Soil Biology & Biochemistry 101 (2016) 74–84

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Biogeochemical drivers of microbial community convergence across actively retreating glaciers

Sarah C. Castle [a, *], Diana R. Nemergut [b], A. Stuart Grandy [c], Jonathan W. Leff [d, e], Emily B. Graham [f], Eran Hood [g], Steven K. Schmidt [d], Kyle Wickings [h], Cory C. Cleveland [a]

[a] Department of Ecosystem and Conservation Sciences, University of Montana, Missoula, MT 59812, USA
[b] Department of Biology, Duke University, Durham, NC 27708, USA
[c] Department of Natural Resources and the Environment, University of New Hampshire, Durham, NH 03824, USA
[d] Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA
[e] Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO 80309, USA
[f] Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99354, USA
[g] Environmental Science Program, University of Southeast Alaska, Juneau, AK 99801, USA
[h] Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA

ARTICLE INFO

Article history:
Received 19 February 2016
Received in revised form
10 July 2016
Accepted 11 July 2016

Keywords:
Microbial succession
Soil carbon chemistry
Bacteria
Convergence
16S rRNA gene sequencing
454

ABSTRACT

The ecological processes that influence biogeographical patterns of microorganisms are actively debated. To investigate how such patterns emerge during ecosystem succession, we examined the biogeochemical drivers of bacterial community assembly in soils over two environmentally distinct, recently deglaciated chronosequences separated by a distance of more than 1300 km. Our results show that despite different geographic, climatic, and soil chemical and physical characteristics at the two sites, soil bacterial community structure and decomposer function converged during plant succession. In a comparative analysis, we found that microbial communities in early succession soils were compositionally distinct from a diverse group of mature forest soils, but that the differences between successional soils and mature soils decreased from early to late stages of succession. Overall differences in bacterial community composition between sites were explained by soil pH. However, within-site successional patterns – leading to community convergence across sites at the latest stage of succession – were explained by alternate factors such as soil organic carbon and soil organic matter chemistry, which were correlated to bacterial community structure across both glacial and mature forest soils.

1. Introduction

The ecological processes that contribute to biogeographic patterns of organisms and communities are actively debated (Vellend, 2010), particularly for microorganisms (Martiny et al., 2006, 2011; Burke et al., 2011; Nemergut et al., 2013). Recent studies have demonstrated that microbial communities are selected for across different habitat types (Lozupone and Knight, 2007; Nemergut et al., 2011), and within one habitat type – soil – researchers have pointed to the importance of deterministic drivers such as contemporary environmental conditions (Hanson et al., 2012) to explain the distribution of microorganisms.

At the regional to global scale, much of our understanding of the relationship between microbial community structure and environmental factors comes from studies conducted at a single point in time. For example, soil pH has been shown to strongly correlate with soil bacterial community composition across moderate to large pH ranges (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010). Yet in many locations, current environmental conditions differ from the historical conditions that may have contributed to microbial community assembly (Hanson et al., 2012), and assemblages likely reflect the combined influences of historic (Hawkes and Keitt, 2015) and contemporary conditions. Soil development, which is influenced by the activity of soil microorganisms, takes place over decades to millennia, precluding simultaneous measurement of microbial dynamics and evolving ecosystem properties. To overcome this issue and resolve the mechanisms driving community assembly in concert with

* Corresponding author. Present Address: 495 Borlaug Hall, 1991 Upper Buford Circle, Saint Paul, MN 55108, USA.
E-mail address: sccastle@umn.edu (S.C. Castle).

http://dx.doi.org/10.1016/j.soilbio.2016.07.010
0038-0717/

changing soil properties, plant ecologists have used space-for-time substitution approaches to indirectly examine the process of ecological succession – the progressive replacement of organisms following major disturbances that reset ecosystem development (Chapin et al., 1994; Walker and del Moral, 2003). More recently soil chronosequences, like those that develop in proglacial systems, have been used to identify the processes and mechanisms of microbial successional change (e.g., Jumpponen, 2003; Kandeler et al., 2006; Nemergut et al., 2007; Schmidt et al., 2008; Sattin et al., 2009; Carlson et al., 2010; Jumpponen et al., 2012; Knelman et al., 2012; Brown and Jumpponen, 2014; Jangid et al., 2013; Knelman et al., 2014).

For microbial communities undergoing primary succession, key questions remain as to whether community assembly processes occur in predictable ways across different sites. That is to say, do early successional microbial communities start out with the same community members conducting the same functions and further, do communities follow the same or different successional trajectories through ecosystem development? Comparative studies have shown that the trajectories of plant primary succession can vary, culminating in compositionally divergent, convergent, or parallel development of plant communities (Fig. S1; Samuels and Drake, 1997; Walker and del Moral, 2003). Convergent succession may reflect strong selective processes such as environmental filtering and species interactions that cause initially different communities to become more similar through time (Samuels and Drake, 1997; Walker and del Moral, 2003) in terms of growth form (Nilsson and Wilson, 1991), species traits (Wilson and Whittaker, 1995; Fukami et al., 2005; Raevel et al., 2012; Helsen et al., 2013), and less commonly community composition (Raevel et al., 2013). By contrast, divergent succession reflects the importance of stochastic events, priority effects, dispersal barriers, and/or environmental filters that increase in strength or change over time; such effects cause initially similar communities to become different with time (McCune and Allen, 1985; Walker and del Moral, 2003; Fukami et al., 2005). In addition, mixed patterns of convergence and divergence have been observed (Inouye and Tilman, 1995). For example several studies have found instances where species composition diverged, while life history and functional traits converged (Fukami et al., 2005; Helsen et al., 2013). Finally, persistently different environmental conditions and/or early stochastic events may preclude divergence or convergence, resulting in parallel successional trajectories in which initial plant community differences also persist through time (Walker and del Moral, 2003). Given the high abundance, high rates of dispersal, short generation times, and the immense phylogenetic and physiological diversity of microorganisms, the processes influencing microbial succession may operate in fundamentally different ways than those influencing plant succession (Fierer et al., 2010; Nemergut et al., 2013). Yet, successional patterns are still relatively unknown for soil microorganisms (Fierer et al., 2010), and few studies have attempted analogous comparisons across multiple primary succession gradients (c.f. Lazzaro et al., 2009).

Here, we used a space-for-time approach (Walker et al., 2010) to compare soil bacterial community development in two actively developing glacial forefields separated by a distance of more than 1300 km: Easton Glacier, WA, USA and Mendenhall Glacier, AK, USA. Since the processes shaping biological succession may result in different patterns of compositional and functional development (Fukami et al., 2005), we assessed both phylogenetic and metabolic characteristics of microbial communities. Next, in a comparative analysis, we examined the successional trajectories of developing glacial communities in light of a group of mature forest soils (Lauber et al., 2009) – thought to represent an advanced stage of succession for both sites. Given the strong dependence of microbial communities on soil characteristics, we hypothesized that initially unique soil properties across sites (i.e., soil pH) would contribute to differences in microbial community composition (H1). However, we also hypothesized that the processes of ecosystem development and plant colonization would influence soil properties in predictable ways across sites – altering soil pH, increasing carbon (C) and nitrogen (N) – and thus would promote similarity among soil bacterial communities through time (H2). Specifically, we hypothesized that plant colonization would lead to an increase in the abundance and chemical diversity of organic C resources in soil, which would support a more compositionally and functionally diverse soil decomposer community (H3).

2. Materials and methods

2.1. Study sites and soil collection

Easton Glacier is located on the southern flank of Mt. Baker in the Mt. Baker-Snoqualmie National Forest, Washington USA (48°44 N, 121°50 E; 1637 m.a.s.l.). Easton experiences 198 cm of precipitation annually (1984–2009 mean, NADP Site WA19) and temperatures that span 3.9–13.6 °C (1961–1990 mean annual minimum/maximum temperature, Western Regional Climate Center ID 458715). The dominant parent material at Easton Glacier is volcanic andesite (Tabor et al., 2003). The Mendenhall Glacier is located in the Tongass National Forest of southeast Alaska, USA (58°26 N, 134°33 E; 75 m.a.s.l.) and is characterized as a perhumid maritime climate with 153 cm of precipitation annually (2004–2009 mean, NADP Site AK02) and temperatures ranging from −1 °C to 8 °C (1965–1980 mean annual minimum/max temperature, Western Regional Climate Center ID 504110). The dominant parent material at the Mendenhall Glacier is metamorphic, composed of plagioclase, quartz and hornblende (Alexander and Burt, 1996). Historical terminus locations have been well documented for both sites (Harper, 1993; Motyka et al., 2003; Pelto and Brown, 2012).

At each site, we established five transects of different successional stages parallel to the glacier terminus. In order to account for different rates of glacial retreat across sites, transect locations were selected on the basis of time-since-retreat and percent plant cover. Specifically, plant colonization occurs rapidly at Mendenhall Glacier and we observed sparse plant cover (<5%) occurring in substrates that were only ~5 years old. By contrast, at Easton Glacier the first signs of plant colonization were observed in ~25 year old substrates (personal observation). At each site, transect 1 was nearest to the glacial terminus and was characterized by newly exposed, disorganized glacial till that was ~0–1 year in age, transect 2 represented unvegetated till ~5 years old, transect 3 included some element of soil crusting (lichens, mosses, cyanobacterial crust) and sparse vegetation (average < 5%), transect 4 represented ~25–50% patchy plant cover, and transect 5 had 50–75% semi-continuous plant cover. Soil crust and percent plant cover were assessed visually. Plant species composition differed between the glacial chronosequences (Alexander and Burt, 1996; Whelan, 2013), though, successional endpoints for both gradients included coniferous tree species. At the Easton Glacier, the initial plant communities of transect 3 were composed of mosses, grasses, rushes, and forbs. Plant cover at transect 4 was composed of moss, grasses, rushes, forbs, including heather (*Phyllodoce empetriformis*), Tolmie saxifrage (*Micranthes tolmiei*), partridge foot (*Luetkea pectinata*), pearly everlasting (*Anaphalis margaritacea*), as well as small Mountain Hemlock (*Tsuga mertensiana*) saplings. Later plant communities (transect 5) also included abundant Lupine (*Lupinus latifolius*), a putative nitrogen-fixing plant species, and larger *T. mertensiana*. At the Mendenhall Glacier, initial plant communities of transect 3

included mosses and grasses. Plant cover at transect 4 included moss, grasses, fireweed (Epilobium spp.), small nitrogen-fixing (*Alnus sinuata*) and non nitrogen-fixing shrubs (Populus spp., Salix spp.), and small Sitka spruce seedlings (*Picea sitchensis*). More developed plant communities at Mendenhall Glacier included larger shrubs and larger conifer saplings. Photoautotrophic microorganisms (i.e., cyanobacteria) were present in soils, but not abundant (<5%), thus the sites were categorized as heterotrophic successional sequences (Fierer et al., 2010).

During the late summer and early fall of 2010, we sampled surface soils (0–5 cm) at nine randomly selected points along each of the five transects at each site. Samples were collected into polyethylene bags using a small trowel. The collection trowel was sterilized with ethanol and wiped with paper towels between every contact with the soil. Following collection, samples were transported on ice to the Terrestrial Ecosystem Ecology Laboratory at the University of Montana, Missoula, MT, USA. The nine individual samples from each transect were randomly assigned to three groups each containing three samples. Individual samples were mixed at equal masses to create the three distinct pooled samples per transect. Prior to processing, each pooled sample was homogenized and hand-sieved of rocks and roots (4 mm × 4 mm) while taking precautions to prevent between-sample contamination. Soil subsamples were frozen for molecular analysis (−80 °C). The catabolic response profiling experiment was conducted immediately with fresh soil. Remaining soils were dried at 60 °C and pulverized for C chemistry, total C, and total N, dried at 105 °C for gravimetric water content analysis, and were air-dried for soil pH measurements.

2.2. Mature sample acquisition and processing

To examine microbial community succession and convergence in a broad context, we compared recently deglaciated soils to a small previously published dataset of mature forest soils (Lauber et al., 2009) – thought to represent an advanced stage of plant succession for Easton and Mendenhall Glaciers. From the authors of the Lauber et al. (2009) study, we obtained archived surface soil samples (0–5 cm) collected from conifer and mixed forest sites across North America (hereafter referred to as the 'mature soil dataset'). Of the 33 forest biome soils initially included in Lauber et al., 2009 study, we selected ten samples representing a range of climatic and soil chemical characteristics, including pH. Frozen samples were sent on dry ice to the Soil Biogeochemistry and Fertility Lab at the University of New Hampshire where they were lyophilized and ground in preparation for C chemistry analysis. Prior to our analyses, samples had been continuously frozen (−80 °C) since the time of initial processing.

2.3. DNA extraction from glacial soil

DNA was extracted from soil samples following standard manufacturers protocols for bulk DNA extractions using the Mo Bio PowerSoil™ kit (Mo Bio Laboratories, Inc., Carlsbad, USA). A modified lysis buffer solution (2 mL 1 M EDTA, 5 mL 1 M $NaHPO_4$, 1 mL 1 M TrisHCl, 1% SDS, 1.9 mL Ultra Pure DI H_2O) was used to increase DNA extraction efficiencies from the volcanic Easton Glacier soils and the modification was used for Mendenhall Glacier soils as well.

2.4. 16S rRNA gene sequence analyses of glacial bacterial communities

DNA was processed as previously described in Nemergut et al. (2010). PCR amplification of bacterial 16S rRNA genes from genomic DNA was conducted using a universal bacterial 27F and 338R primer set (Hamady et al., 2008). PCR reactions for each sample were performed in triplicate using conditions described by Fierer et al. (2008). PCR products were pooled per sample and cleaned with Mo Bio UltraClean-htp PCR Clean-up kits (Mo Bio Laboratories, Inc., Carlsbad, CA). Pyrosequencing of gene amplicons was completed by 454 Life Sciences (Bradford, USA).

We performed sequence processing using the QIIME pipeline (Caporaso et al., 2010) and, as was done in Leff et al. (2012), sequences were not denoised. A total of 24,523 sequences were retained for analysis after processing and quality filtering. Operational taxonomic units (OTUs) were clustered at a 97% sequence similarity level using UCLUST (Edgar, 2010). Chloroplast and mitochondria sequences were removed. To equalize sequence numbers in each sample, we used QIIME's single rarefaction script to rarefy to 108 sequences per sample. After rarefaction our dataset contained 1948 unique OTUs in 30 samples. We note that such low numbers of 16S rRNA gene sequences have been similarly reported for other primary successional soils (Nemergut et al., 2007; Sattin et al., 2009; Knelman et al., 2012, 2014). We assigned taxonomic identity using the RDP classifier against the Greengenes coreset database in QIIME. To calculate pairwise distances between samples, we calculated weighted UniFrac distance (Lozupone and Knight, 2007), which accounts for phylogeny. We examined patterns of bacterial community change with succession at high taxonomic ranks (phyla and sub-phyla level), as organisms within phyla may share common life history strategies (Fierer et al., 2007). For all taxon-based analyses, we removed groups that comprised less than 1% of the community for both sites. Alpha diversity is presented as Shannon (H′), richness (observed OTUs), and phylogenetic diversity. Raw sequence data are available via Figshare (10.6084/m9.figshare.3384694).

2.5. Mature soil sequence data retrieval and analyses

For the ten mature forest samples analyzed for soil C chemistry, we downloaded corresponding quality filtered bacterial 16S rRNA gene sequences and soil metadata originally presented in Lauber et al. (2009) from the publically available QIIME database (now hosted on the Qiita database; http://qiita.ucsd.edu). To compare glacial soils to the mature dataset, pre-processed sequences from the QIIME database and sequences pre-processed in-house were combined for downstream analysis as described above including rarefying all samples to 108 sequences. Sequences from glacial soils were generated using the same primers and molecular protocols used by Lauber et al. (2009).

2.6. Catabolic response profiling of soil decomposers

We assessed the functional potential of soil heterotrophic communities using short-term respiration responses of microbial communities to 24 organic C substrates (Degens and Harris, 1997). Substrates originated from eight different C chemical classes and included two amines (L-glutamine, urea), five amino acids (glycine, histidine, L-asparagine, L-glutamic acid, glucosamine), three carbohydrates (fructose, glucose, sucrose), five carboxylic acids (citric acid, DL-lactic acid, fumaric acid, pentadecanoic acid, α-ketoglutaric acid), one nucleic acid (DNA), three polysaccharides (amylopectin, amylose, glycogen), two proteins (BSA, casein), and several recalcitrant substrates (chitin, humic acid, lignin). Rates of basal respiration were assessed using deionized water for each set of samples. Carbon substrates were prepared to a concentration of 900 mM C in deionized water and were adjusted to pH ~6 with HCl or NaOH. In 60 mL glass vials, we added 4 mL of C substrates individually to 4 g field moist subsamples of each soil sample. Vials were sealed with

Teflon septa, briefly vortexed to slurry contents, and incubated at 20 °C for 24 h in total darkness on a slowly rotating orbital shaker. Following the incubation period, vial headspace was mixed, sampled, and CO_2 concentrations were measured by gas chromatography (Shimadzu GC 2014; Columbia, USA). CO_2 fluxes were calculated on the basis of dry soil (μg CO_2-C g dry soil^{-1} hour^{-1}) and basal respiration (water-only amended samples) was subtracted from substrate-amended responses. Multivariate analyses were based on the calculation of the proportional respiration response of each substrate relative to the cumulative response to all substrates (Leff et al., 2012). Finally, we created an index of catabolic function for each sample by calculating Euclidean distances based on proportional respiration responses to each compound.

2.7. Soil chemical characterization

Available inorganic nitrogen (NH_4^+ and NO_3^-) was extracted using 2 M KCl (Mulvaney, 1996) and was measured colorimetrically (Weatherburn, 1967; Doane and Horwath, 2003) using a microplate reader (Biotek, Winooski, USA). Microbial biomass C and N were measured using a 0.5 M K_2SO_4 extraction on chloroform fumigated and unfumigated samples (Horwath and Paul, 1994) and were analyzed on a Shimadzu TOC-VCPN (Shimadzu, Columbia, MD). Microbial biomass C and N were calculated as the difference between fumigated and unfumigated samples. Total C and N were measured on pulverized samples with a CHN elemental analyzer 1110 (CE Instruments, Wigan, UK) and values were verified with an acetanilide standard (10.36% N; 71.09% C). Inorganic C was assessed with a coulometer (UIC Inc., Joliet, IL) and we subtracted inorganic C from total C to infer soil organic C for each sample. Soil pH was determined using a digital pH meter (Fisher Scientific, Pittsburgh, USA) using a 1:2 ratio of soil to water. All soil chemical properties are expressed in terms of soil dry mass.

2.8. Soil organic carbon chemistry

For glacial soils and ten mature forest soils, we assessed soil C chemistry using pyrolysis-gas chromatography mass spectrometry (py-GCMS) following procedures outlined by (Grandy et al., 2009). Dried and pulverized subsamples were pulse-pyrolyzed at 600 °C on a Pyroprobe 5150 (CDS Analytical, Inc, Oxford, USA). Pyrolyzed materials were transferred to a GC (GC Ultra, Thermo Scientific, West Palm Beach, USA) for separation and were quantified by a mass spectrometer (Polaris Q, Thermo Scientific, West Palm Beach, USA). Compounds were identified using Automated Mass Spectral Deconvolution and Identification System (AMDIS V 2.65) and peaks were verified using the NIST mass spectral library (Grandy et al., 2007, 2009; Grandy and Neff, 2008). Additional peak identification was done using previously published compound libraries (Stewart et al., 2011; Stewart, 2012). We calculated the relative abundances of compounds as the peak area of each compound divided by the sum of peak areas of all identified compounds and grouped C compounds by chemical class (e.g., lignin, lipid, N-bearing compounds, phenolic, polysaccharide, protein, and compounds of unknown origin).

2.9. Statistical analyses

Multivariate statistics were conducted using the packages Ecodist v.1.2.3 (Goslee and Urban, 2007) and Vegan v.2.0–5 (Oksanen et al., 2010) in R v.3.1.1 (R Core Team, 2014). Visualizations of community composition and catabolic function were done using non-metric multidimensional scaling (NMDS) using the nmds function in R. We used permutational vector fitting (999 permutations; Jongman et al., 1995) using a multiple linear regression technique and the envfit function in R to assess relationships between NMDS ordinations of community structure and the relative abundance of bacterial phyla and subphyla. Using the same envfit function, we assessed relationships between NMDS ordinations of soil C chemistry and the relative abundance of soil C functional classes. Between-site differences in community composition, catabolic response, and soil C chemistry were assessed using variance partitioning in non-parametric MANOVA's and the adonis function (999 permutations). We assessed the homogeneity of variances of bacterial communities by transect (β-dispersion) by conducting a multivariate Levene's type test (Fierer et al., 2008) using a median betadisper function in R (Anderson et al., 2006) followed by a one-way ANOVA with transect as the grouping factor. The average weighted UniFrac distance of bacterial communities was calculated on a pairwise basis from each of the glacial samples to each of the samples in the mature forest dataset. For each site, we tested for significant differences in average UniFrac distance with transect as a grouping factor using one-way ANOVA. One-way ANOVA analyses were performed to assess how catabolic evenness, community diversity metrics, and major taxonomic groups changed through succession. Catabolic evenness was calculated using the Simpson-Yule index approach, $\Sigma = 1/pi^2$, wherein pi is equal to the respiration response to a given individual substrate divided by the total sum of respiration responses to all substrates (Magurran, 2004). We conducted permutational MANOVAs to assess the correlative relationship between bacterial community structure, catabolic function, and soil properties using mantel tests with Spearman's rank correlations (10,000 permutations). The weighted UniFrac distance metric was used for community structure analyses and Euclidean distance matrices were used for individual soil variables and catabolic response profiles. An index of soil C chemistry was created for each sample by calculating Bray-Curtis distances based on relative abundances of each C class (Leff et al., 2012). Prior to analyses, soil chemical data and catabolic response profiles were log transformed and soil C chemistry was arcsine-square root transformed to meet the assumptions of normality.

3. Results and discussion

3.1. Soil chemical properties

Many of the major state factors that contribute to soil formation (i.e., parent material, climate, plant species, time; Amundson and Jenny, 1997) differed between Easton and Mendenhall Glaciers. As a result, key soil properties that frequently correlate with microbial community structure (Fierer and Jackson, 2006; Cleveland et al., 2007; Lauber et al., 2009; Rousk et al., 2010; Ramirez et al., 2012) showed minimal overlap between sites (Fig. 1). Initially and throughout succession, soil pH at Easton (range: 4.1–5.5) was consistently lower than Mendenhall (range: 8.1–8.4). However, while Easton soils incrementally accrued organic C through time (range: 0.4–10.4 mg g soil^{-1}), Mendenhall soils were initially C-rich (4.0 mg g soil^{-1}), were C-poor in mid-succession (2.0 mg g soil^{-1}), and accrued C into late succession (3.7 mg g soil^{-1}). Previous studies have attributed Mendenhall Glacier's non-linear pattern of soil organic C accrual to the presence of ancient woody debris (Sattin et al., 2009). Although soil N (total N, NH_4^+) generally increased with time at both sites, N pools were as much as an order of magnitude larger at Easton. By contrast, an index of soil C chemistry reflecting relative abundances of C compound classes (i.e., aromatic, lignin, lipid, N-bearing, phenol, polysaccharide, protein) was not significantly different between Easton and Mendenhall Glaciers (PERMANOVA $r^2 = 0.076$, $P = 0.94$). Rather, at both sites, soil C chemistry was initially similar at the onset of succession and followed a similar trajectory over time (Fig. 2a) despite

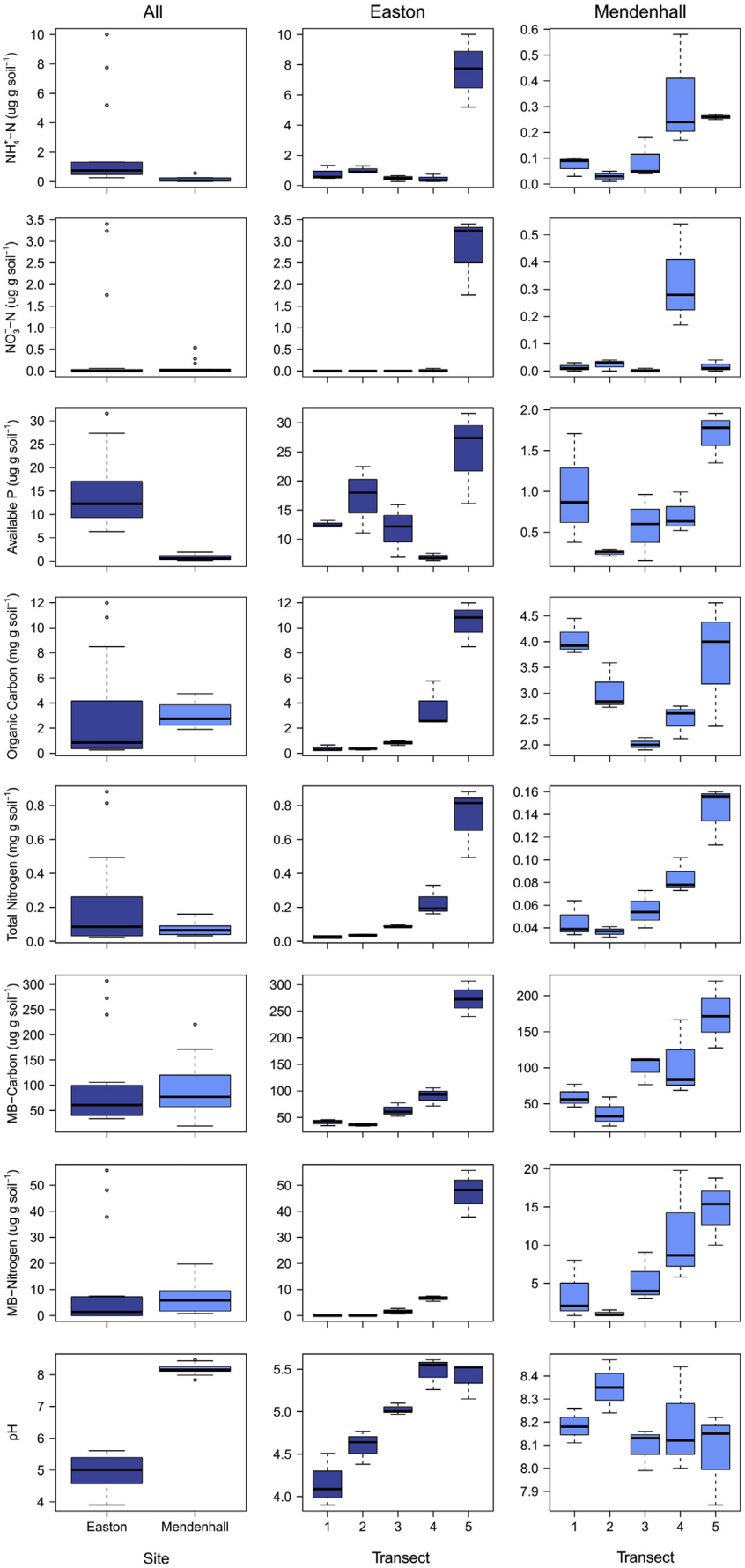

Fig. 1. Soil biogeochemical characteristics of Easton and Mendenhall Glaciers. Boxplot lines represent medians with 95% confidence intervals. Stages of succession are indicated by numbers: 1 (0–1 year old soil), 2 (5 year old soil), 3 (biological soil crust cover), 4 (25–50% plant cover), and 5 (50–75% plant cover). Soil NH_4^+-N, NO_3^--N, available P, microbial biomass C (MB-C), and microbial biomass N (MB-N) are presented as μg g soil^{-1}. Total N and organic C are presented as mg g soil^{-1}. Soil pH was determined using a 1:2 ratio of soil to water. For each transect (n = 3).

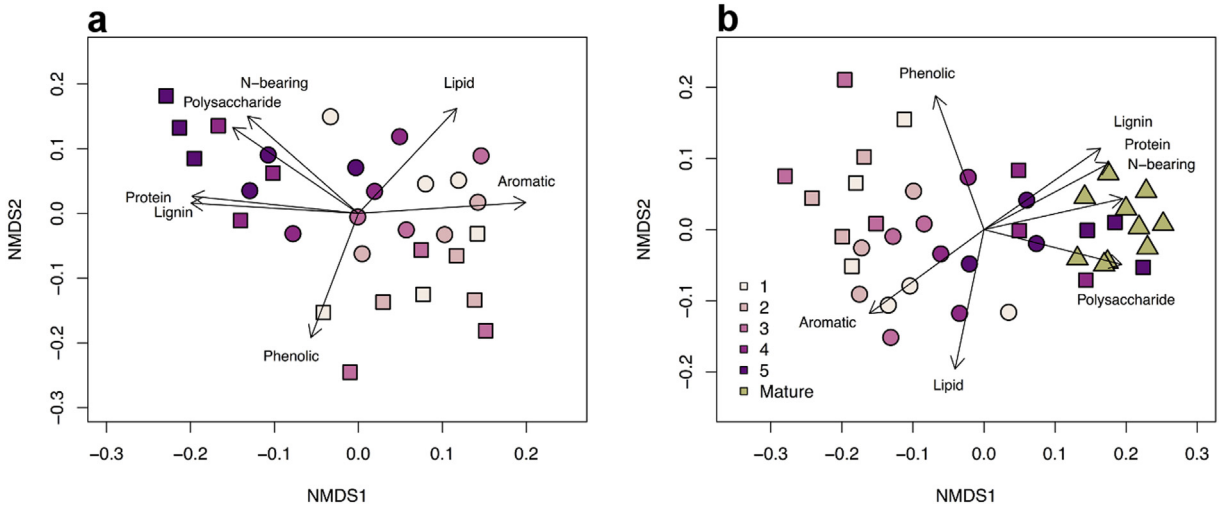

Fig. 2. Non-metric multidimensional scaling of soil organic carbon chemistry (arcsine square-root transformed Bray-Curtis distances) from (a) Easton Glacier (squares) and Mendenhall Glacier (circles). (b) Soil C chemistry for Easton Glacier, Mendenhall Glacier, and a selection of 10 mature forest soils (triangles; Lauber et al., 2009). Successional stage is indicated by color: 1 (0–1 year old soil), 2 (5 year old soil), 3 (biological soil crust cover), 4 (25–50% plant cover), and 5 (50–75% plant cover). Vectors indicate relative abundances of soil C compounds that were significantly correlated the first two dimensions. A permutational MANOVA analysis indicated that there was no difference in C chemistry between Easton and Mendenhall sites ($R^2 = 0.076$, $P = 0.094$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

differences in total soil organic C content (Fig. 1) and plant species composition. In fact, we observed strong differences in soil C chemistry between vegetated and unvegetated soils regardless of site (PERMANOVA $r^2 = 0.19$, $P \leq 0.004$; Fig. 2a). Finally, as young glacial soils developed, soil C chemistry converged with that of mature forest soils (Fig. 2b; Fig. S2) collected from a large geographical range and diverse environmental conditions (see Lauber et al., 2009). From young to mature soils, shifts in soil C chemistry were associated with increasing relative abundances of N-bearing, polysaccharide, and protein based compounds (Fig. 2b; Fig. S2).

3.2. Bacterial community structure and decomposer function

The glacial forefields of Easton and Mendenhall harbored compositionally and functionally distinct soil microbial communities (Fig. 3). Non-metric ordination analysis of weighted Unifrac distances revealed significant differences in bacterial community composition between the two glacial sites (PERMANOVA $r^2 = 0.18$, $P \leq 0.001$; Fig. 3a), regardless of successional stage. Within each site, bacterial communities were compositionally different among the five successional stages (PERMANOVA Easton: $r^2 = 0.38$, $P \leq 0.001$; Mendenhall: $r^2 = 0.19$, $P \leq 0.001$; Fig. 3a; Fig. S3a, b) and bacterial community composition shifted in a stepwise manner, providing support for the idea that bacterial succession occurred during ecosystem development (Fig. 3a; Fig. S3a, b). Across successional stages, differences in community composition were related to shifts in the relative abundances of specific bacterial phyla and sub-phyla (Table S1). Namely at Easton Glacier, compositional shifts reflected decreases in initially abundant *Betaproteobacteria*, as previous studies at developing glacial sites have noted (Jangid et al., 2013), and increases in relative abundances of *Acidobacteria*, *Bacteroidetes*, and *Verrucomicrobia* (Fig. S3a, Table S1). At Mendenhall Glacier, the initially abundant candidate division WPS-2 declined through succession, while *Acidobacteria* increased in relative abundance (Fig. S3b, Table S1). Finally, similar to results from other primary successional chronosequences (Nemergut et al., 2007; Sattin et al., 2009), we observed that local (alpha) diversity of bacterial communities was significantly lower in young soils compared to old soils for Easton Glacier (Table S1), and to a lesser degree for Mendenhall Glacier.

Consistent with phylogenetic data, community function (catabolic potential of bacterial and fungal decomposers) was significantly different across the two glacial sites (PERMANOVA $r^2 = 0.47$, $P \leq 0.001$; Fig. 3b). Further, within each site, catabolic function changed significantly through succession (PERMANOVA Easton: $r^2 = 0.51$, $P \leq 0.001$; Mendenhall: $r^2 = 0.59$, $P \leq 0.001$; Fig. 3b, Fig. S3c, d). Specifically, early successional communities were able to utilize a limited number of compounds, while decomposers of later successional stages were increasingly able to utilize a wide range of compounds to a similar degree (Table S1; catabolic evenness, sensu Degens et al., 2001).

3.3. Successional trajectories of microbial structure and function

While early successional microbial communities from across the two glacial sites started out with the relatively different community members (Fig. 3a) conducting different functions (Fig. 3b), we observed that through time and with increasing soil development community similarity increased. Specifically, variances in bacterial community structure ($P = 0.003$; Fig. 3c) and decomposer function ($P = 0.0008$; Fig. 3d) between Mendenhall and Easton soils were significantly lower in the more advanced successional stages compared to the younger stages.

To examine trajectories of microbial succession in a broad context, we compared bacterial community composition in the recently deglaciated soils to that of mature forest soils (Lauber et al., 2009) – thought to represent an advanced successional stage for both glacial sites. A pairwise comparison of soil bacterial community composition indicated that average phylogenetic distance between deglaciated soils and the mature forest soil dataset was greater in young than in more developed glacial soils for both Easton ($P \leq 0.001$) and Mendenhall glaciers ($P \leq 0.001$; Fig. 4a, b). Further, NMDS ordination of weighted Unifrac distances showed strong compositional differences in bacterial communities among mature and glacial soils along the NMDS2 axis (Fig. 5a). Vector fitting suggested that bacterial community differences were highly correlated to discriminative shifts in *Acidobacteria*, a relatively abundant member of mature forest soils that was present in low abundances in young glacial soils, whereas the opposite was true

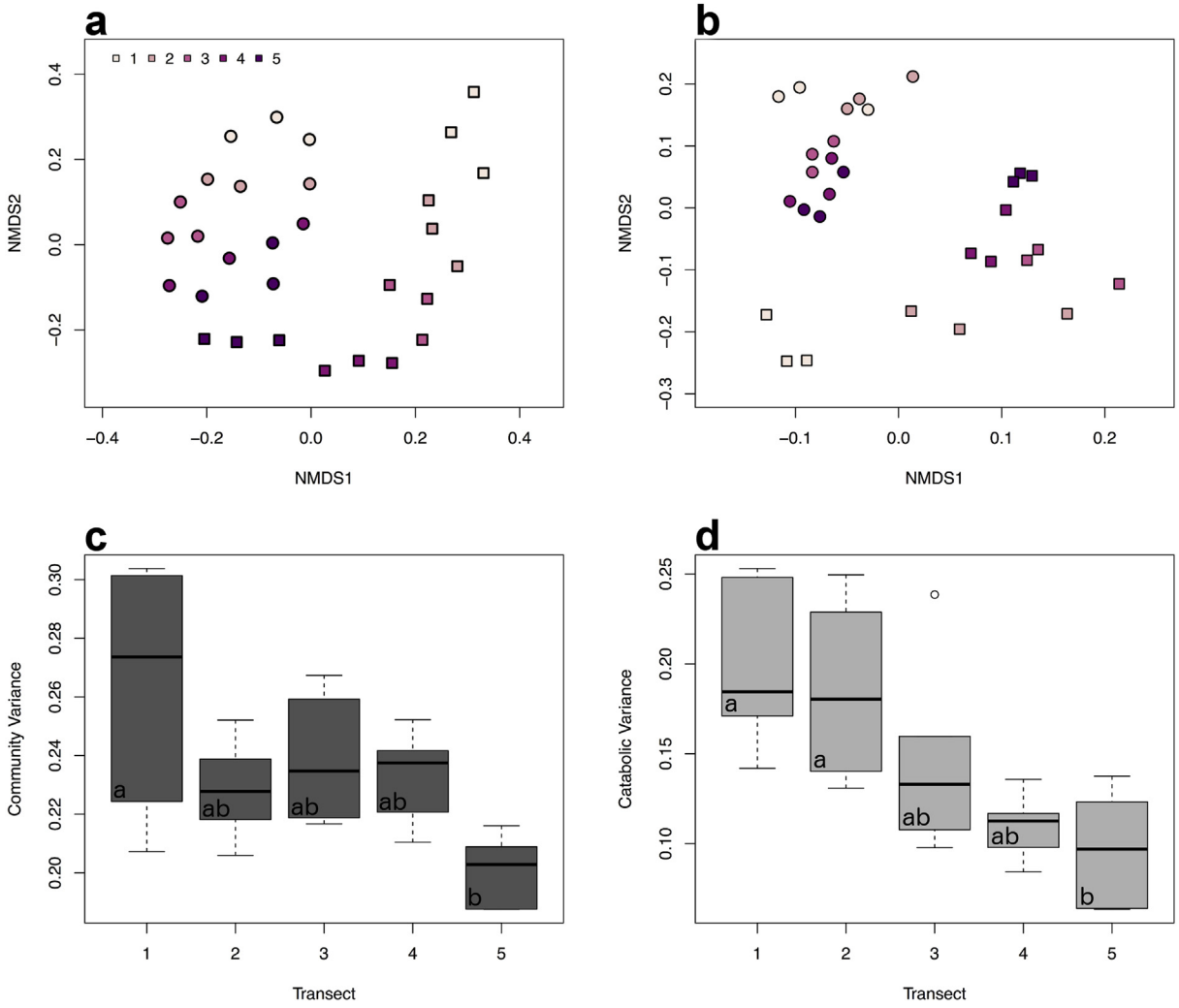

Fig. 3. Non-metric multidimensional scaling of (a) bacterial community structure based on weighted UniFrac distances and (b) decomposer catabolic function based on log transformed Euclidean distances. Clustering of points and permutational MANOVA's indicated that community structure ($R^2 = 0.18$, $P \leq 0.001$) and catabolic function ($R^2 = 0.47$; $P \leq 0.001$) were significantly different between Easton Glacier (squares) and Mendenhall Glacier (circles). Successional stage is indicated by color: 1 (0–1 year old soil), 2 (5 year old soil), 3 (biological soil crust cover), 4 (25–50% plant cover), and 5 (50–75% plant cover). Median based β-dispersion tests indicated that (c) variance in community structure (weighted UniFrac; $P = 0.003$) and (d) variance in catabolic function (Euclidean distance; $P = 0.0008$) were significantly different for early- and late succession soil communities. Letter in panels (c) and (d) represent significant differences in variances across successional stages based on one-way ANOVAs and pairwise Tukey tests adjusted for multiple comparisons. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for *Betaproteobacteria* (Fig. 5a; Table S1). Though the dominance of some taxa may reflect the shallow sequence depths at which samples were compared (rarefaction = 108 sequences per sample), our results suggest that successional convergence occurred for the developing bacterial communities of Easton and Mendenhall glaciers – but also as glacial communities developed, they became increasingly similar to communities in the mature soil dataset. Convergence during succession has been reported for soil bacteria within a single deglaciating site (Brown and Jumpponen, 2014), and results from this study suggest that soil microbial community composition and function may converge even across biogeochemically disparate sites.

3.4. Biogeochemical correlates of microbial structure and function

Despite general differences in the ways in which environmental characteristics developed through time between Easton and Mendenhall Glaciers (Fig. 1), we expected that relationships between soil properties and soil communities at the two sites would be consistent through succession. Contrary to our expectation, the strength and significance of relationships (permutational MANOVAs) among soil properties and community attributes varied between the two glacial sites (Table 1). When each site was assessed independently, we found few relationships that were consistently significant for both glacial sites: soil organic C correlated with community composition and total soil N correlated with catabolic function. As well, an index of soil C chemistry was consistently correlated with bacterial composition and catabolic function for both glacial sites (Table 1). By contrast, soil pH, a known driver of soil microbial communities (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010), was the strongest correlate of bacterial community composition and catabolic function for Easton Glacier, for the two glacial sites combined, but was not a significant correlate for Mendenhall soils. This result reflects progressive increases in soil pH at Easton Glacier through succession (Fig. 1) and the overall differences in soil pH values between the two glacial sites (Fig. 1; Fig. 5b). Had soil pH at Mendenhall Glacier changed appreciably with time, we may have expected similarly significant

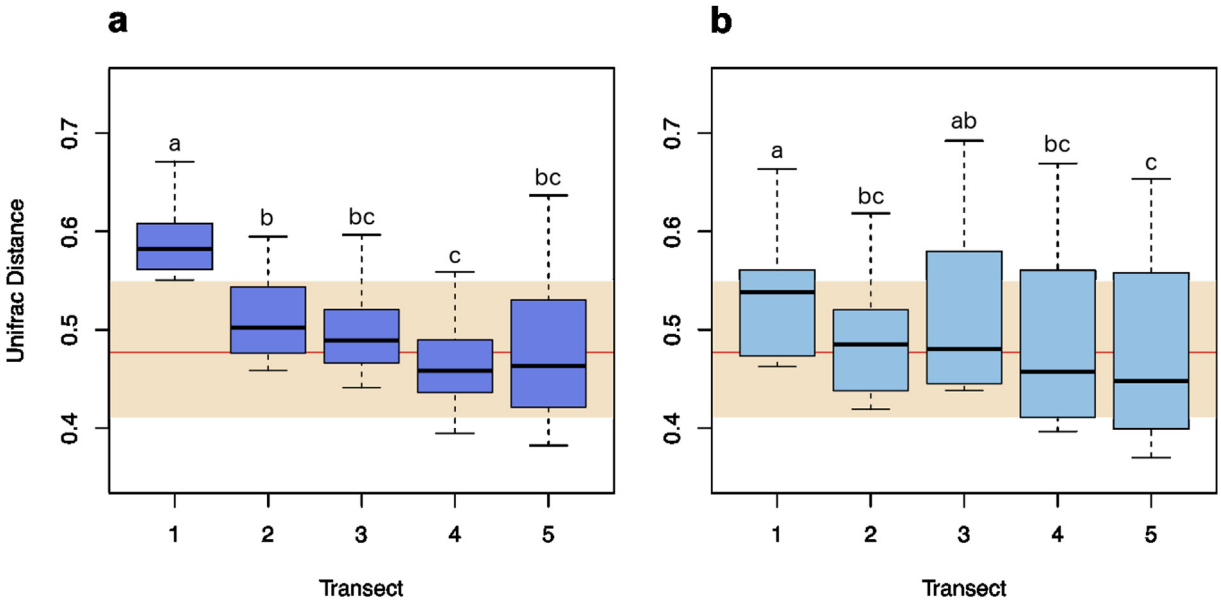

Fig. 4. Pairwise weighted UniFrac distances between glacial soils (this study) and mature forest soils (Lauber et al., 2009). Average UniFrac distance of soil bacterial communities decreases with successional age for (a) Easton Glacier ($P < 0.0001$) and (b) Mendenhall Glacier ($P < 0.0001$). The solid red line indicates the mean weighted UniFrac distance between different mature forest soils with 95% confidence intervals (orange band). Stages of succession are indicated by transect numbers: 1 (0–1 year old soil), 2 (5 year old soil), 3 (biological soil crust cover), 4 (25–50% plant cover), and 5 (50–75% plant cover). Different letters indicate significant differences among successional stages based on one-way ANOVAs and pairwise Tukey tests adjusted for multiple comparisons.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

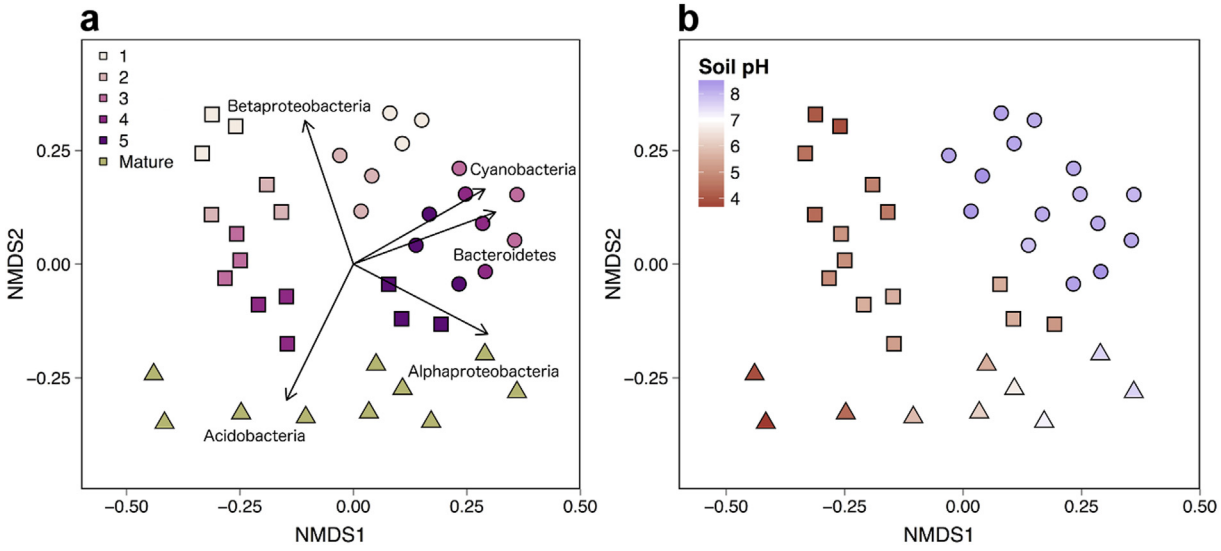

Fig. 5. Non-metric multidimensional scaling (NMDS) of bacterial community structure (Unifrac) for the Easton Glacier (squares), Mendenhall Glacier (circles), and mature forest soils (triangles; Lauber et al., 2009). For (a) colors indicate different successional stages. Stages of succession are indicated by transect numbers: 1 (0–1 year old soil), 2 (5 year old soil), 3 (biological soil crust cover), 4 (25–50% plant cover), and 5 (50–75% plant cover). Vectors show relative abundances of phyla and sub-phyla that were significantly correlated with the first two NMDS dimensions. For (b) colors indicate soil pH values.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

correlations for this site as well. However, in light of subtle pH differences (Fig. 1), the strong evidence for compositional and functional succession at Mendenhall Glacier (Fig. 3) indicates the importance of alternate factors in microbial successional dynamics for this site. Finally, when mature forest soils were examined alone and in combination with developing glacial soils, soil C chemistry (in addition to pH and organic C pool size) correlated with microbial community composition (Table S2).

In support of the MANOVA results (Table 1), we observed that compositional differences in bacterial communities between glacial sites and within mature forest soils, occurring along the NMDS1 axis of ordinations (Fig. 5a), paralleled overall differences in soil pH across sites (Fig. 5b). By contrast, within-site successional changes and broad differences among the glacial and mature forest soil communities occurred along the NMDS2 axis of ordinations (Fig. 5a), which appeared to relate to soil characteristics including organic C content and the relative abundances of different soil C functional classes (Fig. S4). Finally, Mendenhall Glacier's

Table 1
Bacterial community structure and catabolic function Spearman's rank correlations (ρ values) with soil biogeochemical characteristics for Easton and Mendenhall Glaciers.

	Easton (n = 15)		Mendenhall (n = 15)		Combined sites (n = 30)	
	ρ	*P*	*ρ*	*P*	*ρ*	*P*
Community Structure						
NH_4^+	0.20	0.068	0.13	0.14	**0.27**	**0.0008**
NO_3^-	**0.27**	**0.044**	−0.24	0.96	−0.12	0.90
PO_4^{3-}	0.15	0.11	**0.25**	**0.019**	0.41	**<0.0001**
Organic C	**0.48**	**0.002**	**0.21**	**0.032**	**0.54**	**<0.0001**
Total N	**0.47**	**0.003**	−0.004	0.51	**0.22**	**0.011**
pH	**0.67**	**<0.0001**	−0.016	0.53	**0.66**	**<0.0001**
Carbon Chemistry	**0.42**	**0.0014**	**0.21**	**0.045**	**0.32**	**<0.0001**
Catabolic Function						
NH_4^+	0.14	0.15	**0.31**	**0.013**	**0.36**	**0.0003**
NO_3^-	0.20	0.096	−0.09	0.72	−0.10	0.87
PO_4^{3-}	−0.019	0.52	**0.24**	**0.032**	0.59	**<0.0001**
Organic C	**0.39**	**0.008**	0.07	0.23	**0.57**	**<0.0001**
Total N	**0.37**	**0.011**	**0.36**	**0.007**	0.12	0.06
pH	**0.68**	**<0.0001**	0.19	0.084	**0.81**	**<0.0001**
Carbon Chemistry	**0.44**	**0.0013**	**0.21**	**0.04**	**0.29**	**0.0006**

Mantel tests produced Spearman's rank correlations (ρ) and *P* values using weighted UniFrac distances for community structure, Bray-Curtis dissimilarities for community function and carbon chemistry, and Euclidean distances for all other soil characteristics. Significant correlations are shown in bold.

consistently high soil pH supported high bacterial alpha diversity relative to Easton Glacier (Table S1). This finding corroborates previous research showing a strong positive relationship between bacterial alpha diversity and soil pH (Lauber et al., 2009; Fierer and Jackson, 2006).

3.5. Mechanisms of microbial convergence

Taken together, our data suggest that the factors shaping microbial community assembly during primary succession change through time. First, soil pH appeared to play a strong role in structuring differences in community composition and diversity across glacial sites and among the mature forest soils (Fig. 5b; Table 1; Table S2). Yet, within glacial sites and through time, factors including the size and chemistry of the soil organic C pools were closely and consistently correlated with bacterial structure and decomposer function (Fig. S4; Table 1; Table S2). One possible explanation for the simultaneous convergence of soil C chemistry (Fig. 2) and soil microbial communities among both glacial (Fig. 3) and mature soils (Fig. 5a) is that contributions of contemporary plant-derived C compounds increased through ecosystem development. In other words, plant community colonization contributes to shifts in soil C chemistry that support a more metabolically active and diverse microbial community (Zak et al., 2003; Orwin et al., 2008). Increased microbial processing of litter-derived organic matter, in turn, likely enhances soil C concentrations and chemical complexity (Wickings et al., 2012) contributing to further shifts in the relative distribution of C compound types (Grandy and Neff, 2008; Cotrufo et al., 2013). For example, proteins and polysaccharides, two classes of soil compounds that are often microbially-derived (Kiem and Kögel-Knabner, 2003; Grandy et al., 2007), were relatively more abundant in older glacial and mature forest soils (Fig. S2). Such changes in soil C chemistry may increase niche diversity and offers a mechanism for feedbacks between microbes and soil C that enhance microbial diversity and may lead to convergence through succession. Thus, while studies have suggested the importance of microbes in structuring plant communities during succession (Schmidt et al., 2008), our results point to the importance of plant colonization and subsequent plant-microbe interactions in structuring soil C abundance, chemistry and ultimately soil microbial communities in developing ecosystems.

Soil bacterial community composition was strongly related to catabolic potential – a functional measure of the entire decomposer community – at Easton (ρ value: 0.77, $P < 0.0001$) and Mendenhall Glaciers (ρ value: 0.44, $P = 0.0004$). The strong structure-function relationship was somewhat surprising given that our analysis excluded multiple domains of life, namely archaea and fungi, which contribute to decomposition in soils and are known to undergo successional shifts in developing glacial systems (Zumsteg et al., 2012). Previous research in proglacial soils has demonstrated that over short successional gradients, microbial communities transition from bacterial to fungal dominance as SOM accumulates (Ohtonen et al., 1999; Bardgett et al., 2007). While such a shift in dominance may cause bacteria to progressively fill non-primary decomposer niches, these results provide support for the idea that during succession, bacterial communities appear to assemble based on selection processes, in part, related to soil C resources. Finally, because these sites were relatively similar in terms of their successional endpoints (i.e., conifer forests) the quality and chemistry of litter inputs to decomposer communities may have contributed to the patterns observed here. In order to verify if plant communities do indeed play a strong role in structuring microbial communities through SOM chemistry, further work in other locations would be required.

Overall, our results are consistent with the recent theoretical framework presented by Schimel and Schaeffer (2012) who posited that interactions between microbial communities and soil C strengthen as SOM content increases. Here, we observed the dual convergence of both bacterial community structure and SOM chemistry through time, indicating a stronger relationship between structure and catabolic function in soils with higher organic matter content (i.e., more well-developed soils). Our results suggest that while soil pH may structure soil communities across broad gradients, soil C chemistry may have consistent stabilizing effects on microbial community structure and catabolic function during the early stages of soil development (Wardle, 1998). Our research sheds light on factors contributing to microbial community assembly in developing primary successional systems, but such results may also enhance our ability to predict how microbial communities will respond to and recover from disturbance or environmental change (Devries and Shade, 2013).

Statement of authorship

SC, CC, and JL designed the experiment. SC and JL performed phylogenetic and functional analyses. SG and KW performed soil carbon chemistry analyses. SC conducted statistical analyses. SC, CC, and DN wrote the first draft of the manuscript and all authors contributed to revisions.

Acknowledgements

We thank Noah Fierer and Chris Lauber for providing us with soil samples and Sean O'Neill for assistance with molecular sample processing. We thank Sasha Reed and several anonymous reviewers for their comments on an earlier version of this manuscript. This project was funded the National Science Foundation (NSF DEB-0922306). E.B.G was supported by the Microbiomes in Transition (MinT) Initiative at Pacific Northwest National Laboratory, operated by Battelle for the U.S. Department of Energy (DE-AC05-76RL01830).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.07.010.

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