

Progressive and retrogressive ecosystem development coincide with soil bacterial community change in a dune system under lowland temperate rainforest in New Zealand

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Abstract

Background It is established that plant communities show patterns of change linked to progressive and retrogressive stages of ecosystem development. It is not known, however, whether bacterial communities also show similar patterns of change associated with long-term ecosystem development.

Methods We studied soil bacterial communities along a 6,500 year dune chronosequence under lowland temperate rain forest at Haast, New Zealand. Pyrosequencing of 16S rRNA genes was used to observe structural change in bacterial communities during the process of pedogenesis and ecosystem development.

Results Bacterial communities showed patterns of change during pedogenesis, with the largest change during the first several hundred years after dune stabilization. The most abundant bacterial taxa were *Alphaproteobacteria*, *Actinobacteria* and *Acidobacteria*. These include taxa most closely related to nitrogen-fixing bacteria, and suggest heterotrophic nitrogen input may be important throughout the chronosequence. Changes in bacterial community structure were related to changes in several soil properties, including total phosphorus, C:N ratio, and pH. The *Bacteroidetes*, *Actinobacteria*, *Cyanobacteria*, *Firmicutes*, and *Betaproteobacteria* all showed a

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general decline in abundance as pedogenesis proceeded, while *Acidobacteria*, *Alphaproteobacteria*, and *Plantctomycetes* tended to increase as soils aged.

Conclusions There were trends in the dynamics of bacterial community composition and structure in soil during ecosystem development. Bacterial communities changed in ways that appear to be consistent with a model of ecosystem progression and retrogression, perhaps indicating fundamental processes underpin patterns of below and above-ground community change during ecosystem development.

Keywords Bacterial diversity · Haast chronosequence · Pedogenesis · Soil nutrients · Vegetative succession · 16S rRNA pyrosequencing

Introduction

Aboveground plant communities have received considerable attention in chronosequence studies, but there is little detailed information on parallel changes in belowground communities during long-term ecosystem development. This is surprising given the importance of the soil microbial biomass in regulating nutrient cycling and availability (Turner et al. 2013). Several studies have recently reported the use of PLFA and rRNA genes to assess changes in bacterial community composition during the very early stages of soil development on glacial forelands (Tscherko et al. 2004; Knelman et al. 2012; Wu et al. 2012; Zumsteg et al. 2012). These studies showed that both bacterial and fungal communities are dynamic during the earliest stages (~100 years) of ecosystem development following glacial retreat (Nemergut et al. 2007; Schutte et al. 2009), although patterns of community change did not necessarily relate strongly to changes in soil or vegetation (Ohtonen et al. 1999; Wu et al. 2012; Zumsteg et al. 2012). Longer-term studies have recently been published, observing that bacterial communities mimic patterns of vegetative change and pedogenesis in a northern temperate chronosequence (105 to 5,200 y) in Michigan, USA (Williams et al. 2013). However, it is not known if the same changes take place over the same time spans in bacterial communities found in super-humid climates undergoing rapid pedogenesis and ecosystem development.

Long-term ecosystem development can be described by progressive and retrogressive phases, which

are related to large changes in vegetation and nutrients, especially phosphorus and nitrogen. The decline in soil phosphorus availability as pedogenesis proceeds on stable land surfaces (Walker and Syers 1976) is a key part of the later phase decline in ecosystem biomass and productivity. This occurs through the gradual loss of phosphorus by leaching and erosion, as well as chemical transformations from inorganic into organic forms (e.g. Crews et al. 1995; Parfitt et al. 2005; Turner et al. 2007). In contrast, nitrogen concentrations are typically low during very early stages of pedogenesis and ecosystem development, but increase quickly as a result of high rates of biological nitrogen fixation (e.g. Menge and Hedin 2009). As a consequence, nitrogen availability tends to limit plant communities on young soils, while phosphorus becomes increasingly limiting on old soils (Vitousek and Farrington 1997; Peltzer et al. 2010). These major transitions, during the process of pedogenesis and vegetative change are key parts of ecosystem development and are thus expected to influence developmental-scale dynamics of bacterial communities.

For aboveground communities, the patterns in nitrogen and phosphorus availability during ecosystem development favor nitrogen-fixing plants on young soils (e.g. *Alnus* spp. in the temperate north and *Coriaria* spp. in parts of southern New Zealand), while stress-tolerant plant species adapted to low phosphorus availability are typically found on old, strongly-weathered soils (e.g. conifers in New Zealand and Proteaceae in southwestern Australia; Richardson et al. 2004; Lambers et al. 2010). Eventually, the increasing phosphorus limitation during pedogenesis can lead to retrogression and decline in forest biomass on old soils (Wardle et al. 2004). Because of the importance of bacterial communities in nutrient cycling, bacterial communities may also show patterns of change related to vegetative succession and ecosystem retrogression.

In an older retrogressive chronosequence (Waitutu), Williamson et al. (2005) reported an increase in the fungal to bacterial ratio and a corresponding increase in fungal-feeding to bacterial feeding nematode density. Along the Franz Josef chronosequence, also in New Zealand, Allison et al. (2007) used phospholipid fatty acid analysis to determine a similar increase in the fungal to bacterial ratio during pedogenesis. Activities of phosphorus and carbon-nitrogen cycling enzymes increased and decreased, respectively, along the

chronosequence. The changes in hydrolytic enzyme activities and fungal to bacterial ratios suggest that microbial communities vary during ecosystem development and track changes in the availability of key nutrients during pedogenesis. Whether these shifts correspond to alterations in bacterial community structure through progressive and retrogressive phases of ecosystem development and the process of pedogenesis, however, are not known.

We studied soil bacterial communities along a 6,500 year dune chronosequence under lowland temperate rain forest at Haast, New Zealand. The chronosequence is characterized by rapid pedogenesis under high rainfall, associated with podzol development, low nitrogen, phosphorus depletion, and a shift in tree community composition (Eger et al. 2011;

Turner et al. 2012a, b). The objective of this study was to determine soil bacterial community structure (16S rRNA genes) and diversity (Shannon, Simpson's indices) along the Haast chronosequence. Changes along the chronosequence were used to interpret whether bacterial communities track ecosystem development and concurrent shifts in pedogenesis.

Methods

Site description

The Haast dune system is a foredune progradation chronosequence on the west coast of the South Island of New Zealand (Fig. 1). Detailed descriptions

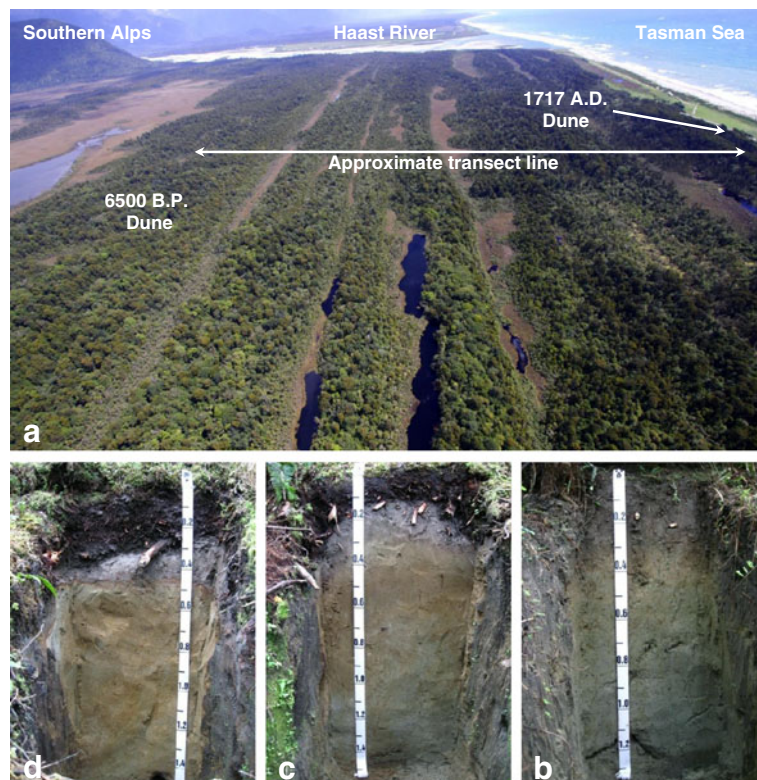


Fig. 1 **a** Aerial view of the Haast Chronosequence looking south towards the Haast River in the distance, with the youngest dunes on the right close to the ocean (indicated by Dune 2 formed following the 1717 A.D. earthquake) and the oldest dunes furthest inland (indicated by the 6500 B.P. dune). The approximate transect line across the sequence is between the two marked dunes. **b** A weakly developed soil (Typic Udipsamment) on a young dune (Dune 4; 517 years B.P.). **c** A

moderately-developed soil (Spodic Udipsamment) on an intermediate-aged dune (Dune 8; 1,826 years B.P.), showing a clear bleached eluvial horizon under a thick organic horizon. **d** A well-developed Spodosol (Typic Placorthod) on an old dune (Dune 12; 3,903 years B.P.), showing a bleached eluvial horizon, a spodic B horizon, and a continuous cemented placic horizon (iron pan). Photo credits: Reproduced from Turner et al. 2012a

of dune system formation are reported elsewhere (Wells and Goff 2006; Wells and Goff 2007). Briefly, dunes are formed following periodic earthquake disturbance on the Alpine Fault, leading to a pulse of sediment transported to the ocean and deposition as a linear dune either side of the mouth of the Haast River (Wells and Goff 2007). There are a total of seventeen dune ridges that occur as generally continuous features across the length of the system, ranging in age from ~180 years to ~6,500 years (Wells and Goff 2007; Turner et al. 2012a,b). Six of these dune ridges were chosen for study of soil bacterial communities.

The Haast system approximates an ideal chronosequence, because parent material, climate, vegetation, and topography are all well constrained. The dunes are comprised of sandy sediments derived from well-foliated schist and the mineralogy of the unweathered sand appears relatively uniform across the chronosequence (Palmer et al. 1986). Mean annual rainfall is 3,455 mm and mean annual temperature is 11.3 °C (New Zealand Meteorological Service, 1983). The chronosequence is under lowland temperate rain forest consisting of a mixed conifer–broadleaf community (see below for details). Topography of the dunes is similar throughout the chronosequence, being linear and generally continuous features, although there is some variation in dune height (Turner et al. 2012a,b).

Summary of changes in soils and vegetation along the chronosequence

Soils develop rapidly to podzols (Spodosols) under the super-humid climate of the west coast of New Zealand (Turner et al. 2012a; Supplementary Table 1). This involves acidification and depletion of base cations in the first few hundred years, formation of an eluvial horizon under a thick organic horizon within 2,000 years, and development of a cemented iron pan within 4,000 years. The early stages of pedogenesis are characterized by a rapid decline in primary mineral phosphate in soil and the accumulation of organic phosphorus, accounting for ~80 % of the total phosphorus in the upper mineral soil. Total soil phosphorus then declines slowly for the remainder of the chronosequence. Nitrogen accumulates rapidly in the early stages of pedogenesis, followed by a slow decline. There appears to be an increasing degree of phosphorus limitation along the chronosequence, as indicated by increasing C:P and N:P ratios and a decline in available phosphate (Fig. 2a).

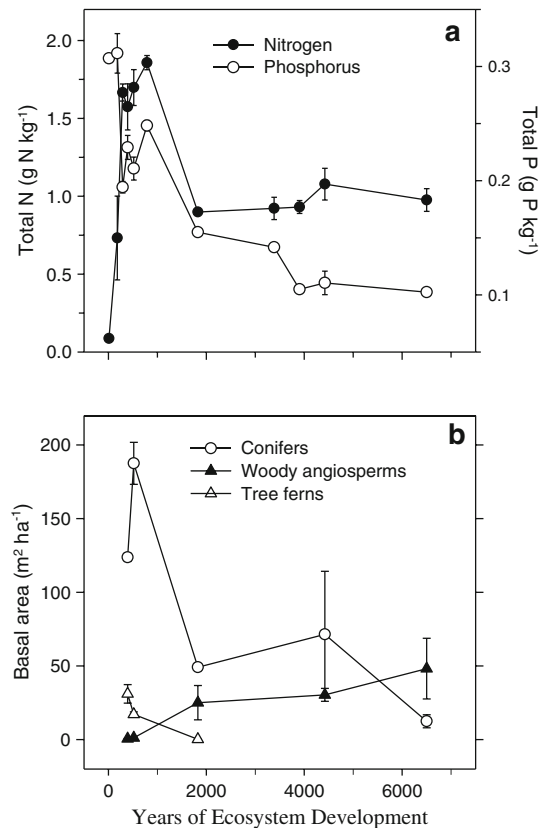


Fig. 2 Summary of changes in soil nutrients and vegetation communities during ecosystem development along the Haast soil chronosequence, New Zealand. **a** Changes in total nitrogen and total phosphorus in mineral soil (0–20 cm), showing the rapid decline in phosphorus and accumulation of nitrogen in the early stages (data from Turner et al. 2012a). **b** Changes in the basal area of conifers (Podocarpaceae), woody angiosperms, and tree ferns (data from Turner et al. 2012b)

There are marked changes in the forest community along the chronosequence (Turner et al. 2012b; Supplementary Table 2; Fig. 2b). Basal area peaks in the early part of the chronosequence and then declines, with *Dacrydium cupressinum* forming monodominant stands on young dunes (but not the youngest dune). Older dunes support a mixed conifer–angiosperm community. Conifers include *Prumnopitys ferruginea*, *Podocarpus hallii*, and *Phyllocladus alpinus*, while woody angiosperm trees include *Weinmannia racemosa* and *Metrosideros umbellata*, as well as shrubs (mainly *Coprosma* spp.). There are abundant tree ferns *Dicksonia squarrosa* and *Cyathea smithii* on young dunes, but they are rare on old dunes. In addition, the ectomycorrhizal tree *Nothofagus menziesii* is conspicuous on older dunes. A number of factors could account

for the changes in the tree community along the chronosequence, although partial Mantel tests indicate that changes in soil nutrients play a key role (Turner et al. 2012b).

Soil sampling

Six dunes ranging in age from 181 years to 6,500 years were sampled. Four replicate plots (5×10 m), separated by ~50 m were established along the crest of each dune. Ten locations within each plot were chosen and soil collected (0 to 20 cm depth) with the use of a 2.5 cm diameter soil probe. The sample bags were frozen immediately in cooler packed filled with dry ice. Thus 24 plots were sampled. Upon arrival in the laboratory, soils were thawed for ~30 min, homogenized through a 2-mm sieve, extraneous roots and organic materials were removed, and the sample refrozen at –80 °C.

DNA extraction and pyrosequencing

For bacterial community analysis, DNA was isolated from 0.5 g of soil from each soil sample using ZR Soil-Microbe DNA™ kit (Zymo Research). After extraction and purification the DNA was inventoried and stored at –80 °C. For pyrosequencing, samples were processed as described earlier (Garcia et al. 2011). Briefly, the 16 rRNA gene fragments were amplified using primers Adaptor(A)-TAG-(515R-NK) and Adaptor(B)-(27F-YM+3), where the A and B sequencing adaptors represent 454 Life Science's FLX adaptors and the TAG is an 8-nt sample –specific barcode for post-sequence sorting of sequences to its affiliate sample. A total of 200 ng amplicon product was obtained for each sample using multiple 25 µl PCR reactions as described in Garcia et al. (2011). A few samples that yielded very low amplification at 20 cycles were amplified using 25 cycle PCR. Our analysis of such variable cycle samples revealed that this did not affect the estimated diversity as long as the total number of sequences used for estimating the diversity was nearly equal. Following gel quantification of amplicons, products from the four replicates of each dune developmental age were pooled in equimolar concentrations and gel eluted using Zymoclean™ Gel DNA Recovery Kit (Zymo Research). The six eluted amplicons were quantified on the Experion® System (Bio-Rad) and pooled in equimolar concentrations to form a single composite sample for pyrosequencing.

This amplicon pool was further purified using the Agencourt AMPure XP system (Beckman Coulter Genomics) and submitted to the Environmental Genomics Core Facility at the University of South Carolina for pyrosequencing on a 454 Life Sciences Genome Sequencer FLX (Roche) machine. A total of 47,401 quality sequences, with up to 5317 sequences from each replicate soil were obtained.

Processing of 16S rRNA gene data

A two-step pipeline was established to analyze the 16S rRNA gene chronosequence data. QIIME (Caporaso et al. 2010) was used to quality trim raw sequences for primers, chimeras and to sort them based on the barcodes. The denoised data was then analyzed in MOTHUR v1.22.0 (Schloss et al. 2009), a software for describing and comparing microbial communities. The sequences were aligned using SILVA reference database and a distance matrix was generated followed by filtering and pre-clustering. Operational Taxonomic Units (OTU) were formed using average - neighborhood algorithm at an evolutionary distance $D=0.03$. Finally, the phylogeny was assigned to representative sequences from OTU using SILVA reference sequences.

Description of the 16S rRNA data

Chimera-Slayer in MOTHUR was used to analyze short-length sequences for chimeras. Potential chimeric sequences were detected, however, according to the programs instructions; some of these sequences were manually tested against the entire NCBI database and none were confirmed to be chimeric. The sequences showed close homology with the investigated genera for the 3' and 5' regions and were thus concluded not to be chimeric. The 260-bp average length reads were submitted to the NCBI Sequence Read Archive according to MIMS standards (SRS394732).

Statistical and multivariate analyses

Bray-Curtis analysis was performed using the PC-ORD software version 4 (MjM Software, Gleneden Beach, OR, USA) as advised by McCune and Grace (2002). The multi-response permutation procedure (MRPP), a nonparametric test, was used to assess differences in bacterial community structure between soil ages. Data was transformed by treatment using the “general

relativization” function to remove the potentially strong influence that absolute abundance can have on community data. Univariate correlations (linear and log-linear) and statistical tests of bacterial diversity and phylogenetic change were conducted using Excel 2003. SigmaPlot version 11.0 (Systat Software, San Jose, CA, USA) was used for graphing. Mantel tests were conducted using PC-ORD to determine if correlations existed between community, vegetative, and soil descriptive data. The standardized Mantel test statistic (r) was calculated based on the Pearson’s product–moment correlation coefficient and significance was assessed using randomization tests with 999 permutations.

Results

Bacterial diversity

Rarefaction curves, which provide a prediction of the number of observed OTU and the number of sequences sampled, failed to plateau, indicating an underestimation of the richness at an evolutionary distance of ~0.03. The Chao1 richness predictor estimated that only 45–58 % of the OTU were actually observed. There were 2943 OTU in the complete data set when grouped at the 97 % similarity level. The most abundant OTU was represented by 5695 sequences and accounted for 12 % of the entire dataset, whereas the top 20 and 120 OTU represented 55 and 85 %, respectively, of the entire data set.

Soil phosphorus declined significantly with age ($p < 0.01$), and was significantly correlated to changes in bacterial richness (Simpson’s 1/D). Nitrogen content, showing a unimodal increase and then decline during ecosystem development, and showed no significant correlation with ecosystem development or index of richness ($p > 0.01$; Table 1; Fig. 3).

Bacterial community structure and ecosystem development

The distribution of the 120 most abundant OTU across the chronosequence showed a significant relationship between bacterial community change and ecosystem development, with a large proportion of the community change explained by the axis of maximum variability using Bray-Curtis ordination (Axis 1=71 %; Fig. 4). The OTU composing the bacterial community in the youngest (181 years B.P.) and next oldest site (392 years B.P.) shared 73 % of the 120 OTU (Whittaker 1972). Bacterial communities in the youngest (181 years B.P.) and the oldest site (6,500 years B.P.) shared 48 % of their dominant taxa. The second axis accounted for 12 % of the variability in the original data set and was most useful for separating the older mature sites. An MRPP was used to compare community structure between the soil ages. Differences were associated with changes occurring during early and late development, with no significant difference between the three mid-age soils (524, 1844, 4422 years B.P.; Fig. 4; $p < 0.01$). There was no significant correlation (Table 2) between

Table 1 Diversity indices based on 16S rRNA gene sequences

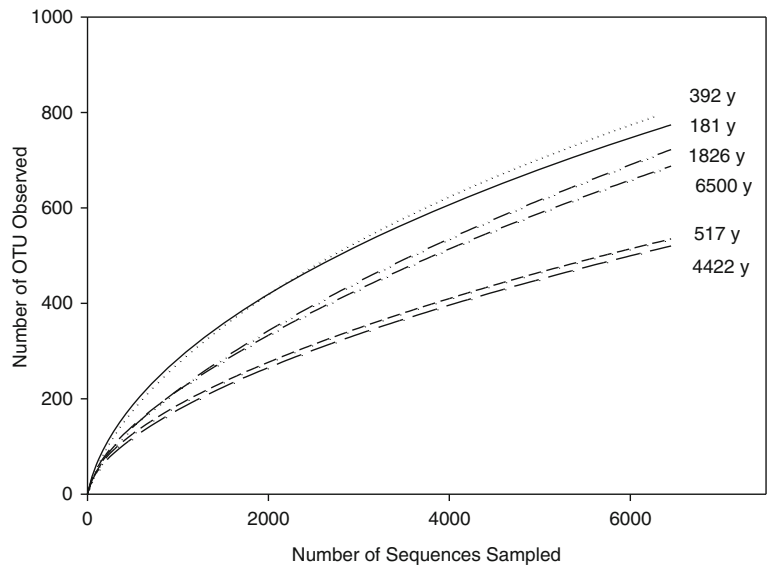
Diversity Index ^{a,b}	Years of Ecosystem Development					
	181	392	517	1826	4422	6500
Number of OTU	760	794	524	726	514	703
Evenness	1.74	1.68	1.55	1.55	1.53	1.61
Richness (ace)	1917	1966	1729	2483	1605	2632
Shannon ^c	5.03	4.88	4.23	4.45	4.14	4.59
Simpson’s reciprocal index (1/D) ^c	50	37	22	26	23	35
Chao 1	1314	1439	1036	1625	1121	1655

^a Calculations based on the Operational Taxonomic Units (OTU) formed at an evolutionary distance of <0.03

^b The calculation of diversity indices for each developmental age was normalized based on 6280 sequences per age. Normalization removes the potential effect of sequence abundance for estimating diversity

^c Bacterial richness for both Simpson’s 1/D and Shannon’s indices declined and showed a significant log-linear relationship with developmental age ($p < 0.01$). No other diversity measure was significantly related to ecosystem development

Fig. 3 Rarefaction curves of the 16S rRNA gene libraries. OTU were formed using the average neighbor algorithm in MOTHUR at a distance of 0.03. This analysis was based on 47,401 quality sequences



bacterial and plant community change. However, mantel tests showed that total phosphorus, carbon, nitrogen, and pH were significantly correlated (Table 2) with change in bacterial community structure.

Phylogenetic affiliation of the 16S rRNA genes

The most abundant bacterial taxa across the chronosequence, in order of abundance, were

Alphaproteobacteria, *Actinobacteria* and *Acidobacteria* (Fig. 5). Collectively, sequences from these three taxa accounted for ~80 % of all sequences. The only bacterial group to change significantly using a log-linear or linear model was *Bacteroidetes*, which declined log-linearly in abundance with developmental age (Fig. 5). Except for the *Alphaproteobacteria*, the relative abundance of taxa varied by at least 2X, when comparing chronosequence ages with the lowest and the highest relative abundance.

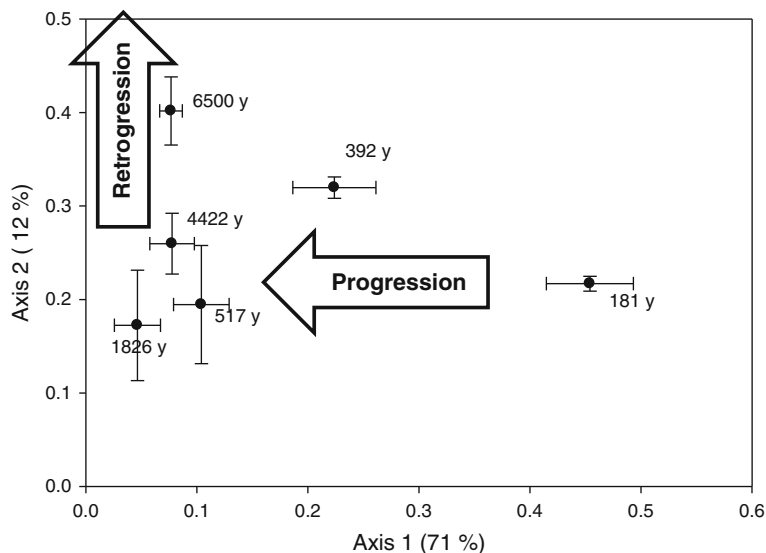


Fig. 4 Bray–Curtis ordination plot, showing the relationship between soil ecosystem development and bacterial community composition and structure. The 120 most abundant OTU, derived from 47,401 rRNA sequences, were used for the ordination. OTU were formed using average neighborhood algorithm

in MOTHUR at a distance of 0.03. Error bars represent standard error ($n=4$). Percentage denotes the amount of variability associated with each axis. Large arrows denote concurrent shifts in bacterial communities with progressive and retrogressive stages of ecosystem development

Table 2 Correlations between the bacterial community structure and selected environmental characteristics during ecosystem development

Variable	Mantel Test ^a	
	<i>r</i> value ^b	<i>p</i> value
pH	0.44	0.0002
Total C	0.59	0.0001
Total N	0.41	0.007
Total P	0.57	<0.0001
C:N	0.75	<0.0001
C:P	0.67	<0.0001
Sand (%)	0.53	0.12
Silt (%)	0.74	0.04
Vegetation (basal area %)	0.76	0.03
Vegetation (individual trees ha ⁻¹)	0.51	0.09

^a Mantel test of relationship between bacterial communities and environmental characteristics

^b Standardized Mantel statistic

Analysis of the closest cultural representatives

The 5 most abundant OTU ($D=0.03$) were matched to their closest cultured representatives. The change in the relative abundance of representative taxa were also described and plotted (Fig. 5) across the chronosequence. The percentage abundance of these OTU varied from non-detectable to ~30 % of the sequences sampled (Fig. 6). The similarity between the representative sequence of an OTU and its closest cultured representative varied between 94–99 %, consisting of *Bauldia consociata*, *Azospirillum lipoferum*, *Methylocella palustris*, *Granulicella pectorans*, and *Mycobacterium heckeshornense*. The genera *Methylocella* and *Azospirillum* are identified as bacteria capable of fixing nitrogen by the National Center for Biotechnology Information (NCBI) database and the broader literature (Dedysh et al. 2000).

Discussion

General patterns of bacterial community change

Only a few previous studies have examined patterns of bacterial change during long-term (>1,000 years) ecosystem development (e.g., Tarlera et al. 2008; Williams et al. 2013; Jangid et al. 2013), but together

with the results reported here, these studies support the idea of bacterial community structure being associated with developmental ecosystem processes such as pedogenesis. A comparison of the Haast (NZ) and Michigan (USA) dune ecosystems, which are described by similar developmental ages but different climates, showed similar abundances of taxa (Williams et al. 2013). For example, the *Acidobacteria* and *Alphaproteobacteria* increasingly dominated the relative abundance of the bacterial community during long-term ecosystem development. In contrast, *Actinobacteria* tended to decline during ecosystem development for both chronosequences. The abundance of *Alphaproteobacteria* was ~50 % of the total community at Haast, but only ~30 % in the Michigan dune chronosequence. This latter finding may be partly attributed to the relatively high abundance of bacterial taxa most closely related to free-living nitrogen-fixing genera (Jangid et al. 2010, 2011; Williams et al. 2013). The soil bacterial communities observed during ecosystem development are strongly dominated by taxa that are closely related to *Azospirillum*, *Methylocella*, *Bauldia*, *Granulicella*, and *Mycobacterium*. The two former taxa are well-represented by N₂-fixing bacteria. Despite some differences, the overall similar dynamics of the bacterial community across several chronosequences that developed under different climates, composed of different genera of plant taxa, and geographically separated by ~15,000 km, suggest that generalizable ecological patterns define bacterial community structure during soil and ecosystem development.

Bacterial community change associated with pedogenesis and phosphorus decline with ecosystem development

One notable shift in bacterial communities not previously observed, was during late stages of ecosystem development. Declining soil phosphorus, changing plant species composition, decline in tree basal area, and increasing C:N:P ratios are evidence of a shift to ecosystem retrogression (Supplementary Table 1,2). Phosphorus decline in particular is a good indicator of an ecosystem shifting into a retrogressive stage of development (Peltzer et al. 2010). The concurrent change in bacterial communities during the retrogressive shift suggests a link between these belowground communities and the changing ecosystem habitat. The role that bacterial communities play in ecosystem processes in older

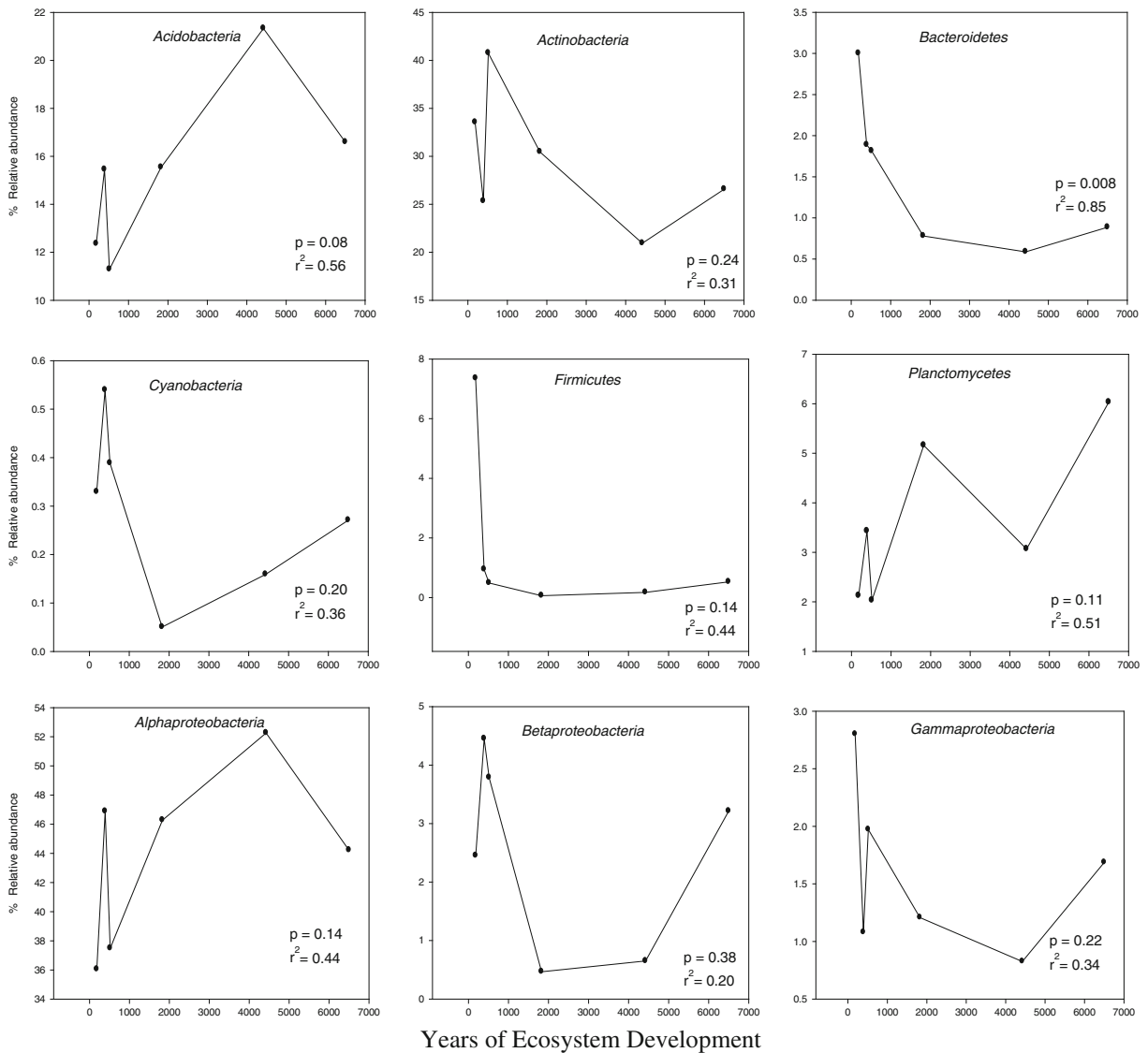


Fig. 5 Relative percentage of nine bacterial taxa across the Haast chronosequence. Each point in the graph is the average ($n=4$) of percent abundance from each sample. Regression co-

efficient and p -value for each taxa is shown. The total number of rRNA sequences acquired for this analysis was 47,401

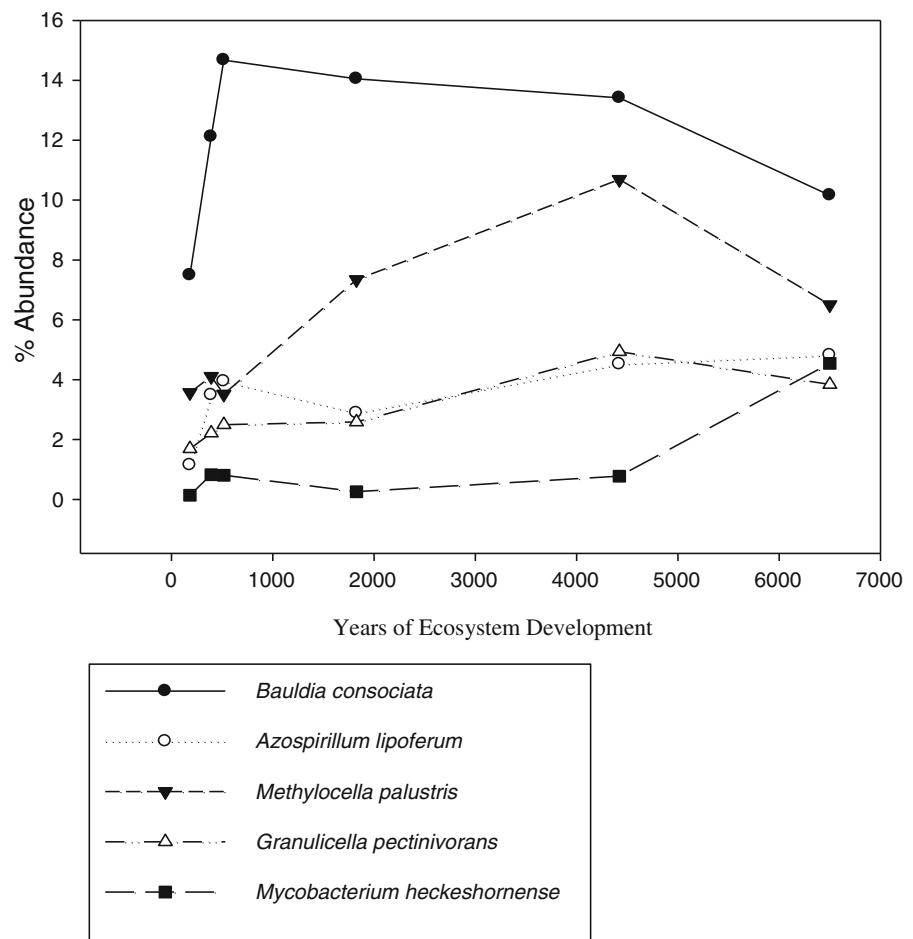
retrogressive stages of ecosystem development thus need further investigation.

Phosphorus availability in ecosystems is thought to be mediated by soil microorganisms, and when plants are limited by soil phosphorus availability during latter phases of ecosystem development, the microbial biomass can be an increasingly important sink contributing to this limitation (Turner et al. 2013). The depletion of phosphorus during ecosystem development could help to explain some of the patterns of bacterial community change and diversity, perhaps facilitated by a

selection of bacteria, such as *Acidobacteria* and *Alphaproteobacteria*, which might efficiently acquire phosphorus from decomposition resistant soil organic pools. Bacterial community change associated with phosphorus depletion during the first several hundred years of pedogenesis have previously noted possible linkages (Mander et al. 2012; Wakelin et al. 2012), but more research is needed to discern how phosphorus impacts bacterial community structure.

Linkages between microbial types and phosphate solubilizing capacity in soil and the root-zone have

Fig. 6 Relationship between the closest cultured representatives from the most abundant OTU based on RDP agent analysis. The five most abundant OTU that showed the highest log-linear relationship with the changing ecosystem were chosen and plotted. OTU were formed using average neighborhood algorithm in MOTHUR at an evolutionary distance of 0.03. Relative abundance was calculated based on the number of sequences found in each OTU relative to the total number collected for that age. The population of sequences used to describe the most abundant OTU was based on 47,401 rRNA sequences



been observed previously (Hariprasad and Niranjana 2009; Deforest and Scott 2010), but whether phosphate-scavenging ability and acquisition are related to bacterial phylogenies and community structures in phosphorus limited ecosystems have rarely been studied (Mander et al. 2012; Wakelin et al. 2012). In particular, while nutrient assimilation genes have been documented in numerous taxa (Rocap et al. 2003), only a few observations have linked the efficiency of enzymes derived from phosphatase genes to particular bacterial taxa. *Bacteroidetes*, for example, may have less efficient *pho* genes than some *Alphaproteobacteria* (*phoX*) (Sebastian and Ammerman 2009). The decline in *Bacteroidetes* and the relative stability of *Alphaproteobacteria* as phosphorus is depleted during soil development is consistent with these observations. These results bring to light the importance of describing how phosphorus and other co-varying nutrients might determine bacterial community

structure and their role in determining the trajectory of ecosystem processes.

Other studies have also noted that phosphorus content in soil correlates with change in bacterial community structure (Beauregard et al. 2010; Michel and Williams 2011). Though there have been few attempts to specifically test if bacterial community variations in soil are related to soil phosphorus concentrations, there is anecdotal evidence in phosphorus limited marine waters that certain types of bacteria are better adapted to conditions of low phosphorus. In lieu of phospholipids, *Trichodesmium* utilizes sulfolipids to help reduce the demand for phosphorus in relatively sulfur rich ocean waters (Dyrman et al. 2005). Though *Trichodesmium* was not noted to occur in our soil samples, it provides an important example of how bacteria can adapt specifically to phosphorus limitation, and evidence that it could be an important factor influencing bacterial communities in soil.

Potential for persistent soil nitrogen accrual throughout the process of ecosystem development

The abundance of bacterial taxa most closely related to heterotrophic diazotrophs (*A. lipoferum*; *M. palustris*) indicate the potential for the persistent role of nitrogen fixation during ecosystem development. It is not known if these bacteria are actually fixing N₂, however, in the absence of plant taxa known to produce root nodules or show symbiosis with bacteria from the genera *Frankia* and *Rhizobia*, the occurrence of these associative diazotrophs would help to explain the accrual and maintenance of soil nitrogen. Low concentrations of nitrogen in most parent materials promote the colonization and dominance of diazotrophic bacteria during early ecosystem development (Walker and Syers 1976), so it is not surprising that diazotrophic bacteria were common in young soils. The dominance of taxa closely related to nitrogen fixing heterotrophs at later stages of the chronosequence, however, is less easily explained.

Though the decline in phosphorus and tendency for increasing N:P ratios is thought to limit plant productivity during latter stages of ecosystem development (Turner et al. 2012a; Eger et al. 2013), it cannot be ruled out that low nitrogen may also co-limit plant productivity. The abundance of taxa closely related to nitrogen-fixing bacteria points to the possibility that nitrogen limitation may favor these microbes and support persistent input of nitrogen throughout ecosystem development. Strong negative feedbacks between diazotrophs with available soil nitrogen result in a relatively low nitrostat setting, “which turns on” N₂-fixation when available nitrogen is scarce and “off” when it is relatively plentiful (Menge and Hedin 2009). This nitrostat hypothesis is related to the sensitivity of nitrogenase activity to de-repression at low levels of inorganic nitrogen, and can help to explain the occurrence of free-living diazotrophs and nitrogen fixation in ecosystems with low levels of available nitrogen (Arp and Zumft 1983). Several other chronosequence studies have noted the prevalence of bacteria closely related to non-photosynthetic free-living nitrogen-fixing bacteria. Nitrogen imports to ecosystems are usually dominated by plant-bacterial N₂-fixing mutualisms during early nitrogen-limited soil development, but high abundance of phyla related to N₂-fixing methane oxidizers have been observed to account for ~16 % of the OTU in a young soil at

Damma glacier (Duc et al. 2009). These results point to the important role of heterotrophic nitrogen fixers and the potentially important trade-offs between nitrogen and phosphorus limitation for determining bacterial community structure, ecosystem productivity and vegetative succession (Wardle et al. 2004; Menge et al. 2012). The relatively high dominance of putative N₂-fixing bacterial rRNA genes need further study to understand their role in nutrient cycles at Haast and other ecosystems (Tarlera et al. 2008; Jangid et al. 2010; Williams et al. 2013).

Conclusion

Trends of bacterial community composition and structure associated with ecosystem development changed in ways consistent with a model of progression and retrogression, suggesting that fundamental processes underpin patterns of above and below-ground community change during ecosystem development. Taxa linked to non-symbiotic nitrogen fixation may be particularly important community members for maintenance of nitrogen pools in the phosphorus-limited ecosystem. Further research into possible links between vegetative and bacterial community succession during ecosystem development will be useful for testing the extent of these plant-bacterial-ecosystem relationships.

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