Microbial Ecology

Microbial Community Succession in an Unvegetated, Recently Deglaciated Soil

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Abstract

Primary succession is a fundamental process in macroecosystems; however, if and how soil development influences microbial community structure is poorly understood. Thus, we investigated changes in the bacterial community along a chronosequence of three unvegetated, early successional soils (~20-year age gradient) from a receding glacier in southeastern Peru using molecular phylogenetic techniques. We found that evenness, phylogenetic diversity, and the number of phylotypes were lowest in the youngest soils, increased in the intermediate aged soils, and plateaued in the oldest soils. This increase in diversity was commensurate with an increase in the number of sequences related to common soil bacteria in the older soils, including members of the divisions *Acidobacteria, Bacteroidetes*, and *Verrucomicrobia*. Sequences related to the Comamonadaceae clade of the Betaproteobacteria were dominant in the youngest soil, decreased in abundance in the intermediate age soil, and were not detected in the oldest soil. These sequences are closely related to culturable heterotrophs from rock and ice environments, suggesting that they originated from organisms living within or below the glacier. Sequences related to a variety of nitrogen (N)-fixing clades within the *Cyanobacteria* were abundant along the chronosequence, comprising 6–40% of phylotypes along the age gradient. Although there was no obvious change in the overall abundance of cyanobacterial sequences along the chronosequence, there was a dramatic shift in the abundance of specific cyanobacterial phylotypes, with the intermediate aged soils containing the greatest diversity of these sequences. Most soil biogeochemical characteristics showed little change along this ~20-year soil age gradient; however, soil N pools significantly increased with soil age, perhaps as a result of the activity of the N-fixing *Cyanobacteria*. Our results suggest that, like macrobial communities, soil microbial communities are structured by substrate age, and that they, too, undergo predictable changes through time.

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Introduction

Primary succession, the establishment and gradual change in plant communities on newly inhabited substrate, is a fundamental ecological process [9, 11, 16] that remains an active focus of both community and ecosystem ecology research (e.g., [7, 8, 13, 55, 57]). Primary succession occurs on newly exposed or deposited substrates, including lava flows, sand dunes, landslides, and glacial till. In systems characterized by continuous substrate inputs, such as active lava flows and receding glaciers, chronosequences result, with older, more weathered soils farther from the substrate source. Here, distance can serve as a proxy for time, and ecosystem development can be investigated along a spatial gradient without the need for long-term observations of the same site.

Chronosequence studies have revealed correlations between plant community composition and substrate age. Integrating these observations with knowledge of plant biology has been key in developing models that describe changes in ecosystem nutrient dynamics and soil

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development through time. For example, it has long been recognized that lichens or plants that form symbiotic associations with nitrogen (N)-fixing microbes, including *Dryas* and *Alnus*, are among the first colonizers of fresh substrate (reviewed in [30]). This observation led to a paradigm that describes nitrogen dynamics in primary successional communities: because N is absent from the new mineral substrate, organisms that fix nitrogen are competitive (and hence abundant) in early stages of succession. Furthermore, the presence of N fixers is tightly linked to the accumulation of soil N [5, 12, 30], and increases in soil nitrogen availability may facilitate colonization by other, later successional species [8, 56].

Because of their ability to fix nitrogen, microorganisms are clearly central to early ecosystem development, and some studies even suggest that microbial activity promotes plant establishment [8, 56] and growth [6]. However, only a few studies have examined the microbial communities and nutrient dynamics in the youngest, unvegetated sections of chronosequences. The investigations that do exist support increases in microbial biomass [47, 48] and microbial activity [54] with soil age. Other studies demonstrate decreases in substrate-induced respiration (SIR) per unit biomass with soil age, suggesting that older communities store more carbon than they respire [34, 41]. Finally, small subunit ribosomal RNA (SSU rRNA) gene fingerprinting suggests both a decrease in diversity and an increase in the microbial community evenness with soil age [48].

Despite the evidence for active, dynamic microbial communities in these barren, unvegetated soils, little data exist on the *types* of microorganisms inhabiting these environments, or how shifts in these microbial communities correlate with soil age. This is likely due to the difficulties inherent in describing microbial community composition, as an estimated less than 1% of microorganisms can be cultured using traditional techniques [2, 35]. However, the recent application of molecular analyses, in particular the use of SSU rRNA gene clone libraries, has allowed for microbial community surveys without the biases associated with cultivation-based techniques. SSU rRNA clone library techniques have been used to examine fungal [24] and archaeal [33] diversity along recently deglaciated chronosequences, but, to our knowledge, no studies have provided detailed, phylogenetic data on the bacterial communities in these developing soils. Thus, we set forth to examine patterns in bacterial community composition along the soil age gradient of a glacial foreland. We sampled unvegetated sites with no visible organic matter along the soil age gradient of the Puca glacier in the Peruvian Andes to address some basic questions about bacterial succession.

First, we were interested in determining if microbial communities, like plant communities, undergo predictable changes in species composition as soil develops. In simpler, less complex microbial systems, including developing biofilms (e.g., [29]) and composting systems (e.g., [43]), microbial communities exhibit successional patterns, and populations are replaced in somewhat consistent and predictable ways. However, the ability to recognize such patterns in soils may be limited by the extreme microscale diversity of soils. Next, we investigated how shifts in the microbial community composition correlate with nutrient dynamics along the chronosequence of unvegetated soils. Although linking populations and processes has been a major focus of macroecology, there is debate about whether or not information on the structure of microbial communities is informative at the ecosystem scale (e.g., [3, 14, 25]). However, it is possible that in unvegetated soil relationships between nutrient dynamics and microbial populations might be more evident. In this region of the Andes, pollen deposition rates on the nearby Quelccaya icecap are among the highest ever recorded [38]. Thus we hypothesized that this allochthonous carbon (C) input would select for heterotrophs in these young soils. In addition, because the C/N ratio of pollen is higher than microbial biomass, and N is absent from new substrate, we hypothesized that heterotrophic nitrogen fixers would be abundant in these soils.

Materials and Methods

Study Sites and Sample Collection. In August 2003, we collected soil samples from the recently exposed forelands of the Puca glacier. This glacier lies in the Laguna Sibinacocha basin, in the Cordillera Vilcanota range in the Peruvian Andes (elevation, ~5000 m) near the Quelccaya ice cap. The moraines in this region are composed of rock with high quartz and calcite content (Anderson and Blum, unpublished data). The forelands of the Puca glacier are largely unvegetated at distances of up to 500 m, with plants covering less than ~10% of the ground. The first vascular colonizers of this terrain are predominately grasses of the genera *Ephedra* and *Calamagrostis*. Precipitation in this area is highly seasonal; of the approximately 1000 mm of annual precipitation, about 60% falls between December and March [15, 52]. Monthly mean temperatures vary by less than 3°C, but diurnal variation changes markedly, ranging from about 12°C in the wet season to about 22°C in the winter dry season. During the dry season, surface soil temperatures at our sites oscillate between –5°C and 25°C [32].

We established three transects *parallel* to the forefront of the Puca Glacier. The transects were chosen to represent regions of progressively older soils, and thus were at distances of 0 m (youngest soil), 100 m (intermediate age), and 500 m (oldest soil) from the glacial forefront. They are hereafter referred to as the 0-,

100-, and 500-m transects. Four replicate surface soil samples (0–5 cm) were then collected along each transect at randomly chosen positions. Each replicate was obtained by marking a 50 × 50 cm area around the collection point, and then bulking three individual soil samples from within this area. These replicate samples are hereafter referred to as 0m-1, 0m-2, 0m-3, 0m-4, 100m-1, and so on. Soils were scooped in sterile conical tubes, immediately placed on ice, and kept in the dark at 0°C for transport to Boulder, CO, USA (approximately 1 week). Soil samples were then divided into two aliquots: one was placed at –80°C for DNA extraction and the other was stored at 4°C for biogeochemical analyses. Along each of the three transects (0, 100, and 500 m), we measured biogeochemical parameters on all four replicate soil samples, and we randomly selected two of these replicates for molecular analyses. Replicate soil samples chosen for molecular analyses were all at distances of at least 25 m (along the transects) from one another. Because these are not soils in the traditional sense, and contain an array of fine particles to fairly large rocks, we did not sieve soils, but we did use soil particles <2 mm for analyses.

Soil Dating. In August 2003, the UTM coordinates of the glacier terminus were 276193 E, 8476734 S (Provisional South American 1956). A sequence of isochrones of glacier terminus location derived from air photo, satellite and ground-based GPS measurements for the period 1931–2003 suggest that the 0 m soils were less than 1 year old, the 100 m soils were 1–4 years old, and the 500 m soils were approximately 20 years old (Seimon, unpublished data).

DNA Extraction, 16S rRNA Gene Clone Libraries, and Sequence Analysis. DNA was extracted from soil samples using a modification of the protocol described by More *et al.* [31]. Soil (0.5 g) was added to 0.3 g of each 0.1-, 0.5-, and 1-mM-sized glass beads with 1 mL phosphate extraction buffer, and agitated for 2 min with a bead beater. Samples were centrifuged at 14,000 rpm for 10 min and the supernatant was removed to a fresh tube. Next, 200 μL of 7.5 mM ammonium acetate was added and samples were incubated on ice for 5 min. Samples were centrifuged at 14,000 rpm for 3 min and the supernatant was then removed to a fresh tube. Samples were extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). DNA was precipitated with 200 μL isopropanol overnight at –20°C. Samples were then centrifuged at 14,000 rpm for 20 min and the supernatant was removed and discarded. DNA pellets were washed with 1 mL of 70% ethanol. Eight separate DNA extractions were pooled and purified over Sepharose 4B (Sigma, St. Louis, MO, USA) packed columns as described by Jackson *et al.* [23].

Approximately 30 ng of DNA was amplified with the primers 515f and 1492r [27]. The reaction conditions consisted of 400 nM each primer, 200 μM each dNTP, and 1.25 U of *Taq* DNA polymerase (Promega, Madison, WI, USA) in *Taq* DNA polymerase buffer containing 2.5 mM $MgCl_2$ (Promega). After an initial denaturation step at 94°C for 1 min, 30 cycles of 94°C for 1 min, 58 ± 5°C for 30 s, and 72°C for 2.5 min with a terminal 10-min extension at 72°C were performed. Polymerase chain reaction (PCR) products from six reactions with different annealing temperatures were pooled and gel purified by using QIAquick gel purification columns (Qiagen, Valencia, CA, USA). Purified products were ligated into the vector pCR 2.1 (Invitrogen, Carlsbad, CA, USA) and transformed into *Escherichia coli* following the manufacturer's instructions.

For each cloning reaction, 96 colonies were inoculated into a 96-well deep-dish plate containing 1.5 mL Luria broth (1% NaCl, 1% tryptone, 0.5% yeast extract) with 50 μg/mL ampicillin. Cultures were agitated at 200 rpm for 14 h at 37°C. Plasmid DNA was extracted by using a modified protocol for 96-well plates based the procedure described by Sambrook and Russell [40]. DNA pellets were air-dried for 20 min and resuspended in 50 μL Tris–HCl (pH 8.5).

Inserted 16S rRNA genes were PCR-amplified from the plasmids by using the primers M13F and M13R (Invitrogen). The reaction conditions consisted of 400 nM each primer, 200 μM each dNTP, and 1.25 U of *Taq* DNA polymerase in *Taq* DNA polymerase buffer containing 2.5 mM $MgCl_2$. After an initial denaturation step at 94°C for 1 min, 30 cycles of 94°C for 1 min, 58°C for 30 s, and 72°C for 2.5 min with a terminal 10-min extension at 72°C were performed. Excess primers and nucleotides were removed by adding 0.5 μL Exonuclease I and 0.5 μL Shrimp Alkaline Phosphatase to 20 μL of PCR reactions and incubating at 37°C for 15 min (New England Biolabs, Beverly, MA, USA). These enzymes were then deactivated with a 15-min incubation at 85°C. The 16S rRNA genes were sequenced with the T7 promoter primer and the M13-9 primer by using the BigDye Terminator Cycle Sequencing kit v. 3.0 (PE Biosystems, Foster City, CA, USA) following the manufacturer's directions. Sequencing products were analyzed at the Iowa State University DNA Sequencing Facility.

Sequences were edited in Sequencher 4.1 (Gene Codes Co., Ann Arbor, MI, USA) and subjected to BLAST searches [1]. 16S rRNA gene sequences were then subjected to chimera check in Ribosomal Database Project (RDP) [10] as well as with Bellerophon [19] and aligned in Dr. Phil Hugenholtz's 16S rRNA ARB database (http://rdp8.cme.msu.edu/html/alignments.html). Approximately six chimeras were detected in each soil sample and excluded from further analyses. Closely

related sequences from the ARB database and from BLAST searches were used as reference taxa for phylogenetic analyses. Alignments were subjected to Bayesian phylogenetic analysis implemented in MRBAYES [20] using the GTR + gamma model of evolution with 1,000,000 generations, sampling trees every 100 generations. Burnin values were determined by plotting the likelihood scores against generation number and retaining trees for which stationarity was evident. Taxonomic affiliations were assigned based on both BLAST matches and Bayesian phylogenetic analysis. OTU abundance was calculated by generating a distance matrix in ARB and importing it into DOTUR [42]. Phylogenetic trees for the *Cyanobacteria* and Betaproteobacteria were constructed in PAUP 4.0b [51] using both the distance and parsimony optimality criterion and bootstrap analysis (1000 replicates). The Shannon index of diversity was calculated using the equation

$$H' = -\sum p_i \ln p_i$$

where p_i is the proportion of cover contributed by the ith species [45]. Phylogenetic diversity was calculated by transforming the sequence data into pairwise distances using the Tamura–Nei model of evolution with α = 0.5, and calculating the average pairwise difference (in % sequence difference) using ARLEQUIN [44]. Adopting different models of sequence evolution when estimating pairwise sequence difference yielded quantitative differences, but did not affect the pattern across sites.

Soil Parameters. For total carbon and nitrogen analyses, soils were ground to a fine powder, and a subsample of each soil was acid-treated for carbonate removal. Briefly, 30 mL of 0.5 N HCl were added to 2 g soil. After an hour at room temperature, soil slurries were centrifuged at 5000 rpm and the acid was removed. Acid-treated soils were then washed twice with diH_2O. All soils (treated and untreated) were dried at 70°C for 18 h. Approximately 30 mg of dried soil was packaged into tin capsules and % C and N was measured by using a Carlo Erba combustion–reduction elemental analyzer (CE Elantech, Lakewood, NJ, USA).

Labile (resin-extractable) phosphorus content was determined on soil samples by using a modification of the protocol described by Tiessen and Moir [53]. First, soil samples were dried at 40°C for 10 h, and 1 g of dried soil was placed in a 50-mL conical tube with 30 mL deionized H_2O and a charged anion-exchange membrane strip. After shaking at 200 rpm for 16 h, resin strips were removed and placed in a 50-mL conical tube, then extracted with 20 mL of 0.5 N HCl. Phosphorus concentrations were determined colorimetrically by using the modified version of the Murphy–Riley method described by Tiessen and Moir [53].

Soil pH was determined in a 1:2 soil/deionized water slurry, by using a digital pH meter (Fisher Scientific, Pittsburgh, PA, USA). Gravimetric soil moisture content was determined by weight loss after 24 h at 60°C.

Statistics. We used one-way ANOVAs (SYSTAT, Version 10) with distance from the glacier as the categorical variable to test for differences in soil C, N, P, pH, moisture, and nutrient ratios along the chronosequence. 16S rRNA gene libraries were compared by using both the FST and P test as described by Martin [28]. In addition, we introduce a new test statistic that quantifies the differences in the phylogenetic composition of microbial communities based on comparing the likelihood scores of individual and combined communities. To do so, we generated neighbor-joining trees for each community after transforming the sequence data into a pairwise distance matrix using the HKY + gamma + invariant sites model of sequence evolution. The gamma rate value and the proportion of invariant sites were estimated to be 0.58 and 0.30, respectively, based on a likelihood analysis of a large number of sequences. Using the same model of sequence evolution, we determined the –ln likelihood for the data using PAUP 4.0b [51]. The same analysis was applied to all pairs of soil 16S rRNA gene libraries. The statistic is

$$D_{AB} = [-\ln L_{AB}/(-\ln L_A + -\ln L_B)] - 0.5,$$

where –ln$L$ is the negative log likelihood score for the data from a given community and A and B are the two data sets for which we can calculate the likelihood of the data given the model of evolution and the topology. If the two communities are identical, namely, that –lnL_A = –lnL_B, then –lnL_{AB}= –lnL_A = –lnL_B and D_{AB} = 0. Phylogenetic similarity of communities was then visualized using neighbor-joining cluster analysis.

Accession Numbers. All sequences from this study were deposited in Genbank. The sequences from the 0M1 library were named with the prefix 0M1_ and were assigned to the accession numbers DQ513833–DQ513909; sequences from the 0M2 library were named with the prefix 0M2_ and were assigned to the accession numbers DQ513910–DQ513971; sequences from the 100M1 library were named with the prefix 100M1_ and were assigned to the accession numbers DQ513972–DQ514041; sequences from the 100M2 library were named with the prefix 100M2_ and were assigned to the accession numbers DQ514042–DQ514115; sequences from the 500M1 library were named with the prefix 500M1_ and were assigned to the accession numbers

DQ514116–DQ514184; sequences from the 500M2 library were named with the prefix 500M2_ and were assigned to the accession numbers DQ514185–DQ514243.

Results

16S rRNA Gene Libraries. Across all six sites sampled, we obtained 411 sequences and detected a total of 181 distinct 16S rRNA phylotypes (>3% sequence difference from all other sequences) representing 15 major bacterial and 2 plastid lineages (Fig. 1). Notably, overall patterns of microbial diversity varied along the chronosequence. For example, phylotype and division-level richness as well as overall phylogenetic diversity of the sequences was highest in the older soils (100 and 500 m) and lowest in the youngest soils (Fig. 1, Table 1). Likewise, the Shannon diversity index implied that evenness increased from the 0 to the 100 m soils; evenness appeared to be similar in the 100 and 500 m soils (Table 1). Lineage per time plots (lower line in Fig. 2) feature a steep initial slope in the youngest soils, implying that many of the individual phylotypes in the 0 m sites are very closely related. In contrast, lineage per time plots for the older soils are more gradual, further supporting the idea that these sites harbored greater phylogenetic diversity than the 0 m soils (Fig. 2; Table 1). Interestingly, although there are no obvious differences in the diversity sampled between the 100 and 500 m soils, the Chao1-derived predicted number of lineages for the 100 m soils is 600, twice as high as the average for the 500 m soils (Fig. 2).

It is important to note here that we have not exhaustively sampled the communities in these soils, which could complicate our interpretations of changes in microbial community diversity over the soil age gradient. In particular, rarefaction analysis reveals that diversity at OTU definitions of <5% sequence difference are undersampled (Fig. 3). However, this analysis also suggests that at an OTU definition of <10% these soils were better-sampled. This trend is also revealed by comparing lineage per time plots (i.e., plotting the number of unique lineages present in a collection of sequences over a range of given phylogenetic distances) with plots of the

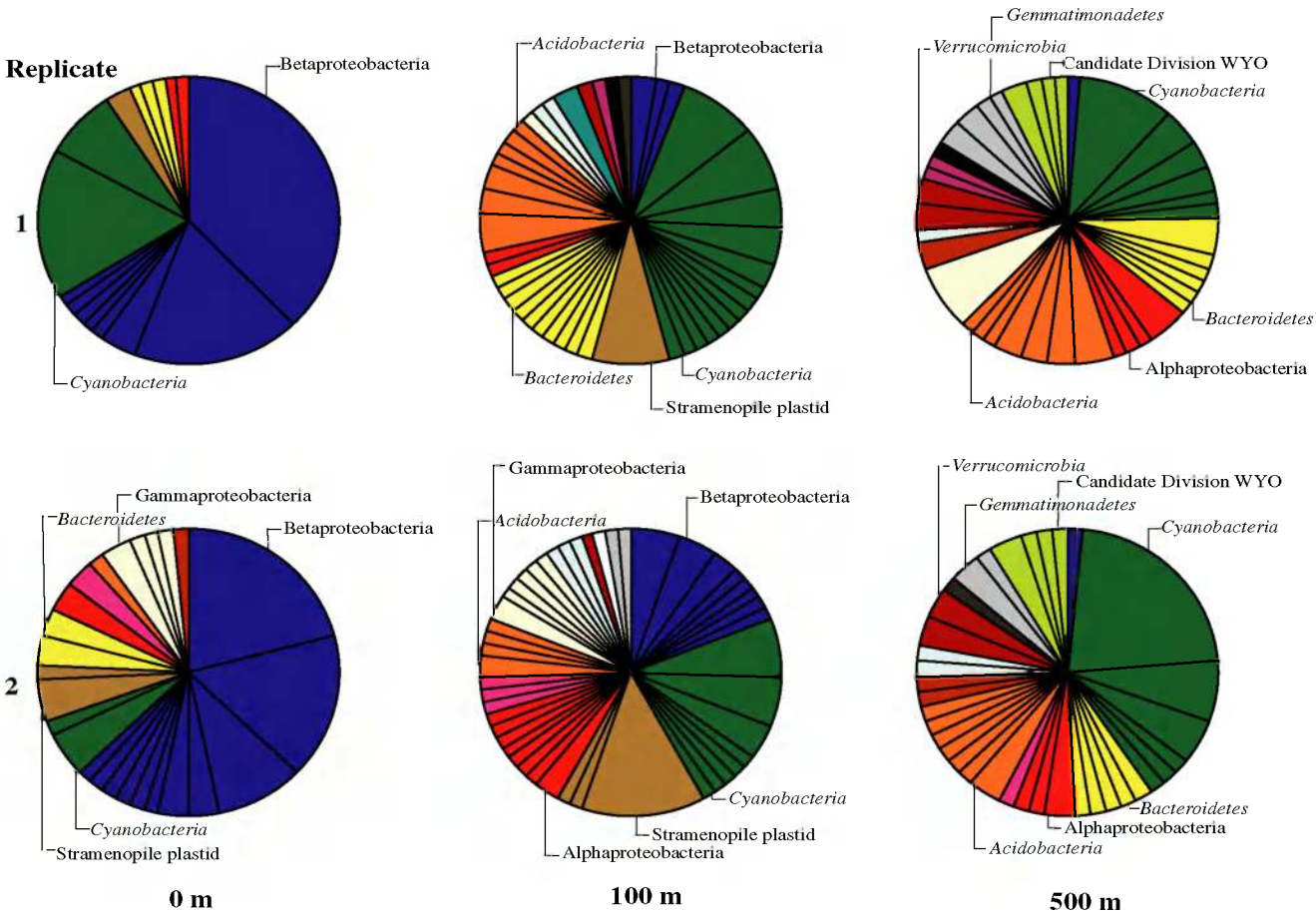

Figure 1. Relative abundance of SSU rDNA phylotypes in clone libraries from different soils. *Colors* represent division- or subdivision-level clades and *black lines* represent individual phylotypes. Number of sequences = 77 (0 m-1), 62 (0 m-2), 70 (100 m-1), 74 (100 m-2), 69 (500 m-1), 59 (500 m-2). Alphaproteobacteria, *Cyanobacteria*, Betaproteobacteria, *Acidobacteria*, *Bacteroidetes*, Stramenopile plastid, *Planctomycetes*, candidate division OP10, Gammaproteobacteria, *Chloroflexi*, *Chlorophyta* plastid, Deltaproteobacteria, *Verrucomicrobia*, *Deinococcus*, *Actinobacteria*, *Gemmatimonadetes*, candidate division WYO.

Table 1. Diversity indices for the six clone libraries

	0 m-1	*0 m-2*	*100 m-1*	*100 m-2*	*500 m-1*	*500 m-2*
Divisions (or proteobacterial subdivisions)	5	9	12	11	12	12
Phylotypes (>3 % sequence difference)	16	25	42	47	39	38
Shannon index	0.8	3.9	4.9	4.8	5.1	4.6
Phylogenetic diversity	18.8	18.9	31.2	28.2	32.3	32.9

nonparametric diversity estimator Chao1 over a range of genetic distances (Fig. 2). These comparisons show that although is clear that we have not sampled the complete diversity at distances of less than 0.1 (10% sequence difference), the lines do converge at a distance of about 0.1 for each soil. This implies that at this phylogenetic distance—somewhere between "genus" and "division" level differences—we have sampled enough diversity to make fairly accurate claims about the diversity in these soils.

The frequencies of sequences related to specific clades varied markedly along the chronosequence (Fig. 1). Most

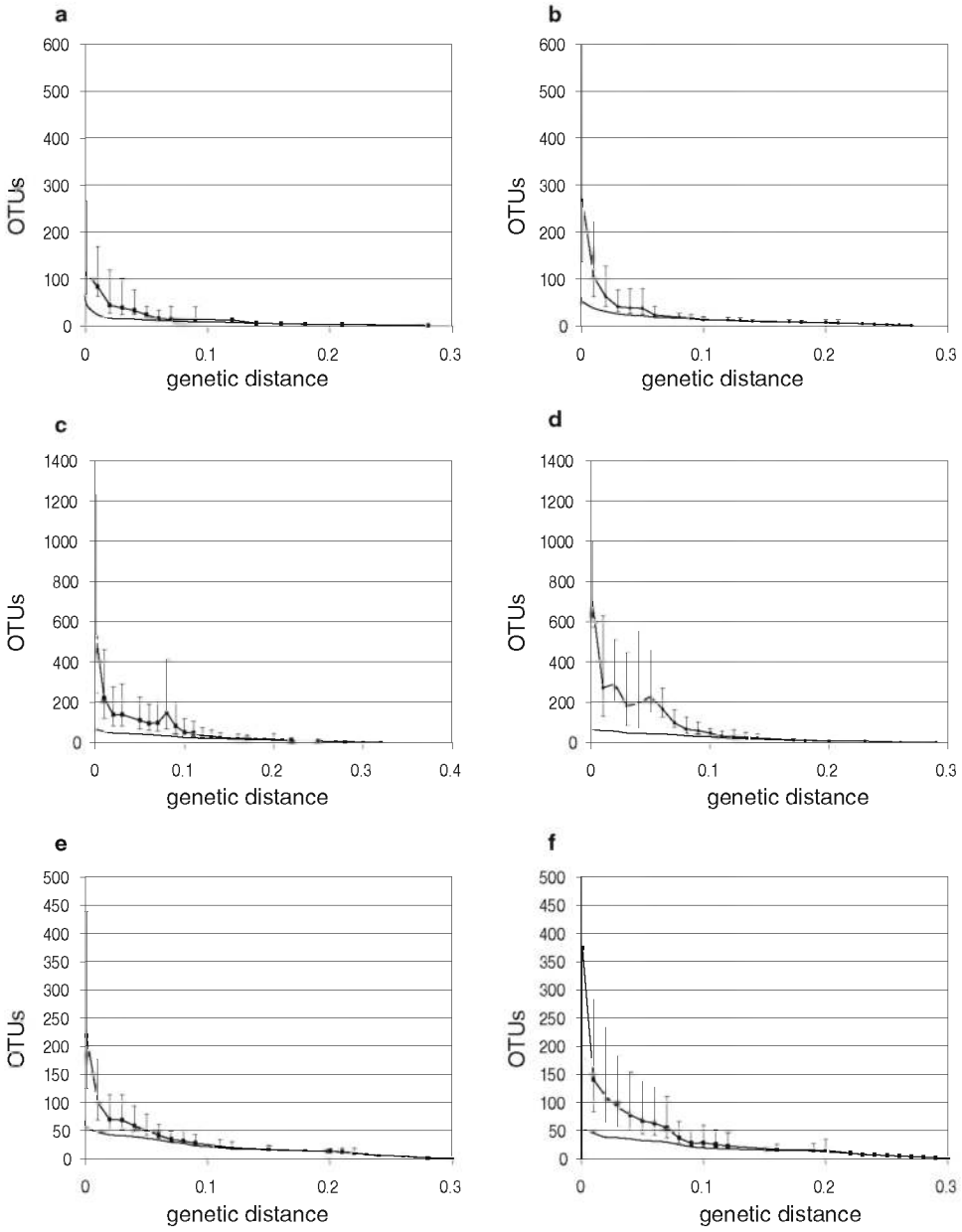

Figure 2. Lineage per time plot (*lower line*) and estimated diversity plots (*upper line* with error bars) for the six samples. (a) 0 m-1, (b) 0 m-2, (c) 100 m-1, (d) 100 m-2, (e) 500 m-1, (f) 500 m-2. OTUs and the Chao1 diversity estimate were calculated in DOTUR [42].

dramatically, sequences related to the Betaproteobacteria dominated in the youngest soil (63–66% of total sequences), decreased in abundance in the 100 m soil (6–19%), and were rare in the oldest soil (1–3%). The intermediate-aged and oldest soils harbored more sequences related to common soil bacteria, including *Acidobacteria*, *Verrucomicrobia*, and *Bacteroidetes*. These older soils also appeared to contain more of the most divergent lineages sampled in this study, including sequences related to the candidate division WYO.

Although it appeared that some patterns in the data were consistent between replicates (Figs. 1 and 2; Table 1), our sampling scheme did not allow us to test for significant differences using traditional statistics. To address this, we applied the FST statistic and the *P* test, two statistical tools developed to compare clone library data [28]. These tests of whether pairs of communities are composed of significantly different pools of phylogenetic diversity revealed that the two samples from the oldest soils were statistically indistinguishable using both hierarchical analysis of variance (FST statistic) and phylogenetic character mapping (*P* test) (data not shown). Samples from the youngest soils harbored similar phylogenetic diversity (a nonsignificant FST test when corrected for multiple comparisons) but contained different sets of clades (significant *P* test), a pattern that reflects the presence of different, but very closely related, clades in the two samples (data not shown). All other

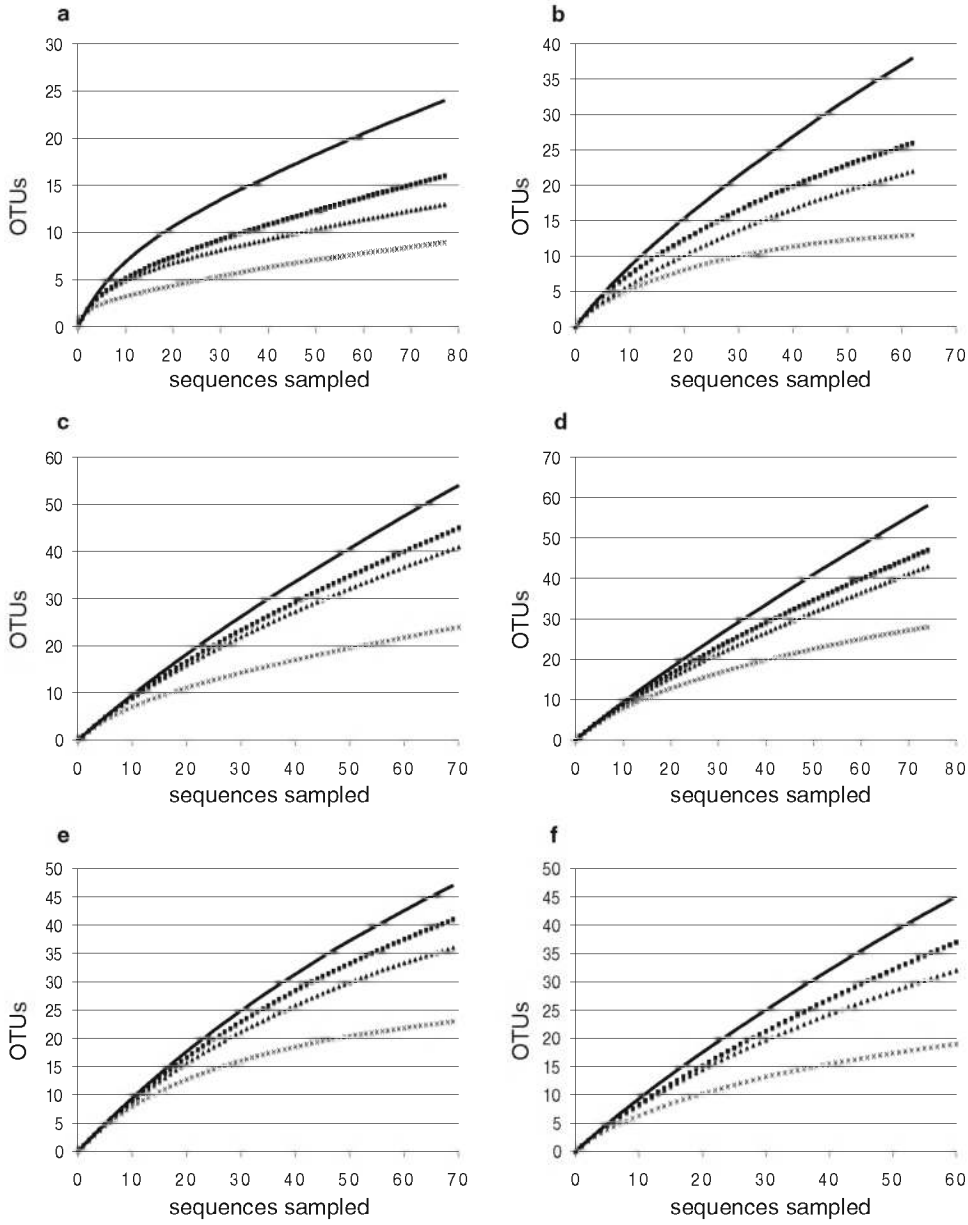

Figure 3. Rarefaction curves constructed from various OTU definitions (sequence differences: bold line = 1%; squares = 3%; triangles = 5%; hatch marks = 10%) for the six samples. (a) 0 m-1, (b) 0 m-2, (c) 100 m-1, (d) 100 m-2, (e) 500 m-1, (f) 500 m-2. OTUs were calculated in DOTUR [42].

pairwise comparisons for both tests were significant, implying the other soils sampled harbored different communities of bacteria (data not shown).

Next, we used a likelihood-based method for estimating the phylogenetic distance between communities, and we summarized the similarities using cluster analysis (Fig. 4). The results mirrored the FST and *P* tests, and the 0 m soils grouped together to the exclusion of all other samples, suggesting that these replicate samples from the youngest soils harbored similar lineages. Likewise, the 500 m soils grouped together to the exclusion of all other libraries, supporting the fact that the replicate samples from the oldest soils contained similar organisms. The two replicates from the 100 m sites, however, were about as different from each other as either was from the 0 or 500 m soils.

Phylogenetic Analyses. We applied a more detailed phylogenetic analysis to the betaproteobacterial sequences, a bacterial lineage that appeared to dramatically decrease in abundance along the soil chronosequence (Fig. 1). Nearly all of the betaproteobacterial sequences from the 0 m soils clustered within the Comamonadaceae group (Fig. 5). The bootstrap support for the exact phylogenetic position of these sequences within this group was weak, likely due to the lack of difference in the 16S rRNA genes of very closely related organisms. However, these sequences appeared to be specifically related to the genera *Variovorax*, *Polaromonas*, and *Rhodoferax*. In addition, these sequences were closely related to two genes obtained from basal ice of the John Evans Glacier, Nunavut, Canada [49]. Although the 100 and 500 m soils also harbored a few betaproteobacterial lineages, these soils contain lineages that are phylogenetically distinct from those in the 0 m soil (Fig. 5). This suggests that not only does the abundance of the Betaproteobacteria shift considerably along the soil age gradient, but also that the types of Betaproteobacteria in the oldest soils are different from the younger soils (Fig. 5).

With the exception of the 0 m-2 library, sequences related to the Cyanobacteria comprised at least 20% of the genes in each clone library (Fig. 1); therefore, we applied detailed phylogenetic analyses to these sequences as well (Fig. 6). There were different types of cyanobacterial sequences in the clone libraries from these sites, and the diversity appeared to shift notably along the chronosequence. In the 0 m soils, the majority of sequences were related to *Chamaesiphon subglobosus* within the Chroococcales, and *Leptolyngbya* sp. SV1-MK-52 within the Oscillatoriales. The diversity of cyanobacterial sequences increased in the 100 m soils, and included sequences related to both the Oscillatoriales and the Nostocaceae. The 500 m soils contained a less diverse suite of cyanobacterial genes, including an abundant sequence of uncertain phylogenetic position, which appears to be distantly related to a 16S rRNA gene from a seafloor sediment sample (Fig. 6).

Soil Nutrient and Physical Parameters. Nutrient pools in these soils were low, approximately 1 mg g^{-1} organic carbon, 0.1 mg g^{-1} nitrogen and 2 μg g^{-1} resin-extractable P, whereas pH and inorganic C concentrations were relatively high in all soils (Table 2). There was a significant increase in soil N content along the chronosequence. In addition, there was a decreasing, but nonsignificant trend in resin-extractable P along the soil age gradient. Water content in all soils was low, and the 500 m soils were significantly drier than the other soils at the time of sampling. Soil C/N ratios did not significantly vary along the chronosequence (Table 2).

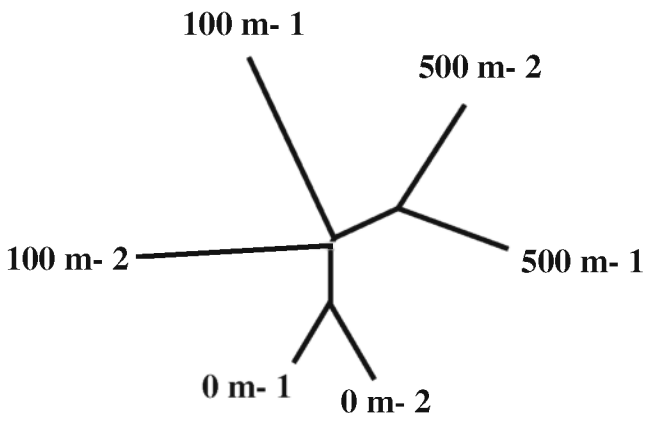

Figure 4. Neighbor-joining topology summarizing the pairwise differences in the phylogeny of each of the communities based on the likelihood test statistic. Branch lengths are proportional to the phylogenetic difference between communities.

Discussion

Although most soil biogeochemical characteristics showed little change along the ~20-year soil age gradient (Table 2), our molecular phylogenetic analyses revealed dramatic shifts in the associated microbial community composition along the chronosequence (Figs. 1, 2, 3, 4, 5 and 6; Table 1). Our results suggested both significant changes in microbial community composition along the chronosequence, as well as an overlap in the replicate communities of both the youngest and the oldest soils (Fig. 4). In addition, our results suggest that autotrophic N fixers are abundant in these soils (Figs. 1 and 6), and may be important in the biogeochemistry of newly deglaciated, unvegetated soils. Below, we further elaborate on how specific information about microbial community composition can advance our understanding about general successional patterns in this system.

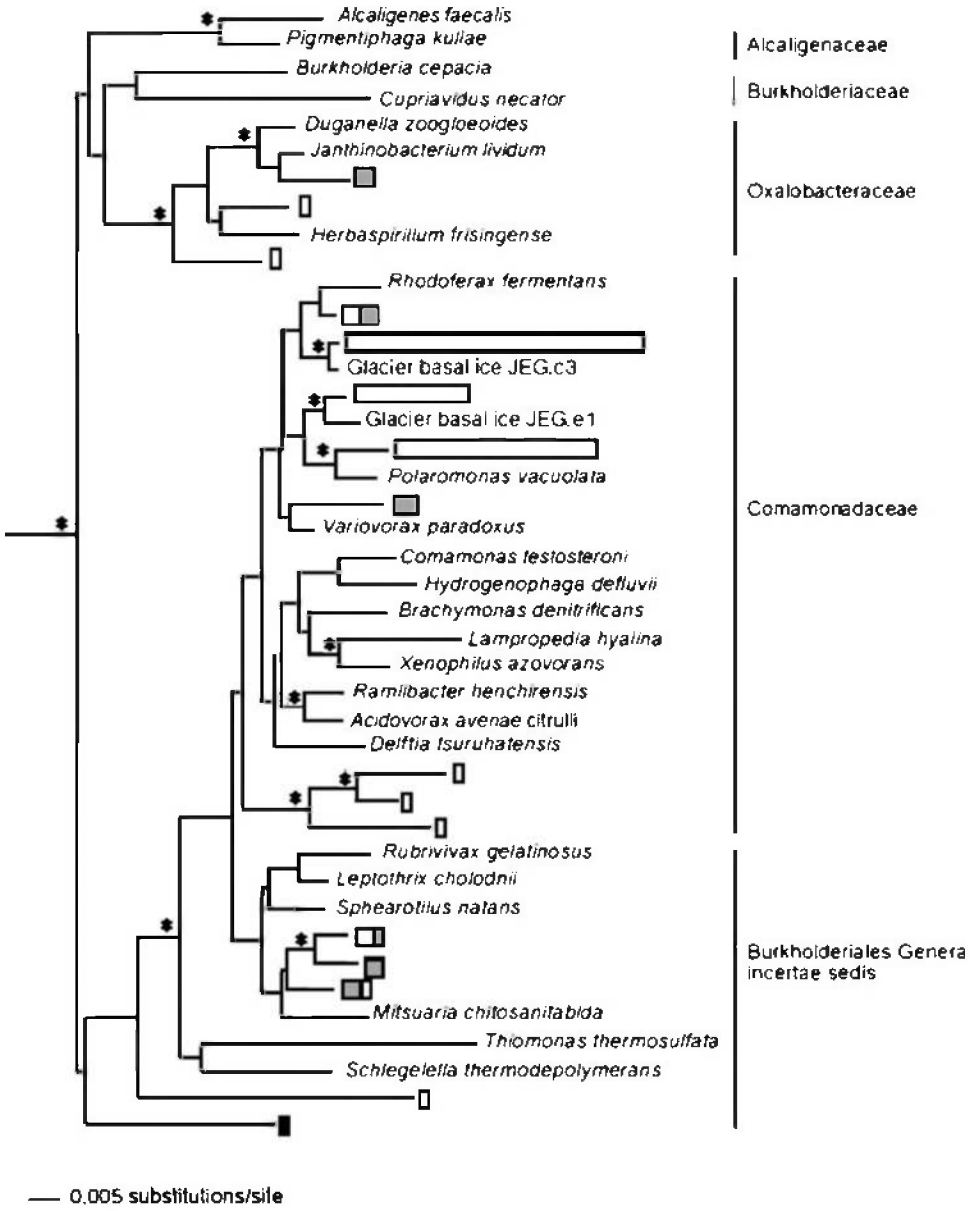

Figure 5. Neighbor-joining phylogenetic tree showing representative sequences from the clones related to the Betaproteobacteria. Rectangles represent sequences from this study. The relative size of the rectangle symbolizes the number of sequences in the 0 m (white), 100 m (dotted), and 500 m (black) soils. Tree is rooted with *Rhodobacter* sp. HTCC515 (AY584573) and *Sphingomonas oligophenolica* (AB018439). *: A parsimony and distance bootstrap value of 80 or higher. Accession numbers: *Alcaligenes faecalis* (AY667065), *Pigmentiphaga kullae* (AF282916), *Burkholderia cepacia* (AY099314), *Cupriavidus necator* (AF191737), *Duganella zoogloeoides* (D14256), *Janthinobacterium lividum* (AY581141), *Herbaspirillum frisingense* (AY043372), *Rhodoferax fermentans* (D16212), Glacier basal ice JEG.C3 (DQ228395), Glacier basal ice JEG.e1 (DQ228403), *Polaromonas vacuolata* (U14585), *Variovorax paradoxus* (AF532868), *Comamonas testosteroni* (AJ606336), *Hydrogenophaga defluvii* (AJ585993), *Brachymonas denitrificans* (D14320), *Lampropedia hyalina* (AY291121), *Xenophilus azovorans* (AF285414), *Ramlibacter henchirensis* (AF439400), *Acidovorax avenae* citrulli (AF137506), *Delftia tsuruhatensis* (AJ606337), *Rubrivivax gelatinosus* (AM086242), *Leptothrix discophora* (X97070), *Sphearotilus natans* (Z18534), *Mitsuaria chitosanitabida* (AY856841), *Thiomonas thermosulfata* (U27839), *Schlegelella thermodepolymerans* (AY538709).

General Patterns in Microbial Diversity. The number of unique sequences represented in the clone libraries and the Shannon index of diversity doubled between the 0 and 100 m soils, and then plateaued in the 500 m soils (Fig. 1, Table 1). Similarly, the number of bacterial divisions and phylogenetic diversity increased by approximately 60% between the 0 and 100 m and leveled off in the 500 m soils (Table 1). This initial increase in diversity (from 0 to 100 m) corroborates other estimates of functional diversity based on microbial

processes and enzyme activity in recently deglaciated soils [54]. In addition, it is similar to the well-documented increases in plant community diversity observed in successional soils [30], and a recent study that demonstrated an increase in archaeal diversity along a successional gradient [33]. Our results are not consistent with a ribosomal fingerprinting study, which suggest a decrease in microbial diversity with soil age in early successional soils [48]. However, clone library techniques are more sensitive to sequence variation in the 16S rRNA molecule, and therefore may be a more accurate reflection of the community dynamics. This increase in the diversity of the microbial community may be attributable to an increase in the diversity and amount of energy and carbon inputs into the system, both in the forms of available light and organic material from deposition, permitting growth of a broader suite of microorganisms. In addition, changes in microecological interactions (e.g., competition) could result in a more diverse microbial community.

Interestingly, Jumpponen [24] showed a decrease in fungal diversity along an early successional, unvegetated gradient of Lyman glacier in the North Cascade Mountains (Washington, USA). He attributed this decrease in diversity to the presence of a diverse, but dormant, spore bank in younger soils that was subsequently displaced by a less diverse, but active fungal community. Additionally, Jumpponen [24] observed high spatial heterogeneity in the fungal communities in recently deglaciated soils, as the majority of the 37 phylotypes that he sequenced were found in only 1 of the 16 soil samples that he examined. This may suggest that fungal community assembly is more stochastic or variable than bacterial succession in early successional soils.

Microbial Dispersal. Sequences related to the Betaproteobacteria were most dominant in the youngest soils, and their abundance decreased markedly with soil age. Phylogenetic analysis placed the majority of the sequences from the 0 and 100 m soils in the Comamonadaceae cluster of the Betaproteobacteria (Fig. 5). Organisms from this clade have been cultured from several "frozen" environments, including glacial ice cores [46] and permanent Antarctic lake ice [17]. In addition, related sequences have been found in 16S rRNA gene libraries from subglacial ice above Lake Vostok, Antarctica [37], as well as from underneath the Bench Glacier in Alaska

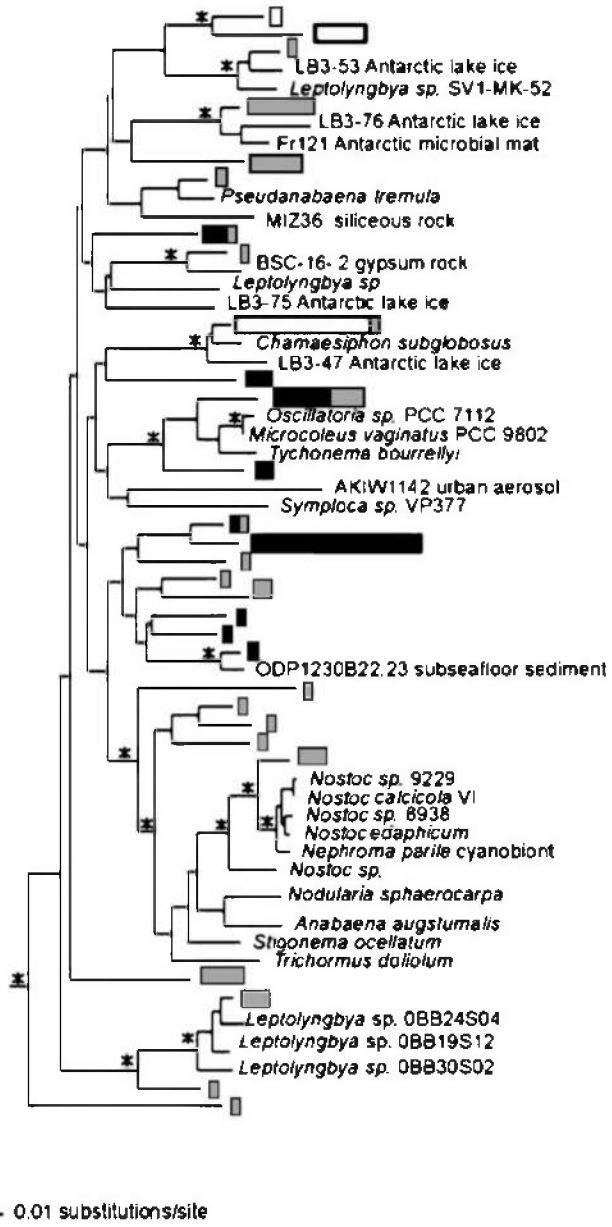

Figure 6. Neighbor-joining phylogenetic tree showing representative sequences from the clones related to the Cyanobacteria. Rectangles represent sequences from this study. The relative size of the rectangle symbolizes the number of sequences in the 0 m (white), 100 m (dotted), and 500 m (black) soils. Tree is rooted with *Rhodobacter* sp. HTCC515 (AY584573) and *Sphingomonas oligophenolica* (AB018439). *: A parsimony and distance bootstrap value of 80 or higher. Accession numbers: LB3-53 (AF076159), *Leptolyngbya* sp. SV1-MK-52 (AY239604), LB3-76 (AF076158), Fr121 (AY151728), *Pseudanabaena tremula* (AF218371), MIZ36 (AB179527), BSC-16-2 (AY422697), *Leptolyngbya* sp. (X84809), LB3-75 (AF076164), *Chamaesiphon subglobosus* (AY170472), LB3-47 (AF076163), *Oscillatoria* sp. PCC 7112 (AB074509), *Microcoleus vaginatus* PCC 9802 (AF284803), *Tychonema bourrellyi* (AB045897), AKIW1142 (DQ129645), *Symploca* sp. VP377 (AF306497), 66863799 (AB177177), *Nostoc* sp. PCC 9229 (AY742451), *Nostoc calcicola* VI (AJ630448), *Nostoc* sp. 8938 (AY742454), *Nostoc edaphicum* (AJ630449), *Nephroma parile* cyanobiont (AF506257), *Nostoc* sp. (AF027655), *Nodularia sphaerocarpa* (AJ781149), *Anabaena augstumalis* (AJ630458), *Stigonema ocellatum* (AJ544082), *Trichormus doliolum* (AJ630455), *Leptolyngbya* sp. 0BB24S04 (AJ639893), *Leptolyngbya* sp. 0BB19S12 (AJ639895), *Leptolyngbya* sp. 0BB30S02 (AJ639892).

Table 2. Biogeochemical parameters for the three soils

Sample (m)	*Approx. age (years)*	*Organic C*	*N*	*P*	*pH*	*% H_2O*	*C/N*
0	< 1	1.29 (0.34)^a	0.08 (0.00)^a	2.52 (0.85)^a	7.5 (0.2)^a	9.16 (1.23)^a	15.3 (3.20)^a
100	1–4	1.14 (0.18)^a	0.09 (0.00)^a	1.71 (0.17)^a	7.6 (0.1)^a	9.81 (0.90)^a	12.9 (2.23)^a
500	20	1.59 (0.63)^a	0.11 (0.01)^b	1.53 (0.36)^a	7.5 (0.1)^a	2.54 (2.26)^b	15.3 (7.50)^a

Organic *C* and *N* are expressed in $mg \cdot g^{-1}$, *P* is the resin-extractable fraction expressed in $\mu g \cdot g^{-1}$. Numbers in parentheses are standard deviations, values followed by the same letter are not significantly different from one another ($P < 0.05$). For each measurement, $n = 4$.

and John Evans Glacier in Nunavut, Canada [49]. Other sequences from the Comamonadaceae cluster have been obtained from inside a deep sea rock [21], as well as from the vadose zone of a California Mollisol [26].

The presence of close relatives in rock and ice environments suggests that these bacteria may be living in or under the glacier, and that melting glacial ice or exposure of subglacial rock may seed the youngest soils with organisms from the Comamonadaceae. If so, this may explain the fact that the youngest soils appeared to harbor different, but very closely related clades in the two samples (Figs. 1 and 4). If these organisms originated from within or beneath the glacier, they could have been physically and genetically isolated for hundreds to thousands of years, allowing for local speciation events. This hypothesis is in agreement with other studies that demonstrated both functional and phylogenetic differentiation of bacteria from geographically isolated frozen environments [50].

On average, the phylotypes from the youngest soils were much more closely related to other sequences in Genbank than the phylotypes from the older soils (average BLAST score ~1700 bits in the 0 m soils; ~1400 bits in all older soils). This pattern is primarily driven by the dominance of the Betaproteobacteria in the youngest soils; if these phylotypes are removed from the analyses the average BLAST score averages ~1400 bits in the 0 m soils as well. Some of the very closely related betaproteobacterial sequences in Genbank are from other frozen environments around the world (Fig. 5), suggesting that geographically separated ice environments may select for similar suites of organisms. However, many of the closely related betaproteobacterial sequences are from other types of environments, ranging from drinking water to contaminated soils. This may indicate that early microbial colonizers are more "cosmopolitan", and are replaced by more endemic species along the soil age gradient.

Nutrient Dynamics. The Comamonadaceae is a phenotypically diverse group of organisms (Fig. 5), which makes functional predictions for this abundant group difficult. Some of the cultured relatives of these organisms are autotrophs, and can fix carbon photosynthetically [39] or using H_2 as an electron source [22]. However, most other isolates from the Comamonadaceae are heterotrophs [58], and are capable of metabolizing both recalcitrant [36] and more labile [46] carbon sources. The high rates of pollen deposition in this region [38] may provide an important carbon source for these organisms in an otherwise C-limited environment. Additionally, closely related organisms that have been isolated from similar environments have been cultured on organic carbon sources [17, 46], suggesting that these bacteria live a heterotrophic lifestyle. This is similar to macrobial successional patterns, where heterotrophs (insects) are often among the first organisms to arrive in recently deglaciated soils [18].

Photosynthetic autotrophs appeared to be common in all of the soil samples that we analyzed. For example, sequences related to the Cyanobacteria were abundant in most of the soils (Fig. 1). In addition, sequences similar to stramenopile plastids were prominent in the 100 m libraries, comprising 16% and 8% of genes from these soils (Fig. 1). The importance of photosynthesis in early succesional soils is obvious; in the absence of a large pool of organic carbon, C-fixing organisms would clearly have an advantage over heterotrophs. The significance of photosynthesis in early successional communities is also supported by work in aquatic systems [4] that show increasing chlorophyll *a* abundance with distance from a glacier. Interestingly, although there was an increasing trend, there was no significant difference in the organic C pools along the chronosequence (Table 2). This suggests that although these carbon-fixing organisms appear to make up a large portion of the microbial community, they are not adding detectable amounts of fixed carbon to these soils. However, many Cyanobacteria can live both autotrophically and mixotrophically, and the actual biogeochemical roles of the organisms represented by these sequences in these soils are unknown. The lack of a significant increase in organic C pools also suggests that although rates of deposition are high in this region, cumulative pollen inputs do not result in significant increases in soil organic matter in this time frame.

Our data also contain some interesting parallels to N cycling in plant successional communities. The significance of N fixation in the ecosystem dynamics of young plant communities is well established, and our work demonstrates that free-living N fixation may be important prior to plant establishment. For example, phylogenetic analysis (Fig. 6) showed that the overwhelming majority of Cyanobacteria in these soils are closely

related to heterocystous (e.g., *Anabaena*, *Nostoc*) and nonheterocystous (e.g., *Oscillatoria*, *Microcoleus*) nitrogen fixers. If these organisms have the ability to fix nitrogen, this could explain the significant increase in N in the older soils (Table 2). Indeed, recent work from our laboratory (Reed and Schmidt, unpublished data) shows that N fixation (acetylene reduction) rates in the 100 m soils are much higher than in the 0 m soils. However, it should be emphasized that the organisms responsible for this activity are still unknown, and that N fixation may also be driven by heterotrophic organisms in this system. Many of the 16S rRNA genes that we sequenced from these soils are only distantly related to cultured organisms, and may originate from organisms that are capable of nitrogen fixation.

Finally, the 16S rRNA gene library data from this study may explain results from other studies reporting a microbial shift from r to K selection (decrease in culturability) [47] along the successional gradient. Cyanobacteria would not have been cultured using the culture conditions and media used in the Sigler *et al.* [47] study. In addition, our later successional soils (Fig. 1) show an increase in other, difficult-to-culture groups including *Acidobacteria*, candidate division WYO, *Gemmatimonadetes*, and *Verrucomicrobia*, possibly explaining this observed switch from r- to K- selected microorganisms with increasing soil age. Bacteria from these groups are common soil bacteria, but are difficult to isolate using traditional culturing techniques. This may suggest that these organisms utilize complex carbon substrates that are more likely to be present in older, more developed soils, and that are not traditionally used in culturing efforts. Alternatively, this may support that the growth of these bacteria requires interactions with other types of bacteria, which would in turn make them very difficult to isolate in pure cultures.

Macroscale controls over microbial processes have long been a focus of ecology; however, the relationship between microbial populations and processes is poorly understood. This work demonstrates that there may be environmental-level influences on bacterial community succession, and that these patterns of community replacement may parallel plant community development. For example, our study suggests that, like macrobial heterotrophs, microbial heterotrophs are likely to be among the first colonizers of new soils. In addition, biogeochemical and sequence analyses suggest that nitrogen fixation is important in these very young, barren soils, much as it is in the early stages of plant community development. Finally, the initial increases in microbial community diversity observed in this study are similar to patterns seen in plant communities. Thus, despite the small-scale heterogeneity of soil environmental conditions, microbial populations are likely controlled, to some extent, by factors which also regulate macrobial communities.

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