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2   Full Title: Biogeochemical drivers of microbial community convergence across actively
3   retreating glaciers
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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 1,300 kilometers. 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.

67　1. INTRODUCTION
68
69　The ecological processes that contribute to biogeographic patterns of organisms and

70　communities are actively debated (Vellend 2010), particularly for microorganisms

71　(Martiny et al. 2006; Burke et al. 2011; Martiny et al. 2011; Nemergut et al. 2013).

72　Recent studies have demonstrated that microbial communities are selected for across

73　different habitat types (Lozupone and Knight 2007; Nemergut et al. 2011), and within

74　one habitat type – soil – researchers have pointed to the importance of deterministic

75　drivers such as contemporary environmental conditions (Hanson et al. 2012) to explain

76　the distribution of microorganisms.

77　　　At the regional to global scale, much of our understanding of the relationship

78　between microbial community structure and environmental factors comes from studies

79　conducted at a single point in time. For example, soil pH has been shown to strongly

80　correlate with soil bacterial community composition across moderate to large pH ranges

81　(Fierer and Jackson 2006; Lauber et al. 2009; Rousk et al. 2010). Yet in many locations,

82　current environmental conditions differ from the historical conditions that may have

83　contributed to microbial community assembly (Hanson et al. 2012), and assemblages

84　likely reflect the combined influences of historic (Hawkes and Keitt 2015) and

85　contemporary conditions. Soil development, which is influenced by the activity of soil

86　microorganisms, takes place over decades to millennia, precluding simultaneous

87　measurement of microbial dynamics and evolving ecosystem properties. To overcome

88　this issue and resolve the mechanisms driving community assembly in concert with

89　changing soil properties, plant ecologists have used space-for-time substitution

90　approaches to indirectly examine the process of ecological succession – the progressive

91 replacement of organisms following major disturbances that reset ecosystem

92 development (Chapin et al. 1994; Walker and del Moral 2003). More recently soil

93 chronosequences, like those that develop in proglacial systems, have been used to

94 identify the processes and mechanisms of microbial successional change (e.g.,

95 Jumpponen 2003; Kandeler et al. 2006; Nemergut et al. 2007; Schmidt et al. 2008; Sattin

96 et al. 2009; Carlson et al. 2010; Jumpponen et al. 2012; Knelman et al. 2012; Brown and

97 Jumpponen 2013; Jangid et al. 2013; Knelman et al. 2014).

98   For microbial communities undergoing primary succession, key questions remain

99 as to whether community assembly processes occur in predictable ways across different

100 sites. That is to say, do early successional microbial communities start out with the same

101 community members conducting the same functions and further, do communities follow

102 the same or different successional trajectories through ecosystem development?

103 Comparative studies have shown that the trajectories of plant primary succession can

104 vary, culminating in compositionally divergent, convergent, or parallel development of

105 plant communities (Fig. S1; Samuels and Drake 1997; Walker and del Moral 2003).

106 Convergent succession may reflect strong selective processes such as environmental

107 filtering and species interactions that cause initially different communities to become

108 more similar through time (Samuels and Drake 1997; Walker and del Moral 2003) in

109 terms of growth form (Nilsson and Wilson 1991), species traits (Wilson and Whittaker

110 1995; Fukami et al. 2005; Raevel et al. 2012; Helsen et al. 2013), and less commonly

111 community composition (Raevel et al. 2013). By contrast, divergent succession reflects

112 the importance of stochastic events, priority effects, dispersal barriers, and/or

113 environmental filters that increase in strength or change over time; such effects cause

114   initially similar communities to become different with time (McCune and Allen 1985;

115   Walker and del Moral 2003; Fukami et al. 2005). In addition, mixed patterns of

116   convergence and divergence have been observed (Inouye and Tilman 1995). For example

117   several studies have found instances where species composition diverged, while life

118   history and functional traits converged (Fukami et al. 2005; Helsen et al. 2013). Finally,

119   persistently different environmental conditions and/or early stochastic events may

120   preclude divergence or convergence, resulting in parallel successional trajectories in

121   which initial plant community differences also persist through time (Walker and del

122   Moral 2003). Given the high abundance, high rates of dispersal, short generation times,

123   and the immense phylogenetic and physiological diversity of microorganisms, the

124   processes influencing microbial succession may operate in fundamentally different ways

125   than those influencing plant succession (Fierer et al. 2010; Nemergut et al. 2013). Yet,

126   successional patterns are still relatively unknown for soil microorganisms (Fierer et al.

127   2010), and few studies have attempted analogous comparisons across multiple primary

128   succession gradients (c.f. Lazzaro et al. 2009).

129   Here, we used a space-for-time approach (Walker et al. 2010) to compare soil

130   bacterial community development in two actively developing glacial forefields separated

131   by a distance of more than 1,300 kilometers: Easton Glacier, WA, USA and Mendenhall

132   Glacier, AK, USA. Since the processes shaping biological succession may result in

133   different patterns of compositional and functional development (Fukami et al. 2005), we

134   assessed both phylogenetic and metabolic characteristics of microbial communities. Next,

135   in a comparative analysis, we examined the successional trajectories of developing

136   glacial communities in light of a group of mature forest soils (Lauber et al. 2009) –

137   thought to represent an advanced stage of succession for both sites. Given the strong

138   dependence of microbial communities on soil characteristics, we hypothesized that

139   initially unique soil properties across sites (i.e., soil pH) would contribute to differences

140   in microbial community composition (H1). However, we also hypothesized that the

141   processes of ecosystem development and plant colonization would influence soil

142   properties in predictable ways across sites – altering soil pH, increasing carbon (C) and

143   nitrogen (N) – and thus would promote similarity among soil bacterial communities

144   through time (H2). Specifically, we hypothesized that plant colonization would lead to an

145   increase in the abundance and chemical diversity of organic C resources in soil, which

146   would support a more compositionally and functionally diverse soil decomposer

147   community (H3).

148

149   2. MATERIALS AND METHODS

150   **2.1. Study sites and soil collection**

151   Easton Glacier is located on the southern flank of Mt. Baker in the Mt. Baker-Snoqualmie

152   National Forest, Washington USA (48°44 N, 121°50 E; 1,637 m.a.s.l.). Easton

153   experiences 198 cm of precipitation annually (1984-2009 mean, NADP Site WA19) and

154   temperatures that span 3.9 to 13.6°C (1961-1990 mean annual minimum/maximum

155   temperature, Western Regional Climate Center ID 458715). The dominant parent

156   material at Easton Glacier is volcanic andesite (Tabor et al. 2003). The Mendenhall

157   Glacier is located in the Tongass National Forest of southeast Alaska, USA (58°26 N,

158   134°33 E; 75 m.a.s.l.) and is characterized as a perhumid maritime climate with 153 cm

159   of precipitation annually (2004-2009 mean, NADP Site AK02) and temperatures ranging

160   from -1°C to 8°C (1965-1980 mean annual minimum/max temperature, Western

161   Regional Climate Center ID 504110). The dominant parent material at the Mendenhall

162   Glacier is metamorphic, composed of plagioclase, quartz and hornblende (Alexander and

163   Burt 1996). Historical terminus locations have been well documented for both sites

164   (Harper 1993; Motyka et al. 2003; Pelto and Brown 2012).

165   At each site, we established five transects of different successional stages parallel

166   to the glacier terminus. In order to account for different rates of glacial retreat across sites,

167   transect locations were selected on the basis of time-since-retreat and percent plant cover.

168   Specifically, plant colonization occurs rapidly at Mendenhall Glacier and we observed

169   sparse plant cover (< 5%) occurring in substrates that were only ~5 years old. By contrast,

170   at Easton Glacier the first signs of plant colonization were observed in ~25 year old

171   substrates (personal observation). At each site, transect 1 was nearest to the glacial

172   terminus and was characterized by newly exposed, disorganized glacial till that was ~0-1

173   year in age, transect 2 represented unvegetated till ~5 years old, transect 3 included some

174   element of soil crusting (lichens, mosses, cyanobacterial crust) and sparse vegetation

175   (average < 5%), transect 4 represented ~25-50% patchy plant cover, and transect 5 had

176   50-75% semi-continuous plant cover. Soil crust and percent plant cover were assessed

177   visually. Plant species composition differed between the glacial chronosequences

178   (Alexander and Burt 1996; Whelan 2013), though, successional endpoints for both

179   gradients included coniferous tree species. At the Easton Glacier, the initial plant

180   communities of transect 3 were composed of mosses, grasses, rushes, and forbs. Plant

181   cover at transect 4 was composed of moss, grasses, rushes, forbs, including heather

182   (*Phyllodoce empetriformis*), Tolmie saxifrage (*Micranthes tolmiei*), partridge foot

183   (*Luetkea pectinata*), pearly everlasting (*Anaphalis margaritacea*), as well as small

184   Mountain Hemlock (*Tsuga mertensiana*) saplings. Later plant communities (transect 5)

185   also included abundant Lupine (*Lupinus latifolius*), a putative nitrogen-fixing plant

186   species, and larger *T. mertensiana*. At the Mendenhall Glacier, initial plant communities

187   of transect 3 included mosses and grasses. Plant cover at transect 4 included moss,

188   grasses, fireweed (Epilobium spp.), small nitrogen-fixing (*Alnus sinuata*) and non

189   nitrogen-fixing shrubs (Populus spp., Salix spp.), and small Sitka spruce seedlings (*Picea*

190   *sitchensis*). More developed plant communities at Mendenhall Glacier included larger

191   shrubs and larger conifer saplings. Photoautotrophic microorganisms (i.e.*,* cyanobacteria)

192   were present in soils, but not abundant ($< 5\%$), thus the sites were categorized as

193   heterotrophic successional sequences (Fierer et al. 2010).

194   During the late summer and early fall of 2010, we sampled surface soils (0-5 cm)

195   at nine randomly selected points along each of the five transects at each site. Samples

196   were collected into polyethylene bags using a small trowel. The collection trowel was

197   sterilized with ethanol and wiped with paper towels between every contact with the soil.

198   Following collection, samples were transported on ice to the Terrestrial Ecosystem

199   Ecology Laboratory at the University of Montana, Missoula, MT, USA. The nine

200   individual samples from each transect were randomly assigned to three groups each

201   containing three samples. Individual samples were mixed at equal masses to create the

202   three distinct pooled samples per transect. Prior to processing, each pooled sample was

203   homogenized and hand-sieved of rocks and roots (4mm $\times$ 4mm) while taking precautions

204   to prevent between-sample contamination. Soil subsamples were frozen for molecular

205   analysis (-80°C). The catabolic response profiling experiment was conducted

206 immediately with fresh soil. Remaining soils were dried at 60°C and pulverized for C

207 chemistry, total C, and total N, dried at 105°C for gravimetric water content analysis, and

208 were air-dried for soil pH measurements.

209

210 **2.2 Mature sample acquisition and processing**

211 To examine microbial community succession and convergence in a broad context,

212 we compared recently deglaciated soils to a small previously published dataset of mature

213 forest soils (Lauber et al. 2009) – thought to represent an advanced stage of plant

214 succession for Easton and Mendenhall Glaciers. From the authors of the Lauber et al.

215 (2009) study, we obtained archived surface soil samples (0-5 cm) collected from conifer

216 and mixed forest sites across North America (hereafter referred to as the 'mature soil

217 dataset'). Of the 33 forest biome soils initially included in Lauber et al. 2009 study, we

218 selected ten samples representing a range of climatic and soil chemical characteristics,

219 including pH. Frozen samples were sent on dry ice to the Soil Biogeochemistry and

220 Fertility Lab at the University of New Hampshire where they were lyophilized and

221 ground in preparation for C chemistry analysis. Prior to our analyses, samples had been

222 continuously frozen (-80°C) since the time of initial processing.

223

224 **2.3. DNA extraction from glacial soil**

225 DNA was extracted from soil samples following standard manufacturers protocols for

226 bulk DNA extractions using the Mo Bio PowerSoil^{TM} kit (Mo Bio Laboratories, Inc.,

227 Carlsbad, USA). A modified lysis buffer solution (2mL 1M EDTA, 5mL 1M $NaHPO_4$,

228 1mL 1M TrisHCl, 1% SDS, 1.9mL Ultra Pure DI H_2O) was used to increase DNA

229 extraction efficiencies from the volcanic Easton Glacier soils and the modification was

230 used for Mendenhall Glacier soils as well.

231

232 **2.4. 16S rRNA gene sequence analyses of glacial bacterial communities**

233 DNA was processed as previously described in Nemergut and others (2010). PCR

234 amplification of bacterial 16S rRNA genes from genomic DNA was conducted using a

235 universal bacterial 27F and 338R primer set (Hamady et al. 2008). PCR reactions for

236 each sample were performed in triplicate using conditions described by Fierer et al.

237 (2008). PCR products were pooled per sample and cleaned with Mo Bio UltraClean-htp

238 PCR Clean-up kits (Mo Bio Laboratories, Inc., Carlsbad, CA). Pyrosequencing of gene

239 amplicons was completed by 454 Life Sciences (Bradford, USA).

240 We performed sequence processing using the QIIME pipeline (Caporaso et al.

241 2010) and, as was done in Leff et al. (2011), sequences were not denoised. A total of

242 24,523 sequences were retained for analysis after processing and quality filtering.

243 Operational taxonomic units (OTUs) were clustered at a 97% sequence similarity level

244 using UCLUST (Edgar 2010). Chloroplast and mitochondria sequences were removed.

245 To equalize sequence numbers in each sample, we used QIIME's single rarefaction script

246 to rarefy to 108 sequences per sample. After rarefaction our dataset contained 1948

247 unique OTUs in 30 samples. We note that such low numbers of 16S rRNA gene

248 sequences have been similarly reported for other primary successional soils (Nemergut et

249 al. 2007; Sattin et al. 2009; Knelman et al. 2012; Knelman et al. 2014). We assigned

250 taxonomic identity using the RDP classifier against the Greengenes coreset database in

251 QIIME. To calculate pairwise distances between samples, we calculated weighted

252 UniFrac distance (Lozupone and Knight 2007), which accounts for phylogeny. We

253 examined patterns of bacterial community change with succession at high taxonomic

254 ranks (phyla and sub-phyla level), as organisms within phyla may share common life

255 history strategies (Fierer et al. 2007). For all taxon-based analyses, we removed groups

256 that comprised less than 1% of the community for both sites. Alpha diversity is presented

257 as Shannon (H'), richness (observed OTUs), and phylogenetic diversity. Raw sequence

258 data are available via Figshare (10.6084/m9.figshare.3384694).

259

260 **2.5. Mature soil sequence data retrieval and analyses**

261 For the ten mature forest samples analyzed for soil C chemistry, we downloaded

262 corresponding quality filtered bacterial 16S rRNA gene sequences and soil metadata

263 originally presented in Lauber et al. (2009) from the publically available QIIME database

264 (now hosted on the Qiita database; http://qiita.ucsd.edu). To compare glacial soils to the

265 mature dataset, pre-processed sequences from the QIIME database and sequences pre-

266 processed in-house were combined for downstream analysis as described above including

267 rarefying all samples to 108 sequences. Sequences from glacial soils were generated

268 using the same primers and molecular protocols used by Lauber et al. (2009).

269

270 **2.6. Catabolic response profiling of soil decomposers**

271 We assessed the functional potential of soil heterotrophic communities using short-term

272 respiration responses of microbial communities to 24 organic C substrates (Degens and

273 Harris 1997). Substrates originated from eight different C chemical classes and included

274 two amines (L-glutamine, urea), five amino acids (glycine, histidine, L-asparagine, L-

275 glutamic acid, glucosamine), three carbohydrates (fructose, glucose, sucrose), five

276 carboxylic acids (citric acid, DL-lactic acid, fumaric acid, pentadecanoic acid, α-

277 ketoglutaric acid), one nucleic acid (DNA), three polysaccharides (amylopectin, amylose,

278 glycogen), two proteins (BSA, casein), and several recalcitrant substrates (chitin, humic

279 acid, lignin). Rates of basal respiration were assessed using deionized water for each set

280 of samples. Carbon substrates were prepared to a concentration of 900 mM C in

281 deionized water and were adjusted to pH ~6 with HCl or NaOH. In 60 mL glass vials, we

282 added 4mL of C substrates individually to 4g field moist subsamples of each soil sample.

283 Vials were sealed with Teflon septa, briefly vortexed to slurry contents, and incubated at

284 20°C for 24 hours in total darkness on a slowly rotating orbital shaker. Following the

285 incubation period, vial headspace was mixed, sampled, and CO_2 concentrations were

286 measured by gas chromatography (Shimadzu GC 2014, Columbia, USA). CO_2 fluxes

287 were calculated on the basis of dry soil (μg CO_2-C g dry soil^{-1} hour^{-1}) and basal

288 respiration (water-only amended samples) was subtracted from substrate-amended

289 responses. Multivariate analyses were based on the calculation of the proportional

290 respiration response of each substrate relative to the cumulative response to all substrates

291 (Leff et al. 2012). Finally, we created an index of catabolic function for each sample by

292 calculating Euclidean distances based on proportional respiration responses to each

293 compound.

294

295 **2.7. Soil chemical characterization**

296 Available inorganic nitrogen (NH_4^+ and NO_3^-) was extracted using 2M KCl (Mulvaney

297 1994) and was measured colorimetrically (Weatherburn 1994; Doane and Horwath 2003)

298   using a microplate reader (Biotek, Winooski, USA). Microbial biomass C and N were

299   measured using a 0.5 M K_2SO_4 extraction on chloroform fumigated and unfumigated

300   samples (Horwath and Paul 1994) and were analyzed on a Shimadzu TOC-VCPN

301   (Shimadzu, Columbia, MD). Microbial biomass C and N were calculated as the

302   difference between fumigated and unfumigated samples. Total C and N were measured

303   on pulverized samples with a CHN elemental analyzer 1110 (CE Instruments, Wigan,

304   UK) and values were verified with an acetanilide standard (10.36% N; 71.09% C).

305   Inorganic C was assessed with a coulometer (UIC Inc., Joliet, IL) and we subtracted

306   inorganic C from total C to infer soil organic C for each sample. Soil pH was determined

307   using a digital pH meter (Fisher Scientific, Pittsburgh, USA) using a 1:2 ratio of soil to

308   water. All soil chemical properties are expressed in terms of soil dry mass.

309

310   **2.8. Soil organic carbon chemistry**

311   For glacial soils and ten mature forest soils, we assessed soil C chemistry using pyrolysis-

312   gas chromatography mass spectrometry (py-GCMS) following procedures outlined by

313   (Grandy et al. 2009). Dried and pulverized subsamples were pulse-pyrolyzed at 600°C on

314   a Pyroprobe 5150 (CDS Analytical, Inc, Oxford, USA). Pyrolyzed materials were

315   transferred to a GC (GC Ultra, Thermo Scientific, West Palm Beach, USA) for separation

316   and were quantified by a mass spectrometer (Polaris Q, Thermo Scientific, West Palm

317   Beach, USA). Compounds were identified using Automated Mass Spectral

318   Deconvolution and Identification System (AMDIS V 2.65) and peaks were verified using

319   the NIST mass spectral library (Grandy et al. 2007; Grandy and Neff 2008; Grandy et al.

320   2009). Additional peak identification was done using previously published compound

321 libraries (Stewart et al. 2011; Stewart et al. 2012). We calculated the relative abundances

322 of compounds as the peak area of each compound divided by the sum of peak areas of all

323 identified compounds and grouped C compounds by chemical class (e.g., lignin, lipid, N-

324 bearing compounds, phenolic, polysaccharide, protein, and compounds of unknown

325 origin).

326

327 **2.9. Statistical analyses**

328 Multivariate statistics were conducted using the packages Ecodist v.1.2.3 (Goslee and

329 Urban 2013) and Vegan v.2.0-5 (Oksanen et al. 2010) in R v.3.1.1 (The R Core Team

330 2014). Visualizations of community composition and catabolic function were done using

331 non-metric multidimensional scaling (NMDS) using the nmds function in R. We used

332 permutational vector fitting (999 permutations; Jongman et al. 1995) using a multiple

333 linear regression technique and the envfit function in R to assess relationships between

334 NMDS ordinations of community structure and the relative abundance of bacterial phyla

335 and subphyla. Using the same envfit function, we assessed relationships between NMDS

336 ordinations of soil C chemistry and the relative abundance of soil C functional classes.

337 Between-site differences in community composition, catabolic response, and soil C

338 chemistry were assessed using variance partitioning in non-parametric MANOVA's and

339 the adonis function (999 permutations). We assessed the homogeneity of variances of

340 bacterial communities by transect (β-dispersion) by conducting a multivariate Levene's

341 type test (Fierer et al. 2008) using a median betadisper function in R (Anderson et al.

342 2006) followed by a one-way ANOVA with transect as the grouping factor. The average

343 weighted UniFrac distance of bacterial communities was calculated on a pairwise basis

344   from each of the glacial samples to each of the samples in the mature forest dataset. For

345   each site, we tested for significant differences in average UniFrac distance with transect

346   as a grouping factor using one-way ANOVA. One-way ANOVA analyses were

347   performed to assess how catabolic evenness, community diversity metrics, and major

348   taxonomic groups changed through succession. Catabolic evenness was calculated using

349   the Simpson-Yule index approach, $\Sigma = 1/pi^2$, wherein pi is equal to the respiration

350   response to a given individual substrate divided by the total sum of respiration responses

351   to all substrates (Magurran, 2004). We conducted permutational MANOVAs to assess the

352   correlative relationship between bacterial community structure, catabolic function, and

353   soil properties using mantel tests with Spearman's rank correlations (10,000

354   permutations). The weighted UniFrac distance metric was used for community structure

355   analyses and Euclidean distance matrices were used for individual soil variables and

356   catabolic response profiles. An index of soil C chemistry was created for each sample by

357   calculating Bray-Curtis distances based on relative abundances of each C class (Leff et al.

358   2012). Prior to analyses, soil chemical data and catabolic response profiles were log

359   transformed and soil C chemistry was arcsine-square root transformed to meet the

360   assumptions of normality.

361

362   3. RESULTS AND DISCUSSION

363   **3.1. Soil chemical properties**

364   Many of the major state factors that contribute to soil formation (i.e., parent material,

365   climate, plant species, time; Amundson and Jenny 1997) differed between Easton and

366   Mendenhall Glaciers. As a result, key soil properties that frequently correlate with

367   microbial community structure (Fierer and Jackson 2006; Cleveland et al. 2007; Lauber

368   et al. 2009; Rousk et al. 2010; Ramirez et al. 2012) showed minimal overlap between

369   sites (Fig. 1). Initially and throughout succession, soil pH at Easton (range: 4.1 – 5.5) was

370   consistently lower than Mendenhall (range: 8.1 – 8.4). However, while Easton soils

371   incrementally accrued organic C through time (range: 0.4 – 10.4 mg g soil^{-1}), Mendenhall

372   soils were initially C-rich (4.0 mg g soil^{-1}), were C-poor in mid-succession (2.0 mg g soil^{-}

373   ^{1}), and accrued C into late succession (3.7 mg g soil^{-1}). Previous studies have attributed

374   Mendenhall Glacier's non-linear pattern of soil organic C accrual to the presence of

375   ancient woody debris (Sattin et al. 2009). Although soil N (total N, NH_4^+) generally

376   increased with time at both sites, N pools were as much as an order of magnitude larger at

377   Easton. By contrast, an index of soil C chemistry reflecting relative abundances of C

378   compound classes (i.e., aromatic, lignin, lipid, N-bearing, phenol, polysaccharide,

379   protein) was not significantly different between Easton and Mendenhall Glaciers

380   (PERMANOVA $r^2 = 0.076$, $P = 0.94$). Rather, at both sites, soil C chemistry was initially

381   similar at the onset of succession and followed a similar trajectory over time (Fig. 2a)

382   despite differences in total soil organic C content (Fig. 1) and plant species composition.

383   In fact, we observed strong differences in soil C chemistry between vegetated and

384   unvegetated soils regardless of site (PERMANOVA $r^2 = 0.19$, $P \leq 0.004$; Fig. 2a). Finally,

385   as young glacial soils developed, soil C chemistry converged with that of mature forest

386   soils (Fig. 2b; Fig. S2) collected from a large geographical range and diverse

387   environmental conditions (see Lauber et al. 2009). From young to mature soils, shifts in

388   soil C chemistry were associated with increasing relative abundances of N-bearing,

389   polysaccharide, and protein based compounds (Fig. 2b; Fig. S2).

390

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; 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; 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.

413   Consistent with phylogenetic data, community function (catabolic potential of

414 bacterial and fungal decomposers) was significantly different across the two glacial sites

415 (PERMANOVA $r^2 = 0.47$, $P \leq 0.001$; Fig. 3b). Further, within each site, catabolic

416 function changed significantly through succession (PERMANOVA Easton: $r^2 = 0.51$, $P \leq$

417 0.001; Mendenhall: $r^2 = 0.59$, $P \leq 0.001$; Fig. 3b, Fig. S3c, d). Specifically, early

418 successional communities were able to utilize a limited number of compounds, while

419 decomposers of later successional stages were increasingly able to utilize a wide range of

420 compounds to a similar degree (Table S1; catabolic evenness, sensu Degens et al. 2001).

421

422 **3.3. Successional trajectories of microbial structure and function**

423 While early successional microbial communities from across the two glacial sites started

424 out with the relatively different community members (Fig. 3a) conducting different

425 functions (Fig. 3b), we observed that through time and with increasing soil development

426 community similarity increased. Specifically, variances in bacterial community structure

427 ($P = 0.003$; Fig. 3c) and decomposer function ($P = 0.0008$; Fig. 3d) between Mendenhall

428 and Easton soils were significantly lower in the more advanced successional stages

429 compared to the younger stages.

430   To examine trajectories of microbial succession in a broad context, we compared

431 bacterial community composition in the recently deglaciated soils to that of mature forest

432 soils (Lauber et al. 2009) – thought to represent an advanced successional stage for both

433 glacial sites. A pairwise comparison of soil bacterial community composition indicated

434 that average phylogenetic distance between deglaciated soils and the mature forest soil

435 dataset was greater in young than in more developed glacial soils for both Easton ($P \leq$

436 0.001) and Mendenhall glaciers ($P \leq 0.001$; Fig. 4a, b). Further, NMDS ordination of

437 weighted Unifrac distances showed strong compositional differences in bacterial

438 communities among mature and glacial soils along the NMDS2 axis (Fig. 5a). Vector

439 fitting suggested that bacterial community differences were highly correlated to

440 discriminative shifts in *Acidobacteria*, a relatively abundant member of mature forest

441 soils that was present in low abundances in young glacial soils, whereas the opposite was

442 true for *Betaproteobacteria* (Fig. 5a; Table S1). Though the dominance of some taxa may

443 reflect the shallow sequence depths at which samples were compared (rarefaction = 108

444 sequences per sample), our results suggest that successional convergence occurred for the

445 developing bacterial communities of Easton and Mendenhall glaciers – but also as glacial

446 communities developed, they became increasingly similar to communities in the mature

447 soil dataset. Convergence during succession has been reported for soil bacteria within a

448 single deglaciating site (Brown and Jumpponen 2014), and results from this study suggest

449 that soil microbial community composition and function may converge even across

450 biogeochemically disparate sites.

451

452 **3.4. Biogeochemical correlates of microbial structure and function**

453 Despite general differences in the ways in which environmental characteristics developed

454 through time between Easton and Mendenhall Glaciers (Fig. 1), we expected that

455 relationships between soil properties and soil communities at the two sites would be

456 consistent through succession. Contrary to our expectation, the strength and significance

457 of relationships (permutational MANOVAs) among soil properties and community

458 attributes varied between the two glacial sites (Table 1). When each site was assessed

459   independently, we found few relationships that were consistently significant for both

460   glacial sites: soil organic C correlated with community composition and total soil N

461   correlated with catabolic function. As well, an index of soil C chemistry was consistently

462   correlated with bacterial composition and catabolic function for both glacial sites (Table

463   1). By contrast, soil pH, a known driver of soil microbial communities (Fierer and

464   Jackson 2006; Lauber et al. 2009; Rousk et al. 2010), was the strongest correlate of

465   bacterial community composition and catabolic function for Easton Glacier, for the two

466   glacial sites combined, but was not a significant correlate for Mendenhall soils. This

467   result reflects progressive increases in soil pH at Easton Glacier through succession (Fig.

468   1) and the overall differences in soil pH values between the two glacial sites (Fig. 1; Fig.

469   5b). Had soil pH at Mendenhall Glacier changed appreciably with time, we may have

470   expected similarly significant correlations for this site as well. However, in light of subtle

471   pH differences (Fig. 1), the strong evidence for compositional and functional succession

472   at Mendenhall Glacier (Fig. 3) indicates the importance of alternate factors in microbial

473   successional dynamics for this site. Finally, when mature forest soils were examined

474   alone and in combination with developing glacial soils, soil C chemistry (in addition to

475   pH and organic C pool size) correlated with microbial community composition (Table

476   S2).

477   In support of the MANOVA results (Table 1), we observed that compositional

478   differences in bacterial communities between glacial sites and within mature forest soils,

479   occurring along the NMDS1 axis of ordinations (Fig. 5a), paralleled overall differences in

480   soil pH across sites (Fig. 5b). By contrast, within-site successional changes and broad

481   differences among the glacial and mature forest soil communities occurred along the

482 NMDS2 axis of ordinations (Fig. 5a), which appeared to relate to soil characteristics

483 including organic C content and the relative abundances of different soil C functional

484 classes (Fig. S4). Finally, Mendenhall Glacier's consistently high soil pH supported high

485 bacterial alpha diversity relative to Easton Glacier (Table S1). This finding corroborates

486 previous research showing a strong positive relationship between bacterial alpha diversity

487 and soil pH (Lauber et al. 2009; Fierer and Jackson 2006).

488

489 **3.5. Mechanisms of microbial convergence**

490 Taken together, our data suggest that the factors shaping microbial community assembly

491 during primary succession change through time. First, soil pH appeared to play a strong

492 role in structuring differences in community composition and diversity across glacial

493 sites and among the mature forest soils (Fig. 5b; Table 1; Table S2). Yet, within glacial

494 sites and through time, factors including the size and chemistry of the soil organic C

495 pools were closely and consistently correlated with bacterial structure and decomposer

496 function (Fig. S4; Table 1; Table S2). One possible explanation for the simultaneous

497 convergence of soil C chemistry (Fig. 2) and soil microbial communities among both

498 glacial (Fig. 3) and mature soils (Fig. 5a) is that contributions of contemporary plant-

499 derived C compounds increased through ecosystem development. In other words, plant

500 community colonization contributes to shifts in soil C chemistry that support a more

501 metabolically active and diverse microbial community (Zak et al. 2003, Orwin et al.

502 2008). Increased microbial processing of litter-derived organic matter, in turn, likely

503 enhances soil C concentrations and chemical complexity (Wickings et al. 2012)

504 contributing to further shifts in the relative distribution of C compound types (Grandy

505   and Neff 2008, Cotrufo et al. 2013). For example, proteins and polysaccharides, two

506   classes of soil compounds that are often microbially-derived (Kiem and Kögel-Knabner

507   2003; Grandy et al. 2007), were relatively more abundant in older glacial and mature

508   forest soils (Fig. S2). Such changes in soil C chemistry may increase niche diversity and

509   offers a mechanism for feedbacks between microbes and soil C that enhance microbial

510   diversity and may lead to convergence through succession. Thus, while studies have

511   suggested the importance of microbes in structuring plant communities during succession

512   (Schmidt et al. 2008), our results point to the importance of plant colonization and

513   subsequent plant-microbe interactions in structuring soil C abundance, chemistry and

514   ultimately soil microbial communities in developing ecosystems.

515   Soil bacterial community composition was strongly related to catabolic potential –

516   a functional measure of the entire decomposer community – at Easton (ρ value: 0.77, $P <$

517   0.0001) and Mendenhall Glaciers (ρ value: 0.44, $P = 0.0004$). The strong structure-

518   function relationship was somewhat surprising given that our analysis excluded multiple

519   domains of life, namely archaea and fungi, which contribute to decomposition in soils

520   and are known to undergo successional shifts in developing glacial systems (Zumsteg et

521   al. 2012). Previous research in proglacial soils has demonstrated that over short

522   successional gradients, microbial communities transition from bacterial to fungal

523   dominance as SOM accumulates (Ohtonen et al. 1999; Bardgett et al. 2007). While such

524   a shift in dominance may cause bacteria to progressively fill non-primary decomposer

525   niches, these results provide support for the idea that during succession, bacterial

526   communities appear to assemble based on selection processes, in part, related to soil C

527   resources. Finally, because these sites were relatively similar in terms of their

528   successional endpoints (i.e., conifer forests) the quality and chemistry of litter inputs to

529   decomposer communities may have contributed to the patterns observed here. In order to

530   verify if plant communities do indeed play a strong role in structuring microbial

531   communities through SOM chemistry, further work in other locations would be required.

532   Overall, our results are consistent with the recent theoretical framework presented

533   by Schimel and Schaeffer (2012) who posited that interactions between microbial

534   communities and soil C strengthen as SOM content increases. Here, we observed the dual

535   convergence of both bacterial community structure and SOM chemistry through time,

536   indicating a stronger relationship between structure and catabolic function in soils with

537   higher organic matter content (i.e., more well-developed soils). Our results suggest that

538   while soil pH may structure soil communities across broad gradients, soil C chemistry

539   may have consistent stabilizing effects on microbial community structure and catabolic

540   function during the early stages of soil development (Wardle et al. 1998). Our research

541   sheds light on factors contributing to microbial community assembly in developing

542   primary successional systems, but such results may also enhance our ability to predict

543   how microbial communities will respond to and recover from disturbance or

544   environmental change (Devries and Shade 2013).

545

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860

FIGURE LEGENDS

862 **Fig. 1:** Soil biogeochemical characteristics of Easton and Mendenhall Glaciers. Boxplot

863 lines represent medians with 95% confidence intervals. Stages of succession are indicated

864 by numbers: 1 (0-1 year old soil), 2 (5 year old soil), 3 (biological soil crust cover), 4 (25-

865 50% plant cover), and 5 (50-75% plant cover). Soil NH_4^+-N, NO_3^--N, available P,

866 microbial biomass C (MB-C), and microbial biomass N (MB-N) are presented as µg g

867 soil^{-1}. Total N and organic C are presented as mg g soil^{-1}. Soil pH was determined using a

868 1:2 ratio of soil to water. For each transect (n = 3).

869

870 **Fig. 2:** Non-metric multidimensional scaling of soil organic carbon chemistry (arcsine

871 square-root transformed Bray-Curtis distances) from (a) Easton Glacier (squares) and

872 Mendenhall Glacier (circles). (b) Soil C chemistry for Easton Glacier, Mendenhall

873 Glacier, and a selection of 10 mature forest soils (triangles; Lauber et al. 2009).

874 Successional stage is indicated by color: 1 (0-1 year old soil), 2 (5 year old soil), 3

875 (biological soil crust cover), 4 (25-50% plant cover), and 5 (50-75% plant cover).

876 Vectors indicate relative abundances of soil C compounds that were significantly

877 correlated the first two dimensions. A permutational MANOVA analysis indicated that

878 there was no difference in C chemistry between Easton and Mendenhall sites (R^2 =

879 0.076, P = 0.094).

880

881 **Fig. 3:** Non-metric multidimensional scaling of (a) bacterial community structure based

882 on weighted UniFrac distances and (b) decomposer catabolic function based on log

883 transformed Euclidean distances. Clustering of points and permutational MANOVA's

884   indicated that community structure ($R^2 = 0.18$, $P \leq 0.001$) and catabolic function ($R^2 =$

885   0.47; $P \leq 0.001$) were significantly different between Easton Glacier (squares) and

886   Mendenhall Glacier (circles). Successional stage is indicated by color: 1 (0-1 year old

887   soil), 2 (5 year old soil), 3 (biological soil crust cover), 4 (25-50% plant cover), and 5

888   (50-75% plant cover). Median based β-dispersion tests indicated that (c) variance in

889   community structure (weighted UniFrac; $P = 0.003$) and (d) variance in catabolic

890   function (Euclidean distance; $P = 0.0008$) were significantly different for early- and late

891   succession soil communities. Letter in panels (c) and (d) represent significant

892   differences in variances across successional stages based on one-way ANOVAs and

893   pairwise Tukey tests adjusted for multiple comparisons.

894

895   **Fig. 4:** Pairwise weighted UniFrac distances between glacial soils (this study) and

896   mature forest soils (Lauber et al. 2009). Average UniFrac distance of soil bacterial

897   communities decreases with successional age for (a) Easton Glacier ($P < 0.0001$) and (b)

898   Mendenhall Glacier ($P < 0.0001$). The solid red line indicates the mean weighted

899   UniFrac distance between different mature forest soils with 95% confidence intervals

900   (orange band). Stages of succession are indicated by transect numbers: 1 (0-1 year old

901   soil), 2 (5 year old soil), 3 (biological soil crust cover), 4 (25-50% plant cover), and 5

902   (50-75% plant cover). Different letters indicate significant differences among

903   successional stages based on one-way ANOVAs and pairwise Tukey tests adjusted for

904   multiple comparisons.

905

906   **Fig. 5:** Non-metric multidimensional scaling (NMDS) of bacterial community structure

907   (Unifrac) for the Easton Glacier (squares), Mendenhall Glacier (circles), and mature

908   forest soils (triangles; Lauber et al. 2009). For (a) colors indicate different successional

909   stages. Stages of succession are indicated by transect numbers: 1 (0-1 year old soil), 2 (5

910   year old soil), 3 (biological soil crust cover), 4 (25-50% plant cover), and 5 (50-75%

911   plant cover). Vectors show relative abundances of phyla and sub-phyla that were

912   significantly correlated with the first two NMDS dimensions. For (b) colors indicate soil

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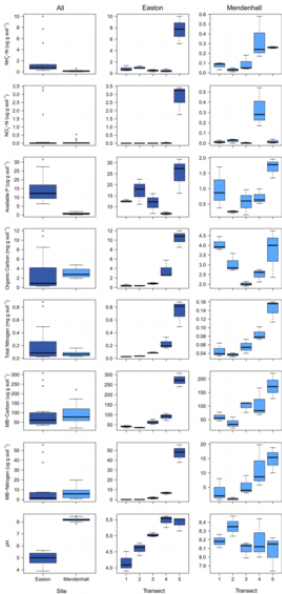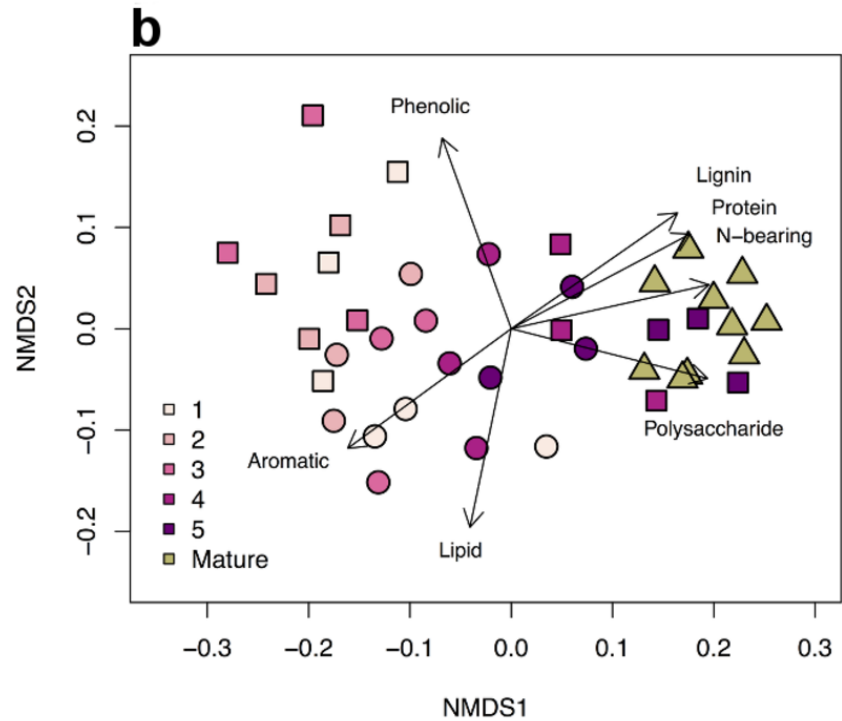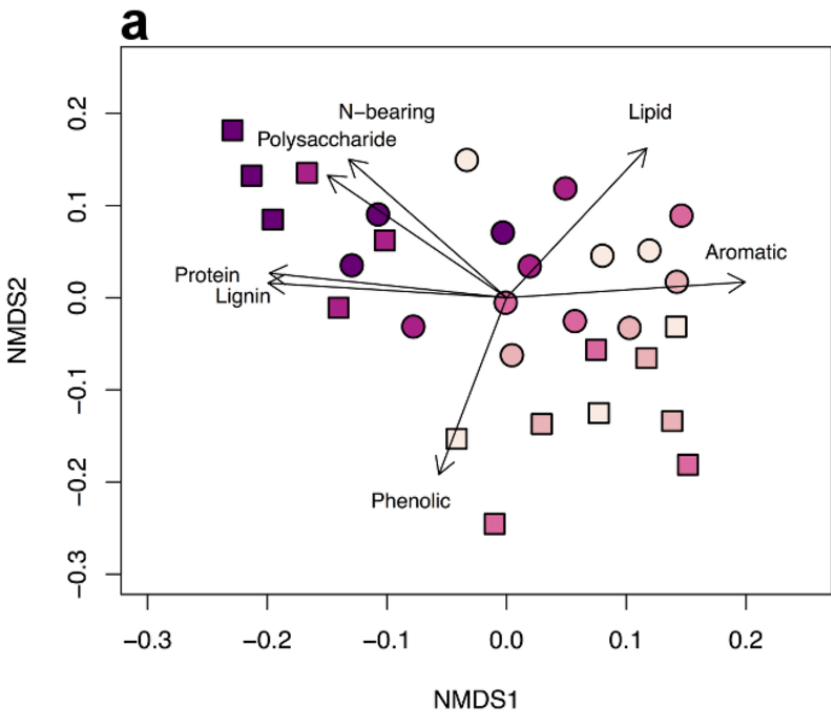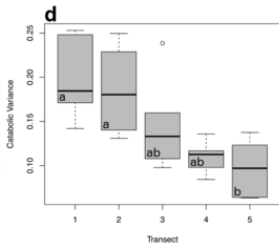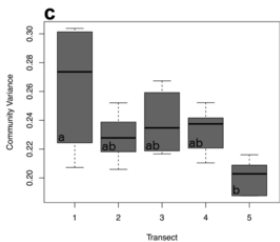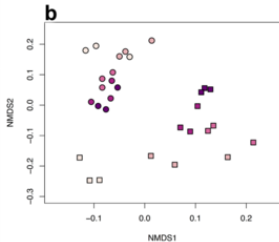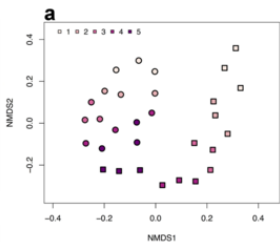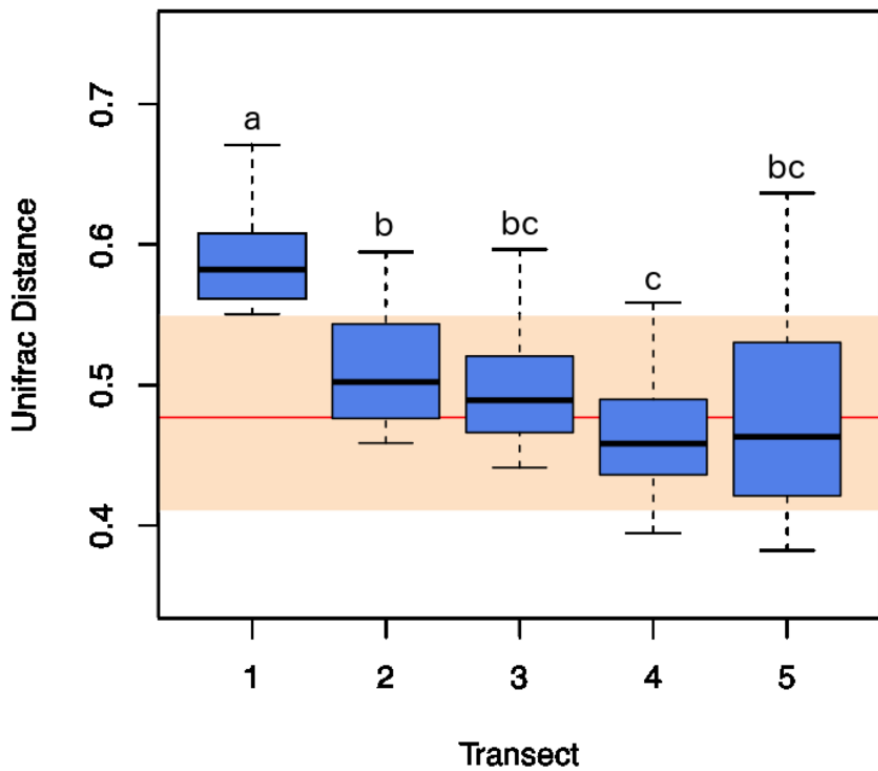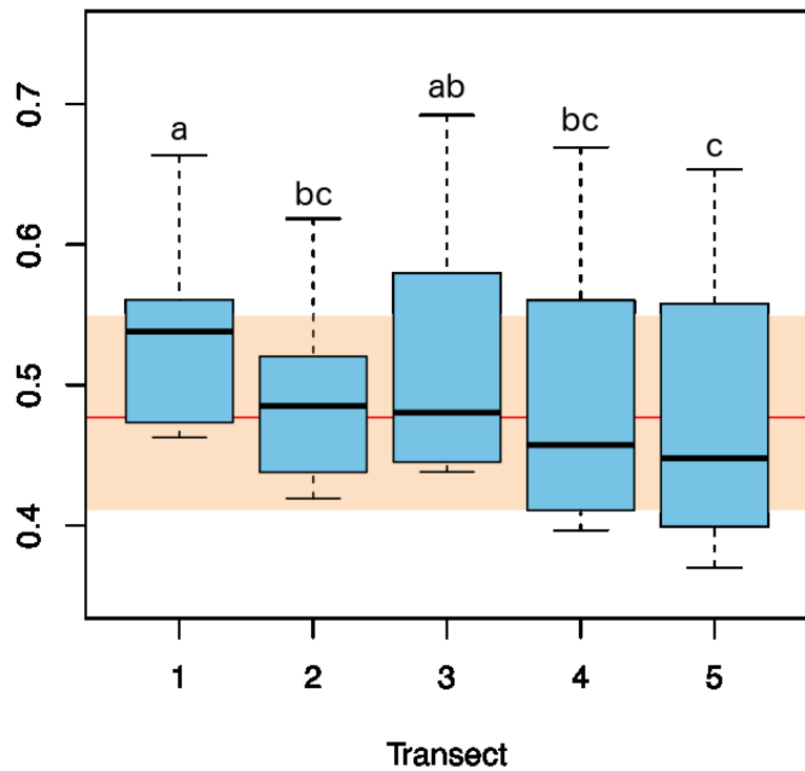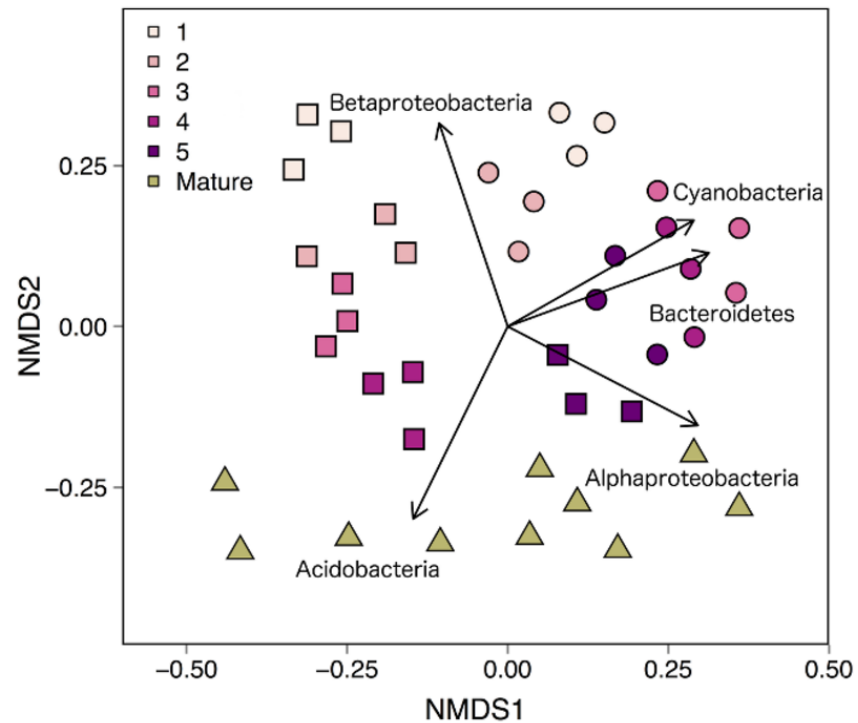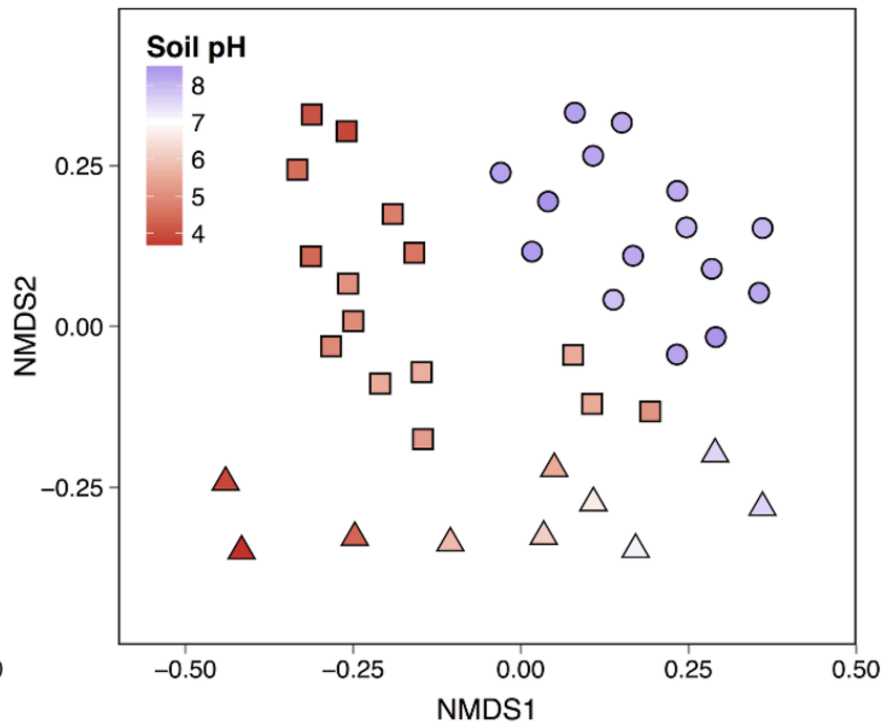
Table 1: Bacterial community structure and catabolic function Spearman's rank correlations (ρ values) with soil biogeochemical characteristics for Easton and Mendenhall Glaciers.

	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.