Soil bacterial communities are shaped by temporal and environmental filtering: evidence from a long-term chronosequence

Zachary Freedman[1]* and Donald R. Zak[1,2]
[1]*School of Natural Resources & Environment and*
[2]*Department of Ecology and Evolution, University of Michigan, Ann Arbor, MI 48109, USA.*

Summary

Soil microbial communities are abundant, hyper-diverse and mediate global biogeochemical cycles, but we do not yet understand the processes mediating their assembly. Current hypothetical frameworks suggest temporal (e.g. dispersal limitation) and environmental (e.g. soil pH) filters shape microbial community composition; however, there is limited empirical evidence supporting this framework in the hyper-diverse soil environment, particularly at large spatial (i.e. regional to continental) and temporal (i.e. 100 to 1000 years) scales. Here, we present evidence from a long-term chronosequence (4000 years) that temporal and environmental filters do indeed shape soil bacterial community composition. Furthermore, nearly 20 years of environmental monitoring allowed us to control for potentially confounding environmental variation. Soil bacterial communities were phylogenetically distinct across the chronosequence. We determined that temporal and environmental factors accounted for significant portions of bacterial phylogenetic structure using distance-based linear models. Environmental factors together accounted for the majority of phylogenetic structure, namely, soil temperature (19%), pH (17%) and litter carbon:nitrogen (C:N; 17%). However, of all individual factors, time since deglaciation accounted for the greatest proportion of bacterial phylogenetic structure (20%). Taken together, our results provide empirical evidence that temporal and environmental filters act together to structure soil bacterial communities across large spatial and long-term temporal scales.

Introduction

Identifying the processes that structure biotic communities has generated scientific discourse over the past century; it is presently fueled by our emerging understanding of the ecological forces shaping microbial communities, especially those in highly diverse habitats like soil (Fukami *et al.*, 2010; Cline and Zak, 2014; Talbot *et al.*, 2014). Soil microbes mediate biogeochemical cycles of global importance (van der Heijden *et al.*, 2008); therefore, understanding the ecological forces that shape microbial communities is key for understanding how biogeochemical cycles may respond to human-induced environmental change. Evidence is mounting that both temporal (i.e. dispersal limitation, priority effects) and environmental (i.e. soil pH, C:N) factors can structure soil microbial communities (Fierer and Jackson, 2006; Hanson *et al.*, 2012; Ramirez *et al.*, 2012; Nemergut *et al.*, 2013; Talbot *et al.*, 2014). The resulting filter-type models (Loreau *et al.*, 2001; Vellend, 2010; Martiny *et al.*, 2011; Talbot *et al.*, 2014) predict that over large temporal scales, ecological drift may structure dispersal-limited communities by stochastic local extinctions, and also can contribute to bacterial genome evolution (Kuo *et al.*, 2009). Conversely, well-dispersed microbial propagules may enter the local species pool to counteract ecological drift (e.g. transport in air or on dust; Pearce *et al.*, 2009; Favet *et al.*, 2013). Environmental filters may then modify microbial community composition through the loss of species unable to acclimate and compete in a changing environment (Keddy, 1992). To date, determination of temporal and environmental filters on microbial communities have mainly assayed low-diversity environments, e.g. hot springs, recently deglaciated soils and subsurface soils (Whitaker *et al.*, 2003; Stegen *et al.*, 2013; Brown and Jumpponen, 2014); or the magnitude of ecological forces at various spatial scales, e.g. local, continental and global (Martiny *et al.*, 2011; Talbot *et al.*, 2014). Consequently, there is limited experimental support that environmental and temporal filters act together to shape microbial communities in the hyper-diverse soil environment, particularly across large spatial (i.e. regional to continental) and temporal (i.e. centuries to millennia) scales.

Glacial retreat presents an opportunity to observe sites that vary in age, but have similar ecological and edaphic attributes, thereby providing a natural experiment to determine the combined effects of temporal and environmental filters in structuring present-day biotic communities (Brown and Jumpponen, 2014; Cline and Zak, 2014). To investigate the ecological forces that shape highly diverse bacterial communities in soil, we quantified bacterial phylogenetic structure across a 4000-year glacial chronosequence in the Upper Great Lakes Region of North America. In this region, the retreat of the Laurentide ice sheet *c.* 14 000 years ago occurred in a south to north direction. We selected four northern hardwood forest stands along this chronosequence, in which new land was exposed over 4000 years, and similar soils and forests have developed to date (Burton *et al.*, 1991). Geographic distance between sites, serving as a proxy for time since deglaciation, enabled us to investigate the influence of time on the structure of present-day bacterial communities. Furthermore, long-term environmental monitoring (i.e. nearly two decades) enabled us to determine the effects of subtle climatic gradients on bacterial phylogenetic structure, as well as to identify and quantify confounding environmental variation along the chronosequence.

If temporal filters structure bacterial community composition in our chronosequence, then a correlation should exist between bacterial community composition and time since deglaciation (i.e. geographic distance between sites). However, if environmental filters structure bacterial communities, differences in bacterial community composition should be correlated with environmental gradients across sites, not time since deglaciation. Moreover, if both temporal and environmental filtering structure bacterial communities, temporal and environmental factors should account for different portions of bacterial phylogenetic structure. To test these hypotheses, we analysed forest floor bacterial phylogenetic structure using Pacific Biosciences high-throughput deoxyribonucleic acid (DNA) sequencing technology (Schadt *et al.*, 2010), thereby enabling us to pursue high-resolution phylogenetic analyses on high-quality DNA sequences of greater length than afforded by other popular sequencing technologies (Fichot and Norman, 2013). Here, we provide evidence that temporal and environmental filters act together to shape soil bacterial communities over centuries to millennia.

Results

Defining a glacial chronosequence

We selected four forest stands along a long-term glacial chronosequence, which spans the north–south geographic range of the northern hardwood forests in the
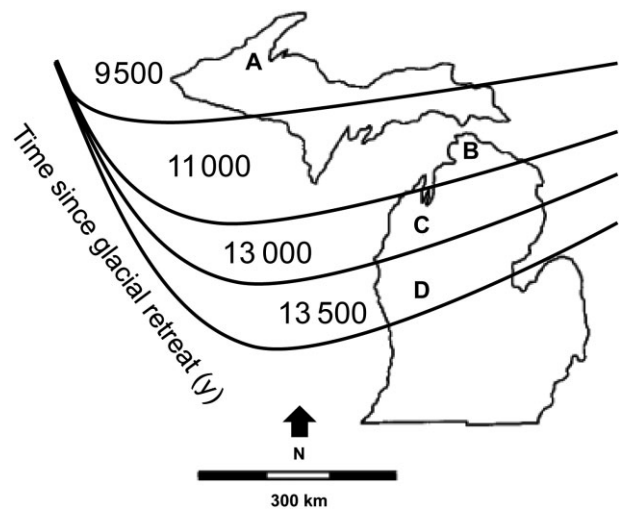

Fig. 1. The geographic distribution of the study sites in lower and upper Michigan. In this region, the retreat of the Wisconsin glacier *c.* 14 000 years ago occurred in a south to north direction as shown.

Great Lakes region of North America (Fig. 1; Burton *et al.*, 1991). The southernmost site (site D) was freed from glacial ice approximately 13 500 years ago (ya), and pollen records indicate sugar maple forest establishment 10 000 ya (Evenson *et al.*, 1976; Davis, 1983). Site C, located 83 km north of site D, was ice free ~ 13 000 ya, followed by maple forest establishment 9000 ya. One-hundred and fifty kilometres north of site C lies site B, which was uncovered approximately 11 000 ya, with sugar maple forest establishment 7000 ya. Finally, the northernmost site A is found 343 km northwest of site B, was ice free 9500 ya, followed by forest establishment 6000 ya. All sites are floristically and edaphically similar and fall along a climatic gradient (Table 1 and Table S1; Burton *et al.*, 1991; Zak *et al.*, 2008). In each stand (*n* = 4), three 30 m by 30 m replicate plots were established (*n* = 12). The biogeochemical, climatic and floristic characteristics of these plots are well characterized, which enabled us to quantify potentially confounding environmental variation along the chronosequence. Since 1987, we have recorded daily air temperature, soil moisture and soil temperature, as well as annual or semi-annual determinations of tree biomass by species, litter biomass, production and biochemistry. All long-term data are available at the Michigan Gradient website (http://www.webpages.uidaho.edu/nitrogen-gradient/).

Bacterial community composition differs along the glacial chronosequence

Bacterial community structure across the chronosequence was determined using high-throughput DNA sequencing of polymerase chain reaction (PCR)-amplified

Table 1. Temporal, physical and biogeochemical characteristics of four forest stands along a glacial chronosequence.

Characteristic	Factor	Site A	Site B	Site C	Site D
Temporal					
	Time since glacial retreat (y)	9500	11 000	13 000	13 500
Physical					
	Soil temperature (°C)	7.2 ± 0.08	7.9 ± 0.20	8.3 ± 0.04	9.1 ± 0.11
	Soil moisture (%)	25.2 ± 0.02	30.3 ± 0.01	38.7 ± 0.04	33.7 ± 0.03
Biogeochemical					
	Soil pH	4.6 ± 0.17	4.7 ± 0.07	4.4 ± 0.07	4.6 ± 0.25
	Leaf litter N content (g N m^{-2})	14.3 ± 2.7	32.1 ± 7.1	25.9 ± 3.2	40.6 ± 8.1
	Leaf litter C content (g N Kg^{-1})	458.2 ± 1.4	456.4 ± 0.52	453.6 ± 0.88	455.2 ± 1.3
	Leaf litter C:N	63.7 ± 2.6	57.1 ± 6.1	52.9 ± 2.1	43.4 ± 0.66
	Leaf litter mass (g m^{-2})	412.8 ± 18.6	396.4 ± 13.6	591.0 ± 51.7	550.3 ± 30.6
	Forest floor turnover (y)	2.2 ± 0.03	4.9 ± 0.56	5.2 ± 0.86	6.5 ± 1.4

Values presented are the average $\pm$ SE of three experimental plots per each site.
Plant cover data can be found in Table S1.

16S ribosomal ribonucleic acid (rRNA) gene fragments (500 bp; V1-V3 region). Prior to quality control (QC), we obtained 224 331 16S rRNA gene sequences, after QC, 106 435 sequences remained (53% removed). Sequence loss through each step of our pipeline is summarized in Table S2. In all, 18 180 operational taxonomic units (OTUs) were generated at 95% sequence similarity to assay the relative effects of temporal and environmental filters on bacterial community composition at a taxonomically broad (i.e. genus) level. For all analyses, the dataset was rarefied to 5000 sequences per plot across the four forest stands.

Along the chronosequence, bacterial communities differed by taxonomic affiliation, alpha diversity (Shannon index (H′) and Chao1 richness) and beta diversity (phylogenetic structure; abundance weighted UniFrac distance). The bacterial community was dominated by the phyla *Proteobacteria* ($41.2 \pm 0.9\%$ of community; Table S3), *Bacteroidetes* ($20.9 \pm 0.7\%$) and *Actinobacteria* ($16.3 \pm 0.4\%$). The relative proportions of bacterial phyla were largely consistent across the chronosequence. However, *Proteobacteria* increased in relative proportion ($P < 0.02$) from north to south in the chronosequence, whereas the relative proportion of *Bacteroidetes* and *Verrucomicrobia* declined ($P < 0.05$). Among the *Proteobacteria*, the relative abundances of *Alphaproteobacteria* and *Betaproteobacteria* increased from north to south in the chronosequence ($P < 0.05$). Conversely, the relative abundance of *Deltaproteobacteria* declined from north to south ($P < 0.02$). Bacterial alpha diversity was lowest in the youngest site A (H′ 6% lower; Chao1 69% lower; $P < 0.05$; Fig. S1) but similar across the three southern-most sites. Beta diversity estimations indicated bacterial communities were phylogenetically distinct across the chronosequence [weighted UniFrac distance; permutational multivariate analysis of variance (PerMANOVA); Pseudo-F = 3.7; $P < 0.01$]. Furthermore, all sites differed from one another by pairwise PerMANOVA

(Table S4; $P < 0.05$), with the exception of the oldest southernmost sites, C and D ($P = 0.11$). There was no difference in community heterogeneity across sites permutational test for homogeneity of multivariate dispersions (PERMDISP; $P > 0.10$).

To determine which OTUs were driving compositional differences along the chronosequence, ordinations were obtained from principle coordinates analysis (PCoA) of Bray–Curtis dissimilarity derived from the full OTU abundance matrix (Fig. 2). Our PCoA clearly differentiated bacterial communities along the chronosequence, wherein sites aligned by time since deglaciation along the second principle coordinate ($\rho = 0.89$, $P < 0.01$). Here, 235 OTUs, representing 20% of the community were significantly ($P < 0.05$) and positively correlated with principle coordinate 2 (PCo2). Operational taxonomic units attributable to the phyla *Actinobacteria* (17%; Table S5), *Bacteroidetes* (16%) and *Proteobacteria* (32%) accounted for a substantial proportion of the OTUs significantly correlated with
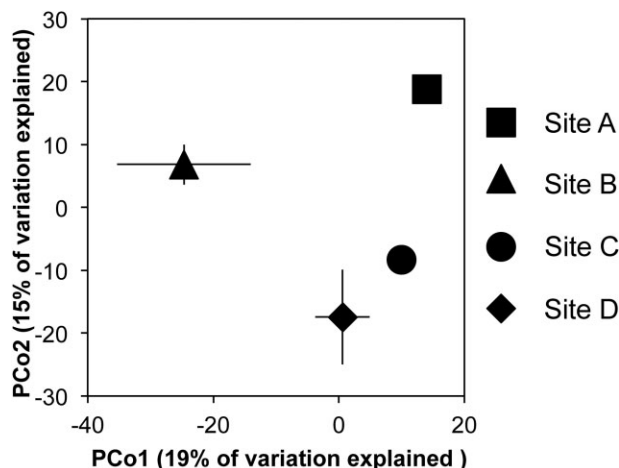

Fig. 2. Ordinations obtained from PCoA of Bray–Curtis dissimilarity derived from all OTUs. Pairwise significance is presented in Table S1.

PCo2. OTUs attributable to *Sphingobacteria* accounted for 87% of significantly correlated OTUs in the *Bacteroidetes* phylum. Order *Actinomycetales* account for 73% of actinobacterial OTUs driving compositional differences among sites in our chronosequence (Table S6). Among the *Proteobacteria*, orders *Rhizobiales* (15%; *alpha*-class), *Rhodospirillales* (13%; *alpha*), *Myxococcales* (15%; *delta*) and *Xanthomonadales* (12%; *gamma*) accounted for the majority of correlated OTUs. No other factor (Table 1) exhibited significant correlations to principle coordinate 1 (PCo1) or PCo2.

Modeling temporal and environmental filters on bacterial phylogenetic structure

To elucidate the contribution of temporal and environmental filters in structuring bacterial communities, we first determined which broad characteristics (e.g. biogeographic, physical, plant community and temporal) were correlated with bacterial phylogenetic structure along the chronosequence. Environmental distance matrices were calculated from data separated into four groups: (i) biogeochemical, (ii) physical, (iii) plant community composition and (iv) temporal (i.e. geographic distance, a surrogate for time). Individual factors comprising each characteristic can be found in Table 1 and Table S1. Using RELATE (a non-parametric form of Mantel test; Clarke and Ainsworth, 1993), we modeled phylogenetic distance (i.e. abundance-weighted UniFrac) as a function of environmental and temporal variation across our long-term chronosequence. Both temporal and physical characteristics were correlated with bacterial phylogenetic structure ($P < 0.05$; Table 2), whereas the correlation of biogeochemical factors with bacterial phylogenetic structure was moderately significant ($P = 0.10$). Plant community composition was not significantly correlated to bacterial community structure ($P = 0.20$).

Distance-based linear models (DistLM; Legendre and Legendre, 1998) were then used to determine: (i) which individual factors (Table 1) within each correlated charac-

Table 3. Proportion of bacterial phylogenetic distance accounted for by individual biogeochemical, physical or temporal factors by marginal DistLM.

Factor	Pseudo-F	Proportion
Biogeochemical		
Soil pH	2.1*	0.17
Litter N	1.7	0.14
Litter C	1.2	0.11
Litter C:N	2.0**	0.17
LLFFTO[a]	1.6	0.14
Litter mass[b]	2.4*	0.19
Physical		
Soil temp[b]	2.3*	0.19
Soil moisture	2.0*	0.16
Temporal		
Distance(Km)	2.5*	0.20

a. Leaf litter turnover.
b. Litter mass and soil temperature were collinear predictive variables ($\rho > 0.7$), thus, litter mass was not included in subsequent analyses.
*$P < 0.05$; **$P < 0.10$.

teristic accounted for significant portions of bacterial phylogenetic structure, and (ii) if the phylogenetic structure explained by each factor is shared or distinct. To ensure robust and inclusive models, we chose to include individual factors that were at least moderately significant ($P < 0.10$) from preliminary tests. Marginal DistLM determined geographic distance (i.e. time since deglaciation), when considered alone, accounted for a significant amount of phylogenetic distance (20%; Table 3). Among environmental factors, soil pH (17%), litter mass (19%), soil temperature (19%) and soil moisture (16%) also accounted for significant proportions of bacterial phylogenetic distance ($P < 0.05$; Table 3); the contribution of litter C:N (17%; $P = 0.06$) was marginally significant.

To determine if each individual factor accounted for a different or similar portion of bacterial phylogenetic structure, 'forward' DistLM model building was implemented using the adjusted R^2 criterion. Prior to model building, Draftsman Plots (Clarke and Ainsworth, 1993) were calculated to test for collinearity between predictive variables. Among predictor variables determined significant by marginal DistLM, litter mass and soil temperature emerged as significantly collinear ($\rho \geq 0.7$); thus, only soil temperature was included in subsequent analyses. To verify the model output, we varied the order of inclusion of each factor in the model (Table 4 and Table S7). Among environmental factors, soil pH (+ 17%; $P = 0.02$) and litter C:N (+ 18%; $P = 0.03$) both accounted for significant and distinct portions of phylogenetic distance when they were included first; no additional phylogenetic distance was accounted for with the inclusion of average soil temperature or soil moisture. The opposite was true when the order of environmental factor introduction to the model was reversed; the addition of average soil temperature

Table 2. Correlations of broad ecological characteristics to bacterial phylogenetic structure by RELATE[a].

	Characteristic	*Rho*
Environmental	Biogeochemical	0.20***
	Physical	0.38*
	Plant cover	0.06
Temporal	Distance	0.47*

a. Rho values were calculated from abundance-weighted UniFrac Distance.
Individual factors that are grouped into each characteristic can be found in Table 1, factors grouped in plant cover can be found in Table S1.
*$P < 0.01$; **$P < 0.05$; ***$P < 0.10$.

Table 4. Proportion of, and cumulative bacterial phylogenetic distance accounted for by individual biogeochemical, physical or temporal factors by sequential DistLM.

Variable	Adjusted R^2	Pseudo-F	Variance explained	
			Proportion	Cumulative
+Soil pH	0.09	2.1*	0.17	0.17
+Litter C:N	0.21	2.6*	0.18	0.36
+Avg. Soil Temp.	0.22	1.1	0.08	0.43
+Avg. Soil Moist.	0.26	1.4	0.10	0.53
+Distance (Km)	0.43	3.5*	0.17	0.70

The 'forward' DistLM procedure was used with the adjusted R^2 selection criterion. Significance indicates the addition of the variable significantly increases the proportion of phylogenetic structure accounted for in the model.
*$P < 0.05$.

(+ 19%; $P = 0.01$) and moisture (+ 15%; $P = 0.04$) accounts for different and significant proportions of bacterial phylogenetic structure when introduced first, whereas no additional phylogenetic structure was accounted for with the addition of pH and C:N. After the inclusion of all environmental factors, the addition of geographic distance (time) significantly improved the model, which accounted for a significantly greater portion of bacterial phylogenetic structure than when distance was excluded (+ 17%; $P = 0.02$).

The 'best' DistLM model building procedure was used to determine the combination of individual factors (Table 1) that accounted for the greatest proportion of phylogenetic structure across the chronosequence, wherein factor addition was evaluated stepwise and was based on sufficient improvement in the model's adjusted R^2. Geographic distance was the single best predictive factor (Table 5). In addition to distance, soil temperature (two-factor model) and soil moisture (three-factor model) emerged as the best predictor variables. The overall best model included four factors; distance, soil temperature, moisture and pH (adjusted $R^2 = 0.47$). The addition of litter C:N did not sufficiently increase the predictive power of the model.

Discussion

Consistent with the filter-type model of microbial community assembly (Loreau *et al.*, 2001; Martiny *et al.*, 2006; Talbot *et al.*, 2014), we provide empirical evidence that temporal and environmental filters act together to structure soil bacterial communities across a 4000-year glacial chronosequence. Across these scales, the effects of historical processes (e.g. dispersal limitation) can be difficult to distinguish from effects of environmental factors known to shape microbial community composition (e.g. pH, C:N; Martiny *et al.*, 2011; Cline and Zak, 2014; Talbot *et al.*, 2014). Here, we identified and quantified temporal

and environmental filters across a long-term glacial chronosequence with sequential DistLM model building, wherein phylogenetic distance was best explained through a combination of environmental factors and geographic distance, our proxy for time since glacial retreat (Tables 3–5 and Table S7). Together, environmental factors accounted for 53% of bacterial phylogenetic structure, indicating that bacterial communities across the chronosequence are mainly shaped by environmental heterogeneity (Table 4). Temporal factors also accounted for a significant proportion of phylogenetic distance, 17% of which was distinct from all other factors (Tables 3 and 4), suggesting the presence of metagenetic artifacts of historical processes in present-day bacterial communities. Indeed, it has been proposed that both temporal and environmental filters guide community assembly; although, experimental evidence has largely been limited to 'low diversity' environments, e.g. fresh water, hot springs, wastewater and early successional glacial soils, (Whitaker *et al.*, 2003; Ostman *et al.*, 2010; Ferrenberg *et al.*, 2013; Brown and Jumpponen, 2014). Our results are consistent with a recent study in subsurface soil that determined ~ 33–57% of bacterial phylogenetic structure was primarily due to selection (i.e. environmental factors), and ~ 35–57% was due to dispersal limitation and drift (i.e. temporal factors; Stegen *et al.*, 2013). Here, we derived novel insight into hyper-diverse soil bacterial community assembly and determined that temporal and environmental factors accounted for 17% and 53% of bacterial phylogenetic structure, respectively, along a long-term glacial chronosequence.

Our temporal factor, i.e. time since deglaciation, accounted for a significant portion of bacterial phylogenetic structure, supporting the hypothesis that ecological drift from historical processes (e.g. dispersal limitation, priority effects) partially shape present-day soil bacterial communities. Considering our experimental

Table 5. Results from 'best' model selection procedure presented for each number of predictor variables.

Number variables	Adjusted R^2	R^2	Predictor variables
1	0.12	0.20	Distance
2	0.25	0.38	Distance
			Soil temperature
3	0.39	0.56	Distance
			Soil temperature
			Soil moisture
4	0.47	0.67	Distance
			Soil temperature
			Soil moisture
			Soil pH
5	0.45	0.70	Distance
			Soil temperature
			Soil moisture
			Soil pH
			Litter C:N

design, we were able to determine the contributions of broad temporal filters to bacterial phylogenetic structure, but we could not derive insight into specific historical processes. Despite this limitation, there is reason to believe that both priority effects and dispersal limitation are driving factors in shaping bacterial phylogenetic structure along the chronosequence. Bacteria are globally distributed by wind and on dust (Pearce *et al.*, 2009; Favet *et al.*, 2013) and the resulting biogeographic patterns are thought to result from combinations of dispersal limitation, priority effects, competition for resources, as well as environmental heterogeneity (Whitaker *et al.*, 2003; Papke and Ward, 2004). Our study sites lie along the west coast of Michigan (Fig. 1), where prevailing westerly winds come across large water bodies (i.e. Lake Michigan and Lake Superior) before deposition of airborne propagules. Given our chronosequence is oriented perpendicular to prevailing winds, we infer that each site was colonized by wind-blown propagules that originated from the same regional species pool as the Laurentide ice sheet began its retreat across the region ~ 14 000 ya (Eisenlord *et al.*, 2012). If there were no priority effects or limits to dispersal, we would expect each site to have similar bacterial communities, because they constantly were colonized from the same regional species pool. Additionally, we would expect differences in bacterial phylogenetic structure to be driven by environmental, and not temporal gradients; this was not the case. Therefore, we conclude that ecological drift resulting from priority effects and subsequent barriers to dispersal partially shaped bacterial community structure in our long-term glacial chronosequence.

It is possible that a proportion of phylogenetic structure attributable to temporal filters in our models may represent unmeasured confounding environmental variation. In our study, we quantified environmental heterogeneity attributable to environmental factors of recognized importance; soil C, N, C:N, pH, moisture and temperature and found that they accounted for the majority of bacterial phylogenetic structure (Table 4 and Table S4; Pietikainen *et al.*, 2005; Fierer and Jackson, 2006; Lauber *et al.*, 2008; Zak *et al.*, 2008; Garbeva and de Boer, 2009; Castro *et al.*, 2010; Ramirez *et al.*, 2012). Soil pH, litter C:N and soil temperature and moisture accounted for 69% of phylogenetic distance when each was considered separately, and 53% when allowing for overlap in variation among factors (Tables 3, 4, and Table S7). Soil bacterial communities can also be altered by heterogeneity in plant composition (Knelman *et al.*, 2012). However, in our study, small differences in plant composition had no effect on bacterial phylogenetic structure across the chronosequence. Given the robust environmental and floristic characterization across our chronosequence, it is unlikely that a significant proportion of phylogenetic structure attributable to temporal filters in our models can be ascribed to unmeasured environmental heterogeneity.

It has been implicated that both temporal and environmental filters can affect microbial community function (Knelman *et al.*, 2012; Freedman *et al.*, 2013; Talbot *et al.*, 2014). In this study, we determined OTUs attributable to *Alphaproteobacteria* and *Gammaproteobacteria* drove the majority of compositional differences across the chronosequence (Fig. 2; Tables S5 and S6). Among these OTUs, *Rhizobiales* accounted for 15% of correlated proteobacterial OTUs and 48% of the community. Members of the order *Rhizobiales* are core members of root microbiota (Schlaeppi *et al.*, 2014) and are well-known for their N_2-fixation capacity when in symbioses with leguminous plants, although evidence for N_2-fixation while free living has been observed (Ludwig, 1984). Among the *Actinobacteria* phylum, compositional differences were driven by the order *Actinomycetales* (accounting for 80% of correlated *Actinobacteria*). *Actinomyceteales* are ecologically important in soil, and have been linked to lignocellulose decay and humus formation (Zimmermann, 1990; Wohl and McArthur, 1998). Operational taxonomic units attributable to order *Spingobacteriales* (Phylum *Bacteroidetes*) accounted for 14% of correlated OTUs. *Spingobacteriales* have been associated with exopolysaccharide and lignin decomposition and are prevalent during plant seedling development (Green *et al.*, 2006; Taylor *et al.*, 2012). Many OTUs responsible for compositional differences along the chronosequence are of functional importance in forest floor, indicating that historical processes may affect the functional potential of microbial communities, and furthermore, how these communities may respond to human-induced environmental change (Fukami *et al.*, 2010; Talbot *et al.*, 2014).

Improvements in high-throughput DNA sequencing technologies may lead to an improved understanding of microbial biogeographic patterns, especially in highly diverse habitats like soil. A meta-analysis of studies aimed to determine environmental and temporal 'filters' on microbial community assembly ($n = 54$) found those studies that utilized 16S rRNA pyrosequencing (often at more coarse scales of phylogenetic resolution) were less likely to detect metagenetic artefacts of historical processes than studies using whole-genome or Sanger sequencing-based technologies (Hanson *et al.*, 2012). This was consistent with several studies that found finer, rather than coarse scales of taxonomic resolution led to more robust evidence of historical processes shaping microbial communities (Cho and Tiedje, 2000; Martiny *et al.*, 2009; Schauer *et al.*, 2010). In this study, we utilized a conservative OTU definition (95% similarity) to assay the effects of temporal and environmental filters on a broad bacterial taxonomic resolution (i.e. genus).

Regardless, we determined that temporal and environmental factors accounted for 17% and 53% of bacterial phylogenetic structure respectively. Furthermore, our models accounted for a markedly greater proportion of bacterial community structure as compared with the findings of the above-mentioned meta-analysis, which determined geographic distance and environmental heterogeneity accounted for, on average, 10.3% and 26% of variation in bacterial community structure respectively (Hanson *et al.*, 2012). Taken together, our results indicate recent improvements to high-throughput DNA sequencing technologies (often affording high quality sequences > 500 bp) may lead to a greater understanding of microbial biogeographic patterns and the processes that drive them.

Conclusions

Our study supports the hypothesis that bacterial communities are partially structured by historical processes and environmental filters over time scales of centuries to millennia. Using distance-based linear models, we determined that both temporal (17%) and environmental factors (59%) accounted for different portions of bacterial community phylogenetic structure along a long-term glacial chronosequence. Metagenetic artefacts of historical processes have been previously observed in soil Actinobacteria (Eisenlord *et al.*, 2012), and fungi (Cline and Zak, 2014) along the chronosequence we studied, and together with the evidence presented here, support the notion that long-term ecological mechanisms shape the structure of highly diverse soil microorganisms on the scale of centuries to millennia. Together, our results highlight the importance of temporal and environmental filtering as ecological forces, which together, act to shape community composition of soil bacterial communities in temperate forest ecosystems.

Experimental procedures

Site description and sample collection

Forest floor samples were collected from four sugar maple (*Acer saccharum* Marsh.) dominated northern hardwood forest stands in lower and upper Michigan, USA (Fig. 1). The stands were selected from 31 candidate sites based on multivariate similarity of plant community composition, stand age and soil properties (Table 1; Braun, 1950; Burton *et al.*, 1991; Macdonald *et al.*, 1991). The thin Oi horizon is composed of sugar maple leaf litter, and the thicker Oe horizon is interpenetrated by a dense root mat. The soils are sandy (85–90%), well-drained, isotic, frigid Typic Haplorthods of the Kalkaska series.

Forest floor sampling was performed in May 2012. In each stand ($n = 4$), samples were collected from three 30 m by 30 m replicate plots. In each plot ($n = 12$), 10 random 0.1 m by 0.1 m forest floor samples (Oe/Oa horizons) were collected after removing the Oi horizon. All samples were composited within each plot and homogenized by hand in the field. The samples were transported on ice to the University of Michigan, where they were stored at −80°C.

DNA extraction

Genomic DNA was extracted from 2.5 g of forest floor samples (1 per plot; $n = 12$) using the PowerMax Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) and was purified using the PowerClean DNA Clean-up kit (MoBio) following manufacturer's instructions. Extracted DNA quality was determined using an ND8000 Nanodrop (Thermo Scientific, Waltham, MA, USA) and quantified by Quant-iT PicoGreen (Invitrogen, Carlsbad, CA, USA), according to manufacturer's instructions, on a Synergy HT fluorimeter (BioTek, Winooski, VT, USA). All DNA was stored at −80°C.

PCR amplification, high-throughput sequencing

PCR amplifications were performed in triplicate using the Expand High Fidelity PCR System (Roche, Indianapolis, IN, USA) on a Mastercycler ProS thermocycler (Eppendorf, Hauppauge, NY, USA) and were pooled prior to purification. Bacterial primers 27F and 519R (Lane, 1991) were used to amplify approximately 500 bp (V1–V3 region) of the 16S rRNA gene from each plot ($n = 12$). The reaction mixture contained 1 μM primers, 3 mM $MgCl_2$, 200 μM deoxyribonucleotides (dNTPs) 3 μL bovine serum albumin (10 μg/μL) and two units of expand high-fidelity *taq* polymerase (v. 20; Roche, Indianapolis, IN, USA). Polymerase chain reaction conditions included an initial denaturation stage of 95°C for 10 min, then 25 cycles of 95°C for 30 s followed by 1 min each at 55°C and 72°C. All products were purified using the MinElute PCR Purification Kit (Qiagen, Valencia, CA, USA). Libraries created from PCR products were pooled in equimolar concentrations by plot and were sequenced on the PacBio-RS II system (Pacific Biosciences, Menlo Park, CA, USA) at the University of Michigan DNA Sequencing Core, using C2 chemistry and standard protocols (Eid *et al.*, 2009). In this study, we utilized PacBio circular consensus technology, which can generate at least 99.5% sequence accuracy for DNA fragments up 500 bp (Travers *et al.*, 2010).

DNA sequence processing

Fastq files of the dataset used in this analysis have been deposited to National Center for Biotechnology Information under project accession number SRR1382191. Sequences were processed using the PBH5TOOLS package (Pacific Biosciences) and MOTHUR (Version 1.31.1; (Schloss *et al.*, 2009). Initial quality control measures removed any sequence with a consensus fold coverage < 5, average quality score < 25 (50 bp rolling window), anomalous length (< 450 or > 550 bp), an ambiguous base, > 8 homopolymers or a > 1 bp mismatch to either the barcode or primer (Marshall *et al.*, 2012; Freedman and Zak, 2014). High-quality reads were de-replicated and aligned with the SILVA ribosomal database (Quast *et al.*, 2013) using k-mer

searching (8-mers) with Needleman–Wunsch global, pairwise alignment methods (Needleman and Wunsch, 1970) and checked for chimeras using UCHIME (Edgar *et al.*, 2011).

Analysis of bacterial community structure

Operational taxonomic units were selected at 95% sequence similarity to determine any effects of temporal and environmental filters on bacterial phylogenetic structure at a broad (i.e. genus) taxonomic level. Bacterial 16S rRNA gene sequences were taxonomically assigned with the SILVA database using a Bayesian classifier (Wang *et al.*, 2007), using a bootstrap cut-off of 80.

Assemblage diversity was estimated using the Shannon Index (H′; Shannon and Weaver, 1963) and richness using the Chao1 estimator (Chao, 1984); significance was determined by a one-way analysis of variance (ANOVA), combined with a protected Tukey's honestly significantly different test of means (HSD; SPSS Statistics, Version 20, IBM, Armonk, NY, USA).

Pairwise distances between 16S rRNA gene assemblages across the chronosequence were determined by phylogenetic structure, i.e. the abundance weighted UniFrac distance (Lozupone and Knight, 2005). UniFrac measures the sum of unique branch length attributable to one site or the other, but not both. Phylogenetic analyses were performed using a maximum likelihood tree generated using representative sequences from each OTU in FastTree (Price *et al.*, 2009). Calculation of UniFrac distance and all downstream analysis were performed using MOTHUR and PRIMER (Version 6, Primer-E, Plymouth, UK).

To further test the hypothesis that temporal and environmental filtering shape soil bacterial communities, differences in phylogenetic structure were statistically tested by PerMANOVA (Anderson, 2001). Permutational multivariate analysis of variance allows multivariate information to be partitioned according to the experimental design and determines significance by permutation. A distance-based test for homogeneity of multivariate dispersions (PERMDISP; Anderson, 2004) was used to determine if any observed compositional response was driven by differences in metagenetic heterogeneity between sites. Ordinations were obtained from PCoA calculated from Bray–Curtis dissimilarity of OTU abundance tables (Legendre and Legendre, 1998), from which, Spearman correlations were calculated to ascertain which OTUs contributed to any observed compositional shift.

Determination of temporal and environmental filters on bacterial phylogenetic structure

More than 50 environmental factors have been characterized in each site composing our chronosequence (http://webpages.uidaho.edu/nitrogen-gradient). For all analyses, we chose a parsimonious subset known to influence soil bacterial communities: soil temperature (Pietikainen *et al.*, 2005; Castro *et al.*, 2010), moisture (Castro *et al.*, 2010), pH (Fierer and Jackson, 2006), C (Garbeva and de Boer, 2009), N (Zak *et al.*, 2008; Ramirez *et al.*, 2012), C:N (Lauber *et al.*, 2008), plant composition (Batten *et al.*, 2006; Knelman *et al.*, 2012) and historical processes (i.e. dispersal limitation, pri-

ority effects; Whitaker *et al.*, 2003; Talbot *et al.*, 2014). In doing so, we reduce the probability of spurious correlations between bacterial community composition and ecologically irrelevant variables.

The RELATE (a non-parametric Mantel-type test) test was first used to determine which broad characteristics (e.g. biogeochemical, physical, plant cover and temporal) were significantly correlated to bacterial phylogenetic structure (Clarke and Ainsworth, 1993). RELATE is similar to the Mantel test for similarity of two matrices (Mantel, 1967), except that rank correlations (ρ), rather than standard Spearman correlations are calculated between all elements of the respective dissimilarity matrices. Significance is determined by permutation to a null model (999 runs). Environmental distance matrices were calculated from data separated into four characteristics: (i) biogeochemical, (ii) physical, (iii) plant community composition and (iv) temporal (i.e. geographic distance, a surrogate for time). The biogeochemical dataset included leaf litter N content (g N m^{-2}), C content (g C Kg litter^{-1}), C:N, turnover (years), total litter mass and soil pH, and contained the plot means averaged across 2005–2009 (Table 1). Physical characteristics included average annual soil temperature and soil matric potential from 1988 to 2009 (Table 1). Plant community composition was determined as the relative dominance of overstory species at each plot based on basal area (Table S1). Biogeochemical and physical dissimilarity matrices were calculated from Euclidian distances of log-transformed data, whereas the Bray–Curtis metric (Legendre and Legendre, 1998) was used to quantify plant community dissimilarity. All environmental data may be found at the Michigan Nitrogen Deposition Gradient Study webpage (http://www.webpages .uidaho.edu/nitrogen-gradient/).

Distance-based linear model building (Legendre and Legendre, 1998) was used to determine which individual factor, or combination of factors, account for the greatest proportion of observed phylogenetic structure, and if the phylogenetic structure explained by each factor is shared or distinct. Prior to DistLM model building, all significant individual factors (e.g. geographic distance, litter N, etc.) within characteristics deemed at least moderately ($P < 0.10$) significant by RELATE were combined to a single matrix and transformed into z-scores. The adjusted R^2 selection criterion was selected for all DistLM procedures. Marginal DistLM was first used to determine which factors accounted for a significant proportion of phylogenetic structure when taken alone, ignoring all other factors. Prior to multiple regression, Draftsman Plots (Clarke and Ainsworth, 1993) were used to test for collinearity between significant factors, wherein, significant collinearity was defined at $\rho \geq 0.70$. The 'forward' procedure within DistLM was then implemented using all moderately significant ($P < 0.10$) individual factors from marginal DistLM. The 'forward' method uses sequential factor introduction to the model to determine whether the proportion of phylogenetic structure accounted for by each factor is shared or distinct to others. Lastly, we used the 'best' model building procedure, which utilizes all possible combinations of factors to determine which combination of factors account for the greatest proportion of bacterial phylogenetic structure; factor addition was evaluated stepwise and was based on sufficient improvement in the model's adjusted R^2.

Acknowledgements

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Supporting information

Additional Supporting information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Chao1 richness and Shannon diversity (H′) of soil bacterial communities along the chronosequence. *$P < 0.05$ by Tukey's HSD.

Table S1. Relative abundance of plant composition based on basal areas of overstory tree species.

Table S2. Sequences lost in quality control pipeline.

Table S3. Taxonomic composition of saprotrophic soil Bacteria along a long-term glacial chronosequence.

Table S4. Pairwise PerMANOVA results.

Table S5. Taxonomic classification of OTUs with significant correlations to PCo2.

Table S6. Taxonomic classification of OTUs attributable to orders *Actinobacteria* and *Proteobacteria* with a significant correlation to PCo2.

Table S7. Proportion of, and cumulative bacterial phylogenetic distance accounted for by individual biogeochemical, physical or temporal factors by sequential DistLM.