Bacterial communities in soil mimic patterns of vegetative succession and ecosystem climax but are resilient to change between seasons

Mark A. Williams,*, Kamlesh Jangid, Shankar G. Shanmugam, William B. Whitman

1. Introduction

Microorganisms are key drivers of global biogeochemical cycles and are considered the most abundant and diverse organisms on earth (Torsvik et al., 2002; Falkowski et al., 2008). Despite their vast diversity, the biogeography of microbes on small and large scales is poorly understood (Ranjard et al., 2010). Previous studies have shown that environmental factors, such as quantity and quality of available soil carbon (Blaalid et al., 2011), nitrogen (Eaton et al., 2008) and season (Williams, 2007) influence soil microbial community structure. Studies of bacterial community change during early (100 y) ecosystem development adjacent to retreating glaciers have generally shown that bacterial communities are highly dynamic during early ecosystem development (Nemergut et al., 2007; Schütte et al., 2009), but that patterns of change are not always easy to discern or to relate to soil and vegetative properties (Wu et al., 2012; Zumsteg et al., 2012). Over shorter-time scales, in contrast, bacterial communities have been shown to be relatively static between seasons and across a variety of land use types (Jangid et al., 2008, 2010). How bacterial communities become structured over seasons and during the natural process of soil accrual, weathering and nutrient transformations (pedogenesis) over much longer time scales could provide clues to the underlying mechanisms and feedbacks that regulate community assembly (Green and Bohannan, 2006; Green et al., 2008).

Numerous studies have researched above-ground and below-ground feedbacks, for example, implicating soil microbes as...
important determinants of plant diversity and productivity (Ehrenfeld et al., 2005; Kara et al., 2008; Van Der Heijden et al., 2008). Above-ground plant community composition, in turn has a major influence on the microbial community by driving changes in litter quality, soil acidity and soil moisture (Porazinska et al., 2003; Kardol et al., 2007; Mitchell et al., 2011; Zhang et al., 2011). It is not well-known, however, how these processes of long-term ecosystem development, vegetative succession, and pedogenesis are related to changes in soil microbial communities (Wardle et al., 2012). If above- and below-ground subsystems are linked and feedback, then patterns of bacterial and plant community change should also show relationships with pedogenesis over hundreds, and thousands of years of ecosystem development. Describing how bacterial communities change during ecosystem development could thus provide fundamental insight into above and below-ground linkages and ultimately whether these feedbacks underlie patterns of community and ecosystem function.

Differences in bacterial community composition and diversity across a series of developmental sand-dune soil chronosequences bordering northern Lake Michigan and ranging in age from ~105 to 4010 years since deposition were studied. A pyrosequencing-based approach was used to characterize phylogenetic changes in soil bacterial communities across the chronosequence. The primary hypothesis was that bacterial communities would follow patterns of change associated with the relatively long-term processes of pedogenesis and primary vegetative succession. The effect of season was also tested to observe whether shorter-term environmental change regulates bacterial community structure. Succession is defined as a non-random “pattern of change” in communities through time (Wardle et al., 2012).

2. Materials and methods

2.1. Site description

The study site consists of a series of beach-dune ridges bordering Lake Michigan (N 45.72729, W 84.94076), and located in Emmet County of northern lower Michigan. Periods of unusually moist conditions altering with dry spells associated with swelling and receding of Lake Michigan formed a series of ~108 elolian deposited dune ridges running parallel to the shoreline with depositional ages from present day to ~4500 years (Lichter, 1995). The dune ridges have a parent material originating from glacial deposits and Paleozoic bedrock underlying the lake basin. Fine sands deposited on the lake shore are dominated by quartz but contain numerous other minerals in minor quantities. The youngest soils (~100 y) are mapped as dunes which then develop into Deer Park sands (soil series) and described taxonomically as mixed, frigid, Spodic Udipsamments. The oldest soils (>1475 y) tend to be mapped to the Roscommon series, and are mixed, frigid Mollic Psammaquents. The chronology of the dunes was estimated using Accelerated Mass Spectroscopy (AMS) radiocarbon dating from each dune (Lichter, 1995). The ridges are approximately 2.5 km long, 10–30 m wide, and vary between 3 and 5 m high to 15 m high parabolic dunes inland (Lichter, 1998a). A previous study (Lichter, 1998b) has shown that patterns of primary succession with grasses and shrubs on younger dunes change into mixed coniferous forests that dominate older dunes.

2.2. Soil and vegetative sampling

Five replicate soil samples were taken at (10-m) intervals across transects (35–120 m) along each dune’s crest. From each sampling location, 5–6 sub-sample cores were collected from the incipient A–E horizon (0–15 cm, 5-cm dia.), homogenized, stored in sterile Whirlpak® bags, and frozen immediately in coolers with dry ice. Five replicate samples were also collected along the beach to simulate the material that might be the source of the dune ridges (time zero). Soils were thawed briefly (25–30 min) to clean extraneous roots and other organic materials by passage through a 4-mm sieve. Samples were collected in August (Summer) and December (Winter) of 2008.

Along each replicate soil age, plant species composition, tree density, and percentage canopy cover was measured (5 × 20 m²). The tree composition and density were measured by counting the number of species within the sampling area and measuring DBH (Diameter Breast Height), respectively. Understory species cover was measured at five random locations within the sampling area using a 1 sq. m quadrat. Two quadrats were randomly placed on each sampling spot along each replicated age. Understory species were identified and their percentage cover was estimated by visual observation as agreed by two observers. For the vegetation data, species that were rare (occurred in 3 or fewer plots) were removed. The tree species canopy cover was estimated by fitting the DBH measurement into a conifer crown radius model (Dequiedt et al., 2011).

2.3. Soil characteristics

Soil organic matter content was measured by loss on ignition (560 °C) in dry soils (104 °C) by subtracting the mass difference before and after ignition. Carbon content on dry soils was measured using a Elementar vario Max CN. Carbonate content was estimated as described by Amundson et al. (1988). The mineralizable C was estimated by measuring the cumulative CO2-C released over 1 month. Incubations were carried out using 1 L canning jars filled with 100 g of soil (~0.03 MPa) and sealed with a lid fitted with a rubber septum. Soil pH was measured on 1:2 soil and 0.01 M CaCl2 mixture. Soil extractable cations were extracted and analyzed according to the Mehlich-3 extraction protocol (Maron et al., 2011, Table 1).

2.4. DNA extraction and pyrosequencing

Total community DNA was extracted from 0.5 g of soil using ZR Soil Microbe DNA Kit (Zymo Research, Orange, California) with minor modifications in the manufacturer’s protocol as described in Garcia et al. (2011), inventoried, and stored at –80 °C. PCR amplification of the bacterial 16S rRNA V3 region, purification and processing for pyrosequencing was carried out using primers and conditions as described by Garcia et al. (2011). Samples were initially denatured at 95 °C for 3 min, then amplified by using 22 cycles of 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 1 min. Samples were further purified to remove PCR inhibitors using OneStep™ PCR Inhibitor Removal Kit (Zymo Research). The final mixed amplicon pool was submitted to the Environmental Genomics Core Facility at the University of South Carolina for pyrosequencing with Roche® GS FLX sequencing (Branford, CT, USA), yielding 108,273 reads (260-bp average length).

2.5. Processing of 16S rRNA gene data

A two-step pipeline was established to analyze the 16S rRNA gene sequence data. QIIME (Caporaso et al., 2010) was used to quality trim the raw sequences for primers and chimeras and to sort them based on the barcodes. The denoised data were then analyzed using MOTHUR v1.22.0 (Schloss et al., 2009). The sequences were aligned using SILVA reference database and a distance matrix was generated in MOTHUR followed by filtering
and pre-clustering. Operational Taxonomic Units (OTUs) were formed using the average-neighborhood algorithm at an evolutionary distance $D = 0.03$. From this, the indices of diversity and richness were calculated (Table 2). Finally the phylogeny was described using the method of Whittaker (1972) to identify bacterial taxa; and taxonomy to describe plant taxa, were calculated using the method of Whittaker (1972) to identify bacterial taxa; and taxonomy to describe plant taxa, were calculated using the method of Whittaker (1972) to identify bacterial taxa; and taxonomy to describe plant taxa.

### 2.6. Analysis of the closest cultural representatives

The percentage abundance of each OTU across the chronosequence was calculated based on the relative abundance of each OTU with the total number of sequences associated with that age. The five most dominant OTUs were then studied to better define the ecological role and possible impacts that pH or other driving variables might have on community structure. In order to achieve that, the five most abundant OTUs described by an increasing linear or log-linear relationship with age were plotted. The taxonomic affiliations of the OTUs were determined by finding its closest cultural representative in RDP using RDP agent. The sequence similarity between each sequence and the closest cultured representative was between 87 and 96%.

### 2.7. Bray–Curtis and multivariate analyses

Bray–Curtis analysis of the 200 most abundant OTUs ($D \leq 0.03$) and the 13 woody plants was performed using the PC-ORD software version 4 (MJM Software, Gleneden Beach, OR, USA) as advised by McCune and Grace (2002). The Shannon index was calculated using the “general relativization” function to remove the potentially strong influence that absolute abundance can have on community data. The Multi-response permutation procedure (MRPP), a nonparametric test, was used to assess differences in bacterial community structure between soil ages and seasons ($P < 0.01$).

### 3. Results

#### 3.1. Changes in soil characteristics during ecosystem development

A number of soil characteristics changed along the chronosequence. Soil Ca and Mg levels decreased in a log-linear pattern and were concurrent with declining pH (7.6–3.5) as soils aged from younger to older soils across the chronosequence (Table 1). Soil organic matter and total soil organic C (but not mineralizable C) decreased along the chronosequence from younger to older soils ($r = 0.76$; $P < 0.05$; Table 1). Soil Na ($\sim 149 \mu g/g$) and P ($\sim 4 \mu g/g$), in contrast, did not change with soil development. Patterns of change, particularly declining concentrations of mineral nutrients and soil organic matter were common observations during pedogenesis.

#### 3.2. Microbial community structure and ecosystem development

The distribution of the 200 most abundant OTUs in the beach sand samples and across the chronosequence from summer and winter samples indicated two primary patterns related to Axis 1

### Table 1

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Ca (µg/g)</th>
<th>K (µg/g)</th>
<th>Mg (µg/g)</th>
<th>pH</th>
<th>SOC (%)</th>
<th>SOM (%)</th>
<th>Mineralizable C (µg/g)</th>
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<tbody>
<tr>
<td>105</td>
<td>1314 a</td>
<td>16 a</td>
<td>110 a</td>
<td>7.6 a</td>
<td>0.32 a</td>
<td>0.58 a</td>
<td>76 a</td>
</tr>
<tr>
<td>155</td>
<td>762 b</td>
<td>20 a</td>
<td>156 b</td>
<td>7.1 b</td>
<td>0.29 ab</td>
<td>0.45 ab</td>
<td>121 a</td>
</tr>
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<td>210</td>
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<td>5.7 c</td>
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<td>0.30 bc</td>
<td>169 a</td>
</tr>
<tr>
<td>450</td>
<td>141 c</td>
<td>22 a</td>
<td>20 c</td>
<td>3.6 d</td>
<td>0.39 c</td>
<td>0.19 c</td>
<td>134 a</td>
</tr>
<tr>
<td>845</td>
<td>109 c</td>
<td>24 a</td>
<td>12 c</td>
<td>3.2 d</td>
<td>0.10 d</td>
<td>0.19 c</td>
<td>129 a</td>
</tr>
<tr>
<td>1475</td>
<td>116 c</td>
<td>24 a</td>
<td>11 c</td>
<td>3.6 d</td>
<td>0.20 e</td>
<td>0.15 c</td>
<td>108 a</td>
</tr>
<tr>
<td>2385</td>
<td>137 c</td>
<td>25 a</td>
<td>13 c</td>
<td>3.6 d</td>
<td>0.13 d</td>
<td>0.18 c</td>
<td>127 a</td>
</tr>
<tr>
<td>3210</td>
<td>110 c</td>
<td>22 a</td>
<td>10 c</td>
<td>3.7 d</td>
<td>0.19 e</td>
<td>0.14 c</td>
<td>85 a</td>
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<td>4010</td>
<td>108 c</td>
<td>24 a</td>
<td>8 c</td>
<td>3.5 d</td>
<td>0.26 ab</td>
<td>0.15 c</td>
<td>153 a</td>
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</table>

* Calculations based on the Operational Taxonomic Units (OTUs) formed at an evolutionary distance of $<0.03$. The results shown here are from the summer samples.

# Table 2

<table>
<thead>
<tr>
<th>Diversity index</th>
<th>105 y</th>
<th>155 y</th>
<th>210 y</th>
<th>450 y</th>
<th>845 y</th>
<th>1475 y</th>
<th>2385 y</th>
<th>3210 y</th>
<th>4010 y</th>
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<td>N0</td>
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<td>4779</td>
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<td>4779</td>
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<tr>
<td>S'</td>
<td>1969</td>
<td>2352</td>
<td>2259</td>
<td>950</td>
<td>1225</td>
<td>1027</td>
<td>674</td>
<td>977</td>
<td>815</td>
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<tr>
<td>Evenness</td>
<td>2.01</td>
<td>2.00</td>
<td>1.99</td>
<td>1.75</td>
<td>1.73</td>
<td>1.70</td>
<td>1.70</td>
<td>1.78</td>
<td>1.72</td>
</tr>
<tr>
<td>Richness (ace)</td>
<td>15,557</td>
<td>27,093</td>
<td>28,193</td>
<td>6320</td>
<td>11,929</td>
<td>7463</td>
<td>7463</td>
<td>7545</td>
<td>4433</td>
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<tr>
<td>Shannon</td>
<td>6.63</td>
<td>6.73</td>
<td>6.68</td>
<td>5.22</td>
<td>5.34</td>
<td>5.11</td>
<td>4.80</td>
<td>5.33</td>
<td>5.00</td>
</tr>
<tr>
<td>1/D0</td>
<td>205</td>
<td>134</td>
<td>125</td>
<td>58</td>
<td>53</td>
<td>43</td>
<td>45</td>
<td>72</td>
<td>52</td>
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<tr>
<td>Chao 1</td>
<td>7372</td>
<td>12,736</td>
<td>10,863</td>
<td>3253</td>
<td>5045</td>
<td>3423</td>
<td>3183</td>
<td>3671</td>
<td>2380</td>
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</table>

* Calculations based on the Operational Taxonomic Units (OTUs) formed at an evolutionary distance of $<0.03$. The results shown here are from the summer samples.

* Total soil organic carbon (SOC) and total soil organic matter (SOM) from summer samples.
and Axis 2 (Fig. 1). The first pattern (Axis 1) showed a strong relationship between the bacterial community structure and ecosystem development. Along axes 2, a lesser but important part of the structural variability was related to differences associated with the beach sand and the chronosequence. There was a seasonal effect (winter vs summer) on the community structure of the beach sand communities, however, there was no difference in the pattern of bacterial community structure with season across the chronosequence. Similarly, there was no season x age effects on bacterial community structure (P = 0.45). Because there were clear differences between the beach sand and soils along the chronosequence, the chronosequence samples were re-analyzed without the beach sand and without separation of winter and summer samples (Fig. 2a). Bacterial communities showed patterns of change across the chronosequence during early ecosystem development (< 845 y) but changed little during latter (845–4010 y) ecosystem development.

A one-dimensional solution accounted for almost 80% of the community variability across the chronosequence (Fig. 2a). This change in bacterial OTU distribution was strongly related to several environmental variables. Environmental variables with the greatest correlation to change in bacterial community structure determined using Bray-Curtis included pH (r = 0.97) and SOM (r = 0.87; Table 3). The partial mantel test results also showed a similar relationship between bacterial community structure and pH (P < 0.0001, r = 0.82) and SOM (P < 0.0001, r = 0.58).

3.3. Change in plant community composition with ecosystem development

Change in percent cover of thirteen dominant plant species (including herbs, shrubs and hardwood trees) across the chronosequence was investigated. The change in plant community structure was greater during early compared to late ecosystem development (Fig. 2b). Generally speaking, dune-building grass species were replaced by evergreen shrubs and these were then replaced by mixed pine forests. This shift in early-succession to late-succession plant species happened at ~ 450 years of soil and ecosystem development (Fig. 2b), when the early-succession species began to disappear and the mixed pine forest began to develop. Early succession was thus defined by considerable turnover of plant species. Indeed, plant community composition in the young dunes (105–155 y) was completely different from communities observed at 210 y, which were again taxonomically different from those >450 y of ecosystem development. Once the forest matured, the plant species composition stabilized and there was no major change in the plant community structure during late ecosystem development (Fig. 2b; P = 0.59). A mantel test of the relationship indicated a correlation between vegetation and bacterial communities along the developmental gradient (P < 0.000001; r = 0.56). This overall trend was comparable with the change in the most abundant bacterial OTUs wherein the biggest change in the bacterial community was also during the initial stages of soil development (Fig. 2a).

3.4. Phylogenetic changes across the ecosystem gradient

The chronosequence gradient showed a number of changes in phyla but were generally dominated by the abundance and
dynamics of Acidobacteria, Actinobacteria, and Alphaproteobacteria, comprising 71% of all the sampled sequences (Fig. 3a,b,g). Other less abundant phyla (<4%) were Bacteroidetes, Cyanobacteria, Firmicutes, Planctomycetes, Betaproteobacteria, and Gammaproteobacteria (Fig. 3c–f, h, i). Between early (<450 y) and late (>450 y) ecosystem development, Acidobacteria increased approximately 6-fold from around 4% to ~30%. Actinobacterial abundance declined, in contrast, from around 60 to ~35% during this same time. The gradient of ecosystem development was also described by changes in low abundance taxa, with Bacteroidetes and Firmicutes, for example declining and Planctomycetes and Gammaproteobacteria increasing 4-fold. Cyanobacterial abundance declined from 5% to less than 0.5% following 210 y of ecosystem development. Patterns of change in bacterial phyla, overall, generally mimicked patterns shown using OTU.

The five most abundant OTUs that showed linear or log-linear increases in abundance with soil age were shown to vary from non-detectable during early ecosystem development and to represent up to ~4.5% of the total sequences during latter ecosystem development (Fig. 4). Two of the closest matches (Ribosomal Database Project; RDP) belonged to genera within the phylum Acidobacteria, and the other three belonged to Proteobacteria, Planctomycetes and Actinobacteria. Four out of five of these matches are documented acidophiles and include Edaphobacter modestus, Singulisphaera acidiphila, Acidobacterium capsulatum and Methylovivibula ligni.

The distribution of bacterial phyla in the freshly deposited beach sand was different from those along the chronosequence. Cyanobacteria was the dominant phyllogenetic group (25%) followed by Alpha-Proteobacteria (22%) and Betaproteobacteria (20%). Actinobacteria and Acidobacteria were less abundant with only 9 and 4% of the phyla, respectively. Approximately 46% of the OTUs were unique to the freshly deposited beach sand and not found in the chronosequence. Assessing the closest cultured representatives of the 5 dominant OTUs from these unique OTUs showed that three of the closest matches were Acidobacteria and the other two belonged to Proteobacteria and Firmicutes. The abundance of Cyanobacteria was thus a major reason for differences between the beach sand and the chronosequence.

### 3.5. Bacterial diversity indices

Soil aging across the chronosequence resulted in declining bacterial diversity (Shannon and Simpson’s reciprocal indexes) with the largest declines during initial soil development (Table 2). For example, diversity declined from 212 to 58 (Simpson’s 1/D) during 105–450 years of soil development. The Chao 1 indicator predicted that only 20–37% of the OTUs were actually observed during sampling. The rarefaction curves showed the number of observed OTUs as a function of sequences sampled, and are generally supportive of the calculations provided by the diversity indices (Fig. 5). When grouped at the 97% similarity level, there were 19,893 OTUs in the complete data set. These OTUs contained 15,013 singletons and 1900 doubletons, representing 75–9.5%, respectively, of the OTU. The decline in bacterial richness (Table 2) showed a similar change associated with soil pH, SOM and vegetation; the biggest decline coincided with the youngest (105 y, 155 y and 210 y) and changed very little in mature soils. Aging of the soil across the chronosequence thus resulted in declining bacterial community diversity and richness.

### 4. Discussion

The goal of this study was to understand the patterns of bacterial community change during ecosystem development in the sandy oligotrophic soils of Wilderness Park (WP). Overall, bacterial and plant communities and soil properties changed synchronously during pedogenesis and ecosystem development and thus support the major hypothesis of the study. Bacterial communities, however, did not change between winter and summer sampling, suggesting that bacterial community structure is not strongly tied to relatively short-term seasonal changes that include altered belowground C allocation and soil temperatures. The study supports a model of soil bacterial communities synchronized to pedogenesis, relatively dynamic during hundreds of years during early ecosystem development, but stable during thousands of years during latter ecosystem development.

#### 4.1. Role of vegetation and pedogenesis in bacterial community structure and composition

Bacterial communities showed patterns of change related to both aboveground vegetative dynamics and belowground pedogenesis during early ecosystem development (Fig. 2a,b; Table 3). In particular, vegetative community structure tracked pedogenic losses in Ca, Mg, and declining soil pH. Plants and plant communities can directly affect the selection of bacterial taxa found in rhizospheres (e.g. Huguet and Rudgers, 2010) and they can alter the chemical and microbial soil properties that change soil habitats in ways that indirectly affect bacterial community structure (Van Breemen and Finzi, 1998; Kuramae et al., 2011). Organic acids produced by plants, for example, can accelerate the acidification of the upper mineral soil, for example (Kelly et al., 1998; Ehrenfeld et al., 2005; Kara et al., 2008, Table 1). Hence, the synchronized patterns of change between plant and bacterial communities and pedogenesis are consistent with both indirect and direct models of plant–bacterial interaction. Studies across chronosequences are not the ideal venue to test for plant–bacterial interactions, however, it is plausible to gain insights into them. Studies that include a test of both soil and plant effects generally have shown that soil type accounts for a much larger proportion of bacterial community variability than do plants (Givvan et al., 2003; Jangid et al., 2011; Kuramae et al., 2011). It is notable that plant communities turn-over and replace one another to form completely new assemblages several times during early ecosystem development (<450 y), while bacterial communities, in contrast, typically share >80–90% of their taxa between adjacent ages (Fig. 2a,b). Rather than direct plant effects, indirect plant-driven pedogenesis may be the primary driver of bacterial community change during ecosystem development.

### Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pearson correlation*</th>
<th>Mantel test*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r²</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>r²</td>
<td>P-value</td>
</tr>
<tr>
<td>SOM (%)</td>
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<td></td>
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<td></td>
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<td>Total N (%)</td>
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<td></td>
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<td>pH</td>
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<td></td>
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<td>Ca</td>
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<td></td>
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<td>Mg</td>
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<td>&lt;0.0001</td>
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<td></td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total C (%)</td>
<td>0.56</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Pearson correlations between the ordination scores of the axis explaining maximum variability (78% from the original OTU distribution data (Fig. 1a)) from the summer samples of the non-metric dimensional scaling ordination and selected environmental characteristics.

* Mantel test of relationship between bacterial communities and environmental characteristics.

* Pearson’s correlation coefficient.

* Standardized Mantel statistic.
Climate has previously been studied as an important determinant of bacterial communities (Castro et al., 2009), and though it cannot be completely ruled out, biogeographical studies suggest that climate is not a major factor in influencing bacterial community structure in soil (Lauber et al., 2009; Chu et al., 2010). Bacterial community patterns of change during pedogenesis at WP are also consistent with community change at climatically different locations (Tarlera et al., 2008; Georgia, USA). For example, communities at these different locations show a similar pattern of change in many phyla (e.g. Acidobacteria) during ecosystem development. Once established, similar patterns of bacterial community structural stability have also been shown to develop (Tarlera et al., 2008) and thus suggest that the patterns of community change are not strongly regulated by differences in climate. Because plant communities in the two geographically isolated (MI and GA) chronosequences are also different, it is hypothesized that plants, regardless of plants species, mediate pedogenesis and indirectly contribute to changes in soil bacterial community structure. It should be noted, however, that climate may ultimately be found to influence bacterial communities when a higher resolution molecular clock other than 16S rRNA genes are used.

4.2. Effect of season on soil bacterial communities

Bacterial community structure in soil was unchanged despite the potential for considerable differences in plant C allocation, temperature, and water activity (Bardgett et al., 1999; Griffiths et al., 2003) between winter and summer. Microbial activities, in contrast to these observations of bacterial community structure, are strongly altered as a result of both seasonal and plant mediated
fundamental nature that plant C has for energy.

In light of the nature of the 16S rRNA gene may make it a relatively insensitive average neighbor algorithm in MOTHUR at a distance of 0.03.

Fig. 5. Rarefaction curves of the 16S rRNA gene libraries. OTUs were formed using the average neighbor algorithm in MOTHUR at an evolutionary distance of 0.03.

OTUs were formed using average neighborhood algorithm in MOTHUR at an evolutionary distance of 0.03 and the abundance was calculated based on the number of sequences found in each OTU relative to the total number collected for that age.

Fig. 4. Relationship between closest cultural representatives from the most abundant OTUs based on RDP agent analysis. 5 OTUs that showed the highest log-linear relationship with the changing ecosystem were chosen and plotted. OTUs were formed factors (e.g. Williams et al., 2000; Parker and Schimel, 2011). The nature of the 16S rRNA gene may make it a relatively insensitive determinant of change of a small active soil community. Indeed, rRNA rather than rRNA genes may be suited for assessing changes in community assembly to detect change in the relative size of rRNA gene pools (DeAngelis and Firestone, 2012). In light of the fundamental nature that plant C has for energy flow in these oligotrophic ecosystems, the stability of the bacterial communities across seasons and across latter stages of soil and ecosystem development are nevertheless rather striking and speak to the need to develop a clearer conceptual picture of bacterial community dynamics in soil ecosystems.

Bacterial communities have been previously described as dynamic across seasons and in response to environmental change. These studies, however, have typically assessed physiological or biomass pools that are more indicative of energy flow than community composition (Bardgett et al., 1999; Steenwerth et al., 2006; Yao et al., 2011; Lazzaro et al., 2012). Phospholipid fatty acid analysis (PLFA) has been shown to be dynamic indicators of microbial (bacterial and fungal) community changes to water content and season (e.g. Williams, 2007), however, interpretation of PLFA dynamics differs from that of rRNA genes. Often described as an indicator of microbial community structure, PLFA is also sensitive to changing microbial physiology. It is thus not surprising that different patterns of change within the same experiment are observed for PLFA and rRNA gene based characterizations of soil communities (Jangid et al., 2008, 2010). The stability of the 16S rRNA gene fits with other findings that they are relatively stable between seasons, however this can vary somewhat based on land-use history (Jangid et al., 2008, 2010). The resiliency of the rRNA gene to change across seasons, nevertheless, may be an important clue to the ecological strategies or adaptations that bacterial communities utilize to survive in soil.

4.3. Relationships between soil bacterial communities and soil properties across the ecosystem development gradient

Rather than succession per se, the comparatively straightforward mechanism of pH as a driver of bacterial community structure has been reported in several studies (Männistö et al., 2007; Hartman et al., 2008; Chu et al., 2010; Rousk et al., 2010). These studies point to the potential for a direct link between soil pH and bacterial community change in soils. The ecological mechanisms conferring this change and driving these relationships, however, may be more complicated. During the first ~450 years, for example, the weathering of Ca and Mg carbonates from the upper mineral soil horizons were key contributors to soil acidification and the decline of soil pH from ~7.6 to 3.6 (Table 1). Mineral weathering was likely accelerated by the release of organic acids by plant roots and the decomposition of coniferous litter material (Berendse, 1998; Huggett, 1998; Lichter, 1998a). Though pH may be an important predictor of soil community composition, it is not clear-cut if it is a direct determinant and driver of community change.

To test the role that pH may have on community structure, the five most abundant clones that showed linear or log-linear increases (P < 0.05) in abundance with increasing acidity (declining pH) were matched to the closest neighbor in RDP. Clues to the changes occurring during soil pedogenesis were expected to shed light on possible mechanisms of community change. Interestingly, the closest matches included E. modestus, S. acidiphila, A. capsulatum and M. ligni. Four of these five OTUs are known for their acidophilic nature and derived from diverse phyla (Acidobacteria, Actinobacteria and Planctomycetes). The physiological importance of pH for microbial physiology in soil is well known (Högberg et al., 2007), but recently it has also been shown that specific soil bacteria have a narrow growth tolerance to pH that may impact their survival in soils (Fernández-Calviño et al., 2011). These results are thus consistent with a model of pH as a driver of bacterial communities (Lauber et al., 2008; Rousk et al., 2010). These results can also help to explain the reduced diversity of other taxa along the soil development gradient and provide evidence that pH can directly impact bacterial community composition and structure.
4.4. Assessing the source of diversity

The eolian deposits of the WP sand dune soils are associated with the recession of lake water from beaches during climatic periods of drought and high wind. The transported sediments, subsequently stabilized by plant colonization, thus might carry bacterial communities that serve as colonizers during ecosystem succession ([Fig. 1]). The communities of these sedimentary deposits are considerably different than those found along the soils of the developing ecosystem but do share some common members (~45%) and could thus contribute to the bacterial communities observed along the chronosequence. Approximately 30% of the beach sand bacteria were derived from the phyla Cyanobacteria, and these taxa in particular, become very rare when herbaceous and woody plants begin to dominate the ecosystem. Whether some of the community members along the chronosequence are descendants of those derived from the beach sediments is not known; however, there is the possibility that some of the bacterial taxa in the developing soils may have persevered. These observations bring to light questions about the importance of colonization and the role that colonizers play during the development of ecosystems.

4.5. Conclusion

Soil bacterial communities showed similar patterns of change to those observed for plant communities and to the process of pedogenesis over several hundred years of early ecosystem development. It is hypothesized that plants primarily impact bacterial communities through an indirect mechanism of plant-accelerated pedogenesis. For example, plant driven increases in soil acidity may be responsible for the increasing abundance of acidophilic taxa during soil pedogenesis. There were no changes in bacterial community structure and composition between summer and winter, a result suggesting resilience to relatively short-term changes in the environment. In mature soils, furthermore, stable soil bacterial community structures develop and mimic the structural stability of aboveground plant communities, which mimics latter stages (climax) of ecosystem development before retrogression and without secondary disturbances. The strong linkages between bacterial communities and pedogenesis suggest that unless pedogenic properties are altered, bacterial communities may be resilient to changes derived, for example, from disturbance, invasion by plant species, or climate change.

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