional age, thus encompassing a greater proportion of the succession sequence. Although this observation is in disagreement with most theories of succession that predicted increases in evenness with succession age, we propose the following explanation. Since bacteria of low relative abundance and broad niche specialization characterize the early succession environment, a state of low

spatial competition and nutrient surplus may prevail. This is because low resource consumption resulting from low population density can potentially result in adequate to high nutrient availability [7]. Under these conditions, both dominance and evenness in those dominant populations is possible, which is reflected in the relatively high estimations of species richness and evenness for the 0-y soil. The opposite is true for late succession communities, where high cellular density, competition for space and resources, and narrow niche specialization create dominance-limiting conditions. For example, the relatively nutrient-rich late succession soils exhibited an approximately 100-fold increase in bacterial abundance over the youngest soil but only a 30-fold (Transect-1) or 80-fold (Transect-2) increase in total carbon, resulting in a decrease in the carbon-to-cell ratio. Thus, as exhibited by the 100-y soils, a decreased number as well as a shift toward an uneven distribution of dominant types occurred.

Often, rank-abundance plots of a succession series will be fitted to a specific distribution model in order to demonstrate differences in community organization. We have avoided this practice, as apparent changes in distribution as described by model fitting might not reflect significant changes in patterns of abundance [53].

A Call for a New Model

It is apparent that the use of conventional succession theories that are based upon observation of plant populations to describe bacterial succession should be questioned and further examined. In order to accurately describe the succession of bacterial communities, fundamental differences between bacteria and plants developing in succession environments must be addressed. The physical attributes of the plants themselves (dispersivity of individuals, longevity, maturation time, ultimate size) are often component of many plant succession theories, and one might expect that these same attributes could be applied to bacteria succession as well. However, these criteria appear to be restricted to plant succession and are likely not pertinent to bacterial communities. For instance, seed dispersal is a major mechanism for the translocation and survival of succession plant species [6]. Dispersal of soil bacteria might not be of significance in the forefield environment because they are often resistant to translocation, owing to the production of adhesive exopolymeric substances, and also to their preferred location in small, protected pore spaces [35], although wind- and rain

splash-aided dispersal [26] as well as earthworm-mediated translocation [8] of bacteria is known to occur. Next, an increase in plant size (up to several orders of magnitude for primary vs late succession species) is often associated with increased succession age [15]. Because bacteria living in oligotrophic environments will optimize their surface-to-volume ratio through a decrease in size, one would expect bacteria present in late succession soils to also follow this trend and thus exhibit an increased biovolume relative to bacteria in early succession soils. This hypothesis was rejected by Battin et al. [4], who used flow cytometry to show that there was no difference in cell size distribution along a glacial stream chronosequence. Furthermore, our microscopic observations of Dammaglacier forefield soil bacteria confirmed this finding, as we could not visually detect differences in cell sizes based on soil age (data not shown). Finally, the importance of longevity as a discriminatory characteristic of succession plants has also been proposed [1]. Again, plants may exhibit up to two orders of magnitude of age difference along a succession sequence, but bacterial turnover in soil is most likely occurring in a period of days and such large variation in turnover rate has not been observed in soils [3].

Based on this evidence, it is apparent that alternative criteria are necessary to describe the succession of bacteria populations. Whereas the visualization, counting, and estimation of plant species and individuals can be performed with relative accuracy, the concept of individuals in microbial ecology is obscure [57] and necessitates the need for future research to address methods useful in abundance estimation, especially of low-abundance species.

Conclusions

We have shown that bacterial assemblages in the glacier forefield environment have the potential to yield valuable information concerning primary succession. The succession of dominant bacterial phylotypes was dynamic yet orderly, exhibiting detectable changes during post-glaciation where noticeable changes in plant populations were undetected (e.g., 0-y soil vs 10-y soil, plant data unpublished). Based on the results presented here, we accept our first hypothesis, as there was an apparent shift from r- to K-strategists with successional age. Although the shift in C:T ratio could simply indicate a metabolic change at the cellular level caused by adaptation in resource allocation strategy, the results of molecular fingerprinting suggested

that community-level changes were also occurring. Increased community evenness was associated with increased successional age and was opposite to the trend commonly observed in plant populations. Thus, we reject our second hypothesis. We are aware that the co-occurrence of an r- vs K-continuum and a community structure change does not imply that the two factors are functionally correlated. However, it does reveal for the first time the reorganization of succession populations of bacteria from both a structural and functional perspective.

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References

- Adam G, Duncan H (2001) Development of a rapid and sensitive method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol Biochem* 33:943–951
- Atlas RM (1984) Diversity of microbial communities. In: Marshall KC (ed) *Advances in Microbial Ecology*, vol 7. Plenum Press, New York pp 1–47
- Baath E (1998) Growth rates of bacterial communities in soils at varying pH: a comparison of the thymidine and leucine incorporation techniques. *Microb Ecol* 36:316–327
- Battin TJ, Wille A, Sattler B, Psenner R (2001) Phylogenetic and functional heterogeneity of sediment biofilms along environmental gradients in a glacial stream. *Appl Environ Microbiol* 67:799–807
- Borneman J, Triplett EW (1997) Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Appl Environ Microbiol* 63:2647–2653
- Chapin FS, Walker LR, Fastie C, Sharman LC (1994) Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecol Mono* 64:149–175
- Cheeson P, Huntley N (1997) The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *Amer Nat* 150:519–553
- Clegg CD, Anderson JM, Lappin-Scott HM, van Elsas JD, Jolly JM (1995) Interactions of a genetically modified *Pseudomonas fluorescens* with the soil-feeding earthworm *Octolasion cyaneum* (Lumbricidae) *Soil Biol Biochem* 27:1423–1429
- Dang H, Lovell CR (2000) Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes. *Appl Environ Microbiol* 66:467–475
- DeLeij FAAM, Whipps JM, Lynch JM (1993) The use of colony development for the characterization of bacterial communities in soil and on roots. *Microb Ecol* 27:81–97
- Eichner CA, Erb RW, Timmis KN, Wagner-Döbler I (1999) Thermal gradient gel electrophoresis analysis of bioprotection from pollutant shocks in the activated sludge microbial community. *Appl Environ Microbiol* 65:102–109
- Erschbamer B, Bitterlich W, Raffl C (1999) The vegetation as an indicator of soil development on the glacier foreland of the Rotmoosferner (Obergurgl, Oetzal, Northern Tyro) *Ber Naturwiss Med Wereins Innsbrook* 86:107–122
- Felske A, Akkermans ADL (1998) Spatial homogeneity of abundant bacterial 16S rRNA molecules in grassland soils. *Microb Ecol* 36:31–36
- Felske A, Wolterink A, van Lis R, de Vos WE, Akkermans ADL (2000) Response of a soil bacterial community to grassland succession as monitored by 16S rRNA levels of the predominant ribotypes. *Appl Environ Microbiol* 66:3998–4003
- Finegan B (1984) Forest succession. *Nature* 312:109–114
- Frenot Y, Gloaguen JC, Cannavacciuolo M, Bellido A (1998) Primary succession on glacier forelands in the subantarctic Kerguelen Islands. *J Veg Sci* 9:75–84
- Gadgil M, Solbrig OT (1972) The concept of r- and K selection: evidence from wildflowers and some theoretical considerations. *Amer Nat* 106:14–31
- Garland JL, Cook KL, Adams JL, Kerkhof L (2001) Culturability as an indicator of succession in microbial communities. *Microb Ecol* 42:150–158
- Gaspar ML, Cabello MN, Pollero R, Aon MA (2001) Fluorescein diacetate hydrolysis as a measure of fungal biomass in soil. *Cur Microbiol* 42:339–344
- Gelsomino A, Keijzer-Wolters AC, Cacco G, van Elsas JD (1999) Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. *J Microbiol Meth* 38:1–15
- Guilbaut GC, Kramer DN (1964) Fluorometric determination of lipase, acylase, alpha- and gamma-chymotrypsin and inhibitors of these enzymes. *Anal Chem* 36:409–412
- Helm DJ, Allen EB, Trappe JM (1996) Mycorrhizal chronosequence near Exit Glacier, Alaska. *Can J Bot* 74:1496–1506
- Hu S, van Bruggen AHC (1997) Microbial dynamics associated with multiphasic decomposition of ¹⁴C-labeled cellulose in soil. *Microb Ecol* 33:134–143
- Jackson CR, Roden EE, Churchill PF (1998) Changes in bacterial species composition in enrichment cultures with various dilutions of inoculum as monitored by denaturing gradient gel electrophoresis. *Appl Environ Microbiol* 64:5046–5048
- Jackson CR, Churchill PF, Roden EE (2001) Successional changes in bacterial assemblage structure during epilithic biofilms development. *Ecology* 82:555–566

26. Jones DG (1998) *The Epidemiology of Plant Diseases*. Kluwer Academic Publishers, Dordrecht, The Netherlands
27. Konopka A, Bercot T, Nakatsu C (1999) Bacterioplankton community diversity in a series of thermally stratified lakes. *Microb Ecol* 38:126–135
28. Kozdroj J, van Elsas JD (2000) Application of polymerase chain reaction-denaturing gradient gel electrophoresis for comparison of direct and indirect extraction methods of soil DNA used for microbial fingerprinting. *Biol Fert Soils* 31:372–378
29. MacArthur RH, Wilson EO (1967) *The Theory of Island Biogeography*. Princeton University Press, Princeton, NJ
30. Matthews JA (1992) *The Ecology of Recently Deglaciated Terrain: A Geological Approach to Glacier Forelands and Primary Succession*. Cambridge University Press, Cambridge, UK
31. Murray A, Hollibaugh JT, Orrego C (1996) Phylogenetic compositions of bacterioplankton from two California estuaries compared by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Appl Environ Microbiol* 62:2676–2680
32. Nakatsu CH, Torsvik V, Ovreas L (2000) Soil community analysis using DGGE of 16S rDNA polymerase chain reaction products. *Soil Sci Soc Am J* 64:1382–1388
33. Nübel U, Garcia-Pichel F, Kühl M, Muyzer G (1999) Quantifying microbial diversity: morphotypes, 16S rRNA genes and carotenoids of oxygenic phototrophs in microbial mats. *Appl Environ Microbiol* 65:422–430
34. Odum EP (1969) The strategy of ecosystem development. *Science* 164:262–270
35. Paul EA, Clark FE (1996) *Soil Microbiology and Biochemistry*. Academic Press, San Diego
36. Pennanen T, Strömmer R, Markkola A, Fritze H (2001) Microbial and plant community structure across a primary succession gradient. *Scand J For Res* 16:37–43
37. Pianka ER (1970) On r- and K-selection. *Amer Nat* 104:592–597
38. Pickett STA (1976) Succession: an evolutionary interpretation. *Amer Nat* 110:107–119
39. Rotman B, Papermaster BW (1966) Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic esters. *Proc Natl Acad Sci USA* 55:134–141
40. Schauer M, Massana R, Pedros-Alio C (2000) Spatial differences in bacterioplankton composition along the Catalan coast (NW Mediterranean) assessed by molecular fingerprinting. *FEMS Microbiol Ecol* 33:51–59
41. Schipper LA, Degens BP, Sparling GP, Duncan LC (2001) Changes in microbial heterotrophic diversity along five plant successional sequences. *Soil Biol Biochem* 33:2093–2103
42. Schnittler M (2001) Ecology of myxomycetes of a winter-cold desert in western Kazakhstan. *Mycologia* 93:653–669
43. Schoenly KG, Justo Jr HD, Barrion AT, Harris MK, Bottrell DG (1998) Analysis of invertebrate biodiversity in a Philippine farmer's irrigated rice field. *Environ Entomol* 27:1125–1136
44. Sigler WV, Zeyer J (2002) Microbial diversity and activity along the forefields of two receding glaciers. *Microb Ecol* 43:397–407
45. Sigler WV Molecular and cultural assessment of copiotrophic bacteria in the forefield of a receding glacier. In: Bottarin R, Tappeiner U (eds) *Interdisciplinary Mountain Research*. Blackwell Science, Berlin
46. Smith RIL (1991) Bryophyte propagule banks: a case study of an Antarctic fellfield soil. In: Miles J, Walton DH (eds) *Primary Succession on Land*. Blackwell Scientific Publications, Oxford pp 123
47. Sneath PHA, Sokal RR (1973) *Numerical Taxonomy*. W. H. Freeman Co., San Francisco
48. Sponseller RA, Benfield EF, Valett HM (2001) Relationships between land use, spatial scale and stream macroinvertebrate communities. *Freshwat Biol* 46:1409–1424
49. Stephan A, Meyer AH, Schmid B (2000) Plant diversity affects culturable soil bacteria in experimental grassland communities. *J Ecol* 88:988–998
50. Suzuki MT, Giovannoni SJ (1996) Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl Environ Microbiol* 62:625–630
51. Tiedje JM, Asuming-Brempong S, Nüsslein K, Marsh TL, Flynn SJ (1999) Opening the black box of soil microbial diversity. *Appl Soil Ecol* 13:109–122
52. Tilman D (1985) The resource ratio hypothesis of succession. *Amer Nat* 125:827–852
53. Tokeshi M (1993) Species abundance patterns and community structure. *Adv Ecol Res* 24:111–186
54. Torsvik V, Goksoyr J, Daae FL (1990) High diversity in DNA of soil bacteria. *Appl Environ Microbiol* 56:782–787
55. Wintzingerode FV, Goebel UB, Stackebrandt E (1997) Determination of microbial diversity in environmental samples: Pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev* 21:213–229
56. Wilson JB, Wells TCE, Trueman IC, Jones G, Atkinson MD, Crawley MJ, Dodd ME, Silvertown J (1996) Are there assembly rules for plant species abundance? An investigation in relation to soil resources and successional trends. *J Ecol* 84:527–538
57. Zarda B, Hahn D, Chatzinotas A, Schoenhuber W, Neef A, Amann R, Zeyer J (1997) Analysis of bacterial community structure in bulk soil by *in situ* hybridization. *Arch Microbiol* 168:185–192