THE IMPACT OF COLONIZER PLANTS ON BACTERIAL COMMUNITY STRUCTURE AND FUNCTION IN EARLY SUCCESSIONAL SOILS OF A GLACIAL FOREFIELD

by

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B.A., Northwestern University, 2008

A thesis submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment
of the requirement for the degree of
Master of Arts
Department of Ecology and Evolutionary Biology
2011
This thesis entitled:
The Impact of Colonizer Plants on Bacterial Community Structure and Function in Early Successional Soils of a Glacial Forefield
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Date ______________

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
Knelman, Joseph E. (M.A., Ecology and Evolutionary Biology)  
The Impact of Colonizer Plants on Bacterial Community Structure and Function in Early Successional Soils of a Glacial Forefield  
Thesis directed by Assistant Professor Diana R. Nemergut

Through litter inputs, root exudates, and the resulting changes in soil chemistry, plants directly interact with the soil microbial community. Recent research on plant-microbe interactions suggests that soil microbial community structure and function play an integral role in plant community succession through both positive and negative feedbacks; yet, plant-microbe dynamics along a successional gradient have not been well-studied. My study in the recently exposed soils of the Mendenhall Glacier forefield near Juneau, AK, USA examined the development of microbial communities in coordination with the establishment of the first plants. The Mendenhall Glacier features a perhumid climate, with moist soils throughout the year, and nearby vegetation that serves as a propagule source, facilitating relatively rapid plant colonization. I sampled soils under two different plant species (alder, *Alnus sinuata* and spruce, *Picea sitchensis*) and from unvegetated areas. All samples were gathered within a single transect of soils that had been exposed for 6 years. For each sample site soil pH, organic carbon (C), available nitrogen (N), bioavailable (Olsen) Phosphorus (P), microbial biomass C, and nitrogen fixation rates were determined. My research shows specific vegetation type differences in bacterial community structure and the general enrichment of *α-Proteobacteria* in vegetated soils. Soil nutrient and carbon pools did not correlate with bacterial community composition. Interestingly, although pH did not significantly vary by vegetation type, it was the only parameter that correlated with bacterial community structure. My study revealed a significant
correlation between nitrogen fixation rates and bacterial community composition, a feedback with potentially important impacts for the ecology of these environments. Vegetation type explained more variation in differences in bacterial communities than pH, suggesting that plant acidification of soils only partly drive broad shifts in bacterial communities. Plant species-specific differences in bacterial community structure may also relate to the chemical composition of litter and root exudates. Additionally, plant carbon inputs in general likely enhance asymbiotic N-fixer function in these relatively new soils where nitrogen limitations may stifle bacterial growth. My study provides insights into how colonizer plants drive changes in bacterial community structure and function in a glacial forefield, altering bacterial succession and ecosystem development.
ACKNOWLEDGEMENTS

I owe a debt of gratitude to Dr. Diana Nemergut for exceptional support and guidance in my thesis work as well as facilitating a productive work environment replete with collaboration and good spirit. I am deeply thankful for the encouragement to develop my own research interests, which has resulted in an indelible personal intrigue and voice in my work. Thank you to Dr. Steve Schmidt for sharing his expertise, lab resources, and field strategies. I greatly appreciate the input and advice of Dr. Ned Friedman and Dr. Eran Hood in my research. Along with Dr. Cory Cleveland, the aforementioned individuals provided resources for the completed research through their funding sources, for which I am truly grateful. Thank you to Dr. Bill Bowman for challenging me to ask the next question and for being a strong advocate of my goals and plans. Additionally, I owe many thanks to Dr. Matthew Sterenberg for his continuing help in pursuing academic opportunities.

My master’s work is partly a result of the help and camaraderie of the larger Nemergut Lab: Terry Legg, Lee Stanish, Sean O’Neill*, Chris Washenberger, Antonio González Peña, Kate Schimel, and Peter Frey. Equally, thank you to the Townsend Lab and others at INSTAAR and EBIO, specifically Sasha Reed, Will Wieder, Phil Taylor, Samantha Weintraub, and Janet Prevéy, all who made labwork to socializing productive and enriching activities.

As always, thanks to my family for their love and interest in my endeavors and dreams. And last but not least I owe much to the main event… the best friendliest friends, crafters, adventurers, buckhunters, aquarists, burritophiles, gamers, musicians, and fellow graduate students, with an awesome ability to share true joie de vivre: Abbey, Armin, Cameron, Dan, Heidi, Jan, Jules, Sam, Sarah, Stower, and Taryn. Skål!
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CHAPTER I

INTRODUCTION

Soils exposed by receding glaciers are becoming increasingly prevalent amidst current climate change and accelerated glacial loss (Oerlemans 1994, Kintisch 2009). The relative simplicity of post-glacial chronosequence macrobiotic communities and nutrient dynamics has facilitated advances in ecological theory related to soil development, plant functional groups, and species-species interactions (Matthews 1992, Chapin et al. 1994, Crews et al. 1995). Given that all of these phenomena are linked to belowground microbial communities (Wardle 2004, Van Der Heijden et al. 2008, Bennett 2010), the study of microbial community structure and function along soil chronosequences offers the possibility of discovering a new mechanistic understanding of processes ranging from biogeochemical cycling to plant community assembly. Indeed, a nascent body of research is beginning to uncover microbial dynamics and patterns across primary successional chronosequences (Sigler et al. 2002, Nemergut et al. 2007, Schmidt et al. 2008, Schütte et al. 2009).

After deglaciation and prior to plant colonization, microbial communities rapidly colonize newly exposed soils and have important implications for soil development and biogeochemical cycling (Nemergut et al. 2007). For example, studies in the new landscape exposed by 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 concomitant accretion of
soil nitrogen (N) pools in unvegetated soils (Nemergut et al. 2007). Schmidt et al. (2008) observed soil stabilization in the same site in coordination 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 chronosquence, has been documented as an integral component to ecosystem processes ranging from biogeochemistry to pedogenesis.

While microbial succession in the newest, unvegetated soils of deglaciated chronosequences plays a salient role in ecosystem development, plant colonization dramatically alters the trajectory of microbial community composition and function. Plants directly impact microbial communities through C inputs such as root exudates and litter inputs, for example (Grayston et al. 1998, Bardgett and Walker 2004, Bardgett et al. 2005). Past studies have examined the relative influence of vegetation and soil properties on microbial community structure and activity in recently deglaciated landscapes. Such studies have illuminated shifts between bacterial and fungal dominated soils, increases in microbial metabolic function and efficiency, increases in phylotype diversity, and stronger plant influence with more advanced successional stages (Chapin et al. 1994, Ohtonen et al. 1999, Tscherko et al. 2004a, Tscherko et al. 2005, Schütte et al. 2010). In turn, microbes can affect plant performance via interactions ranging from pathogen resistance to nutrient acquisition (Van Der Heijden et al. 2008, Bennett 2010) and can engender historical contingency effects through positive and negative feedbacks for later stages of ecosystem succession (Chapin et al. 1994, Kardol et al. 2007). Through these feedbacks microbes impact plant communities and vice versa.

Plants in recently deglaciated soils must cope with strong abiotic stressors including intense nutrient limitation; plant-microbe interactions are important relationships in
circumventing such constraints. For example, nitrogen is strongly limiting in early primary successional ecosystems, rendering N-availability and N-fixation dynamics a major factor in structuring both microbial and plant communities (Walker and Syers 1976, Matthews 1992, Edwards et al. 2006, Brankatschk et al. 2010). For this reason, nodulated plants with symbiotic N-fixers are often early colonizers of recently deglaciated soils; their activity amends N-pools facilitating later successional phases (Walker and Syers 1976). Although symbiotic nitrogen fixers are thought to have the greatest impact on N-availability in young soils, free-living N-fixers may have an enhanced importance in soils with lower rates of microbial activity (Patra et al. 2007). Recent research has demonstrated strikingly high asymbiotic nifH gene diversity in the Dama glacier forefield in the Swiss Alps as compared to the findings of similar studies in environments ranging from the arctic to temperate forests (Duc et al. 2008). The same study found differences in nifH phylogenetic distributions among different soils, suggesting that differences in early vegetation contributed to shifts in N-fixer community structure. Although higher rates of N-fixation were also observed in rhizosphere soils, the study did not confirm a relationship between plant-influenced bacterial community shifts and N-fixation rates (Duc et al. 2008).

Further work at the Dama glacier by Brankatschk et al. (2010) found that nifH gene copy abundances peak in early successional soils with the presence of the first plant patches. This suggests that ecologically important interactions between initial plant colonizers and bacterial community structure and function occur at the intersection of the unvegetated and vegetated landscapes; however, questions remain about the biotic and abiotic factors structuring microbial communities. In this study, I focused on plant-microbe interactions at this transitional environment within a deglaciated forefield. I hypothesized that: 1) Early plant colonizers
uniquely alter bacterial community structure as compared to unvegetated soils; 2) Asymbiotic N-fixation rates relate to differences in bacterial community structure. I examined soils of the Mendenhall Glacier forefield where I assessed bacterial community structure and asymbiotic N-fixation along with a variety of other 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. Employing high-throughput 454 pyrosequencing to gain an understanding of bacterial community structure in vegetated and unvegetated soils, this work builds on previous research in glacial foregrounds that demonstrates the impact of colonizer plants on bacterial communities using coarser-resolution community profiling techniques (Bardgett and Walker 2004, Tscherko et al. 2004a, Tscherko et al. 2005, Miniaci et al. 2007). I show a direct linkage between colonizer vegetation influence and bacterial community structure in a glacial forefield and demonstrate a relationship between bacterial community structure and nitrogen fixation function. This research thus reveals how early colonizer plants alter bacterial community successional trajectories, and how early colonizer plants impact nitrogen cycling at the transition from unvegetated to vegetated primary succession of these increasingly prominent deglaciated landscapes.
CHAPTER II

RESEARCH MATERIALS AND METHODS

Study site and sampling

My study took place in October 2009 at the Mendenhall Glacier outside of Juneau, Alaska. The Mendenhall Glacier is a low elevation, high-latitude glacier. The glacier extends over 22 km, ending 20 meters above sea level at its terminus, where sampling took place (Motyka et al. 2003, Sattin et al. 2010). The glacial forefield, a result of deglaciation that has been occurring since the Little Ice Age, is comprised of Entisols which are more than 75% granitic (Burt and Alexander 1996). In the youngest soils (< 15 years) size fractions of the < 2 mm portion average 39% sand, 47% silt, and 14% clay (Sattin et al. 2010). The site receives an estimated mean annual precipitation of >2,500 mm and the mean annual temperature in Juneau is between 4.4 and 6.1 °C (Burt and Alexander 1996).

I sampled soils of three types – unvegetated, and beneath spruce and alder seedlings – hereafter referred to as vegetation type, along a single transect of ~6 year-old soils where vascular plants were beginning to colonize the landscape. *Picea sitchensis, Alnus crispa subsp. sinuata*, (Hurd 1971) and to a lesser extent, *Epilobium augustifolium* (spruce, alder, and fireweed) dominate this patchy colonization. Eight replicates of vegetated soil samples were
collected from under both alder and spruce seedlings that were ~0.3-0.7m tall. Surface soil samples (0-5cm) were aseptically collected from locations under the crown of the seedling, near the trunk base. Paired samples were used for the acetylene reduction assay to estimate N fixation. This sampling procedure was repeated for unvegetated soils within the same transect. All soils were free of lichens and crusts and any other vegetation within a 0.75m radius. Soils for DNA and chemical analysis were immediately placed on ice and were passed through a 4 mm mesh-size sieve in Juneau. Subsamples were used for immediate KCl extractions to examine inorganic nitrogen pools (Weaver et al. 1994) or transported at ~0° to Boulder, CO, USA. Subsamples for DNA analysis were kept at -80°C and samples for further biogeochemical analysis were stored at 4°C in Boulder, CO.

DNA extractions and 454 pyrosequencing

DNA was extracted from the 24 soil samples. Mo Bio PowerSoil™ DNA Isolation kits were used according to the manufacturer’s protocols for bulk DNA extractions (Mo Bio Laboratories, Inc., Carlsbad, CA). I PCR-amplified bacterial 16S rRNA genes from the genomic DNA of the 24 soil samples 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’ CGTATCGCCTCCTCGGCATCAGNNNNNNNNNCATGCTGCCTCCCGTAGG AGT-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 respective 16S primer. PCR
reactions for each sample were performed in triplicate with 2µL of 1:1 mixture of sterile water and genomic DNA, 1 µL of the forward primer at 30µM, 2 µL of the reverse primer at 30µM, 1 µL of 25 mM MgCl₂, 9 µL of sterile H₂O, 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.

Soil chemical parameters.

Extractable ammonium and nitrate, Olsen phosphorous (P), pH, %C, total dissolved N and C, and microbial biomass were evaluated for each soil sample. NH₄⁺-N and NO₃⁻-N were measured through extractions of fresh soil with 2M KCl over 18 hours (Weaver et al. 1994). NH₄⁺-N was analyzed colorometrically on an Alpkem autoanalyser (OI Analytical, College Stations, TX, USA). Olsen P bicarbonate extractions were performed on dried, ground soils at the University of Minnesota Research Analytical Laboratory. Briefly, soils were shaken in 20 mL of 0.5 M NaHCO₃, pH 8.5, for a 30 min. Using the molybdate-blue method with ascorbic acid as a reductant (Watanabe and Olsen 1965), filtrate was analyzed through colorimetric methods 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. For total organic C analysis, carbonate removal was first performed on soils ground to a fine powder to remove inorganic C as per Nemergut et al. (2007). Samples of ~50 mg dried, ground soils were
packed into tin capsules, and %C was determined using a Thermo Finnigan EA 1112 Series Flash Elemental Analyzer; (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) (Matejovic 1997). 0.5 M K$_2$SO$_4$ extractions were completed in combination with chloroform fumigation (Jenkinson and Powlson 1976, Brookes et al. 1985) to assess microbial biomass C and as well as total dissolved C and N in pre-fumigation soils. Total organic C was determined by analyzing extracts on a high temperature combustion total CN analyzer (Shimadzu TOCvcpn, Kyoto, Japan.) Microbial C calculations were treated as relative values and not corrected for extraction efficiency.

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 hours over both dark and light hours, but out of direct sunlight (20:00 Oct. 7th to 19:45 Oct. 8th) in Juneau. Headspaces were sampled and injected into preevacuated vacutainer tubes, and transported to Boulder, CO, USA for gas analysis. 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/cm²/h (Reed et al. 2007, Reed et al. 2010).

Pyrosequence and statistical analysis

Pyrosequencing resulted in 67,924 quality short-read 16S SSU rDNA sequences from 23 samples prior to quality check and denoising. Quality check parameters included 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. One unvegetated sample was not sequenced and thus omitted from further analysis. I used QIIME software for analysis of pyrosequencing data (Caporaso et al. 2010). Data were denoised in QIIME using Denoiser, which analyses flowgram data to cluster similar reads in order to remove potentially erroneous sequences (Reeder and Knight 2010). Sequences were then clustered based on operational taxonomic units (OTUs) using the UCLUST method (Caporaso et al. 2010). OTUs were assigned a taxonomic identification using Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) method with a SILVA (Pruesse et al. 2007) generated database. Sequence alignments were made using the NAST (DeSantis et al. 2006a) algorithm against the GreenGenes (DeSantis et al. 2006b) database. Using additional downstream tools in QIIME, a phylogeny was built with the FastTree algorithm (Price et al. 2009), and pairwise beta diversity distance matrices among all samples were also generated based on the weighted UniFrac phylogenetic distance metric (Lozupone et al. 2006, Lozupone et al. 2007). Principal Coordinate Analysis (PCoA) ordinations were generated based on these UniFrac beta diversity matrices in QIIME.
Environmental variables and bacterial taxa relative abundances at the phyla level were checked for normal distributions and homoscedasticity. Nitrogen fixation rates were log transformed to achieve a more normal distribution. To examine relationships between overall 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 weighted UniFrac phylogenetic dissimilarity matrices - 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) among 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. Spearman’s rank correlations were calculated for relative abundance of bacterial taxa and variables correlated with differences in community structure.

Using the vegan package in R (Oksanen et al. 2010), Principal Components Analysis (PCA) was employed to further examine environmental variation among samples along linear axes. Euclidean distance among samples in environmental space 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 correlated with Olsen P and extractable organic C, which were included. Variance partitioning identified the respective contribution of vegetation type and pH, significant explanatory variables, to the variation in bacterial community structure. Here, I 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.
CHAPTER III

RESULTS

Pre-analysis quality checks and Denoiser (Reeder and Knight 2010) analysis resulted in 15,899 16S rRNA gene sequences, or on average 691 sequences per sample with a standard deviation of 99. Major bacterial taxa (phyla and, in the case of Proteobacteria, sub-phyla) making up on average greater than 5% of community composition included Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, α-Proteobacteria, and β-Proteobacteria (Table 1). The PCoA ordination showed clustering of bacterial community composition by vegetation type and a clear differentiation of vegetated and unvegetated communities. This suggested a phylogenetic dissimilarity between the bacterial community structure of vegetated soils and unvegetated soils as well as vegetation-specific differentiation (Figure 1). A permutational ANOVA (PERMANOVA) (Clarke and Gorley 2006) of weighted UniFrac dissimilarity matrices indicated that bacterial community composition is significantly different between all vegetation types (Table 2).
Table 1. Relative Abundances of Bacteria Taxa >5% of total community (% of total community) and Soil Parameters

<table>
<thead>
<tr>
<th>Bacterial Taxa</th>
<th>Vegetated</th>
<th>Unvegetated</th>
<th>Vegetated vs. Unvegetated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spruce</td>
<td>Alder</td>
<td>Unvegetated</td>
</tr>
<tr>
<td>Acidobacteria</td>
<td>6.897 ± 0.51</td>
<td>9.23 ± 2.1</td>
<td>10.57 ± 1.3</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>7.995 ± 0.62</td>
<td>10.17 ± .87</td>
<td>8.248 ± 1.2</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>14.95 ± 1.01</td>
<td>11.27 ± 1.6</td>
<td>12.68 ± 0.68</td>
</tr>
<tr>
<td>alpha-Proteobacteria</td>
<td>26.02 ± 0.88</td>
<td>25.34 ± 1.3</td>
<td>22.18 ± 1.1</td>
</tr>
<tr>
<td>beta-Proteobacteria</td>
<td>20.64 ± .011</td>
<td>18.24 ± 0.61</td>
<td>20.95 ± 1.1</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>6.055 ± 1.9</td>
<td>7.615 ± 1.4</td>
<td>4.811 ± 1.8</td>
</tr>
<tr>
<td>Rhizobiales</td>
<td>13.96 ± 0.77</td>
<td>12.54 ± 0.75</td>
<td>10.07 ± 0.72</td>
</tr>
</tbody>
</table>

Soil Parameters

<table>
<thead>
<tr>
<th></th>
<th>Vegetated</th>
<th>Unvegetated</th>
<th>Vegetated vs. Unvegetated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbial C (mgC/kg)</td>
<td>97.0 ± 15.6</td>
<td>129.0 ± 25.2</td>
<td>113.5 ± 11.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.29 ± .02</td>
<td>7.41 ± .05</td>
<td>7.41 ± .16</td>
</tr>
<tr>
<td>NO₃⁻ (µg N/g soil)</td>
<td>3.70 ± 1.40</td>
<td>1.37 ± .49</td>
<td>1.39 ± .40</td>
</tr>
<tr>
<td>%C (% soil mass)</td>
<td>0.30 ± .05</td>
<td>0.25 ± .02</td>
<td>0.23 ± .03</td>
</tr>
<tr>
<td>Extract. Org. C (mg/kg soil)</td>
<td>97.7 ± 15.8</td>
<td>68.3 ± 8.7</td>
<td>41.0 ± 5.1</td>
</tr>
<tr>
<td>Total Dissolved N (mg/kg soil)</td>
<td>8.6 ± 1.2</td>
<td>4.8 ± .7</td>
<td>3.9 ± .8</td>
</tr>
<tr>
<td>Nfix (ng Nfixed/cm2/h)</td>
<td>0.37 ± .04</td>
<td>1.48 ± .72</td>
<td>0.20 ± .02</td>
</tr>
<tr>
<td>Extractable NH₄⁺-N (µg N/g soil)</td>
<td>1.10 ± .21</td>
<td>0.30 ± .04</td>
<td>0.31 ± .04</td>
</tr>
<tr>
<td>Olsen P (ppm)</td>
<td>14.3 ± 1.6</td>
<td>8.5 ± 1.0</td>
<td>9.41 ± .6</td>
</tr>
</tbody>
</table>
Figure 1. Principal Coordinates Analysis of UniFrac Bacterial Community Dissimilarity.

Samples are colored and labeled by vegetation type. The plot shows grouping of bacterial communities by vegetation type. U=Unvegetated (red), A=Alder (blue), S=Spruce (green).
Table 2. Significant results of Mantel-like RELATE tests and PERMANOVA tests.

<table>
<thead>
<tr>
<th>UniFrac Phylogenetic Community Dissimilarity</th>
<th>RELATE tests</th>
<th>PERMANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rho</td>
<td>p</td>
</tr>
<tr>
<td>pH</td>
<td>0.195</td>
<td>0.047</td>
</tr>
<tr>
<td>Nitrogen Fixation</td>
<td>0.261</td>
<td>0.04</td>
</tr>
</tbody>
</table>
I also evaluated broad-scale differences between bacterial taxa relative abundances in vegetated vs. unvegetated soils. Only α-Proteobacteria were significantly different between vegetated and unvegetated soils, being significantly enriched in vegetated soils (Welch Two Sample t-test, t=-2.67, p=.0195) (Table 1). Furthermore, at the order level, Rhizobiales, which in itself comprised greater than 5% of bacterial communities sampled, was significantly enriched in vegetated soils (Welch Two Sample t-test, t=3.5, p=.0037) (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 enrichment of Rhizobiales in spruce versus unvegetated soils (p<0.05). Although additional contrasts were suggestive, none revealed any significant differences for the bacterial taxa examined (Table 1).

For soil properties, differences were evaluated both between vegetated and unvegetated soils in general, and between each of the three vegetation types. Vegetated soils contained significantly higher levels of extractable organic C, total dissolved N, ammonium, and nitrogen fixation rates compared to unvegetated soils (Welch Two Sample t-test, p < 0.05) (Table 1). In addition, pH was lower in vegetated than unvegetated soils (t=-1.805, p < 0.1) (Table 1). Notably, pH significantly negatively correlated with ammonium, total dissolved N, extractable organic C, Olsen P, and positively correlated with microbial C (Table 3). In contrasting each of the vegetation types to each other using Tukey’s HSD a-posteriori ANOVA tests, spruce had significant increases in % organic C and extractable organic C compared to unvegetated soils (p<.05). Spruce also exhibited significantly higher total dissolved N, NH₃, and P pools compared to both unvegetated and alder soils (Table 1).

While a comparison of N-fixation in vegetated vs. unvegetated soils revealed a significantly greater rate of N-fixation in vegetated soils (Table 1), the a-posteriori Tukey’s HSD
Table 3. Correlation Analysis of Environmental Variables (Pearson Product Moment Correlation Coefficient)

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>N-fixation</th>
<th>NH₄⁺</th>
<th>NO₂/NO₃⁻</th>
<th>Total Dissolved N</th>
<th>Extractable Organic C</th>
<th>Soil % C</th>
<th>Microbial C</th>
<th>Olsen P</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>---</td>
<td>-0.0751</td>
<td>- .479*</td>
<td>-0.205</td>
<td>-.535**</td>
<td>-.472*</td>
<td>0.0131</td>
<td>.465*</td>
<td>-.529**</td>
</tr>
<tr>
<td>N-fixation</td>
<td>-----</td>
<td>-0.0651</td>
<td>0.0192</td>
<td>0.0589</td>
<td>0.238</td>
<td>.541*</td>
<td>-0.0887</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₃</td>
<td></td>
<td>.596**</td>
<td>0.368</td>
<td>.759***</td>
<td>0.147</td>
<td>-0.358</td>
<td>.825***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂/NO₃⁻</td>
<td></td>
<td>.072</td>
<td>.644***</td>
<td>-0.138</td>
<td>-0.385</td>
<td>0.403</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dissolved N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.526**</td>
<td>0.00687</td>
<td>-0.147</td>
<td>0.341</td>
<td></td>
</tr>
<tr>
<td>Extractable organic C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0332</td>
<td>-.507*</td>
<td>.572**</td>
<td></td>
</tr>
<tr>
<td>Soil % C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0759</td>
<td>0.225</td>
<td></td>
</tr>
<tr>
<td>Microbial C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.283</td>
<td></td>
</tr>
<tr>
<td>Olsen P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

¹ Value significance denoted by * p<.05, ** p<.01, ***p<.001
contrasts show only alder to have significantly higher N-fixation rates than unvegetated soils despite nearly a doubling of N-fixation rates in spruce soils as compared to unvegetated soils.

The only soil chemical parameter that was correlated to bacterial community composition was pH (RELATE test, r=0.195, p=0.047) (Table 2). Likewise, nitrogen fixation rates were correlated with bacterial community structure (RELATE test, r=0.261, p=.04) (Table 2). Spearman’s rank correlation analysis indicated that pH was positively correlated with acidobacterial relative abundance (Rho= 0.538, p<0.01) and negatively correlated with the abundance of Bacteroidetes (-0.541, p<0.01) (Table 4). There were no correlations between the relative abundance of bacterial taxa and nitrogen fixation rates.

The orientation among samples in environmental space was further discerned by principal components analysis (Figure 2). Vector lengths show the relative contribution of individual parameters to multivariate variation. Samples generally grouped by vegetation type across axis 1, which described 43.5% of variation. Axis 2 described 19.4% of variation. While alder and unvegetated soils more closely grouped according to pH, spruce was more influenced by differences in soil nutrient pools.

To disentangle the interrelated effect of pH and vegetation type on differences among bacterial communities, I conducted variance partitioning (Table 5). Vegetation type explained the greatest variation (23.27% ) in bacterial community structure. The remaining variability was explained by pH (3.17%), the interaction term (6.49%), and error (67.07%); neither pH nor interactions were significantly related to variation in community composition (p>.05). Given that pH is a significant correlate to bacterial community structure as determined through the Mantel-like RELATE test (Table 2), the variance partitioning suggests that pH is not
Table 4. Bacterial Taxa (>5% of Community) Spearman Rank Correlations with pH

<table>
<thead>
<tr>
<th>Bacterial Taxa</th>
<th>rho</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidobacteria</td>
<td>0.538</td>
<td>0.00805</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>0.134</td>
<td>0.5423</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>-0.541</td>
<td>0.007709</td>
</tr>
<tr>
<td>alpha-Proteobacteria</td>
<td>-0.383</td>
<td>0.07122</td>
</tr>
<tr>
<td>beta-Proteobacteria</td>
<td>0.0456</td>
<td>0.8362</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>-0.0283</td>
<td>0.898</td>
</tr>
<tr>
<td>Rhizobiales</td>
<td>-0.393</td>
<td>0.06325</td>
</tr>
</tbody>
</table>
Figure 2. Principal Components Analysis of Sample Environmental Parameters. Multivariate sample points are colored by vegetation type.
Table 5. Variance Partitioning, Analysis of Pairwise Dissimilarity

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>$\text{Df}$</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>$R^2$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1</td>
<td>0.0064624</td>
<td>0.0064624</td>
<td>0.8033155</td>
<td>0.0317</td>
<td>0.548</td>
</tr>
<tr>
<td>Vegetation Type</td>
<td>2</td>
<td>0.0474434</td>
<td>0.0237217</td>
<td>2.9487656</td>
<td>0.2327</td>
<td>0.003</td>
</tr>
<tr>
<td>pH x Vegetation Type</td>
<td>2</td>
<td>0.0132359</td>
<td>0.006618</td>
<td>0.822657</td>
<td>0.0649</td>
<td>0.589</td>
</tr>
<tr>
<td>Residuals</td>
<td>17</td>
<td>0.1367586</td>
<td>0.0080446</td>
<td></td>
<td>0.6707</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>0.2039004</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
independent from soil vegetation type, which by far explains the most variation in UniFrac distances. All other environmental variables were also tested as factors in the adonis model (Oksanen et al. 2010) and none were significant.
CHAPTER IV

DISCUSSION

Past research in glacial forefields has shown that bacterial communities and associated biogeochemical cycling undergo successional changes in unvegetated soils, before plant colonization (Nemergut et al. 2007, Noll and Wellinger 2008, Schmidt et al. 2008, Schütte et al. 2010). Plant colonization acts as an important transition in the successional trajectory of these dynamic bacterial communities. My research indicates that early colonizer plants at this specific point in the chronosequence significantly alter bacterial community composition. My study not only supports past findings that show broad-scale bacterial community shifts across different plant types in vegetated soils (Bardgett and Walker 2004, Tscherko et al. 2005), but also work that reveals unique bacterial communities associated with different vegetation types (Westover et al. 1997, Grayston et al. 1998). My research shows that plants alter bacterial communities, increasing the relative abundance of α-Proteobacteria, specifically the Rhizobiales (Table 1). Additionally, I show that soil bacterial community structure is unique to each vegetation type. (Table 2, Figure 2)

Past studies have demonstrated inconsistent results as to the influence of plant colonization on early successional bacterial communities. 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 stronger vegetation influence in soils older than 75 years old. This finding was attributed to the 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 quick plant colonization may indicate more favorable conditions for plants, which may explain other findings that show significant vegetation effect on microbial community structure in the pioneering stage (Bardgett and Walker 2004, Edwards et al. 2006). My research also supports this interpretation by showing strong early plant colonizer influence on bacterial community structure in newly exposed soils that are characterized by quick vegetation colonization and relatively less extreme environmental factors (Burt and Alexander 1996).

Not surprisingly, differences in soil chemistry correspond with vegetation type (Figure 2). A variety of other studies across larger soil age gradients of deglaciated chronosequences have shown that these changes in soil chemistry impact bacterial community composition and function (Tscherko et al. 2004b, Edwards et al. 2006). For rhizosphere bacterial communities in the Damma Glacier forefield, Edwards et al. (2006) found that soluble carbon and mineral nitrogen were dominant influences on bacterial communities across the chronosequence. While shifts in nutrient and carbon pools over the chronosequence may correspond to bacterial community structure, my data yielded no correlations within the various chemical parameters measured and bacterial community composition. This does not mean, however, that changes in
C and N pools are not influencing bacterial community composition within the sampled transect. The specific chemical properties of carbon in both litter and exudates, for example, or unmeasured fractions of nutrient pools may influence individual taxa and/or overall microbial community structure (Orwin et al. 2006, Meier and Bowman 2008, Eskelinen et al. 2009). As such, unvegetated soil bacterial communities could be influenced by sources of allochthonous carbon in the soil, distinct in composition from plant inputs. Indeed, previous studies have shown that recently deglaciated soils contain older, ancient pools of soil carbon (Bardgett et al. 2007, Hood et al. 2009, Sattin et al. 2010).

My research indicates that pH is the single measured environmental variable that significantly correlates with bacterial community structure. While pH is one mechanism to describe differences in bacterial community structure, it is not a significant factor in the adonis model (Table 5). Variance partitioning thus indicates that pH is not independent of vegetation type. Variance partitioning confirms that vegetation type describes the highest amount of variation in bacterial community structure, supporting the notion that plants drive decreases in pH along with other factors to uniquely alter bacterial communities when colonizing deglaciated soils.

My results are important in the context of an increasing body of research that suggests that pH may act as a primary driver of bacterial community composition and diversity at a variety of scales. Eskelinen et al. (2009) showed strong correlations between fungal:bacterial ratios, pH, and plant functional type. In a comprehensive 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 among bacterial community structure across samples. Furthermore, contrary to the conventional portrayal of Acidobacteria as purely
acidophilic organisms, my research shows a significant positive correlation between acidobacterial relative abundance and pH (Table 3). This adds to a growing body of work that has revealed a more variable response within the Acidobacteria phylum to shifts in pH (Jones et al. 2009, Ramirez et al. 2010). Such research has also demonstrated positive correlations between the relative abundance of some acidobacterial taxa and pH.

My work also showed evidence for increases in N-fixation rates in vegetated soils (Table 1). These functional changes could be due to changes in soil chemical properties (Table 1) and/or the bacterial community structure in these soils. Indeed, my work shows that community structure correlated with N-fixation rates. While past research has shown shifts in nitrogen metabolism-related enzyme activity and nifH gene abundance and diversity within vegetated sections of deglaciated chronosequences (Tscherko et al. 2004a, Duc et al. 2008, Brankatschk et al. 2010, Töwe et al. 2010), this study establishes a direct linkage between plant-driven shifts in soil properties and nitrogen fixation rates in a glacial forefield.

Thus, my 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. 2008, Brankatschk et al. 2010, Schütte et al. 2010, Töwe et al. 2010). As asymbiotic nitrogen fixers are widely phylogenetically distributed, it is difficult to speculate on the taxa responsible for the increased N-fixation rates (Gnanamanickam 2006). It is worth noting, however, that OTUs of order Rhizobiales, known N-fixers, were significantly enriched in vegetated soils. Such findings are consistent with past studies that document increased abundance of Rhizobiales in association with vegetation (King et al. 2010, Uroz et al. 2010). Although typically attributed to symbiotic N-fixation, work by Buckley et al. (2007) suggests active N-fixation by soil Rhizobiales. This suggested relationship may be obfuscated in
correlation analysis as only a small subset of *Rhizobiales* may fix nitrogen as free-living diazotrophs, and overall N-fixation is attributable to far more phylogenetically dispersed taxa.

While cyanobacteria have been documented to contribute to N-fixation in unvegetated soils of glacial forefields (Schmidt et al. 2008, Schütte et al. 2009), cyanobacteria made up a comparatively small proportion of bacterial communities at the Mendenhall Glacier (Table 3) (Sattin et al. 2010). As plants colonize soils, the role of cyanobacteria is likely diminished as heterotrophs can use plant carbon inputs for biomass and respiration. Thus heterotrophic diazotrophs likely contribute more prominently to overall nitrogen fixation after plants colonize young landscapes. Recent research has documented the highest abundance of *nifH* genes across a glacial chonrosequence in soils associated with early colonizer plants (Brankatschk et al. 2010, Töwe et al. 2010). In accordance with this finding, my study supports the application of an individualistic process model for asymbiotic nitrogen fixation (Walker and Chapin 1987, Matthews 1992) where asymbiotic nitrogen fixation is of particular relevance in ecosystem development at the pioneering stage of succession when plants colonize.

Finally, in agreement with Miniaci et al. (2007), my study shows 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. 2004b, Tscherko et al. 2005, Edwards et al. 2006). My work highlights the need for better definitions of plant zones of influence. Like Miniaci et al. (2007), my 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. The broader range of plant effects on microbial communities
is likely easier to discern in the patchy vegetation in these young soils, yet such an expanded spatial perspective regarding plant feedbacks on soil microbial communities may also exist in more developed soils. Our understanding of how the range of plant influence may change in older soils is minimal. Overall, denser and more diverse vegetation cover and the root-adhering soil sampling, commonly associated with rhizosphere studies, have limited our understanding of the range of plant influence in later stages of the primary successional gradient. My study yields new insights on important interactions between plant colonizers and the trajectory of bacterial community succession, a relationship that has feedback effects on biogeochemistry and the overall primary succession of plant and microbial communities.
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