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ORIGINAL ARTICLE

Bacterial succession in a glacier foreland of the High Arctic

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Succession is defined as changes in biological communities over time. It has been extensively studied in plant communities, but little is known about bacterial succession, in particular in environments such as High Arctic glacier forelands. Bacteria carry out key processes in the development of soil, biogeochemical cycling and facilitating plant colonization. In this study we sampled two roughly parallel chronosequences in the foreland of Midre Lovén glacier on Svalbard, Norway and tested whether any of several factors were associated with changes in the structure of bacterial communities, including time after glacier retreat, horizontal variation caused by the distance between chronosequences and vertical variation at two soil depths. The structures of soil bacterial communities at different locations were compared using terminal restriction fragment length polymorphisms of 16S rRNA genes, and the data were analyzed by sequential analysis of log-linear statistical models. Although no significant differences in community structure were detected between the two chronosequences, statistically significant differences between sampling locations in the surface and mineral soils could be demonstrated even though glacier forelands are patchy and dynamic environments. These findings suggest that bacterial succession occurs in High Arctic glacier forelands but may differ in different soil depths.

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Introduction

There is compelling evidence that glaciers are retreating in many mountainous areas of the world due to global warming, and, if left unabated, up to one quarter of the existing mountain glacier ice will disappear by 2050 (Fitzharris, 1996). Indeed, Arctic ecosystems may be disproportionately affected by global warming because average Arctic temperatures have increased at almost twice the global average rate over the past 100 years (Bernstein *et al.*, 2007). As glacier retreat occurs, terrestrial habitats are exposed, and the bacteria present have key roles in the ensuing development of soil, biogeochemical cycling and facilitating colonization by plants, but little is known about these processes.

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Succession is simply defined as changes in biological communities over time (Begon *et al.*, 1996; Brown and Lomolino, 1998). Most studies of biological succession in glacier forelands have focused on plant communities (Godwin, 1929; Huston and Smith, 1987; Drake, 1991; Matthews, 1992; del Moral and Jones, 2002; Hodkinson *et al.*, 2003) and only a few studies have investigated succession in animal (Kaufmann, 2001; Hodkinson *et al.*, 2003) and microbial communities (Sigler and Zeyer, 2002; Sigler *et al.*, 2002; Jumpponen, 2003; Tscherko *et al.*, 2003; Nicol *et al.*, 2005). Previous efforts to assess changes in the structure of bacterial communities in Arctic soils over time have been limited by the analytical methods used. Most studies have employed microscopy (Vestal, 1993; Wynn-Williams, 1993) or assessed changes in biomass and catabolic potential (Walker and del Moral, 2003). For example, Bardgett (2000) found that bacterial biomass is large compared to fungal biomass in the early stages of succession in glacial moraines, but fungal biomass increases over time, probably due to their higher tolerance for low pH.

Such studies are valuable because they give an indication of which organisms dominate a given environment, and if used in conjunction with respiration measurements, insight to the energy flow through the ecosystem can be obtained (Insam and Haselwandter, 1989). However, no information is gained on changes in community structure over time and, therefore, these studies provide little insight to bacterial succession *per se*. For example, although the bacterial biomass may decrease over time it cannot be assumed that the species composition or the rank abundances of species remain unchanged over time. In addition, a decrease in biomass does not necessarily imply that functionally important members have been lost from the ecosystem. Finally, although light microscopy is informative for some groups of bacteria such as cyanobacteria (Miles and Whalton, 1993), this is generally not the case for the large majority of bacterial species that cannot be distinguished based on cell morphology alone.

Some studies to examine microbial succession in glacier forelands have used molecular methods to assess changes in microbial community structure (Schipper *et al.*, 2001; Sigler and Zeyer, 2002; Sigler *et al.*, 2002; Jumpponen, 2003; Nicol *et al.*, 2005; Nemergut *et al.*, 2007), functional diversity (Ohtonen *et al.*, 1999; Deiglmayr *et al.*, 2006; Kandeler *et al.*, 2006) or enzyme activity over time (Tscherko *et al.*, 2003). Studies on bacterial community composition based on the analysis of 16S rRNA gene sequences have shown an increase in phylotype diversity over time following glacier retreat (Nemergut *et al.*, 2007), whereas others have found the opposite (Sigler and Zeyer, 2002; Sigler *et al.*, 2002). The latter findings suggest that the development of bacterial species richness may be the opposite of that observed in plant succession where species richness increases over time (Godwin, 1929; Huston and Smith, 1987; Drake, 1991; Matthews, 1992; del Moral and Jones, 2002; Hodkinson *et al.*, 2003). Bacterial activity was found to transiently increase and then decrease over time (Schipper *et al.*, 2001; Sigler *et al.*, 2002), and functional diversity reaches a steady state after 50 years (Tscherko *et al.*, 2003). Although informative, the relevance of these studies to succession in soils of the High Arctic is not known (Hodkinson *et al.*, 2003).

In this study we sought to determine whether bacterial succession occurs in soils of a glacier foreland in the High Arctic. However, assessing patterns of bacterial diversity in soils is complicated by the heterogeneous nature of these habitats and the patchy distribution of microorganisms within them (Green *et al.*, 2004; Horner-Devine *et al.*, 2004; Green and Bohannan, 2006). This patchiness has been demonstrated on different spatial scales ranging from a few micrometers to meters (Grundmann and Normand, 2000; Oda *et al.*, 2003; Noguez *et al.*, 2005). As a result of the patchiness that occurs on both vertical and horizontal scales in soils, systematic sampling designs including large numbers of replicates are needed to establish whether bacterial succession occurs in terrestrial ecosystems before the patterns observed can then be linked to possible causes. To overcome the difficulties of high spatial heterogeneity in these landscapes, we intensively sampled locations in two roughly parallel chronosequences that represent six time intervals since the glacier receded. The bacterial communities in a total of 117 soil samples were compared based on profiles of terminal restriction fragment length polymorphisms (T-RFLP) of 16S rRNA genes (Liu *et al.*, 1997). Well-established log-linear statistical models were used to test for significant differences among bacterial communities at different stages following glacier retreat, between chronosequences and between soil depths.

Materials and methods

Study site and sampling

The glacier foreland of Midre Lovén glacier near the settlement Ny-Ålesund, West Spitsbergen (74° 81′ N; 10° 35′ E) was chosen as a field site to study bacterial succession. Previous studies characterized the vegetation, invertebrates and soil development (Hodkinson *et al.*, 2001, 2002, 2003). Samples were taken in the beginning of August 2003 along the chronosequence established by Hodkinson *et al.* (2003) and along a second roughly parallel chronosequence located about 25 m away. The second chronosequence was designed to account for differences in bacterial community structure caused by horizontal variation. The drainage streams had most likely not influenced the second chronosequence, and the sampling locations represented the appropriate time stages. We assumed that the two chronosequences were independent of each other because bacterial cells have a cell diameter of only about 1 μm and in comparison, 25 m, the distance between the two chronosequences, can be considered far. Both chronosequences included six sites with a total distance of about 1 km and the distance between sites being roughly 100–200 m. The total time period covered was 150 years and sites 1–6 representing times since glacier retreat of 5, 19, 40, 63, 100 and 150 years, respectively. Hodkinson *et al.* (2003) had previously determined the time since deglaciation of each sampling location using photographs and radiocarbon analysis.

At each sampling location a transect 5 m long was established mostly in the direction of the main chronosequence along which five samples were taken 1 m apart. Each sample had a surface area of $10 \times 10\,cm^2$. Larger gravel was removed before samples were taken. Each sample was divided into a 'surface layer' and a 'mineral soil' subsamples so that differences on a vertical scale could be determined. The surface layer contained a mixture

of vegetation, rhizosphere and bulk soil and did not vary in thickness among sampling sites, whereas the bottom layer included the mineral soil up to 4 cm below the rhizosphere. Mineral soil subsamples were sieved using a mesh with a diameter of 2 mm. In total, 120 samples were collected. They were placed in plastic bags and kept on ice during transport to the laboratory, then stored at −80 °C until they were analyzed.

T-RFLP analysis

Genomic DNA was isolated from 0.5 g of soil samples using a modified procedure based on the UltraClean Fecal DNA Kit (MoBio, Carlsbad, CA, USA). The soil was weighed into sterile 2 ml tubes containing 0.5 g of silicate glass beads (Glenn Mills, Clifton, NJ, USA) and 750 µl of TE (1 mM Tris and 50 mM EDTA) were added. We enzymatically lysed cells using 50 µl lysozyme (10 mg ml^{-1}; Sigma-Aldrich, St Louis, MO, USA) and 25 µl of mutanolysin (2 mg ml^{-1}; Sigma-Aldrich). Afterwards, 60 µl of the solution S1 (lysis solution; MoBio) and 200 µl of IRS (Inhibitor Removal Solution; MoBio, USA) were added followed by a bead-beating step for 3 min at full speed (Biospec Products Inc., Bartlesville, OK, USA). The tubes were centrifuged for 4 min at 13 000 *g* and 450 µl of the supernatants were transferred to a new 2 ml tube before following the protocol from MoBio. The column was incubated for 5 min before eluting the DNA using 50 µl deionized water pH 7, heated to 90 °C. As positive controls, we used cells of *Escherichia coli* K12 MG1655 and *Lactobacillus aviaris* ATCC 43232. The DNA from all samples was cleaned using the Wizard PCR purification kit (Promega, Madison, WI, USA).

The16S rRNA genes in the genomic DNA samples were amplified. PCR reactions with a total volume of 50 µl contained a final concentrations of 1 × buffer (Applied Biosystems, Foster City, CA, USA), 3 mM $MgCl_2$ (Applied Biosystems), 480 µg ml^{-1} bovine serum albumin (BSA; Roche Applied Science, Indianapolis, IN, USA), 200 µM dNTP (Amersham Bioscience, Piscataway, NJ, USA), 0.2 µM forward primer 8fm (AGAGTTTGATCMTGGCTCAG) labeled with VIC, 0.2 µM reverse primer 926r (CCGTCA ATTCCTTTRAGTTT) labeled with 6-carboxyfluorescein, 0.02 U µl^{-1} Taq DNA polymerase (Applied Biosystems), 35.2 µl of PCR-grade water and finally 1.0 µl of DNA template, in a total volume of 50 µl. Amplification of 16S rRNA genes was done using an initial denaturation step at 94 °C for 4 min, followed by 34 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and an extension at 72 °C for 2 min. The final extension was 10 min step at 72 °C. PCR products were cleaned using QIAquick PCR purification kits (QIAgen, Valencia, CA, USA).

Amplicons were digested in 20 µl reactions. For *Alu*I, the mixture contained 2.5 µl of buffer, 0.5 µl of restriction enzyme (10 U µl^{-1}; Promega), 40–50 ng of amplicon and ddH_2O to adjust the total volume to 20 µl. For *Hpa*II, the mixture contained 2.3 µl of buffer, 0.2 µl of 20 mg ml^{-1} BSA, 0.5 µl of restriction enzyme (10 U µl^{-1}; Invitrogen, Carlsbad, CA, USA), 40–50 ng of amplicon and ddH_2O to adjust the total volume to 20 µl. For both enzymes, the digests were incubated in the PTC-100 thermal-cycler (MJ Research Inc., Watertown, MA, USA) for 3 h at 37 °C followed by 20 min at 65 °C, and then stored at 4 °C.

The T-RFLP profiles of each digest were determined separately as described previously (Zhou *et al*., 2007) containing 1 µl of digest and 0.5 µl of ROX 25_1000 standard (Bio Ventures Inc., Murfreesboro, TN, USA). We used an ABI PRISM 3100 DNA Analyzer (Applied Biosystems) with a slight variation of the default run module: an injection voltage of 2 V instead of 1 V. The data from both digests were then combined to form one dataset for each sample.

Statistical analysis

Data processing. 'True' peaks in the electropherogram were identified by distinguishing baseline noise from signal and T-RFLP fragments of comparable sizes from different profiles were aligned to account for analytical errors made in estimating the fragment size using the method described by Abdo *et al*. (2006). We, however, used the nearest neighbor algorithm instead of average linkage to align the profiles.

Clustering. The distance matrix was calculated using Euclidean distance based on the standardized data, referred to as the species profile distance (Legendre and Gallagher, 2001). Hierarchical clustering was then done using average linkage to identify the number of distinct clusters. Similar communities that clustered together were taken to represent a single bacterial community type (distinct cluster). To determine the number of distinct community types we employed the cubical clustering criteria (CCC) index (Sarle, 1983), the pseudo F index (Calinski and Harabasz, 1974) and a statistic that can be transformed to pseudo T^2 developed (Duda and Hart, 1973). Cluster analysis was done using SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

The contingency table and mathematical modeling. To study the effects of time since glacier retreat, distance between chronosequences and soil depths on the bacterial community structure we used the number of samples belonging to each community type (distinct cluster) to construct a contingency table (Table 1, as described in Supplementary material Appendix A). This resulting table consisted of 24 rows (6 times since glacial retreat × 2 chronosequences × 2 soil depths) and columns corresponding to the number of community types identified by cluster analysis.

Eight models were introduced to evaluate the role of time, distance and depth in explaining the observed variation in the data. The simplest of these

models was based on that the bacterial community structures sampled from different time stages since glacier retreat, chronosequences and soil depths did not differ. This model was referred to as the simple-null model. The second time-alone model accounted for the cumulative effect of time since glacier retreat on the bacterial communities alone and presumed that communities from different chronosequences and soil depths did not differ significantly. The third and fourth models accounted for the effects of the distance between chronosequences (chronosequence alone) and different soils depths (depth alone), respectively. The fifth model assumed that the time since glacier retreat and soil depth both influenced the bacterial community structure, but the distance between the two chronosequences did not (time-depth model). Similarly, the sixth and seventh models accounted for the combined effect of time since glacier retreat and distance between the chronosequences (time chronosequence), and distance between chronosequences and soil depth (chronosequence depth), respectively. Finally, the eighth model accounted for the combined the effect of time, chronosequence and depth. The last model was the most parameter rich and hereafter is referred to the saturated model.

We utilized two strategies in selecting the model that fits the data best. The first was a stepwise approach using the likelihood ratio test (Bain and Engelhardt, 1991) and the bootstrap (Efron and Tibshirani, 1993), and the second approach utilized Akaike's information criterion (AIC; Burnham and Anderson, 2002) as the basis for choosing the most appropriate model (Supplementary material Appendices B and C).

Pairwise comparisons. Pairwise comparisons were conducted to identify significant differences between the bacterial communities sampled at sites that reflect different times since glacier retreat. These comparisons were performed using a likelihood ratio test similar to that described in the stepwise comparison (Supplementary material Appendix B). This test involved comparing a null model that assumes bacterial communities from two different times since glacier retreat did not differ to an alternative hypothesis that these communities significantly differed from each other. The construction of the null distribution used to evaluate the significance of the difference between these two models was the same as that used in the stepwise model selection approach described in Supplementary material Appendix C.

Results

We tested whether three factors were associated with changes in the bacterial community structure: time after glacier retreat, the horizontal variation caused by the distance between the chronosequences and vertical variation at two soil depths. The bacterial communities in 117 soil samples were compared by T-RFLP analysis of 16S rRNA genes. Each DNA fragment in the T-RFLP profiles was considered to be a distinct operational taxonomic unit (OTU), and the relative fluorescence of each OTU was assumed to reflect its true proportional abundance. Although this is a useful way to detect differences in community structure, it is limited in so far as a DNA fragment of a given length can be derived from multiple phylotypes (Liu *et al*., 1997) and rare phylotypes are not accounted for. Thus, if the bacterial communities of two or more samples appear identical, it could be that actual differences were not resolved or detected. For these reasons, and because of well-documented PCR biases, we were unable to estimate the absolute or comparative species richness (Blackwood *et al*., 2007; Bent and Forney, 2008). T-RFLP was, however, useful in our study to detect differences in bacterial communities over time because if profiles differ in terms of the fragment sizes present and their relative abundance it implies that the phylotype composition of the communities compared are different.

Testing for significance of time, chronosequence and soil depth

Three significantly different community types among the soils sampled were identified using three different algorithms based on cluster analysis of T-RFLP profiles (Figure 1). Group 2 (G2) consisted only of samples from the surface layer. It included 9 of the 10 replicates taken from the surface layer exposed for 5 and 19 years, respectively, and only 1 or 2 replicates from the sites exposed for 40–150 years. The distribution of community profiles among the community types suggested there were some differences in the structures of bacterial communities along the chronosequences and for the two soil depths sampled.

The data were further analyzed to determine whether time since glacier retreat, soil depth or chronosequence were significantly associated with differences in bacterial community structure. Standard statistical methods such as multivariate analysis of variance (MANOVA) or perMANOVA could not be used because the data were sparse, not normally distributed and did not meet the assumption of equal variance. Other multivariate methods such as principal component analysis would have allowed us to identify similarities among the bacterial communities. These methods would, however, not have provided a means to test the significance of the effect of the time since glacier retreat, distance between chronosequences, and soil depths on the bacterial community structure and whether the effects of these factors were independent from one another. For these reasons we resorted to the stepwise selection of log-linear models and AIC to test whether time since glacier retreat, soil

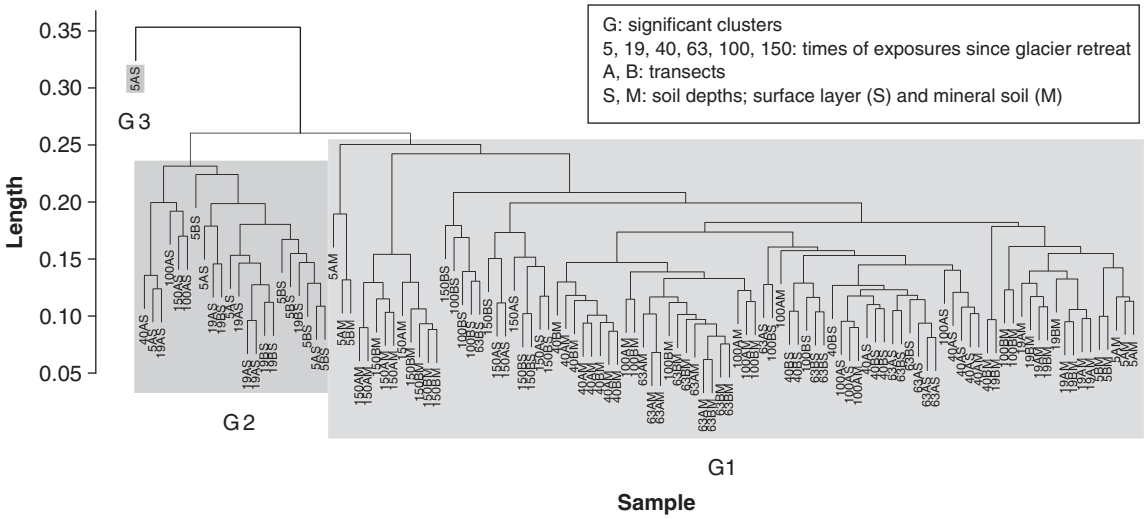

Figure 1 Hierarchical clustering of bacterial communities from times since glacier retreat, chronosequences and soil depths, based on data from terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes. Cluster analysis was done using average linkage based on Euclidean distances using the standardized dataset, and groups (clusters) were determined by cubic clustering criterion (CCC), pseudo F, and pseudo Hotelling T2 (see Abdo *et al.*, 2006). These groups are designated by a 'G' followed by a number. Five samples were taken from each combination of time of exposure since glacier retreat (5–150 years), chronosequences (A and B), and soil depth [(surface layer (S) and mineral soil sample (M)]. Three samples (19BS and two from 5BM) were excluded from the analysis because no T-RFLP profile could be obtained.

Table 1 Contingency table generated based on the community types identified using clustering analysis (shown in Figure 1)

Time (years)	*Chronose-quence*	*Depth*	*Group*		
			1	*2*	*3*
5	A	Surface	0	4	1
		Mineral soil	5	0	0
	B	Surface	0	5	0
		Mineral soil	3	0	0
19	A	Surface	0	5	0
		Mineral soil	5	0	0
	B	Surface	0	4	0
		Mineral soil	5	0	0
40	A	Surface	4	1	0
		Mineral soil	5	0	0
	B	Surface	4	1	0
		Mineral soil	5	0	0
63	A	Surface	5	0	0
		Mineral soil	5	0	0
	B	Surface	5	0	0
		Mineral soil	5	0	0
100	A	Surface	3	2	0
		Mineral soil	5	0	0
	B	Surface	5	0	0
		Mineral soil	5	0	0
150	A	Surface	4	1	0
		Mineral soil	5	0	0
	B	Surface	5	0	0
		Mineral soil	5	0	0

depth or chronosequence had a significant impact on the structure of bacterial communities (Table 1), and whether the effects of these factors were independent from each other (Tables 2 and 3). The results of these statistical analyses showed that a model that took both time since glacier retreat and soil depth into account best fit the data (Table 2, largest likelihood ratio value, 58.45, $P<0.0001$; Table 3, lowest AIC score: 81.02). The analysis also showed that the effects of time since glacier retreat and soil depth were not independent of each other and hence, it was not possible to conclude whether the time of exposure following glacier retreat or soil depth alone were significant (Table 2, time-alone $P<0.0001$; Table 3, AIC = 115.47; and Table 2, depth-alone $P<0.0001$; Table 3, AIC = 96.04). These results work consistent with the results obtained from extended distance based redundancy analysis (data not shown). The bacterial communities of corresponding sites in the two chronosequences did not differ significantly (Tables 2, $P=0.417$; Table 3, AIC = 133.34). This is not surprising because the two chronosequences were chosen in such a way that the drainage streams had not influenced sampling locations, and that the sampling locations represented the appropriate time stages.

Further analyses were done to determine whether the time of exposure alone was a statistically significant variable if the communities in the surface and the mineral soil layers were considered separately. Stepwise model selection and AIC showed that the time since glacier retreat was significantly associated with changes in the structure of bacterial communities in surface and mineral soils in each chronosequence (surface soils: $P<0.0001$, AIC = 101.53; mineral soils: $P<0.0001$, AIC = 128.68), which suggests that succession occurred. This was consistent with the results of cluster analysis (Figure 2). For example, the T-RFLP profiles of communities in surface layer soils from

Table 2 Stepwise model selection using likelihood ratio testing to determine whether the effect of time since glacier retreat, distance between chronosequences and soil depth on the bacterial community structure was significant

Model	*Null model*	*Likelihood ratio*	P *value*[a]
Time alone	Simple null	35.59	<0.0001
Chronosequence alone		1.713	0.417
Depth alone		39.01	<0.0001
Time chronosequence	Time alone	6.27	0.460
Time depth		58.45	**<0.0001**[b]
Time chronosequence depth	Time depth	6.27	0.266

[a]Level of significance: 0.05/3 = 0.008 using Bonferroni adjustment.
[b]Time-depth model fits the data best.

Table 3 Akaike's Information Criterion (AIC) scores for the models tested

Model tested	*AIC score*
Simple null	131.05
Time alone	115.47
Chronosequence alone	133.34
Depth alone	96.04
Time chronosequence	133.19
Time depth	81.02[a]
Depth chronosequence	102.02
Time chronosequence-depth	122.75

[a]Minimum AIC score, therefore, time-depth model best fits the data.

early successional stages mostly clustered in community group GS2, and profiles from midsuccessional stages clustered in community group GS1. Succession seems less pronounced in the mineral soils, though there is still a successional trend. Most of the profiles obtained from the oldest site (150 years) clustered in community group GM4, whereas half the profiles from the two earliest stages in succession clustered in community group GM3.

Pairwise comparisons

Likelihood ratio tests were performed on all pairwise combinations of different time stages since glacier retreat for both the surface layer and mineral soils to determine whether the change in bacterial communities observed was gradual and at what time stages along the chronosequence statistically significant changes had occurred (Table 4). These analyses showed that there were gradual changes in the composition of the bacterial communities along the chronosequences in the surface and less pronounced in the mineral soils. In the surface soils the community structures from sites exposed for 5 and 19 years since glacier retreat were similar to one another as were bacterial communities found at locations exposed for 40–100 years since glacier retreat. Thus, communities from younger sites differed significantly from older sites (40–100 years old), and all bacterial communities differed significantly from the bacterial communities found at the sites exposed for 150 years (Table 4). In the mineral soils, the differences between communities from younger sites and sites exposed for 40–100 years is not as distinct as in the surface layer. The observed changes in community structure were not only based on changes in relative abundances of phylotypes but also on phylotype replacement (data not shown). Our findings suggest that the changes in community structure over time were gradual and that succession occurred in soils from both depths, but the changes observed in the surface and mineral soils differed significantly from each other.

Discussion

Log-linear statistical models were used in this study to show that significant changes in the composition of bacterial communities occurred in surface and mineral soils sampled from two chronosequences in the foreland of Midre Lovén glacier. This is the first time that bacterial succession has been shown to occur in a terrestrial ecosystem of the High Arctic. It is likely that bacteria in these communities exert a strong influence on plant succession and soil development in this ecosystem by altering the physical and biological environment. These impacts may be direct through bacterial-plant root interactions (Kloepper *et al.*, 1999; Gregory, 2007) and through the involvement of bacteria in biogeochemical processes (Cotner and Biddanda, 2002; Inubushi and Acquaye, 2004; Huang *et al.*, 2005).

Chronosequences are commonly used to study succession (Bormann and Sidle, 1990; Matthews, 1992; Avis and Lubke, 1996; Kaufmann, 2001; Walker and del Moral, 2003; Breen and Lévesque, 2006) and they are particularly useful if the changes of interest occur over decades or centuries (Begon *et al.*, 1996; Walker and del Moral, 2003). It is based on the assumption that space can be substituted for time, which implies that every site has essentially the same biotic and abiotic history (Walker and del Moral, 2003; Johnson and Miyanishi, 2008). This is often not the case due to differences in stochastic events and disturbances at the sampling locations (Fastie, 1995; Walker and del Moral, 2003). It is particularly difficult to study succession using

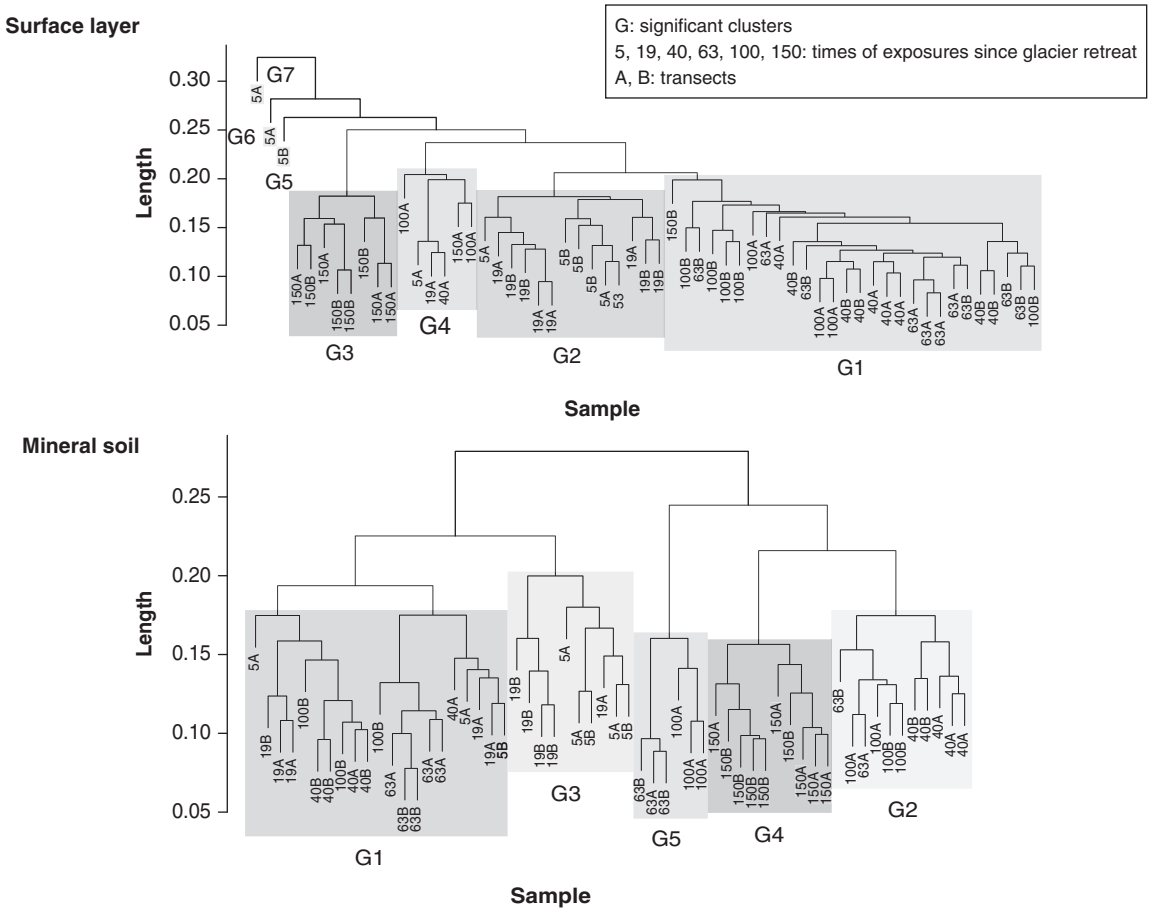

Figure 2 Hierarchical clustering of bacterial communities from surface and mineral soils sampled from the two chronosequences as described in Figure 1. The distinct clusters (groups) identified are designated by a 'G' followed by a number. Five samples were taken from each combination of time of exposure since glacier retreat (5–150 years) and chronosequence (A and B). Three samples (19BS and two from 5BM) were excluded from the analysis because no terminal restriction fragment length polymorphism (T-RFLP) profile could be obtained.

Table 4 Pairwise comparison of microbial community structures among sites

Comparison (years)	*Surface layer*		*Mineral soil*	
	− 2 log likelihood ratio test statistic	P *value*[a]	*−2 log likelihood ratio test statistic*	P *value*[a]
5 vs 19	4.39	0.15	0.28	0.62
5 vs 40	24.95	**<0.0001**	14.15	0.003
5 vs 63	27.73	**<0.0001**	14.15	**0.002**
5 vs 100	23.91	**<0.0001**	16.41	**0.001**
5 vs 150	24.95	**<0.0001**	29.72	**<0.0001**
19 vs 40	23.51	**<0.0001**	13.86	0.001
19 vs 63	26.29	**<0.0001**	13.86	**0.005**
19 vs 100	22.47	**<0.0001**	17.14	**<0.0001**
19 vs 150	23.51	**<0.0001**	33.65	**<0.0001**
40 vs 63	1.44	0.11	5.49	0.10
40 vs 100	0.4	0.46	4.78	0.14
40 vs 150	18.45	**<0.0001**	33.65	**<0.0001**
63 vs 100	3	0.04	1.18	0.58
63 vs 150	21.02	**<0.0001**	33.65	**<0.0001**
100 vs 150	17.62	**<0.0001**	33.65	**<0.0001**

[a]Level of significance: 0.05/15 = 0.0033 using Bonferroni adjustment; significant *P*-values are printed in bold.

chronosequences in High Arctic glacier forelands because they are comparatively unstable due to the thermal and hydrological structure of the polythermal glaciers commonly found in the High Arctic (Harland, 1997; Hodkinson *et al.*, 2003). Thus, glacier streams have reworked a significant

proportion of the foreland independent of the site's age (Hodkinson *et al.*, 2003). In addition, frost heaving, slumping of ice-cored moraines and grazing may disturb the foreland repeatedly (Hodkinson *et al.*, 2003). Our approach was, however, comparatively robust because Hodkinson *et al.* (2003) had identified sites with the least disturbance in the glacier foreland. Thus, our sampling design seemed valid to test whether bacterial succession occurred in the glacier foreland of Midre Lovén glacier.

To some extent it is surprising that any significant differences in the bacterial community structures could be detected because soils are heterogeneous and bacteria have a patchy distribution (Green *et al.*, 2004; Horner-Devine *et al.*, 2004; Green and Bohannan, 2006). Thus, the within-site variation in the glacial foreland was expected to be large. The results of the clustering analysis reflect this within-site variation; none of the samples from a particular location clustered exclusively within a specific group (Figures 1 and 2). However, with extensive sampling we were able to determine that the communities of the youngest sites differed significantly from all other sites and the communities sampled at sites of intermediate age differed from the communities obtained at the oldest site. However, no conclusion can be drawn with regards to the pattern of succession. Considering the environmental conditions of soils and High Arctic glacier forelands, successional change was probably not linear and predictable (Walker and del Moral, 2003). It is more likely that the successional trajectories were deflected by mild disturbances. These disturbances such as freezing and thawing, amount of snow and wind may have had variable impacts on bacterial communities in the soil due to differences in, for example, the micro-topography and spatial structure within a site. As a result, variability exists within each site resulting in a temporal mosaic rather than a uniform environment (Walker and del Moral, 2003). Nevertheless we were able to determine that there was an overall change in the bacterial community structure over time, and thus, succession had occurred.

The factors that affect bacterial succession have not been well studied. Succession in bacterial communities is thought to be at least partly autogenic (Archer *et al.*, 1988; Walker and del Moral, 2003) wherein the metabolic activities of various bacterial populations alter the physical and chemical characteristics of the environment in ways that facilitate the colonization and growth of other bacterial populations. One example of this is nitrogen fixation by cyanobacteria, which increases the levels of ammonia nitrogen in soils and creates conditions suitable for nitrifying bacteria and heterotrophic organisms that are dependent on fixed nitrogen for growth. It is likely that nitrogen fixation is important to succession in the glacier foreland of Midre Lovén glacier. Turicchia *et al.* (2005) described the cyanobacterial community along chronosequence A and they found that the nitrogen-fixing genera *Leptolyngbya* and *Nostoc* are abundant members of the bacterial community at the youngest sites. Other factors that likely affect the species composition of these bacterial communities are changes in the extent of vegetation cover (Rutigliano *et al.*, 2005), plant species diversity (Kuske *et al.*, 2002; Johnson *et al.*, 2003; Tscherko *et al.*, 2004) and the activity of plant communities over time (Lipson *et al.*, 1999; Mukerji *et al.*, 2006). Recently exposed sites in the foreland of Midre Lovén glacier have a high proportion of coarse gravel with patchy cyanobacterial crusts and mosses. Over time the vegetation cover increases to 100% and vascular plant species such as *Carex rupestris* and *Dryas octopetala* are found (Hodkinson *et al.*, 2003). These changes in plant community composition are accompanied by a general increase in the soil organic matter content, nitrogen (Hodkinson *et al.*, 2003) and probably by changes in the quantity and composition of root exudates (Bais *et al.*, 2006; Mukerji *et al.*, 2006). In addition, measurements of soil compounds taken in 2002 indicated that total carbon increased and metal concentrations changed over time; for example, concentrations of magnesium and iron decreased (UME Schütte *et al.*, unpublished data). Although a causal relationship could only be determined by controlled manipulation of the environment, it is likely that both changes in the plant community and soil environment impacted the kind and amount of resources available for bacterial growth and thus altered the ecological niches that could be occupied by immigrant bacteria. Although the immediate effect is evident in surface layers, the habitats at greater depths are probably affected by leached organic matter, which is accompanied by a decrease in pH (Matthews, 1992; Hodkinson *et al.*, 2003). In addition, the physical environments of soils in glacier forelands are dynamic and the changes that occur probably have a strong influence on bacterial succession. For example, pervection (the mechanical movement and downwash of clay, silt and fine particles) occurs following snowmelt, whereas eolian processes translocate fine grain materials to surface depressions to create a patchy landscape (Matthews, 1992). As a consequence of sorting based on particle size the microclimate of bacterial habitats along chronosequences may also be affected because it impacts the retention of heat and water in a given locality. In coarser gravel, air circulation penetrates deeper and water drainage is more rapid, whereas finer-grained substrates retain heat and water more effectively (Matthews, 1992). Although these processes have not been specifically studied in the glacier foreland of Midre Lovén glacier, it is likely that such processes have modified the habitats of bacteria at the site and had a strong influence on bacterial communities in this glacier foreland.

The two chronosequences sampled in this study were chosen to avoid influence by glacial drainage streams, which cause recurring disturbances that reset areas to earlier stages in successional development (Hodkinson *et al.*, 2003). Our results showed that the bacterial communities from comparable locations along the two chronosequences were not significantly different from one another. This suggests that disturbances may have happened to an equal extent at sites along both chronosequences, and that colonization may have occurred from the same species pool (McCune and Allen, 1985). Multiple studies have shown that bacteria are present beneath glaciers (Skidmore *et al.*, 2000; Kastovska *et al.*, 2005; Bhatia *et al.*, 2006), and subglacial sediments form a likely source of species for bacterial succession in glacier forelands. Bacteria could also conceivably immigrate to these sites through the deposition of particulate matter through air currents or precipitation, as well as through glacier runoff (cryoconite holes) and snowmelt, however, the importance of these or other mechanisms are not known. Future studies done to determine the bacterial taxa through analysis of 16S rRNA sequences at the different locations in these chronosequences may provide insight to their origin and functional significance, and controlled manipulations of the environment in the field or in the laboratory may give insights into causes and consequences of bacterial succession.

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