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Soil bacterial community succession during long-term ecosystem development

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Abstract

The physicochemical and biological gradients of soil and vegetative succession along the Franz Josef chronosequence in New Zealand were used to test whether bacterial communities show patterns of change associated with long-term ecosystem development. Pyrosequencing was conducted on soil-derived 16S rRNA genes at nine stages of ecosystem progression and retrogression, ranging in age from 60 to *c.* 120 000 years since glacial retreat. Bray–Curtis ordination indicated that the bacterial communities showed clear patterns of change that were closely aligned with ecosystem development, pedogenesis and vegetative succession (Mantel test; $r = 0.58$; $P < 0.001$). Eighty per cent (80%) of the explained variability in bacterial community structure was observed during the first *c.* 1000 years of development, when bacterial richness (Simpson's 1/*D*) declined from 130 to 30. The relatively high turnover of soil bacterial communities corresponded with an integrative 'plant–microbial successional feedback' model that predicts primarily negative feedbacks between plants and soil bacterial communities during progression and early pedogenesis. Positive feedbacks, similar to those of the plant community, could explain the long periods of community stability during later retrogressive stages of ecosystem development. This hypothesized model provides a consistent description linking belowground communities to ecosystem development and succession. The research, using deep sequencing technology, provides the first evidence for soil bacterial community change associated with the process of long-term ecosystem development. How these bacterial community changes are linked to the processes of primary ecosystem succession is not known and needs further investigation.

Keywords: 16S rRNA pyrosequencing , bacterial diversity , Franz Josef chronosequence , soil development , soil nutrients , vegetative succession

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Introduction

Microbial ecologists are in the nascent stages of developing theories to describe and predict patterns of soil microbial community composition and structure across meaningful ecological scales. In this regard, chronosequences of primary developing ecosystems are natural experiments that can be used to study ecological relationships (Walker *et al.* 2010). Ecological succession and ecosystem development have for decades been used to provide fundamental descriptions of ecosystem processes (McIntosh & Odum 1969; Walker & Syers 1976), but little is known about belowground microbial communities and their relationship to the process of succession. Studies of soil bacterial community change associated with retreating glaciers during early (*c.* 100 years) ecosystem development have shown that bacterial communities

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can be dynamic (Nemergut *et al.* 2007; Schutte *et al.* 2009; Wu *et al.* 2012; Zumsteg *et al.* 2012) but difficult to predict. How soil bacterial communities change during ecosystem development over longer timescales has not been studied during glacial retreat, but could provide clues to the linkages, mechanisms and feedbacks that regulate soil community assembly during the process of long-term ecosystem development.

Gradients of primary development have been used to understand fundamental ecological processes associated with ecosystem change. Conceptually, the aboveground plant community can be viewed as the engine of primary ecosystem development. As the process of vegetative succession proceeds, it is often constrained by the availability of nitrogen. At the same time, root growth helps to liberate available forms of many key nutrients, setting the stage for the accumulation of soil organic matter during early progressive stages of ecosystem development. This early transformation of the ecosystem is fundamental and typically driven by early mutualistic relationships between plants (*Myrica, Alnus* spp.) and symbiotic bacteria (e.g. *Rhizobia*, *Frankia* spp.) that can fix nitrogen (Menge & Hedin 2009; Chaia *et al.* 2010; Walker *et al.* 2010). The progressive stage of ecosystem biomass accrual is eventually, in the absence of disturbance, followed by a retrogressive stage of biomass decline. This stage occurs as a result of long-term losses and re-allocation of key nutrients, especially phosphorus, into biologically resistant forms, which ultimately limits ecosystem productivity (Peltzer *et al.* 2010). As such, the developing soil and vegetative gradient are defined by an interactive set of dynamic biological and chemical feedbacks that are fundamental to the process of succession and ecosystem development. The extent to which soil bacterial communities are patterned by these changes and feedbacks during long-term ecosystem development is not known.

Plants clearly impact the composition and structure of belowground microbial taxa (Kourtev *et al.* 2002, 2003; Bonanomi *et al.* 2005; Singh *et al.* 2007), and microorganisms, in turn, impact the occurrence and survival of plants (Kardol *et al.* 2006). Relatedly, microbial communities have been studied during ecosystem development (Jumpponen *et al.* 2002; Tscherko *et al.* 2004; Nicol *et al.* 2005). However, many unanswered questions remain about the broader extent of direct and indirect community-level linkages between plants and microbes and how they fit into the interactive model of ecosystem development and primary ecological succession (Wardle *et al.* 2004; Peltzer *et al.* 2010).

The 120 000-year-old Franz Josef soil chronosequence was sampled to test whether bacterial communities show patterns of succession during ecosystem development. Within this framework, vegetative succession and pedogenesis were related to changes in bacterial community structure and diversity. It was hypothesized that change in bacterial community structure would closely follow the developing gradient of pedogenesis and vegetative succession during ecosystem development.

Materials and methods

Site description

A series of schist greywacke sediments formed by the outwash of retreating glaciers have formed a 120 000-year chronosequence of developing soils across the western South Island of New Zealand (Almond *et al.* 2001). The yearly annual average temperature is 10.8 °C, and annual rainfall totals 3500–6500 mm. The 47 dominant woody plant species changed appreciably along the chronosequence (Richardson *et al.* 2004). The dominant plant species and their occurrence along the chronosequence collectively represent ~80% of the woody plant cover (Table S1, Supporting information). Bray–Curtis ordination of these data based on canopy cover (%) is shown in Fig. 1a. Vegetative succession was dominated by evergreen angiosperms during the early stages, while conifers become increasingly common during the latter stages, contributing ~60% of the vegetative plant canopy cover on the two oldest sites. Plant biomass peaks at *c.* 5000 years, highlighting the unimodal progressive and retrogressive stages of plant succession (Richardson *et al.* 2004).

Soil sampling

Five 5-m radius replicate plots along a 50-m transect were setup for each of 9 soil ages (60, 130, 280, 530, 1000, 5000, 12 000, 60 000 and 120 000 years). This design allowed for the collection of 5 independent replicates ($n = 5$) from nine plots for a total of 45 soil samples. Within each replicate, 5-m radius plot, a set of five cores was collected using a 6.5-cm-diameter corer from the centre of the plot and from 2 m in each cardinal direction from the plot centre and pooled. These individually pooled soil samples from each replicated plot were collected in plastic bags and stored on ice before transportation to the laboratory (Allison *et al.* 2007). Soils were then sieved through a 4-mm mesh and stored frozen (−20 °C) before DNA-based analysis.

DNA extraction and pyrosequencing of bacterial 16S rRNA genes

Total community DNA was extracted from 0.5 g of soil using ZR Soil Microbe DNA kit (Zymo research, Orange, CA, USA) with minor modifications in the manufacturer's protocol as described in Garcia *et al.* (2011) and

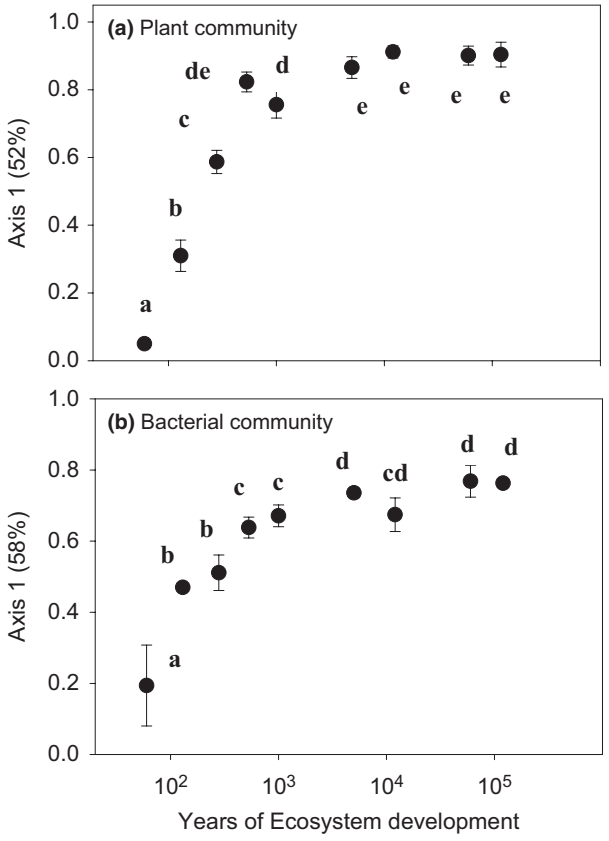

Fig. 1 Bray–Curtis ordination of the age-related (60–120 000 years) change in the 12 dominant (Table S1, Supportimg information) plant taxa based on per cent cover (a) and the change in the bacterial community based on relative abundance of the 250 most abundant OTUs (b). Percentages on the *y*-axis denote an assessment of the variance explained by the multivariate data reduction. The OTUs were formed using the average neighbour algorithm in MOTHUR at a distance of 0.03. Each symbol represents the average of five age-related replicate samples. Significant differences based on multiresponse permutation procedure (MRPP) are noted with different lower case letters ($P < 0.01$).

stored at −80 °C. Overall, PCR amplification of the bacterial 16S rRNA V3 region, purification and processing for pyrosequencing was carried out using barcoded primers and conditions as described by Garcia *et al.* (2011). Briefly, each 25 μL PCR contained 1.25 μL (20–50 ng) of DNA, 12.5 pmol of each primer and 22.5 μL of Platinum® PCR SuperMix High Fidelity (Invitrogen). Samples were initially denatured at 95 °C for 3 min, then amplified by using 20 cycles of 94 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 1 min. Samples that did not amplify were further purified to remove PCR inhibitors using OneStep™ PCR Inhibitor Removal kit (Zymo Research) and the PowerClean® DNA Clean-Up kit (MoBio Laboratories, Inc.). For a few samples with very low amplification, 25 cycles were used. Our analysis of such variable cycle samples revealed that doing this did not affect the estimated diversity as long as the total number of sequences used for estimating the diversity was equal. Following gel quantification of amplicons, products from the replicates of each developmental age were pooled in equimolar concentrations and gel was eluted using Zymoclean™ Gel DNA Recovery kit (Zymo Research). The 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 with Roche® GS FLX sequencing (Branford, CT, USA), yielding 76 555 reads (260-bp average length).

Processing of 16S rRNA gene data

A standardized two-step pipeline was established that used a combination of QIIME version 1.1.0 (Caporaso *et al.* 2010) and MOTHUR version 1.11.0 (Schloss *et al.* 2009). First, QIIME was used to quality trim the 16S rRNA gene sequences to >200-bp length, sort them into individual libraries based on the 8-nt barcodes and followed by denoising (Reeder & Knight 2010). The denoised data were then imported into MOTHUR for further processing. In MOTHUR, sequences were aligned against the SILVA reference database, filtered, preclustered and checked for chimeras. Chimera-Slayer analysis detected 730 potentially chimeric sequences, of which 316 and 132 sequences, respectively, showed >90% and 100% bootstrap support. According to the programs instructions, some of these 132 sequences were manually tested against the entire NCBI database, and none were confirmed to be chimeric as they showed close homology with the same genera for the 3′ and 5′ regions. Hence, it was concluded that the majority of these potentially chimeric sequences were false positives, and the entire data set was used to calculate the distance matrix. Finally, operational taxonomic units (OTUs) were formed using the average neighbour method at an evolutionary distance (D) = 0.03, followed by classification of representative sequences from OTUs using the SILVA reference taxonomy.

The experimental strategy used to sample bacterial communities across five replicates showed consistently low variability (see Fig. 1b) for each plot except in one case. A replicate from the youngest 60-years soil contained low amounts of DNA that resulted in reduced amplification of the 16S rRNA genes. The replicate was removed from subsequent analyses because of low quantity and quality of DNA.

Statistical and sequence analyses

Bray–Curtis ordination (using Sorenson distance) of the 250 most abundant OTUs ($D < 0.03$) and the 47 woody plants was performed using the PC-ORD software version 4 (MjM Software, Gleneden Beach, OR, USA) as advised by McCune & Grace (2002). Data were transformed by treatment using the 'general relativization' function to remove the potentially strong influence that absolute abundance can have on community data. The multiresponse permutation procedure (MRPP), a nonparametric test, was used to assess differences in bacterial community structure between soil ages. Mantel tests were conducted using PC-ORD to determine whether correlations existed between community, vegetative and soil descriptive data (Table S2, Supporting information). Multivariate statistics (Mantel, MRPP) were considered significantly different using an $\alpha < 0.01$. For pairs of samples, coefficients of similarity (S_c) were calculated for both plant and bacterial communities, using the method of Whittaker (1972): $S_c = S_s/(S_a+S_b-S_s)$, where S_s is the number of taxa shared between samples, and S_a and S_b are the number of taxa in the first and second sample, respectively.

Results

Description of the 16S rRNA data

The 16S rRNA gene sequence possessed an 260-bp average length and was submitted to the NCBI Sequence Read Archive according to MIMS standards (SRP006445.2). These formed 4775 OTUs at $D = 0.03$ (Table 1). Each soil age was represented by between 7155 and 11 248 sequences forming 488 to 1420 OTUs per soil age. The most abundant OTUs were represented by 6167 sequences, accounting for ~8% of the entire sequence data set. The top 20 and 250 OTUs represented 48% and ~83% of the entire sequence data set, respectively.

Bacterial community diversity along the ecosystem development gradient

All of the indices for community diversity (Simpson's index, Shannon index and Chao1) declined along the sequence, showing statistically significant (Mantel) relationships with age (Table 1). Variation in sample size can affect the calculation of alpha-diversity indices; however, the effect on β-diversity would be minimal if a random subsample of equal size was taken. To assess the effect of size differences among the libraries on the calculated beta-diversity, the indices (Simpson's $1/D$, Chao1) were also calculated from random subsamples of 100, 200 and 300 sequences from each of the replicate soil samples. These indices showed the same trends as those calculated with the complete libraries, indicating that the variation in sample size did not bias the results. Thus, all quality sequences were included in the subsequent analyses to maximize sample coverage. Moreover, the decline in diversity across the chronosequence was also supported by the rarefaction curves, which are independent of sample size (Fig. 2).

Table 1 Diversity indices for the 16S rRNA sequences according to site age

Diversity index[†]	60 years	130 years	280 years	530 years	1000 years	5000 years	12 000 years	60 000 years	120 000 years	Reg**
N[‡]	7155	8579	7779	7961	11 284	8311	8826	7480	9180	
S[§]	1377	1420	978	953	1035	625	764	488	668	0.81*
Goods coverage	0.90	0.91	0.93	0.94	0.95	0.96	0.96	0.96	0.96	0.80*
Richness (Ace)	3364	3871	2614	2502	2866	1483	1821	1431	2320	0.62*
Shannon (H)	5.99	5.72	5.20	4.99	4.77	4.42	4.46	4.09	4.27	0.84*
$1/D$[¶]	130	83	61	38	38	30	28	26	29	0.63*
Chao1	2508	2686	1851	1709	1998	1090	1411	1031	1414	0.72*

[†]Calculations based on the operational taxonomic units (OTUs) determined at an evolutionary distance of 0.03.
[‡]Number of sequences collected.
[§]Number of OTUs.
[¶]Simpson's reciprocal index.
**Regression between diversity index and ecosystem age using a log-linear model. Significant results are noted by an asterisk (*) ($P < 0.01$).

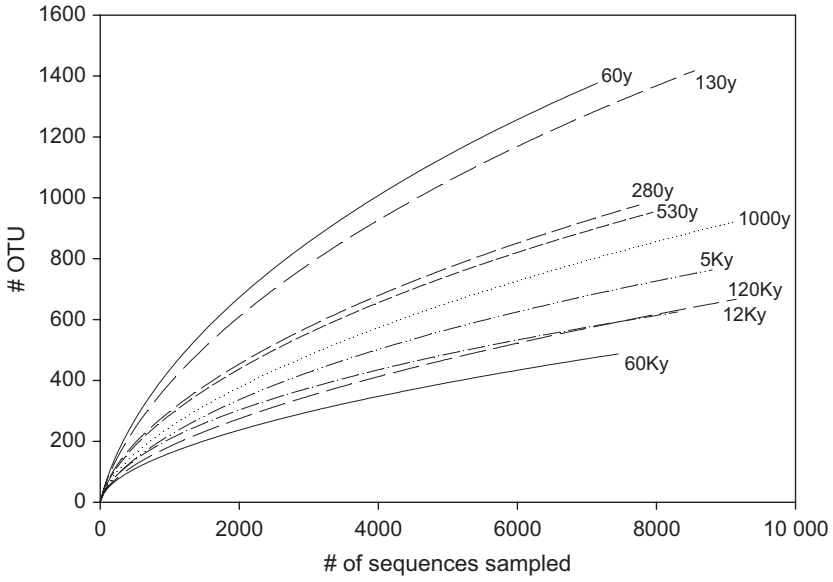

Fig. 2 Rarefaction curves of the 16S rRNA gene libraries. The OTUs were formed using the average neighbour algorithm in MOTHUR at a distance of 0.03. K = 1000.

Bacterial community structure and its association with soil, vegetation and ecosystem properties

Bray–Curtis ordination was used to provide a metric of bacterial community relatedness and explained ~58% of the variability in the original data set using one dimension (Fig. 1b; McCune & Grace 2002). The 250 most abundant bacterial OTUs changed considerably across the chronosequence during the early stages (Fig. 1b; <1000 years) and were significantly correlated (Mantel; $P < 0.001$) with changing levels of phosphorus and pH throughout ecosystem development. The bacterial communities shared only 40% of their dominant members in the youngest (60 years) compared with older soils (>1000 years), as calculated by the method of Whittaker (1972). Bacterial community structure changed much less during the latter stage of ecosystem development. Thus, a multiresponse permutation procedure identified significant differences in community structure only during early but not late ecosystem development (Fig. 1b). The fit of a log-linear model indicated the presence of two main stages of ecosystem development defined by a transition *c.* 530–1000 years.

While there was a significant correlation (Mantel test; $r = 0.58$; $P < 0.001$) between bacterial and plant community change, a closer inspection of the data indicated that the correlation was strongest during early ecosystem development (Fig. 1a). Bacterial community structure varied but remained relatively unchanged during late ecosystem development. Plant community change also slowed during latter ecosystem development. The dominant members in the woody plant community in the youngest site did not overlap with those from the 530-years sites, and the plant communities continued to change with ecosystem development. However, *Weinmannia racemosa* and *Dacrydium cupressinum* were dominant and found consistently throughout the latter stages of the chronosequence (>1000 years), contributing to the observed similarities in plant community structure based on Bray–Curtis ordination.

In terms of the phylogenetic composition, rRNA genes related to Actinomycetes, Alphaproteobacteria, Acidobacteria, Planctomyces and Betaproteobacteria accounted for ~82% of the sequences, representing 36%, 25%, 11%, 5% and 5% of the total sequences, respectively. The relative abundance of the three largest taxa as represented by rRNA genes (Actinomycetes, Alphaproteobacteria and Acidobacteria) was fairly constant across the gradient (Fig. 3). *Frankia*, a genus of Actinomycetes that is capable of nitrogen fixation, was highly abundant during the earliest stages of ecosystem development (*c.* 60 years), correlating with the high abundance of its putative plant host, *Coriaria* (Table S1, Supporting information). The 16S rRNA genes most closely associated with Bacteroidetes, Firmicutes (Bacilli) and other groups such as Verrucomicrobia (data not shown) each accounted, on average, for $\leq 2\%$ of the sequences. However, these least dominant phyla were typically prone to change across the ecosystem gradient. Betaproteobacteria- and Bacteroidetes-related rRNA genes showed significant declines during soil and ecosystem development, almost disappearing completely in the oldest soils. Bacilli-related rRNA genes were abundant early but much less abundant during the latter stages of pedogenesis.

Discussion

Patterns of soil bacterial community change during primary ecosystem succession

Bacterial community dynamics during ecosystem development near retreating glaciers have previously been

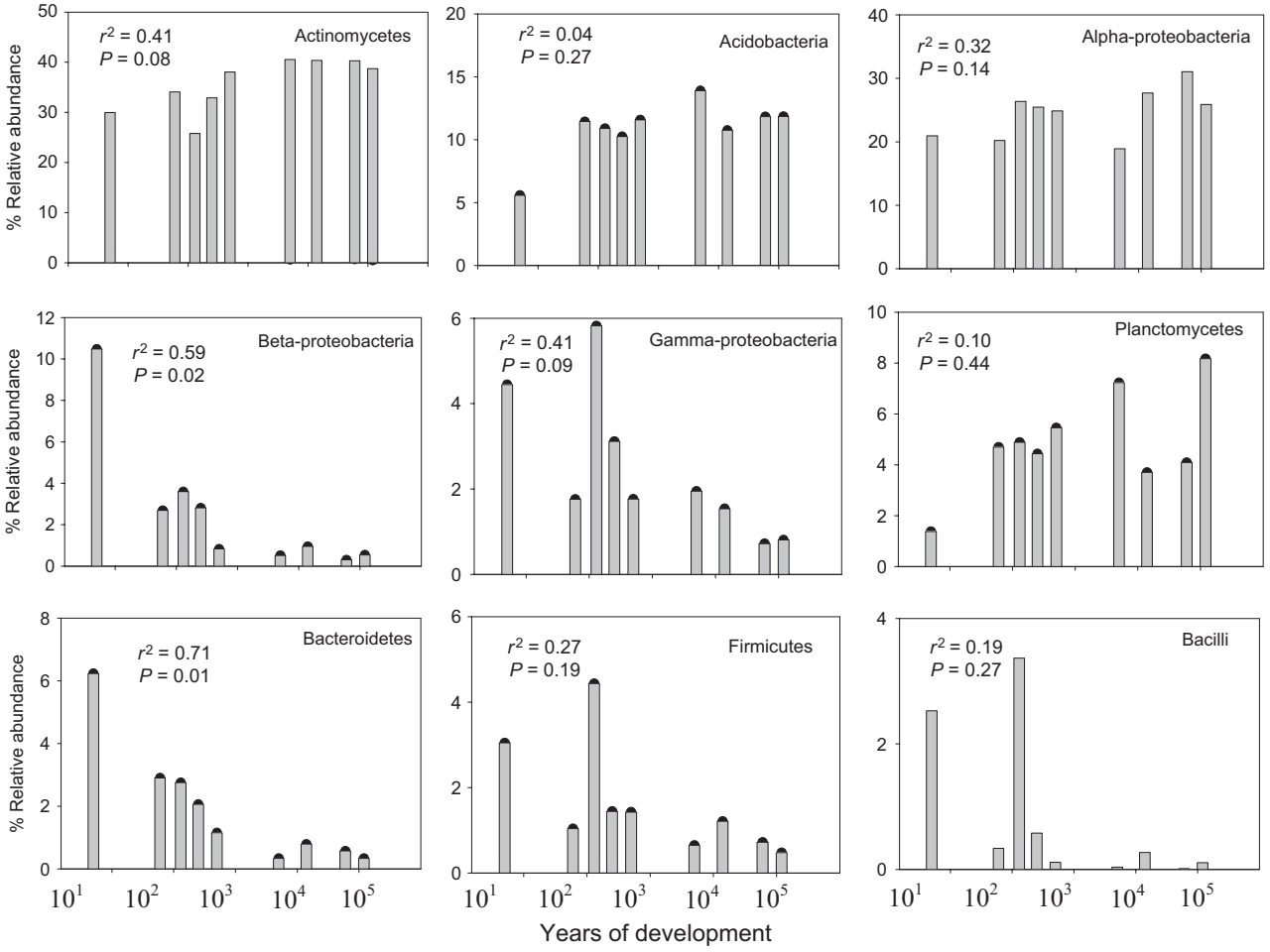

Fig. 3 Relationship between percentage relative abundance of nine individual bacterial phyla across the chronosequence during ecosystem development (60–120 000 years). Each point in the graph is the average (n = 5) of the percentage abundance of each phyla at each stage of development. Regression coefficient and P-value for each phylum are shown. Relative abundance of bacterial phyla across the FJ chronosequence.

studied during the earliest decades of pedogenesis (Nemergut *et al.* 2007; Schutte *et al.* 2009). These studies have reported rapid changes in bacterial communities that are variable but sometimes correlated with soil properties (Wu *et al.* 2012; Zumsteg *et al.* 2012). The long-term nature of the Franz Josef chronosequence greatly extends the temporal extent of change that can be studied to understand bacterial community linkages to the process of succession during ecosystem development (Wardle *et al.* 2004; Peltzer *et al.* 2010). Even with large differences in chronosequence age, bacterial community change during development at Franz Josef and the younger sequences together indicate that community variation is greatest during the earliest years and then slows with ecosystem development. The very high bacterial community turnover during very early development (less than a decade) tends to involve fewer discrete patterns of structural change (Nemergut *et al.* 2007; Wu *et al.* 2012; Zumsteg *et al.* 2012), which might indicate that young stages are more prone to the natural stochasticity associated with colonization. At Franz Josef, in contrast, there was a clear pattern of change during the early stages of development (up to *c.* 1000 years). Bacterial communities thus become more predictable, perhaps a reflection of the stabilizing effect of belowground habitat development during primary ecosystem development.

Bacterial community change in the dune sands of northern Michigan (Wilderness Park) and southern Georgia, USA (Altamaha), showed some similar patterns to Franz Josef (Tarlera *et al.* 2008; Williams *et al.* 2013); however, the periods of correlated change occurred over *c.* 500 years in Michigan (Wilderness Park) and thousands of years in Georgia, suggesting that ecosystems might follow developmental timing that is specific to the conditions of a chronosequence, such

as parent material or climate (Griffiths *et al.* 2011; De Vries *et al.* 2012). Despite this, bacterial community dynamics showed a number of consistent patterns at higher taxonomic levels (phyla, class) across biomes. For example, Betaproteobacteria and Bacteroidetes showed similar patterns of decline as the ecosystems aged. These changes are patterned after the process of ecosystem development and primary succession, applied up to now, mainly to above-ground plant communities (Wardle *et al.* 2004; Bardgett & Wardle 2010).

Nutrients and soil properties as drivers of bacterial community change during ecosystem development

Soil development along the Franz Josef chronosequence illustrates patterns that are typical of a broad range of developmental ecosystems (Stevens 1968; Crews *et al.* 1995; Vitousek & Farrington 1997; Lichter 1998; Turner *et al.* 2007). Nitrogen levels show a very typical increase and plateau early during primary succession, similar to carbon (Allison *et al.* 2007; Menge & Hedin 2009; Menge *et al.* 2012). Although it may limit colonization and ecosystem productivity early, the quick accumulation of N in soil at Franz Josef during the first *c.* 530 years helps to reduce or eliminate N limitation. Before this, N limitation probably drives the colonization process, selecting for dominance by specific types of bacteria and plants. Indeed, early ecosystem development at Franz Josef was described by a classic N-limited response whereby co-colonization and dominance of a plant–microbial mutualistic association was likely responsible for importing large amounts of N and setting the stage for a productive developing ecosystem. At Franz Josef, the symbionts are the plant *Coriaria* (Menge & Hedin 2009) and a bacterium closely related to the N-fixing symbiont *Frankia*. Nitrogen is clearly important to ecosystem productivity during very early ecosystem development, consistent with bacterial community changes that appear synchronized to nitrogen accumulation during this same period.

Phosphorus showed patterns of decline that are typical of long-term ecosystem development (Crews *et al.* 1995; Allison *et al.* 2007; Turner *et al.* 2012). At Franz Josef, phosphorus was highly correlated with change in the structure and diversity of bacterial communities, possibly indicating a link between phosphorus and bacterial community change (Beauregard *et al.* 2010; DeForest & Scott 2010; Wakelin *et al.* 2012). However, this same relationship with phosphorus was not observed in the much younger *c.* 4000-year-old Wilderness Park ecosystem (Williams *et al.* 2013).

A slightly more complex model incorporates the ecosystem paradigm of N and P as key limiting nutrients for biological activity during primary ecosystem succession. Ecosystem development can be viewed in two fairly distinct stages described by ecosystem progression and retrogression, based largely on transitions from N to P limitation with age (Wardle *et al.* 2004; Peltzer *et al.* 2010). There are a number of other important transitions, such as vegetative change, that occur concurrently. The tipping point whereby the ecosystem shifts from progression (nutrient sufficient) to retrogression (nutrient insufficient) at Franz Josef has been identified somewhere *c.* 5000–12 000 year. (Richardson *et al.* 2004). If bacterial communities follow this similar unimodal model, bacterial and other belowground microbial communities would reach a state of relative 'feast' during midecosystem development, when P levels are still relatively high and N levels are accumulating that would be preceded and followed by periods of 'famine'. If the bacterial community was responding to the increasingly favourable conditions early, then it would be logical to expect that the bacterial community would similarly decline or change again during the retrogressive nutrient decline stages of late ecosystem development. However, the bacterial community did not show a similar pattern linked to progression and retrogression. Belowground bacterial community structure may be indirectly related to the effects of progression and retrogression through plant community dynamics, which have been previously linked to nutrient limitation and stress during the development of ecosystems (Richardson *et al.* 2004).

Pedogenesis and bacterial community change during ecosystem development

Over the past several years, pH and bacterial community change have been shown to be well correlated across broad geographic landscapes (Lauber *et al.* 2009; Rousk *et al.* 2010). Although pH correlates well with bacterial community change at Franz Josef and Wilderness Park (Williams *et al.* 2013), it is not well correlated with similar bacterial community changes (e.g. declining bacterial richness and diversity) at the uniformly acidic (pH < 4.5) Altamaha sequence (Tarlera *et al.* 2008). It is thus not clear which processes can simultaneously account for the patterns of community change between these three developmental ecosystems. Pedogenesis is described by complex but predictable chemical and physical changes that correlate with these numerous dynamics during ecosystem development. A pedogenic model describing bacterial communities has the advantage of being a well-described mechanism of change during ecosystem succession (Walker & Syers 1976; Vitousek & Farrington 1997). The pedogenic model is further supported by other studies that have observed covariance between bacterial communities and

pedogenesis-related changes such as soil type, organic C content and texture (Bååth & Anderson 2003; Girvan *et al.* 2003; Högberg *et al.* 2007).

Pedogensis may adequately describe soil bacterial community dynamics during the early stages of ecosystem development. However, pedogenesis continues during the advanced stages of ecosystem development, while bacterial community structure remains relatively unmodified. Pedogenesis may reach a critical developmental point whereby changes in soil properties have less of an effect on bacterial communities. The changes in the bacterial communities themselves may also be resilient to further pedogenic change following *c.* 1000 year, although it is not clear why this would occur.

Fungal to bacterial ratios using phospholipid fatty acids declined two-fold during ageing across the Franz Josef sequence (Allison *et al.* 2007). Declining fungal to bacterial ratios have been linked to declining (five-fold) bacterial and increasing (six-fold) fungal community activity (Rousk *et al.* 2010), suggesting that declining ratios provide an indication of the extent that these microbes contribute to community processes. The established and unvarying structure of the bacterial community during latter ecosystem development was thus consistent with the declining belowground role that bacterial communities play relative to fungi during succession. Very low bacterial activity could slow the turnover of bacterial communities and, assuming high survival rates, support invariant and structurally stable communities that are resilient to immigration and soil-environmental change.

The successional plant–microbial feedback hypothesis and bacterial community change during ecosystem development

The dynamics of the bacterial community were partly related to progression and retrogression and to the shifting contents of nutrients such as nitrogen and phosphorus during ecosystem development. The process of pedogenesis was also shown to covary with bacterial community change, particularly during the early rapid changes of ecosystem development. Although these mechanisms can be used to explain bacterial community dynamics and show merit for incorporating communities into various ecosystem development paradigms, testing of these hypotheses requires further investigation.

A hypothesis described by Kardol *et al.* (2006) explains that plant communities interact differently with belowground biota depending on the stage of plant succession and ecosystem development. They and others have shown evidence that negative plant–microbial feedbacks encourage the replacement of plant species during early succession (Kulmatiski *et al.* 2008). Pathogens, in particular, were hypothesized to accrue in response to early successional species, and this process facilitates plant species replacement (Van der Putten *et al.* 1993, 2001, 2009). This model of rapid vegetative turnover is consistent with the patterns of maximum turnover of soil bacterial communities during the first *c.* 1000 year. Similarly, positive plant–bacterial community feedbacks would be consistent with a stabilizing effect on plant and bacterial communities during latter ecosystem development. Although this explanation has not been discussed explicitly in the context of soil bacterial communities (Tarlera *et al.* 2008; Michel & Williams 2011), the relative stability of bacterial community structure during the advanced stages of ecosystem development mirrors the slower turnover of plant communities. The widespread application of the successional plant–microbial feedback hypothesis needs further verification.

The plant–microbial successional feedback model is consistent with the correlation between vegetative and bacterial community change during ecosystem development. However, this does not have to be the result of direct species–species interactions much like the *Coriaria–Frankia* mutualism observed early during ecosystem development. Rather, it could result from broad changes in plant community functional types (Bardgett & Wardle 2010) that support the growth of specific bacterial communities. It is also worth noting that plant and bacterial communities can indirectly influence one another through a number of mechanisms, which influence soil weathering and pedogenesis (Leyval & Berthelin 1990; Banfield *et al.* 1999; Bonanomi *et al.* 2005; Lambers *et al.* 2009; Knelman *et al.* 2012). Bacterial community and vegetative succession show patterns reminiscent of one another and thus deserve further study to understand the potential feedbacks between them during ecosystem development.

Conclusions

The research, using deep sequencing technology provides the first observations for soil bacterial community change associated with the process of long-term ecosystem development. The results indicate that belowground bacterial communities are linked to the processes of primary ecosystem succession. The 'pedogenesis', 'progression–retrogression' and 'plant–microbial successional feedback' hypotheses provide interrelated mechanisms that explain and incorporate bacterial community change into the paradigm of ecosystem development and succession. The consistency in bacterial community structure observed during the advanced stages of ecosystem development

provides a glimpse into the potential stability of bacterial communities over long time periods. Further research is needed on how to best integrate soil bacterial community dynamics and stability into models of ecosystem development and succession.

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Data accessibility

DNA sequences: NCBI SRA: SRP006445.2. Sample collection metadata, Barcode Information: Associated with NCBI SRA submission. Final DNA sequence assembly: uploaded as online supporting information.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Dominant woody vegetation at each stage of ecosystem development across the Franz Josef chronosequence.

Table S2 Concentrations of Mehlich-3 extractable cations and descriptive soil variables in the mineral soil (0–10 cm depth) across the Franz Josef chronosequence.