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Functional Shifts in Unvegetated, Perhumid, Recently-Deglaciated Soils Do Not Correlate with Shifts in Soil Bacterial Community Composition

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Past work in recently deglaciated soils demonstrates that microbial communities undergo shifts prior to plant colonization. To date, most studies have focused on relatively 'long' chronosequences with the ability to sample plant-free sites over at least 50 years of development. However, some recently deglaciated soils feature rapid plant colonization and questions remain about the relative rate of change in the microbial community in the unvegetated soils of these chronosequences. Thus, we investigated the forelands of the Mendenhall Glacier near Juneau, AK, USA, where plants rapidly establish. We collected unvegetated samples representing soils that had been ice-free for 0, 1, 4, and 8 years. Total nitrogen (N) ranged from 0.00~0.14 mg/g soil, soil organic carbon pools ranged from 0.6~2.3 mg/g soil, and both decreased in concentration between the 0 and 4 yr soils. Biologically available phosphorus (P) and pH underwent similar dynamics. However, both pH and available P increased in the 8 yr soils. Nitrogen fixation was nearly undetectable in the most recently exposed soils, and increased in the 8 yr soils to ~5 ng N fixed/cm^2/h, a trend that was matched by the activity of the soil N-cycling enzymes urease and β-1,4-N-acetyl-glucosaminidase. 16S rRNA gene clone libraries revealed no significant differences between the 0 and 8 yr soils; however, 8 yr soils featured the presence of cyanobacteria, a division wholly absent from the 0 yr soils. Taken together, our results suggest that microbes are consuming allochtonous organic matter sources in the most recently exposed soils. Once this carbon source is depleted, a competitive advantage may be ceded to microbes not reliant on *in situ* nutrient sources.

Keywords: N fixation, microbial community structure, succession, soil enzyme activity

Succession is a fundamental ecological process defined as a directional change in the species composition of communities through time (Krebs, 2001). Primary succession occurs in newly deposited or exposed soils, such as those formed by volcanic eruptions (e.g., del Moral *et al.*, 1995) and glacial retreat (e.g., Crocker and Dickson, 1957). In general, these soils are extremely nutrient poor, with little to no nitrogen (N), organic carbon (C), or available phosphorus (P). As succession proceeds, interactions between this oligotrophic substrate and the climatic and biotic components of the ecosystem result in predictable changes in soil fertility, which are reflected in a variety of ecosystem processes ranging from net primary production to nutrient limitation. Primary succession has been well described for plant communities (e.g., Chapin *et al.*, 1994; Vitousek, 2004) and, although the actual biota vary from site to site, nutrient dynamics appear to explain some consistent shifts in the functional characteristics and life history strategies of plants across geographically, climatically, and geologically disparate primary succession sequences.

Before plants colonize newly exposed substrate, however, microbes may perform important functions critical to initial soil development and nutrient accumulation (Nemergut *et al.*, 2007; Jones *et al.*, 2008). Studies of post-glacial chronosequences have confirmed that microbial biomass and activity increase with soil age in unvegetated soils (Ohtonen *et al.*, 1999; Sigler and Zeyer, 2002; Tscherko *et al.*, 2004). Moreover, shifts in the microbial community structure have been observed over relatively rapid (<10 years) time frames in these plant-free sites (Sigler *et al.*, 2002; Sigler and Zeyer, 2002; Nemergut *et al.*, 2007; Noll and Wellinger, 2008).

Molecular gene surveys, microscopy, and process measurements have been used to reveal the types of microbes that colonize these unvegetated soils and to generate hypotheses about the relationship between structure and function during succession. For example, over an ~80 year chronosequence at the Puca Glacier, Peru, microbial activity, and diversity increased in as few as four years. Here, relatives of N-fixing cyanobacteria were abundant and coincided with increases in soil N pools and N fixation rates (Nemergut *et al.*, 2007; Schmidt *et al.*, 2008). Likewise, Kastovska *et al.* (2005) found that *Nostoc*, an N-fixing cyanobacterium, was abundant in

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recently deglaciated soils and Duc *et al.* (2009) demonstrated the importance of N fixation in the forelands of the Damma glacier. Other studies have shown that the activities of enzymes involved in phosphorus and carbon cycling also undergo significant shifts over unvegetated soil age gradients (Tscherko *et al.*, 2004; Schmidt *et al.*, 2008). Together, these studies show that active microbial communities quickly establish on barren substrates, and that they perform a diversity of biogeochemical functions prior to the establishment of plants or visible soil crusts.

To date, most studies of unvegetated soils have focused on relatively 'long' chronosequences with extended pioneer stages characterized by scattered grasses (Tscherko *et al.*, 2005; Hammerli *et al.*, 2007), and the ability to sample plant-free sites over at least 50 years of development (Tscherko *et al.*, 2004; Nicol *et al.*, 2005; Nemergut *et al.*, 2007). However, some recently deglaciated soils feature rapid plant colonization; thus, questions remain about the relative rate of change in the structure and function of the microbial community in these sites. For example, the low-elevation Mendenhall Glacier, near Juneau, AK, is located in a perhumid environment (Alexander and Burt, 1996). This climate, in combination with the close proximity of a mature spruce forest, greatly expedites plant establishment. Seedlings are scattered in soils as young as 1 year, and alder and spruce are well established in soils within 38 years of their emergence from beneath the glacier (Alexander and Burt, 1996). Here, the foreland features sculpted bedrock with small (0.5~5 m^2) pockets of soil, which are nearly entirely colonized by plants within ~12 years. Thus, to examine if and how microbial communities change prior to plant establishment, we selected unvegetated soils from four transects (0, 1, 4, and 8 years post-deglaciation) and examined element pools, microbial activity, and microbial community composition. We hypothesized that there would be significant functional and phylogenetic shifts in the soil community between the most recently exposed and 8 yr soils. We predicted that changes in measures of elemental fluxes (e.g., N fixation, soil enzyme activities) would correspond with shifts in microbial community composition.

Materials and Methods

Site description

Juneau, Alaska has a mean annual temperature of between 4.4 and 6.1°C and mean annual precipitation at the Mendenhall foreland is estimated to be >2,500 mm (Alexander and Burt, 1996). The Mendenhall Glacier is one of the more than 40 large valley glaciers draining the 3800 km^2 Juneau Icefield, which mantles the northern Coast Range in southeastern Alaska. The Mendenhall Glacier is ~22 km long and flows from 1,600 m above sea level (masl) to 20 masl at its terminus (Motyka *et al.*, 2003). The glacier has been retreating since the end of the Little Ice Age, and has moved 3 km during the last century. The eastern portion of the glacier terminates in Mendenhall Lake, while the lateral portions of the terminus are grounded on a bedrock outcrop where soils consist of unconsolidated glacial till deposits. Both coarse and fine fractions are predominately (>75%) granitic (Burt and Alexander, 1996). The soils along the chronosequence are classified as shallow Entisols and the texture of the <2 mm size fraction averages approximately 39% sand, 47% silt, and 14% clay (C. Cleveland, unpublished data). The first vascular colonizers are *Epilobium latifolium* and *Equisetum variegatum* (Crocker and Dickson, 1957). Our site surveys reveal that approximately 0%, 1%, 2%, and 5% of the total area and approximately 0%, 5%, 25%, and 50% of the area of soil pockets are vegetated at 0, 1, 4, and 8 years post-deglaciation, respectively. Lichens and cyanobacterial crusts are rare features of the unvegetated landscape.

Sampling scheme and collection method

Samples were collected in early June of 2006 from sites ranging from one month (~1 m from the glacier terminus) to 8 yr (~220 m from the glacier face) ice-free. At each location (0, 1, 4, and 8 yr ice-free), a total of five replicate samples (~1-2 m apart) were collected along transects paralleling the glacier terminus (0 yr: 58.435837 N, 134.554673 W, 1 yr: 58.435532 N, 134.554415 W, 4 yr: 58.433402 N, 134.55317 W, 8 yr: 58.433413 N, 134.554346 W). Along each transect, sampling locations were randomly selected. Unvegetated soil (top 10 cm) was aseptically sampled into sterile tubes and transported to the lab on ice. Soils were stored at -20°C (for enzyme activity and biogeochemical analysis) and -80°C (for molecular phylogenetic analysis).

Soil organic carbon, total nitrogen, and pH analyses

Soils were sieved to 2 mm and ground to a fine powder. Inorganic C was removed as described in Nemergut *et al.* (2007). Approximately 45 mg of dried soil were packaged into tin capsules and % C and N by mass were measured using a Carlo Erba combustion-reduction elemental analyzer (CE Elantech, USA). Five replicates were analyzed for each transect, and two analytical replicates were measured for each sample. Soil pH was determined as described by Nemergut *et al.* (2007).

Soil available P analysis

The biologically available P fraction (the sum of the resin- and bicarbonate-extractable P pools of the Hedley fractionation) was extracted using the methods described by Tiessen and Moir (1993). Five replicates were analyzed for each transect, and three analytical replicates were measured for each sample. Extracts were analyzed using the molybdate ascorbic acid method (Kuo, 1996) with an Alpkem autoanalyzer (OI Analytical, USA).

Enzyme assays

The activities of eight extracellular enzymes [β-D-1,4-cellobiosidase (CBD), α-glucosidase (AG), β-glucosidase (BG), β-xylosidase (BXYL), leucine amino peptidase (LAP), acid phosphatase (PHOS), β-1,4-N-acetyl-glucosaminidase (NAG), and urease] were assayed for each sample using the procedures outlined by Saiya-Cork *et al.* (2002) and 50 μl of 200 μM substrate. Assay plates were incubated in the dark at 14°C for 18~20 h (Weintraub *et al.*, 2007; King *et al.*, 2008; Nemergut *et al.*, 2008), the minimum amount of time necessary to observe enzyme activity in cold-adapted soils. Fluorescence and absorbance were measured using a Synergy

HT Multi-Detection Microplate Reader (Biotek, USA).

Nitrogen fixation

The acetylene reduction assay (Hardy *et al.*, 1968) was performed in the field with a method modified from Belnap (1996) described in Reed *et al.* (2007). Ten soil cores were taken along each of the transects, for a total of 40 samples. Rates of acetylene reduction were converted into rates of N fixation using a 3:1 acetylene reduction:N fixation conversion factor (Hardy *et al.*, 1968).

Statistics

Soil biogeochemical parameters, N fixation rates and enzyme activities were compared using one-way ANOVAs and Tukey's *post-hoc* analysis with SPSS 11.0.4 (Chicago, IL, USA). Non-normal data were ln-transformed prior to statistical analysis. For all data, significance was assigned at $\alpha=0.05$.

Molecular phylogenetic analysis

Two replicates (out of five) from the 0 and 8 yr soils were pooled for DNA extraction in two batches, resulting in four total extractions (0A, 0B, 8A, 8B). DNA was extracted from soil samples as described in Nemergut *et al.* (2007).

Approximately 30 ng of DNA were amplified with the primers 27f and 1492r (Lane, 1991). The reaction mixtures consisted of 10 μM each primer, 200 μM dNTPs, 1.25 U of *Taq* DNA polymerase (Promega, USA) in *Taq* DNA polymerase buffer (Promega). After an initial denaturation step at 94°C for 1 min, 30 cycles of 94°C for 1 min, 53±5°C for 30 sec and 72°C for 2.5 min with a terminal 10 min extension at 72°C were performed. Purified PCR products were ligated into the vector TOPO 2.1 (Invitrogen, USA) and transformed into *Escherichia coli* following the manufacturer's instructions. 16S rRNA genes were sequenced as described by Nemergut *et al.* (2007).

Sequences were edited in Sequencher 4.1 (Gene Codes Co., USA), aligned using the NAST aligner available from the Greengenes web interface (DeSantis *et al.*, 2006), and subjected to BLAST searches (Altschul *et al.*, 1990). 16S rRNA gene sequences were then subjected to chimera check in Mallard (Ashelford *et al.*, 2006) and Bellerophon (Huber *et al.*, 2004) using the default parameters. Phylogenetic identity was assigned using both BLAST matches and the Hugenholz taxonomy of closely related genes available in the Greengenes database. We used DOTUR (Schloss and Handelsman, 2005), employing the further neighbor sequence assignment method and a precision level of 100 to

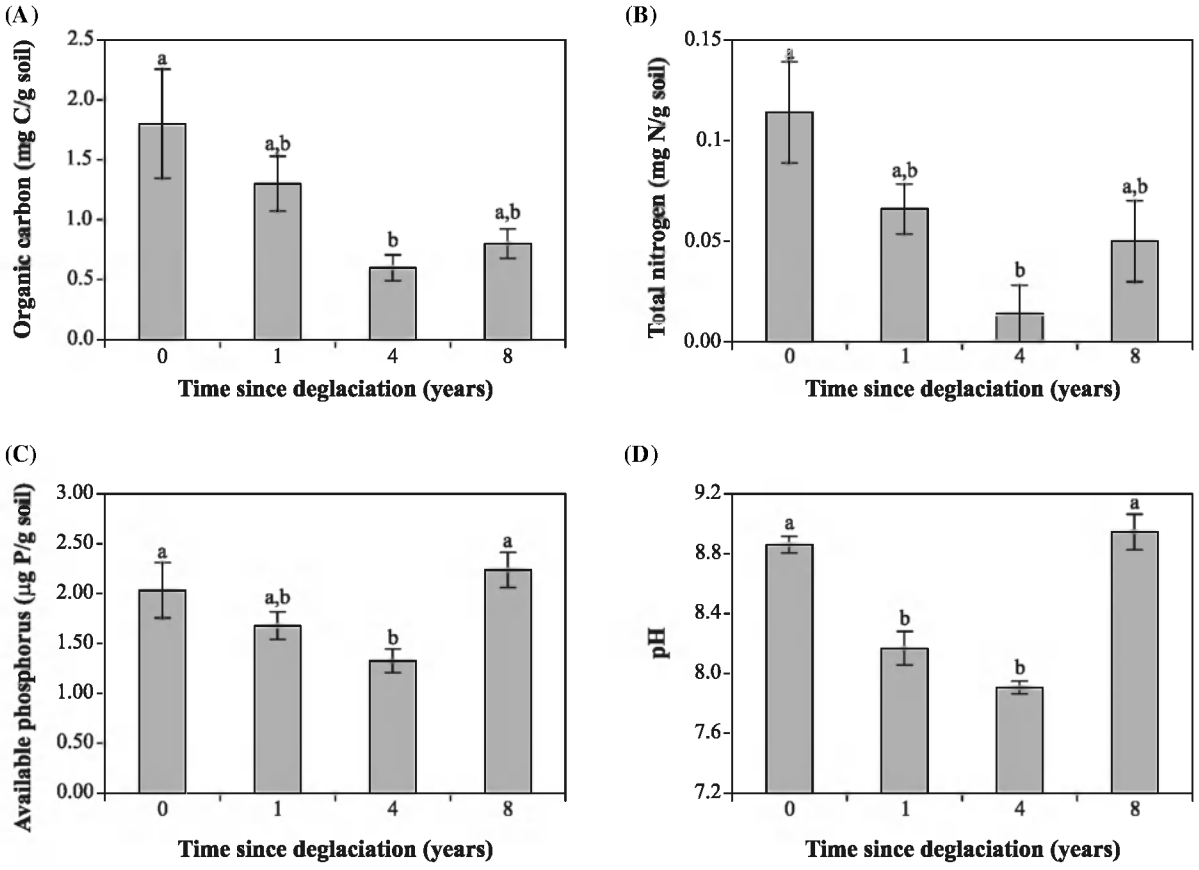

Fig. 1. Soil characteristics along the chronosequence. Graphs show changes in organic carbon (A), total nitrogen (B), available phosphorus (C), and pH (D). The x-axis shows time since deglaciation in years. Different letters indicate values that are significantly different from one another (ANOVA, $P<0.05$). Error bars show standard error.

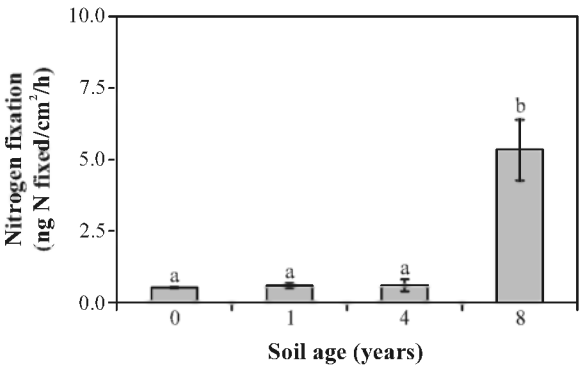

Fig. 2. Nitrogen fixation rates along the chronosequence. The x-axis shows time since deglaciation in years. Different letters indicate values that are significantly different from one another (ANOVA, $P<0.05$). Error bars show standard error.

designate sequences to operational taxonomic units (OTUs), defined as sequences at least 3% different from all others. A phylogenetic tree for the Burkholderiales group of the β-Proteobacteria was constructed in PAUP 4.0b (Swofford, 2001) with close relatives selected from the Greengenes database using both the distance and parsimony optimality criteria and bootstrap analysis (100 replicates). Sequences were deposited in GenBank with the accession numbers GQ396801-GQ397099.

Results

Soil chemical and functional parameters

Four sites were sampled, corresponding to soil ranging from 0~8 yr ice-free. Pools of organic C, total N, and available P in the soils at the Mendenhall Glacier foreland were low relative to other, more developed soils (Fig. 1). Levels of organic C varied from 0.6~2.3 mg/g soil, total N from 0.00~0.14 mg/g soil, and available (resin and bicarbonate extractable) P from 1.4~2.5 μg/g soil. Organic C, total N, and available P pools were all lowest in the 4 yr soils, values that were significantly lower than the 0 yr soils. P pools in the 8 yr soils were significantly higher than in the 4 yr soils (Fig. 1). Soil pH decreased significantly from 8.8 to 7.8 in the first four years, and increased significantly to 8.9 in the 8 yr soils (Fig. 1).

Functional attributes of soil microbial communities over the chronosequence were also examined. Nitrogen fixation was nearly undetectable in the most recently exposed soils, and increased in the 8 yr soils to ~5 ng N fixed/cm^2/h (Fig. 2). Eight soil enzymes were also assayed. CBD and BXYL activities were very low, and showed no significant differences over the chronosequence (data not shown). Enzymes involved in the decomposition of plant material, including AG and BG, were active in these recently exposed, unvegetated soils; however, no significant differences were observed between transects (ANOVA, $P>0.05$) (Table 1). Likewise, LAP and PHOS, two enzymes involved in N and P cycling, respectively, displayed activity; however, due to the extremely high variance among replicates these enzymes revealed no significant changes along the chronosequence (Table 1). The activities of NAG, which catalyzes a reaction involved in chitin and peptidoglycan degradation, and urease, which cleaves urea into ammonium and CO_2, showed significant differences between transects, and displayed the highest activity in the 8 yr soils (Table 1).

16S rRNA gene clone libraries

We constructed two replicate 16S rRNA gene clone libraries from the most recently exposed (0 yr) and longest exposed (8 yr) soils (0A, 0B, 8A, 8B, respectively). Along each transect, two clone libraries were constructed, each consisting of soils bulked from two sampling locations. For each library, approximately 10 sequences were identified as putative chimeras and were removed from subsequent analyses. A total of 480 sequences (0A=134, 0B=136, 8A=109, 8B=101) were obtained from these two soils, with 299 OTUs that were at least 3% different from all other sequences. Rarefaction analysis (Schloss and Handelsman, 2005) suggested that the diversity of all libraries was not adequately captured at the species level (i.e., 3% difference, data not shown). However, recent analyses suggest that the number of clones analyzed here is sufficient to generate consistent microbial community comparisons (Ley *et al.*, 2008).

The sequences obtained represented 19 bacterial divisions and proteobacterial subdivisions (Table 2). Each clone library contained sequences from 13 to 17 divisions or subdivisions, values which did not vary notably between replicates or between transects. β-Proteobacteria was the most common clade represented in all libraries, comprising ~30% of sequences in the 0 yr libraries, and ~20% of sequences in 8 yr libraries. Five clades (β-*Proteobacteria*, *Actinobacteria*, α-*Proteobacteria*, *Bacteroidetes*, and γ-*Proteobacteria*) were highly represented in all samples, and jointly comprised at least 60% of the sequences in all libraries. Some differences were apparent in the less highly represented divisions. For example, sequences related to candidate divisions TM6 and SC3, the *Firmicutes*, and *Nitrospirae* were generally repre-

Table 1. Activity of enzymes along the chronosequence

Soil age (yr)	AG	BG	LAP	PHOS	NAG	Urease
0	0.04 (0.025)	0.66 (0.138)	3.20 (0.602)	1.67 (0.654)	0.07 (0.034) a,b	0.00 (0.000) a
1	0.10 (0.040)	0.40 (0.149)	3.05 (0.761)	1.80 (0.863)	0.00 (0.000) a	23.69 (15.953) a,b
4	0.01 (0.006)	0.18 (0.060)	3.11 (0.336)	1.45 (0.303)	0.01 (0.008) a	4.24 (4.244) a,b
8	0.08 (0.043)	0.43 (0.146)	5.57 (1.393)	4.57 (1.765)	0.40 (0.200) b	87.96 (39.38) b

Values are expressed as nmol product/h/g soil with the exception of urease, which is expressed in μmol product/h/g soil. Numbers in parentheses are standard errors. Abbreviations: AG, α-glucosidase; BG, β-glucosidase; LAP, leucine amino peptidase; PHOS, acid phosphatase; and NAG, β-1,4-N-acetyl-glucosaminidase. Different letters indicate values that are significantly different from one another (ANOVA, $P<0.05$).

Table 2. Relative abundance of 16S rRNA gene phylotypes in clone libraries from different soils. Numbers show percentage of clones from each group in each library

Division or subdivision	0A	0B	8A	8B
Acidobacteria	7	4	15	3
Actinobacteria	16	17	18	10
α-Proteobacteria	11	21	13	16
Bacteroidetes	10	18	6	10
β-Proteobacteria	34	30	22	18
Chlorobi		1		1
Chloroflexi	4	1		9
Cyanobacteria			4	8
γ-Proteobacteria	1			1
Firmicutes		1	1	
γ-Proteobacteria	9	4	4	13
Gemmatimonadetes	1		8	3
Nitrospirae		1	1	1
Candidate Division OP10	1	1	3	3
Planctomycetes	1	1	3	1
Candidate Division SC3				1
Candidate Division TM6	1			1
Candidate Division TM7	2		2	
Verrucomicrobia	1	1	2	2

sented by a single sequence and not consistently detected in all libraries.

Qualitatively, 0A and 0B appeared to harbor similar lineages of bacteria. 8A and 8B also featured many of the same sequences as in the 0 yr soils, but contained 4~8% of sequences that were related to the cyanobacteria, a division not detected in the 0 yr soils. The microbial communities in the 8 yr soils showed more variation between replicate libraries than was apparent in the 0A and 0B soils. For example, 18% of the sequences in the 8A clone library were related to the Actinobacteria, while only 10% of sequences in the 8B library fell into this division. Additionally, sequences related to the *Acidobacteria* comprised 15% of the 8A library, but made up only 3% of the sequences from 8B.

We used two statistical tools to examine differences between 16S rRNA gene clone libraries: UniFrac (Lozupone *et al.*, 2006) and phylogenetic character mapping (P test) (Martin, 2002). When corrected for multiple comparisons, no libraries were significantly different using either metric (data not shown). As no differences were observed between the 0 and 8 yr soils, the intermediate soils were not examined for microbial community composition.

Discussion

Most previous studies of soil microbial community succession in recently deglaciated environments have focused sampling over the first ~100+ years of soil development (Schipper *et al.*, 2001; Sigler *et al.*, 2002; Tscherko *et al.*, 2003; Nicol *et al.*, 2005; Deiglmayr *et al.*, 2006; Kandeler *et al.*, 2006; Allison *et al.*, 2007). At the Mendenhall Glacier foreland, we focused our sampling over the first eight years of exposure to examine the changes in unvegetated soils prior to plant colonization. Despite this relatively short time frame, we observed changes in the elemental pools, pH, and microbial activity (Fig. 1 and 2, Table 1). As has been documented in other deglaciated systems (Schipper *et al.*, 2001; Tscherko *et al.*, 2003; Nemergut *et al.*, 2007) organic C, total N, and available P were 10 to 100 times lower than pools in more developed soils, which typically range in organic C content from 6 to 120 mg/g, total N content from 0.5 to 17 mg/g (Cleveland and Liptzin, 2007) and available P content from 5 to 125 μg/g (Cross and Schlesinger, 1995). Soil pH averaged 8.5, a value 1~3 units higher than other recently deglaciated systems (Schipper *et al.*, 2001; Tscherko *et al.*, 2003; Nemergut *et al.*, 2007). Enzyme activity rates were 1~3 orders of magnitude lower than other, vegetated, cold-adapted soil microbial communities (Weintraub *et al.*, 2007; Nemergut *et al.*, 2008) but were comparable to the low end of activities measured in other unvegetated, recently deglaciated systems (Tscherko *et al.*, 2003; King *et al.*, 2008; Schmidt *et al.*, 2008).

A gradual increase in soil C, N, and available P pools of newly exposed soils has been shown in multiple early successional environments, both before and after plant invasion (Schipper *et al.*, 2001; Sigler *et al.*, 2002; Schmidt *et al.*, 2008). However, these studies focused on examining soils over much longer time scales than the samples in the current study. Our work supports a different pattern in soil biogeochemical parameters over the first 8 years of soil development at the Mendenhall Glacier (Fig. 1). C, N, and P pools first decreased to nearly undetectable levels over the first four years. A similar decrease in soil C, N, and P pools has also been shown in the first 20~30 years of succession at the Ödenwinkel and Rotmoos glaciers (Tscherko *et al.*, 2003). pH displayed a similar pattern; however, we also observed a significant increase in soil pH in the 8 yr soil (Fig. 1). This is an unusual characteristic of recently deglaciated landscapes, which typically feature a steady decrease in pH (but see Hammerli *et al.*, 2007). The reasons

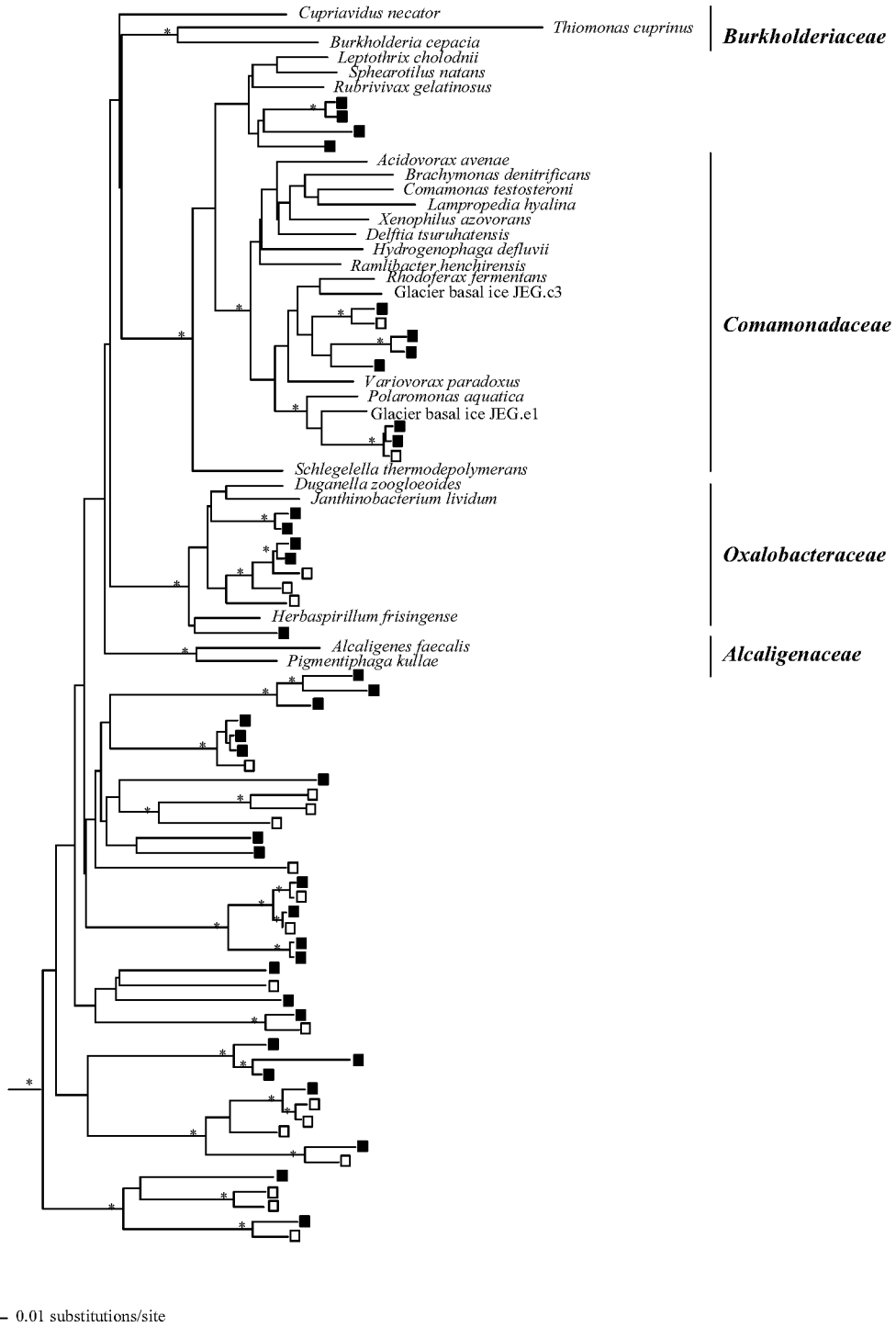

Fig. 3. Neighbor-joining phylogenetic tree showing representative sequences from the clones related to the *Burkholderiales* order of the β-*Proteobacteria*. Squares represent sequences from this study: 0 yr (black), 8 yr (white). Tree is rooted with *Rhodobacter* sp. HTCC515 (AY584573) and *Sphingomonas capsulata* (M59296). * A parsimony or distance bootstrap value of 90 or higher. Accession numbers: *Alcaligenes faecalis* (M22508), *Pigmentiphaga kullae* (AF282916), *Burkholderia cepacia* (X87275), *Cupriavidus necator* (M32021), *Duganella zoogloeoides* (X74914), *Janthinobacterium lividum* (AF174648), *Herbaspirillum frisingense* (AJ238359), *Rhodoferax fermentans* (D16211), Glacier basal ice JEG.C3 (DQ228395), Glacier basal ice JEG.e1 (DQ228403), *Polaromonas aquatica* (AM039831), *Variovorax paradoxus* (CS000359), *Comamonas testosteroni* (M11224), *Hydrogenophaga defluvii* (AJ585993), *Brachymonas denitrificans* (D14320), *Lampropedia hyalina* (AY291119), *Xenophilus azovorans* (AF285414), *Ramlibacter henchirensis* (AF439400), *Acidovorax avenae* (AF078761), *Delftia tsuruhatensis* (AY302438), *Rubrivivax gelatinosus* (M60682), *Leptothrix discophora* (X97070), *Sphearotilus natans* (Z18534), *Thiomonas cuprinus* (U67162), *Schlegelella thermodepolymerans* (AY538709).

for the observed dynamics in pH are unknown, but may reflect cation deposition from marine salts (White *et al.*, 1996) urease activity (Chin and Gorin, 1966), or heterogeneity in the underlying bedrock.

The pools of organic C, total N, and available P in the 0 yr soils indicate that a source of organic matter is available in the new substrate immediately following deglaciation at the Mendenhall Glacier (Fig. 1). Additionally, the activity of enzymes involved in the decomposition of cellulose (Table 1) in the most recently exposed soils supports that, while these soils appear barren, some plant material may be present. Indeed, small pieces of wood are scattered around the forelands of the Mendenhall Glacier, presumably derived from beneath the glacier as they are most abundant near the terminus. This and other carbon in these deglaciated soils could provide a source of organic material following the first few years of soil exposure, as has been shown in other ecosystems (Hodkinson *et al.*, 2001; Bardgett *et al.*, 2007). Interestingly, C, N, and P pools all declined significantly in the intermediate soils (Fig. 1). Likewise, although an ANOVA for all four sites was not significant, a t-test revealed a significant decrease in the activity of BG between the 0 and 4 yr soils (Table 1, $p=0.01$). BG is involved in cellulose degradation, and this pattern may reflect the metabolism of this organic matter over the first few years following deglaciation. It is important to note, however, that other scenarios could cause the observed patterns in soil nutrient pools, including variable rates in allochtonous organic matter deposition along the chronosequence or spatial variation in the organic matter content of glaciated soils.

Sequences related to the Burkholderiales clade of the β-Proteobacteria were abundant in libraries from the 0 yr and the 8 yr soils (Table 2 and Fig. 3). Organisms from within this clade have been shown to be abundant in other early primary successional environments (Nemergut *et al.*, 2007; Noll and Wellinger, 2008). However, other studies have found sequences that cluster within the *Comamonadaceae*, while the sequences from this study appear to fall outside the four described families within the *Burkholderiales* (Fig. 3).

While differences in the microbial community composition were not found to be significant (data not shown), cyanobacteria were absent in the most recently exposed soils but were present in the 8 yr soils (Table 2). These organisms are related to members of the orders Nostocales and Oscillatoriales, known N-fixers (Bergman *et al.*, 1997). The emergence of cyanobacteria coincides with an increase in N fixation activity: from ~0.5 ng N fixed/cm^2/h in the most recently exposed soils, to ~5.3 ng N fixed/cm^2/h at 8 yr (Fig. 2). Thus, although present in low relative abundance, the cyanobacteria in our soils may be functionally important as in other early successional, unvegetated sites (Kastovska *et al.*, 2005; Nemergut *et al.*, 2007; Duc *et al.*, 2009). In addition to autotrophs, we found a few sequences related to heterotrophic N-fixing organisms in our libraries, including relatives of the genus *Zoogloea*, a β-Proteobacterium (Xie and Yokota, 2006). The roles of heterotrophic and autotrophic N-fixers in this ecosystem are unknown and other preliminary results reveal complex dynamics. For example, N fixation rates in the Mendenhall soils were not significantly higher when incubations were exposed to light (Nemergut and Sattin, unpublished data). A *nifH* gene survey could more directly answer questions about the organisms involved in nitrogen fixation in this system.

The dynamics of P availability appear to partially mirror C and N pools in this system, undergoing a significant decrease between 0 and 4 yr; however, P pools also increased significantly between 4 and 8 yr (Fig. 1). Phosphorus is an important regulator of N fixation owing to the high ATP requirement of this process (Sprent and Raven, 1985). This increase in P availability in the 8 yr soil may be important in fueling N-fixing organisms. Likewise, although not significant, phosphatase activity increased nearly three-fold in the 8 yr soil, suggesting an increase in P supply along the chronosequence (Table 1). Thus, heterotrophs present in the most recently exposed soils may exhaust available C and N, ceding a competitive advantage to diazotrophic autotrophs (Table 2) whose activity is fueled through an increase in available P. Together, these shifts may then lead to general increases in N cycling in the 8 yr soils, as evidenced by the higher rates of NAG, urease and N fixation activity (Table 1 and Fig. 2).

Several caveats are worth mentioning for the proper interpretation of these data and for comparison with other studies. Overall, the chronosequence at the Mendenhall Glacier is highly heterogeneous and is characterized by pockets of fine and coarse soils and a great deal of exposed bedrock. The abundance and size of plant seedlings as well as the presence of visible soil crusts may also play important roles in structuring soil microbial community composition and activity. In sampling, patches with seedlings and crusts were avoided, yet it is difficult to know if and how this heterogeneity may have affected our results. Indeed, many soil parameters were highly variable along the chronosequence (Table 1 and Fig. 1 and 2), likely a consequence of this heterogeneity. Additionally, it is worth noting that this study represents a single point in time, and soil microbial communities are known to undergo rapid shifts in both structure and function (Nemergut *et al.*, 2005; Schmidt *et al.*, 2007). Likewise, we only compared the community composition in the 0 and 8 yr soils. Although it is reasonable to believe that these soils should be the most different based on time since exposure, enzyme activities, and the observed dynamics in the soil elemental pools along the chronosequence, it is unknown if the communities in the 1 and 4 yr soils may be significantly different. Finally, because of the highly compressed chronosequence at the Mendenhall, it is difficult to directly compare successional processes in this system with other unvegetated soils that feature slower plant colonization. Thus, it is possible that a different sampling approach in other glacial forelands would reveal patterns similar to what we observed at the Mendenhall Glacier.

As in more extreme environments, deglaciation processes at the Mendenhall Glacier have led to the exposure of seemingly barren soil. However, this low-elevation, temperate, maritime glacier presents different environmental conditions than those in high arctic and alpine environments, leading to rapid plant colonization. In summary, if these soils represent a true chronosequence, there appear to be two functional halves to the unvegetated post-glacial chronosequence in front of the Mendenhall Glacier. Microbial activity in

the first half, between 0 and 4 yr of soil exposure, appears to be driven by the presence of 'ancient' or allochtonous organic matter (Hodkinson *et al.*, 2002). Once this carbon source is depleted, a competitive advantage may be ceded to microbes not reliant on *in situ* nutrient sources. Thus, we see a small relative, but enormous absolute increase in 16S rRNA gene sequences related to photoautotrophic N-fixing cyanobacteria. The presence of these organisms coincides with replenished soil P pools, and increases in indicators of N cycling. Alternatively, as few mechanisms could explain the increase in pH between the 4 and 8 yr soils, this may not represent a true chronosequence, and may suggest that the 8 yr soil followed a trajectory distinct from the other transects. The repeated sampling of the same transects over several years will be necessary to resolve these two explanations for the observed patterns in the structure and function of the microbial community.

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