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Bacterial community structure and function change in association with colonizer plants during early primary succession in a glacier forefield

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ABSTRACT

Plants directly interact with the soil microbial community through litter inputs and root exudates, and these interactions may be particularly important in nutrient poor soils that typically characterize early ecosystem development. However, little is known regarding how plant–microbe interactions may actually drive ecosystem processes in early succession, a perspective this study helps to define. We investigated how soil microbial communities develop and interact with the establishment of the first plants in the recently exposed soils of the Mendenhall Glacier forefield near Juneau, AK, USA. We sampled soils from under two different plant species (alder, *Alnus sinuata* and spruce, *Picea sitchensis*) and from unvegetated areas; all samples were collected along a single soil transect that had been exposed for 6 years. The presence or absence of vegetation as well as the type of plant (i.e., alder vs. spruce) structured the soil microbial community. Furthermore, asymbiotic nitrogen (N) fixation rates, which were greater in vegetated soils, correlated with differences in bacterial community composition. Although soil microbial community composition varied with vegetation type, soil nutrient and carbon (C) pools did not correlate with bacterial community composition. Moreover, pH did not significantly vary by vegetation type, yet it was the only soil parameter that correlated with bacterial community composition. Vegetation type explained more of the variation in bacterial community composition than pH, suggesting that plant acidification of soils only partly explains the observed shifts in bacterial communities. Plant specific differences in bacterial community structure may also relate to the chemical composition of litter and root exudates. Our research reveals differences in the bacterial community composition of vegetated soils, and how such differences may promote shifts in fundamental biogeochemical processes, such as rates of asymbiotic N fixation, in early stages of primary succession where low N availability may limit bacterial and plant growth and thus constrain ecosystem development. As such, this suggests that plant–soil microbe interactions in themselves may drive processes that shape the trajectory of primary succession.

1. Introduction

Studies of plant functional groups, plant species–species interactions and soil development have been central in advancing the ecological theory of primary succession (Connell and Slatyer, 1977; Matthews, 1992; Chapin et al., 1994; Crews et al., 1995). However, as all of these phenomena can involve belowground microbial communities (Wardle, 2004; Van Der Heijden et al., 2008; Bennett, 2010), recent attention has focused on understanding microbial community succession in environments undergoing primary succession. While it is often implied that primary succession begins with plant establishment (Chapin et al., 1994), a nascent body of research has shown the importance of microbial succession across post-glacial chronosequences that occur well before plant colonization (Nemergut et al., 2007; Schmidt et al., 2008).

After deglaciation and prior to plant colonization, microbial communities rapidly colonize recently exposed substrates, catalyzing the earliest stages of soil development and biogeochemical cycling (Nemergut et al., 2007). For example, studies in the emerging

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landscape near the receding Puca Glacier in the Cordillera Vilcanota of southeast Peru found increases in the relative abundance of cyanobacterial phylotypes with soil age. Increases in these phylotypes – closely related to known nitrogen-fixers – corresponded with the accretion of soil nitrogen (N) pools in unvegetated soils (Nemergut et al., 2007). Schmidt et al. (2008) observed soil stabilization in the same site concomitant with increases in cyanobacterial diversity and pigment concentrations in older soils. As such, microbial colonization of deglaciated soils, beginning even in unvegetated portions of the chronosequence, represents a critical first step in setting the ecological, biogeochemical, and pedological trajectories of developing ecosystems.

While microbial succession in the most recently exposed, unvegetated soils of deglaciated chronosequences is an important factor in early ecosystem development, plant colonization dramatically alters soil microbial community composition and function. Plants directly impact microbial communities in many ways, including through the provision of carbon (C) inputs in the form of both root exudates and litter (Grayston et al., 1998; Bardgett and Walker, 2004; Bardgett et al., 2005). A variety of studies have examined shifts in microbial community structure and activity into and across vegetated portions of succession (Sigler et al., 2002; Noll and Wellinger, 2008; Schütte et al., 2009, 2010), but only a subset have directly evaluated plant–microbe interactions (Schimel et al., 1998; Fierer et al., 2001). In recently deglaciated landscapes, such studies examining plant effects have illuminated shifts in the relative abundance of bacteria and fungi, increases in microbial metabolic function, alterations in bacterial community structure, and changes in plant influence with more advanced successional stages (Ohtonen et al., 1999; Tscherko et al., 2004, 2005; Edwards et al., 2006; Miniaci et al., 2007).

In light of mounting evidence that confirms the importance of plant–microbe interactions in shaping plant community dynamics (Reynolds et al., 2003; Kardol et al., 2007; Bever et al., 2010), evaluating the actual *interaction* between soil microbes and plants may serve as an edifying approach to understanding ecosystem development in primary succession. Though previous studies have revealed broad-scale shifts in microbial community structure and activity in relation to plant succession, a gap remains in understanding how the interaction between microbes and plants connects to ecosystem processes that shape the trajectory of primary succession. For example, as plants and microbes in recently deglaciated soils must tolerate strong abiotic stressors including intense nutrient limitation, plant–microbe interactions may help alleviate such constraints. Low N availability strongly limits plant growth in many early primary successional ecosystems, thus N inputs via N fixation strongly influence early successional microbial and plant community dynamics (Matthews, 1992; Chapin et al., 1994; Edwards et al., 2006; Brankatschk et al., 2010). Recent work in the early successional soils of the Damma Glacier found that free-living *nifH* gene abundance peaked with the presence of the first plant patches (Brankatschk et al., 2010). Although symbiotic N-fixers are thought to have the greatest impact on N availability for plants in young soils, this suggests that ecologically important interactions between initial plant colonizers, bacterial community structure, and asymbiotic (i.e., free-living) N fixation may occur at the intersection of the unvegetated and vegetated landscapes.

However, the roles of biotic and abiotic factors in structuring microbial community composition and function during early plant colonization are poorly understood in these developing ecosystems. Thus, in this study, we focused on plant–microbe interactions at this transitional environment within a deglaciated forefield to understand how initial plant–microbe interactions may actually drive ecosystem process rates and therefore subsequent succession. We examined: 1) if (and how) early plant colonizers uniquely alter bacterial community structure and rates of N fixation; 2) the relative influence of biotic (e.g., plant) and soil chemical parameters on microbial community composition; and 3) the relationship between asymbiotic N-fixation rates and bacterial community structure.

2. Research materials and methods

2.1. Study site and sampling

We examined soils of the Mendenhall Glacier forefield where we assessed bacterial community structure and asymbiotic N fixation along with soil chemical parameters. Samples were collected within a single transect of 6-year-old soils where alder, a symbiotic N-fixer, and non-nodulated spruce co-occur in largely unvegetated soils. Our study took place in October 2009 at the Mendenhall Glacier, a low elevation, high-latitude glacier near Juneau, Alaska (58.356° N, −134.527° W). The glacier extends over 22 km, ending 20 m above sea level at its terminus, where sampling took place (Motyka et al., 2003; Sattin et al., 2009). The glacial forefield was formed from an ongoing deglaciation event that has been occurring since the Little Ice Age. Soils are classified as Entisols forming in predominantly granitic tills (Burt and Alexander, 1996). In the youngest sites (<15 years) the <2 mm size fraction soils are loams (Sattin et al., 2009). The site receives an estimated mean annual precipitation of >2500 mm and the mean annual temperature in Juneau varies between 4 and 6 °C (Burt and Alexander, 1996).

We sampled three soil types, hereafter referred to as 'vegetation types' – unvegetated, and beneath spruce and alder seedlings. Avoiding roots, eight replicates of vegetated surface (0–5 cm) soil samples were aseptically collected from locations under the crown and near the trunk base of both alder and spruce seedlings that were ~ 0.3–0.7 m tall. Paired samples, excluding all visible roots, were used for the acetylene reduction assay to estimate N-fixation rates. This sampling procedure was repeated for unvegetated soils within the same transect. All selected soil sampling points were free of lichens, biological crusts and/or any other vegetation within a ~0.75 m radius. Soils sampled for DNA and chemical analysis were transported to the laboratory in Juneau on ice and sieved to 4 mm at the time of collection. One set of soil subsamples was immediately extracted in KCl to determine inorganic N pools (Weaver et al., 1994), and the remaining soil was transported on ice to Boulder, CO, USA. Subsamples for DNA analysis were kept at −80 °C and samples for further biogeochemical analysis were stored at 4 °C.

2.2. Soil chemical parameters

Soil extractable ammonium (NH_4^+) and nitrate (NO_3^-), Olsen (bicarbonate) phosphorous (P), pH, % organic carbon (C), total dissolved N and C, and microbial biomass C and N were measured on each soil sample. NH_4–N and NO_3–N were measured following extractions of fresh soil with 2 M KCl over 18 h (Weaver et al., 1994), and were analyzed colorimetrically on an Alpkem autoanalyser (OI Analytical, College Stations, TX, USA). Olsen P extractions were performed on dried, ground soils at the University of Minnesota Research Analytical Laboratory. Briefly, 1 g of soil was shaken in 20 mL of 0.5 M $NaHCO_3$ (pH 8.5) for 30 min and filtrates were analyzed using molybdate-blue/ascorbic acid (Watanabe and Olsen, 1965) on a PC 900 probe colorimeter (Brinkman Instruments, Westbury, NY). Standard methods as described by Nemergut et al. (2007) were employed to measure soil pH and percent soil moisture.

For total organic C analysis, carbonate removal was first performed on soils to remove inorganic C as per Nemergut et al. (2007). Samples of ~50 mg dried, ground soils were packed into tin capsules, and %C and %N were determined using a Thermo

Finnigan EA 1112 Series Flash Elemental Analyzer; (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) (Matejovic, 1997). 0.5 M K_2SO_4 extractions were completed on soils before and after chloroform fumigation (Jenkinson and Powlson, 1976; Brookes et al., 1985) to assess chloroform labile C in evaluation of microbial C as well as total extractable C and N in pre-fumigation soils. Extracts were analyzed on a high temperature combustion total CN analyzer (Shimadzu TOCvcpn, Kyoto, Japan). Microbial chloroform labile C calculations are reported as relative values and were not corrected for extraction efficiency.

2.3. DNA extractions and 454 pyrosequencing

Mo Bio PowerSoil™ DNA Isolation kits were used according to the manufacturer's protocols for bulk DNA extractions (Mo Bio Laboratories, Inc., Carlsbad, CA). PCR-amplification of bacterial 16S rRNA genes from the genomic DNA of the 24 soil samples was conducted using a highly conserved universal bacterial primer set as described by Hamady et al. (2008). The 27F (5′-*CTATGC* GCCTTGCCAGCCCGCTCAGTCAGAGTTTGATCCTGGCTCAG-3′) and 338R fusion primers (5′-*CGTATC*GCCTCCCTCGCGCCATCAGNNNNNNNNNNNNCATGCTGCCTCCCGTAGGAGT-3′) were employed. For each sample this fusion primer included a 6 bp adapter (CTATGC/CTATGC) to utilize Titanium chemistry, the 454 A/B primer, a unique, error-correcting barcode (denoted NNNNNNNNNNNN), and the 16S rRNA primer. PCR reactions for each sample were performed in triplicate with 2 μL of 1:1 mixture of sterile water and genomic DNA, 1 μL of the forward primer at 30 μM, 2 μL of the reverse primer at 15 μM, 1 μL of 25 mM $MgCl_2$, 9 μL of sterile H_20, and 10 μL of 5 Prime HotMasterMix (5PRIME, Inc. Gaithersburg, MD). PCR reaction conditions followed the protocol of Fierer et al. (2008). The three PCR reaction products per sample were pooled and then cleaned using Mo Bio UltraClean-htp PCR Clean-up kits (Mo Bio Laboratories, Inc., Carlsbad, CA), according to the manufacturer's protocol. 16S rRNA gene amplicons were sent to the Environmental Genomics Core Facility (Engencore) at University of South Carolina for 454 Life Sciences GS FLX Titanium pyrosequencing.

2.4. qPCR

Quantitative PCR was used to estimate the relative abundance of rRNA genes of bacteria (16S) and fungi (ITS-5.8S) as well as the nitrogenase reductase gene, *nifH*. We used the fungal primers ITS1 (TCCGTAGGTGAACCTGCGG) and 5.8S (CGCTGCGTTCTTCATCG), the bacterial primers Eub338f (ACTCCTACGGGAGGCAGCAG) and Eub518r (ATTACCGCGGCTGCTGG) (Fierer et al., 2005) and the bacterial *nifH* primers Pol1 (TGCGAYCCSAARGCBGACTC) and PolR (ATBGCCATCATYTCRCCGGA) (Poly et al., 2001). Reactions were assembled using the Agilent Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies, Santa Clara, CA, USA) and each contained ~10–30 ng of genomic DNA. All reactions were performed in triplicate and a standard curve was run on each plate. Amplifications were performed using a Stratagene Mx3005P QPCR machine (Agilent Technologies, Santa Clara, CA, USA) and data were analyzed using the manufacturer's MxPro software.

2.5. Nitrogen fixation assay

Nitrogen-fixation rates were assessed using the acetylene reduction assay (Hardy et al., 1968), with the specifications described by Reed et al. (2010) as modified from Belnap (1996). Incubations lasted for 23.75 h over both dark and light hours, but out of direct sunlight (20:00 Oct. 7th to 19:45 Oct. 8th) in Juneau, AK. 8 mL of each headspace were sampled and injected into pre-evacuated vacutainer tubes, and transported to Boulder, CO, USA for gas analysis, which occurred three days after headspace collection. Gas chromatography analysis was completed on a Shimadzu 14-A Gas Chromatograph (Shimadzu Corporations, Kyoto, Japan) employing a flame ionization detector (330 °C) and Poropak N column (110 °C; Supelco, Bellefonte, PA, USA). Ethylene standards used to construct a standard curve were first injected into vacutainer tubes and allowed to incubate for the same amount of time as samples to account for minor gas leakage over the transport and processing time as well as possible ethylene contamination from vacutainer stoppers. Acetylene blanks (no soil) and controls (soil and no acetylene) were also analyzed and were consistently undetectable beyond ethylene production from vacutainer stoppers, which was subtracted out of the calculations. The standard curve constructed from 10 and 100 ppm ethylene standards was used to calculate sample concentrations, which were then converted into ngNfixed/cm^2/h (Reed et al., 2007, 2010).

2.6. Pyrosequence and statistical analysis

We filtered pyrosequencing data using the following quality check parameters: a minimum sequence length of 200 to a maximum of 400 base pairs, a maximum of 5 homopolymers, a minimum quality score of 25, and a maximum of ambiguous bases and primer mismatches of 0 using the QIIME software package (Caporaso et al., 2010). One unvegetated sample was not sequenced and thus omitted from further analysis. Data were denoised in QIIME using Denoiser, which analyzes flowgram data to cluster similar reads in order to remove potentially erroneous sequences (Reeder and Knight, 2010). Sequences were then clustered based on representative operational taxonomic units (OTUs) using UCLUST (Caporaso et al., 2010; Edgar, 2010). OTUs were assigned a taxonomic identification using Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) method with the SILVA (Pruesse et al., 2007) database. Sequence alignments were made using the NAST algorithm (DeSantis et al., 2006a) against the GreenGenes database (DeSantis et al., 2006b). Using additional downstream tools in QIIME, a phylogeny was built with the FastTree algorithm (Price et al., 2009), and a pairwise beta diversity distance matrix among all samples was also generated based on the weighted UniFrac phylogenetic distance metric (Lozupone et al., 2006, 2007). A principal coordinate analysis (PCoA) ordination was generated based on this UniFrac beta diversity matrix in QIIME.

Environmental variables and bacterial taxa relative abundances at the phyla level were checked for normality and homoscedasticity. N-fixation rates and qPCR data were log transformed to achieve a normal distribution. To examine relationships between overall bacterial community composition and normalized environmental variables, Primer v6 (Clarke and Gorley, 2006) statistical software was used to perform Mantel-like RELATE tests and determine correlations between community composition – based on the weighted UniFrac phylogenetic dissimilarity matrix – and all environmental variables and nitrogen fixation. Non-parametric, permutational ANOVA (PERMANOVA) was used to test differences in community composition between each vegetation type (i.e., spruce, alder and unvegetated). The statistical computing language, R, (R Development Core Team, 2009), was used to test one-way analysis of variance (ANOVA) of environmental variables and bacterial taxa relative abundances to examine differences in these variables among the three different vegetation types. Pearson product moment correlation coefficients were calculated among all environmental variables, and Spearman's rank correlations were calculated for relative abundance of bacterial taxa and variables correlated with differences in community structure in R.

Using the vegan package in R (Oksanen et al., 2010), Principal Components Analysis (PCA) was employed to further examine the variation in environmental parameters among samples. Euclidean

distance among samples was calculated based on pH, nitrate, total dissolved C and N, percent C, and Olsen P. Extractable NH_4^+–N was removed from PCA analysis as it strongly covaries with Olsen P and extractable organic C, which were included. Variance partitioning identified the amount of variation in bacterial community structure, as measured by the weighted UniFrac metric, that could be attributed to vegetation type and pH; these variables explained a statistically significant amount of variation in bacterial community structure. Here, we used the adonis function in the vegan package of R (Oksanen et al., 2010), to perform a permutational ANOVA based on the weighted UniFrac dissimilarity. The adonis function is advantageous in determining explained versus residual variation as both continuous (pH) and categorical (vegetation type) variables can be included in the proposed explanatory model.

2.7. Accession numbers

Sequence data have been deposited in the MG-RAST database under ID# 4471523.3.

3. Results

3.1. Microbial community structure

Pyrosequencing generated 67,924 quality short-read 16S SSU rDNA sequences from 23 soil samples prior to quality check and denoising. Pre-analysis quality checks and Denoiser analyses (Reeder and Knight, 2010) resulted in 15,899 16S rRNA gene sequences from all soil samples combined, or on average 691 ± 99 sequences per sample. Major bacterial taxa (phyla and, in the case of Proteobacteria, sub-phyla) that represented >5% of community composition included Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, α-Proteobacteria, and β-Proteobacteria (Table 1). The PCoA ordination showed a clear differentiation of vegetated from unvegetated communities, and clustering of bacterial community composition by vegetation type (Fig. 1). This suggested a phylogenetic dissimilarity between the bacterial community structure of vegetated soils and unvegetated soils as well as plant specific differentiation of soil communities (Fig. 1). A permutational ANOVA (PERMANOVA) (Clarke and Gorley, 2006) of the weighted UniFrac dissimilarity matrix indicated that bacterial community composition was significantly different between all vegetation types (Table 2).

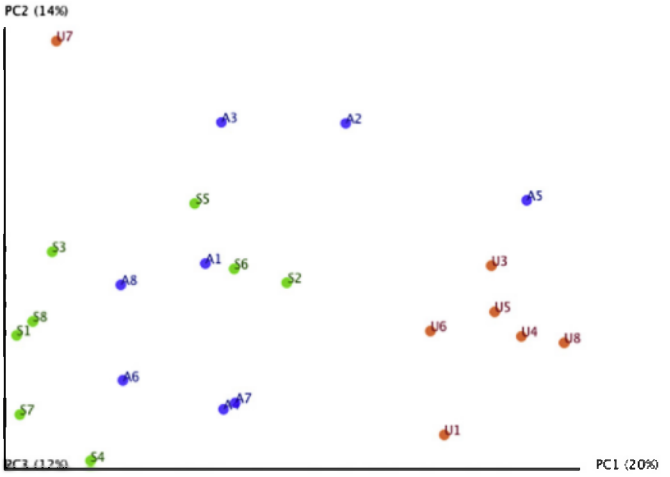

Fig. 1. Principal coordinates analysis of UniFrac bacterial community distances. Points representing bacterial communities are colored by sample type and labeled. The plot shows grouping of bacterial communities by sample type. All PERMANOVA contrasts between vegetation types show significant differences ($P < 0.05$). Red = unvegetated soil, blue = alder soil, green = spruce soil. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

We also evaluated broad-scale differences in the relative abundances of bacterial taxa in vegetated vs. unvegetated soils. Among all taxa, only α-proteobacterial abundance varied significantly between vegetated and unvegetated soils, with greater abundance in vegetated soils (Welch Two Sample *t*-test, $t = -2.67$, $P = 0.02$) (Table 1). Furthermore, the α-Proteobacteria order *Rhizobiales*, which in itself comprised greater than 5% of bacterial communities sampled, was significantly more abundant in vegetated soils (Welch Two Sample *t*-test, $t = 3.5$, $P = 0.004$) (Table 1). One-way ANOVA tests using Tukey's HSD a-posteriori analysis to determine differences in bacterial taxa between each of the three vegetation types only showed a significant increase in the relative abundance of *Rhizobiales* in spruce versus unvegetated soils ($P < 0.05$). Although additional contrasts suggested some additional variation in microbial community abundances among the

Table 1
Relative abundances of bacterial taxa >5% of total community and soil chemical parameters. *P*-Values are given for *t*-tests comparing vegetated with unvegetated soils.

Bacterial taxa (% community)	Vegetated (Mean ± SE)		Unvegetated	Vegetated vs. unvegetated
	Spruce	Alder	Unvegetated	
Acidobacteria	6.897 ± 0.51	9.23 ± 2.1	10.57 ± 1.3	NS
Actinobacteria	7.995 ± 0.62	10.17 ± 0.87	8.248 ± 1.2	NS
Bacteroidetes	14.95 ± 1.01	11.27 ± 1.6	12.68 ± 0.68	NS
α-Proteobacteria	26.02 ± 0.88	25.34 ± 1.3	22.18 ± 1.1	$t = -2.6798$, $p = 0.0195$
β-Proteobacteria	20.64 ± 0.011	18.24 ± 0.61	20.95 ± 1.1	NS
Cyanobacteria	6.055 ± 1.9	7.615 ± 1.4	4.811 ± 1.8	NS
Rhizobiales[a]	13.96 ± 0.77^{A}	12.54 ± 0.75^{AB}	10.07 ± 0.72^{B}	$t = 3.507$, $p = 0.0037$
Soil Parameters				
Microbial C (mgC/kg)	97.0 ± 15.6	129.0 ± 25.2	113.5 ± 11.2	NS
pH	7.29 ± 0.02	7.41 ± 0.05	7.41 ± 0.16	NS
NO_3^- (μg N/g soil)	3.70 ± 1.40	1.37 ± 0.49	1.39 ± 0.40	NS
%C (% soil mass)	0.30 ± 0.05	0.25 ± 0.02	0.23 ± 0.03	NS
Extract. Org. C (mg/kg soil)	97.7 ± 15.8^{A}	68.3 ± 8.7^{AB}	41.0 ± 5.1^{B}	$t = 3.9001$, $p = 0.0008$
Total Dissolved N (mg/kg soil)	8.6 ± 1.2^{A}	4.8 ± 0.7^{B}	3.9 ± 0.8^{B}	$t = 2.4897$, $p = 0.0224$
Nfix (ngNfixed/cm^2/h)	0.43 ± 0.05^{AB}	1.69 ± 0.82^{A}	0.23 ± 0.03^{B}	$t = 3.3192$, $p = 0.0033$
Extractable NH_4^+–N (μg N/g soil)	1.10 ± 0.21^{A}	0.30 ± 0.04^{B}	0.31 ± 0.04^{B}	$t = 2.5219$, $p = 0.0217$
Olsen P (ppm)	14.3 ± 1.6^{A}	8.5 ± 1.0^{B}	9.41 ± 0.6^{B}	NS
% Moisture	20.5 ± 2.1	20.7 ± 1.9	17.2 ± 0.9	NS

Letters denote significant differences in pairwise comparisons ($p < 0.05$).
[a] (also included in α-Proteobacteria).

Table 2
Results of Mantel-like RELATE tests and PERMANOVA tests; all other parameters tested yielded non-significant results.

RELATE tests	UniFrac phylogenetic community dissimilarity		
	Rho	*p*	
pH	0.195	0.047	
Nitrogen Fixation	0.261	0.04	
PERMANOVA	Alder vs. Spruce	Spruce vs. Unveg	Alder vs. Unveg
	$t = 1.357$, $p = 0.015$	$t = 1.6971$, $p = 0.005$	$t = 1.4405$, $p = 0.012$

different sites, none revealed any significant differences for the bacterial taxa examined (Table 1).

The relative abundance of *nifH*, bacterial 16S rRNA, and fungal 18S rRNA genes were compared for each of the soil samples. *nifH* gene copy number was greater in vegetated soils. Spruce soils displayed significantly higher relative abundances of *nifH* genes versus unvegetated soils (Tukey's HSD tests, Table 5), although *nifH* gene relative abundance did not significantly correlate to measured nitrogen fixation activity. Fungal:bacterial ratios were significantly higher in both spruce and alder versus unvegetated soil samples (Table 5).

3.2. Soil parameters

Again, to evaluate both the influence of vegetation in general and also plant species effects, soil properties were evaluated both between vegetated and unvegetated soils, and between each of the three vegetation types. Concentrations of extractable organic C, total dissolved N, NH_4^+, and N-fixation rates were higher in vegetated than unvegetated soils (Welch Two Sample *t*-test, $P < 0.05$) (Table 1), but pH was generally lower in vegetated than in unvegetated soils (Table 1). Notably, pH was significantly negatively correlated with NH_4^+, total dissolved N, extractable organic C, Olsen P, but positively correlated with microbial C ($P < 0.05$). Spruce had significantly higher extractable organic C compared to unvegetated soils (Tukey's HSD a-posteriori ANOVA tests, $P < 0.05$), and significantly higher total dissolved N, NH_4^+, and P pools compared to both unvegetated and alder soils (Table 1). Alder soils were not significantly different from unvegetated soils in any of the measured parameters. While a comparison of asymbiotic N-fixation rates in vegetated vs. unvegetated soils revealed a significantly greater rate of N fixation in vegetated soils (Table 1), at the plant species level, only alder soils had significantly higher N-fixation rates than unvegetated soils despite nearly a doubling of N-fixation rates in spruce soils as compared to unvegetated soils (a-posteriori Tukey's HSD, $P < 0.005$ Table 1).

The orientation among samples in environmental space was further discerned by principal components analysis (Fig. 2). Samples generally grouped by vegetation type across axis 1, which described 43.5% of variation. Axis 2 described 19.4% of variation.

Table 3
Bacterial taxa (>5% of community) Spearman's rank correlations with pH.

Bacterial taxa	Rho	*p*-value
Acidobacteria	0.538	0.0081
Actinobacteria	0.134	0.5423
Bacteroidetes	−0.541	0.0077
α-Proteobacteria	−0.383	0.0712
β-Proteobacteria	0.0456	0.8362
Cyanobacteria	−0.0283	0.898
Rhizobiales	−0.393	0.0633

Table 4
Variance partitioning, analysis of pairwise dissimilarity.

Source of variance	*Df*	SS	MS	F	R^2	*p*
pH	1	0.0064624	0.0064624	0.8033155	0.0317	0.548
Vegetation Type	2	0.0474434	0.0237217	2.9487656	0.2327	0.003
pH × Vegetation Type	2	0.0132359	0.006618	0.822657	0.0649	0.589
Residuals	17	0.1367586	0.0080446		0.6707	
Total	22	0.2039004				

While alder and unvegetated soils more closely grouped according to pH, spruce was more influenced by differences in soil nutrient pools.

3.3. Relationships among bacterial community composition and soil chemistry

The only soil chemical parameter that was correlated with the UniFrac metric used to examine differences in bacterial community structure was pH (RELATE test, $r = 0.195$, $P = 0.047$) (Table 2). Likewise, N-fixation rates were correlated with bacterial community structure (RELATE test, $r = 0.261$, $P = 0.04$) (Table 2). pH was positively correlated with acidobacterial relative abundance (Spearman's: Rho = 0.538, $P < 0.01$) and negatively correlated with the relative abundance of Bacteroidetes (−0.541, $P < 0.01$) (Table 3). There were no significant correlations between the relative abundance of bacterial taxa and N-fixation rates.

To disentangle the interrelated effects of pH and vegetation type on bacterial community composition, we conducted variance partitioning (Table 4). Vegetation type explained the greatest proportion of the variation (23.27%) in bacterial community structure. The remaining variability was explained by pH (3.17%), the interaction term (6.49%), and the residual term (67.07%); however, neither pH nor interactions were significantly related to variation in community composition despite the significant correlation between pH and bacterial community composition ($P > 0.05$). Given that pH was significantly correlated with bacterial community structure as determined through the Mantel-like RELATE test (Table 2), the variance partitioning suggests that pH is not independent from soil vegetation type, which by far explains the most variation in the UniFrac distances among samples. All other environmental variables measured in this study were also tested as factors in the adonis model; however, none of these environmental variables were significantly related to bacterial community structure.

4. Discussion

Past research in glacial forefields has shown that bacterial communities and associated biogeochemical cycling undergo successional changes in unvegetated soils well in advance of plant colonization (Nemergut et al., 2007; Schmidt et al., 2008). However, plant colonization imparts significant influence on the successional trajectory of these dynamic bacterial communities. Our research indicates that early colonizer plants significantly alter bacterial community composition at early successional stages in post-glacial ecosystem development. Our study not only supports past findings that show broad-scale bacterial community shifts in vegetated soils (Bardgett and Walker, 2004; Tscherko et al., 2005), but also reveals plant species effects on bacterial communities (Westover et al., 1997; Grayston et al., 1998). Although microbial communities under both plant types have higher fungal:bacterial ratios than unvegetated soils, bacteria dominate the microbial community in all of these soils. Nonetheless, the observed shifts in fungal:bacterial ratios are congruent with past studies (Bardgett and Walker, 2004; Ohtonen et al., 1999). It is likely that plant C inputs,

Table 5
nifH gene relative abundance and fungal:bacterial rRNA copy numbers.

Ratio	Spruce	Alder	Unvegetated
nifH: Bacterial	1.94E-04 ± 7.42E-05^{A}	1.68E-04 ± 8.13E-05^{B}	7.00E-05 ± 4.61E-05^{B}
Fungal:Bacterial	0.0033 ± 5.67E-04^{A}	0.00296 ± 6.95E-04^{A}	0.000961 ± 2.30E-04^{B}

Letters denote significant differences in pairwise comparisons ($p < 0.05$).

especially more recalcitrant forms, support saprophytic fungi. Furthermore, both studied plants are known to support ectomycorrhizae. Our research demonstrates that plants may either directly or indirectly drive shifts in soil bacterial communities, increasing the relative abundance of α-Proteobacteria, specifically the *Rhizobiales* (Table 1). Additionally, we determined that soil bacterial community structure is unique to each vegetation type (Table 2, Fig. 2).

Past studies have also shown that the effects of plant colonization on early successional bacterial communities are variable. At the Rotmoosferner Glacier in the Otz Valley of Austria, Tscherko et al. (2005) found no significant plant effect on the composition or activity of the rhizosphere microbial communities in soils less than 43 years old. However, they noted that there was a stronger vegetation influence on bacterial communities in soils older than 75 years old. This finding was attributed to the relative strength of abiotic factors as a primary determinant of microbial communities in the harsh pioneer stage environment. However, it is well known that the rate and patterns of soil development vary across distinct deglaciated landscapes based on differences in climates and available biota (Matthews, 1992; Walker and del Moral, 2003). Accounting for unique development of different deglaciated environments, Tscherko et al. (2005) suggested that chronosequences with relatively rapid rates of plant colonization may indicate more favorable conditions for plants, which could explain other findings

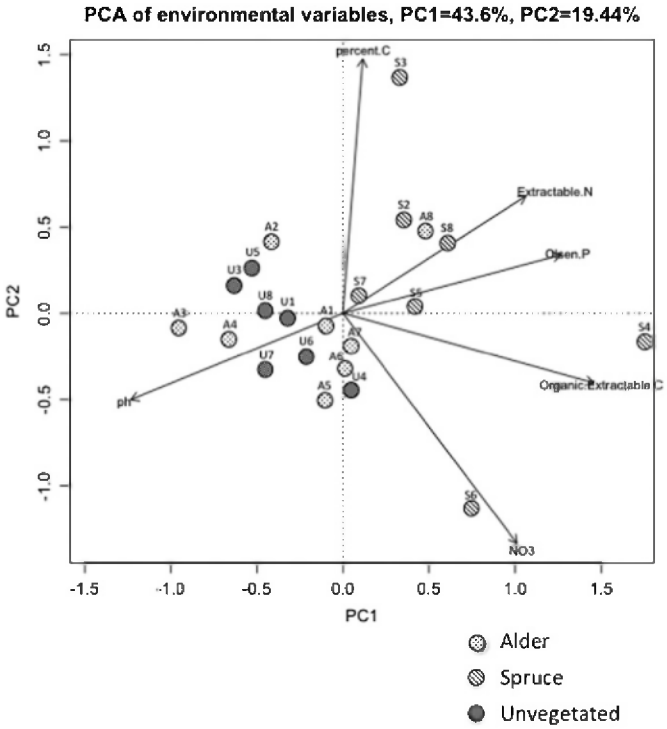

Fig. 2. Principal components analysis of sample environmental parameters. Vector lengths show the relative contribution of individual parameters to multivariate variation. Multivariate sample points are colored by vegetation type and labeled.

that show significant vegetation effects in structuring bacterial communities in the pioneering stage of ecosystem development (Bardgett and Walker, 2004; Edwards et al., 2006). Our research also supports this interpretation by showing early plant colonizer influence on bacterial community structure in newly exposed soils that are characterized by quick plant colonization (Burt and Alexander, 1996).

While it is possible that plants colonize unique sites with pre-existing differences in microbial communities, the observed differences in bacterial community composition, fungal:bacterial ratios, and soil parameters presented are consistent with plant-derived effects that have been widely documented in previous studies. For example, plant-driven enrichment of α-Proteobacteria, and more specifically *Rhizobiales*, are also observed in other environments as well as experimental research using homogenized soils or replicated, randomized block designs to control for natural environmental heterogeneity (Costa et al., 2006; Haichar et al., 2008; King et al., 2010).

Not surprisingly, differences in soil chemistry also corresponded with vegetation type (Fig. 2). A variety of other studies across other deglaciated chronosequences have shown that changes in soil nutrients and C often impact bacterial community composition and function (Tscherko et al., 2004; Edwards et al., 2006). For rhizosphere bacterial communities in the Damma Glacier forefield, Edwards et al. (2006) found that soluble C and mineral N were dominant influences on bacterial communities across the chronosequence. While shifts in nutrient and C pools over the Mendenhall chronosequence may correspond to bacterial community structure, our data yielded no correlations within the various C and nutrient parameters measured and bacterial community composition.

This result, however, does not imply that changes in C and N pools do not influence bacterial community composition within the sampled transect. The standard assays we used to quantify total C and N may have been too general to uncover how changes in the chemistry of these pools influence bacterial community structure. For example, the specific C chemistry of both litter and exudates may influence individual taxa and/or overall microbial community structure (Orwin et al., 2006; Meier and Bowman, 2008; Eskelinen et al., 2009). Unvegetated soil bacterial communities could also be influenced by sources of ancient or allochthonous soil C, distinct in composition from plant inputs. Indeed, previous studies have shown that recently deglaciated soils contain older, ancient pools of soil C (Bardgett et al., 2007; Hood et al., 2009; Sattin et al., 2009). Furthermore, additional abiotic factors and unmeasured plant effects (e.g., shading/light, substrate chemistry, temperature, etc.) may play roles in structuring microbial communities (Horner-Devine et al., 2004; Weber and Bardgett, 2011). Interestingly, despite the relative simplicity of the study system, our research indicates that the complexity of the environmental factors that structure microbial communities in recently deglaciated soils sufficiently masks observable differences using standard soil characterization techniques.

Our research showed that pH was the only measured environmental variable that significantly correlates with bacterial community structure. While pH is one mechanism that could drive differences in bacterial community structure, it was not a significant factor in the adonis model (Table 4). Variance partitioning subsequently established that pH is not independent of vegetation

type, but rather is a vegetation-derived effect, and that vegetation type describes the highest amount of variation in bacterial community structure. Together, these results support the notion that plants drive decreases in pH (e.g., via litter inputs, organic acid exudation, and proton extrusion) and other factors to uniquely alter bacterial communities during early soil colonization and development (Crocker and Major, 1955; Matthews, 1992; Rengel, 2003). For example, Yao et al. (2000) found vegetated tea orchard and forest soils to harbor unique microbial communities only in part due to differences in pH, attributing overall differences to a host of other plant-derived effects including litter and exudate composition and antimicrobial properties.

Even if pH only explains a portion of the plant influence on microbial community structure, our results are important in the context of an increasing body of research that suggests that soil pH may act as a primary driver of bacterial community composition and diversity at a variety of scales. For example, Eskelinen et al. (2009) showed strong correlations between fungal:bacterial ratios, pH, and plant functional type in alpine tundra soils. In a study of bacterial 16S rRNA gene data from 88 samples from North and South America, Lauber et al. (2009) showed that pH explained the most variation in phylogenetic differences in bacterial communities across samples. Furthermore, despite the fact that Acidobacteria are generally considered acidophilic organisms, our research showed a significant positive correlation between acidobacterial relative abundance and pH (Table 3). This provides further evidence suggesting a variable response within the Acidobacteria phylum to shifts in pH (Jones et al., 2009; Ramirez et al., 2010). Our findings that Acidobacteria are less abundant in more acidic soils may suggest that increased organic C availability in the more acidic vegetated soils may reduce the competitive advantages of this generally oligotrophic taxon.

Our work also showed evidence for higher N-fixation rates in vegetated soils that could be driven by differences in soil chemical properties (Table 1) and/or bacterial community structure in these soils (Fig. 1). Indeed, our work confirmed that bacterial community structure correlated with N-fixation rates (Table 2). While past research has shown shifts in N metabolism-related enzyme activity and *nifH* gene abundance and diversity *across* vegetated sections of deglaciated chronosequences (Tscherko et al., 2004; Duc et al., 2009; Brankatschk et al., 2010; Töwe et al., 2010), this study also established a direct linkage between vegetation-related shifts in bacterial community composition and N-fixation rates in a glacial forefield. The lack of a significant correlation between *nifH* gene relative abundance and N-fixation rates could indicate that changes in N-fixation rates relate to shifts in the efficiency and/or community structure of N-fixing organisms rather than relative abundances of asymbiotic N-fixers within soil bacterial communities.

Thus, our work adds to a body of evidence supporting the importance of asymbiotic N fixation in the ecosystem development of low-nutrient landscapes such as glacial foregrounds (Patra et al., 2007; Duc et al., 2009; Brankatschk et al., 2010; Schütte et al., 2010; Töwe et al., 2010). While the low (but still measurable) levels of asymbiotic N fixation may have a limited, indirect impact on plants, they are certainly important for microbial dynamics. As asymbiotic N-fixers are distributed widely throughout the phylogenetic tree, it is difficult to speculate which taxa were responsible for the increased N-fixation rates (Beattie, 2006). It is worth noting, however, that OTUs related to the order *Rhizobiales*, known N-fixers, were significantly more abundant in vegetated soils. Such findings are consistent with past studies that document increased abundance of *Rhizobiales* in association with vegetation (King et al., 2010). Although typically attributed to symbiotic N fixation, work by Buckley et al. (2007) suggests active N fixation by asymbiotic soil *Rhizobiales*. This suggested relationship may be obscured in correlation analysis as only a small subset of *Rhizobiales* may fix N as free-living diazotrophs, and overall N fixation is attributable to far more phylogenetically dispersed taxa that are likely contributing to enhanced rates as well.

While cyanobacteria contribute to N fixation, especially in unvegetated soils of glacial forefields (Schmidt et al., 2008; Schütte et al., 2009), they made up a relatively small proportion of bacterial communities at the Mendenhall Glacier (Table 1) (Sattin et al., 2009). As plants colonize soils, the role of autotrophic cyanobacteria is likely diminished with the increasing availability of plant carbon for heterotrophs. Thus heterotrophic diazotrophs may contribute more prominently to overall N fixation after plants colonize young landscapes. Recent research has documented the highest abundance of *nifH* genes across a glacial chronosequence in soils associated with early colonizer plants (Brankatschk et al., 2010; Töwe et al., 2010). Consistent with this, our study supports the application of an individualistic process model for asymbiotic N fixation (Walker and Chapin, 1987; Matthews, 1992) where asymbiotic N fixation is of particular relevance in early ecosystem development. At the pioneering stage of succession when plants colonize, an increase in C inputs could further accentuate N limitations. As such, heterotrophic N-fixing bacteria could gain a competitive advantage given their ability to escape N limitations while utilizing more C-available soils to fuel this energetically expensive process. Similar dynamics between C availability and heterotrophic N fixation function have been documented in decomposing litter (Vitousek and Hobbie, 2000).

Consistent with the results of Miniaci et al. (2007), our study showed that plant influence on soil bacterial communities is not restricted to root adhering soil particles, as is often used to assess microbes in the rhizosphere environment (Bardgett and Walker, 2004; Tscherko et al., 2004, 2005; Edwards et al., 2006). Our work highlights the need for better definitions of plant zones of influence. Our research further supports the idea that even patchy vegetation can have a broader spatial impact than the immediate rooting zone on microbial community structure and associated biogeochemistry. Possible mechanisms for this expanded area of influence include root exudates, rhizodeposition, litter inputs, and physical alterations of the soil environment.

In summary, by using a high-throughput sequencing approach our study reveals how the trajectory of bacterial community succession is influenced by initial plant colonization in the context of primary succession. Likewise, our work provides insight into how these changes in microbial community structure may drive functional shifts that influence plant community development and succession in these transitional environments. This study, concentrating on a crucial stage of primary succession, is unique in establishing a direct link between vegetation-induced differences in overall microbial community composition and an ecologically important microbial function, rates of asymbiotic nitrogen fixation. Our research suggests that assessing the role of the plant–microbe interaction itself may be an important perspective in understanding ecosystem process rates and dynamics of primary succession.

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