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1 Title:

2 **Long-term changes in soil microbial communities during primary**
3 **succession**

4

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18

19 **Abstract**

20 Soil microbial communities (SMCs) play a critical role in the cycling of carbon and nutrients in
21 terrestrial ecosystems, as well as regulating plant productivity and diversity. However, very
22 little is known about long-term (decades-centuries) structural changes in these communities.
23 The development of aboveground-belowground linkages during century-scale succession is
24 also poorly understood. Our study addressed this knowledge gap by investigating SMC and
25 plant communities undergoing primary succession on an 850-year chronosequence of lava
26 flows in Iceland. We hypothesised that communities of microfungi and bacteria would
27 respond to progressive changes in vegetation and that SMC diversity would increase with
28 terrain age. Soil samples were collected from three lava flows at different stages of primary
29 succession (165, 621 and 852 years after lava flow emplacement). Plant community
30 composition was surveyed as the samples were collected. The composition of the SMCs
31 present in the soil was determined using amplicon pyrosequencing. The physical and
32 chemical properties of the soil were also analysed. The results of the study indicated
33 changes in plant and fungal communities with increasing terrain age. Distinct plant and
34 fungal assemblages were identified on the three sites and both communities became richer
35 and more diverse with increasing terrain age. There was also evidence to suggest the
36 development of mycorrhizal associations on older sites. In contrast, the composition and
37 structure of the bacterial communities did not change systematically with terrain age.
38 Similarly, there were few changes in soil properties: SOM concentrations and pH, both of
39 which have been demonstrated to be important to SMCs, were constant across the
40 chronosequence. These results suggest that plant community composition is significant for
41 fungal communities, but less relevant for bacterial communities. This finding has implications
42 for studies of primary succession and the biogeochemical impact of vegetation change in
43 high-latitude ecosystems.

44

45 Key words: Iceland; soil ecology; community assembly; plant-soil interactions; aboveground-
46 belowground linkages

47

48 **1 Introduction**

49 The changes that occur in plant and animal communities during primary succession
50 (ecosystem development on terrain with no biological legacy) have been studied for decades
51 (Walker and del Moral, 2003). However, very little is known about long-term (decades-
52 centuries) primary succession in soil microbial communities (SMCs). SMCs play a critical role
53 in the cycling of carbon and nutrients in terrestrial ecosystems as well as regulating plant
54 productivity and diversity (van der Heijden et al., 2008). Understanding long-term changes in
55 such communities is therefore essential to efforts to model and manage ecological change,
56 including the restoration of degraded ecosystems (Kirk et al., 2004). Some attention has
57 been given to changes in SMCs (particularly bacteria) over comparatively short periods of
58 time (e.g. Jumpponen, 2003; Nemergut et al., 2007; Schütte et al., 2009; Sigler et al., 2002);
59 the succession of mycorrhizal fungi over relatively short timescales has also received
60 attention (Last et al., 1987). However, technological barriers (specifically, problems in
61 describing the high diversity of microbial communities) have meant that, until recently, the
62 focus has been on aggregate SMC function rather than taxonomy. Furthermore, the studies
63 that have been published are largely based on glacial forelands (Schaaf et al., 2011). This
64 study aims to address this gap by investigating SMC dynamics across an 850-year
65 chronosequence of lava flows in Iceland.

66

67 A lack of comparability between previous studies means that it is difficult to form a clear
68 picture of SMC succession. Most researchers agree that SMC biomass increases during
69 primary succession (Bardgett et al., 2005). There is also some evidence of progressive
70 change in the structure of SMCs during succession. For example, Nemergut et al. (2002)
71 observed increases in SMC diversity with increasing terrain age and Schütte et al. (2003)
72 and Nicol et al. (2005) reported similar patterns for bacterial and archaeal communities,
73 respectively. The functional diversity of SMCs may also increase as primary succession
74 unfolds (e.g. Tscherko et al. 2003). However, these patterns are not universal. For example,
75 Sigler et al. (2002) reported a decrease in bacterial diversity with increasing terrain age.
76 Furthermore, Sigler & Zyer (2002) and Jumpponen (2003, studying fungal communities),

77 could find no evidence of SMC succession; both studies stressed the importance of site-
78 specificity and stochastic effects in SMC assembly. The central research question posed by
79 this study is therefore: do SMCs (specifically soil bacteria and fungi) undergo predictable
80 changes, analogous to those observed in plant communities, during long-term (multi-century)
81 primary succession? Based on existing studies, we hypothesised that:

82

83 H1: changes in SMC composition would parallel changes in above-ground vegetation;
84 hence, there would be progressive change in SMCs with terrain age, with distinct
85 bacterial and fungal communities on sites at different stages of primary succession.

86

87 H2: the taxonomic diversity of fungal and bacterial communities would increase with
88 terrain age, as ecosystem development progressed and new niches (e.g. different
89 types of organic substrate) became available.

90

91

92 **2 Materials and Methods**

93 Soil samples were collected from three lava flows at different stages of primary succession.

94 Plant community composition was surveyed as the samples were collected. The composition
95 of the SMCs present in the soil was determined by amplicon pyrosequencing. The physical
96 and chemical properties of the soil, both of which are important to SMCs, were also
97 analysed.

98

99 **2.1 Study sites**

100 It is clearly difficult to observe long-term ecosystem development directly, necessitating the
101 use of space-for-time substitution (chronosequences) to infer multi-century ecological change
102 (Walker et al., 2010). The use of chronosequences is well-established in ecology and soil
103 science (Matthews, 1992; Stevens and Walker, 1970; Walker et al., 2010; Wardle et al.,
104 2004). This study utilised to a well-dated chronosequence of lava flows on Mt Hekla, Iceland

105 (64° 00' N, 19° 40' W) to investigate long-term (multi-century) changes in SMC composition.
106 The chronosequence has been described in detail in Bjarnason (1991) and Cutler et al.
107 (2008). The Hekla sites are particularly well-suited to chronosequence studies as a) the age
108 of the lava flows is well constrained (to a sub-annual level in some cases); b) the lava flows
109 are close to each other and at the same altitude, ensuring that the sites have similar
110 environmental conditions and accessibility to propagules and c) the lava flows have similar
111 slopes, substrate chemistry and surface physiognomy (Bjarnason, 1991). The region has a
112 cool, maritime climate with mean January and July air temperatures of -1.7°C and 11.0°C,
113 respectively, and a mean annual rainfall of around 1200 mm. The lava flows varied in age
114 (165-852 years) but were otherwise similar in all respects. Younger lava flows do exist (e.g.
115 those emplaced in the 1980 and 1947 CE eruptions of Mt Hekla), but these flows do not have
116 soil cover and were therefore omitted from this study. The lava has been dated by
117 contemporary accounts and tephrochronology (Thorarinsson 1967), giving excellent age
118 constraint. All of the flows have an altitude around 300 m above sea level and are composed
119 of blocky, a'a lava with a similar geochemistry. The survey sites used are characterised by
120 fine, free-draining andosols (Arnalds, 2004). The lava flows undergo slow vegetation
121 succession (Bjarnason, 1991). The vegetation on the younger surfaces is dominated by a
122 thick mat of the moss *Racomitrium lanuginosum* with scattered patches of the pioneer lichen
123 *Stereocaulon vesuvianum*. Vascular plants establish on surfaces ~100 years old and
124 increase in abundance with terrain age. The oldest surfaces are characterised by hardy
125 shrubs, notably willow (e.g. *Salix phylicifolia*), birch (*Betula pubescens*) and ericaceous
126 shrubs such as *Vaccinium* spp. Plant taxonomic richness and diversity increase
127 monotonically across the chronosequence (Cutler, 2010).

128

129 2.2 Sampling

130 Soil samples were collected from lava flows emplaced in 1845, 1389 and 1158 CE (i.e.
131 surfaces that were 165, 621 and 852 years old in August 2010) (Fig. 1). Two transect lines
132 were established on flat sites on each flow. Each transect line comprised three 2 m x 2 m
133 quadrats 10 m apart. Soil samples were collected from two opposing corners of the quadrats,

134 resulting in a total of six samples per transect and 36 samples in total. Sampling was carried
135 out on a scale relevant to the spatial scale of the vegetation (cm to m) and the sampling
136 strategy aimed for a balance between spatial resolution and coverage, within the available
137 resources. A soil core approximately 2 cm in diameter and 10 cm long was collected at each
138 sampling location using a stainless steel corer. The corer was sterilised using a 70% solution
139 of ethanol before and after the collection of each core. The core was immediately placed in a
140 sterile sample bag and stored at -20°C within hours of collection. On the 1389 and 1158
141 flows, there was a clear distinction between an organic layer, primarily formed of partially
142 decomposed moss stems, and an underlying layer with a sandy texture. These two layers
143 were separated in the field and stored in separate sterile bags. Vegetation composition and
144 abundance was recorded for each quadrat, using the Braun-Blanquet scale, with taxonomy
145 following Kristinsson (1998). No fungal fruiting bodies were observed in the quadrats during
146 the sampling.

147

148 Fig. 1: Site plan

149

150 2.3 Soil analysis

151 Grain size distribution was analysed using a laser particle sizer in accordance with the
152 manufacturer's instructions (Malvern Mastersizer 2000, Malvern Instruments Ltd, Malvern,
153 UK). Soil organic matter (SOM) content was determined by loss on ignition (LOI)
154 measurements. Samples were dried at 105°C overnight to drive off moisture, then heated to
155 550°C for four hours to determine the organic content (Heiri et al., 2001). Total
156 concentrations of soil phosphorus, potassium, magnesium and calcium (hereafter, the format
157 [nutrient] is used to indicate nutrient concentrations) were determined by inductively coupled
158 plasma optical emission spectrometry (ICP-OES), using an Optima 2100 DV (Perkin Elmer,
159 Waltham, MA), following digestion in aqua regia (6 ml 70% HCl and 2 ml 70% HNO₃) heated
160 to 105°C (Chen and Ma, 2001). In addition to the soil samples, material from the organic
161 layers present on the 1389 and 1158 sites was also analysed for comparison (five samples
162 from each site). Soil pH was determined using an Orion 410A electronic pH meter (Thermo

163 Fisher Scientific, Waltham, MA): 1 g of soil was mixed with 5 ml of deionised water, shaken,
164 sonicated with ultrasound for 15 mins then left to stand for 30 min. before the measurements
165 were taken.

166

167 2.4 Molecular analysis

168 A modified CTAB extraction method (Rogers and Bendich, 1988) was used to extract
169 microbial DNA from the soil samples. Each core was thawed at room temperature and
170 thoroughly mixed; 500 µl lysis buffer (0.5% SDS, 25 mM EDTA, 20 µg/ml proteinase K) was
171 then added to ~0.4 g subsample of the soil, mixed by inversion and incubated at 55°C for 30
172 minutes, mixing every 10 minutes. 100 µl of 5 M NaCl and 80 µl of 10% CTAB solution at
173 65°C were added and the samples incubated at 65°C for 10 minutes. 680 µl of
174 chloroform:isoamyl alcohol (24:1) was then added; the mixture was shaken to form an
175 emulsion, then centrifuged for 5 minutes at 16 000 x g at 20°C. Nucleic acids were
176 precipitated from the aqueous layer by adding 0.6 volumes of isopropanol. The mixture was
177 left for 2 hours at room temperature, then pelleted by centrifugation for 30 minutes at 14 680
178 x g at 20°C, washed with 100 µl of 70% ethanol and left to air dry for one hour. Pellets were
179 resuspended in 50 µl H₂O and stored at -20°C. The DNA yield of each sample was estimated
180 by analysing 1 µl of extract on a NanoDrop 8000™ multi-sample spectrophotometer (Thermo
181 Scientific, Waltham, MA). Aliquots of 45 µl were drawn from each sample and cleaned with
182 Powerclean kits (MoBio Laboratories Inc, Carlsbad, CA) in accordance with the
183 manufacturer's instructions. The cleaned DNA solutions were then combined according to
184 transect to produce six pooled samples designated HK-1 to HK-6 (i.e. each pooled sample
185 comprised six sub-samples, two from each of the three quadrats in the transect). HK-1 & -2
186 represented the two transects from the 1845 flow (165 years old), HK-3 & -4 the 1389 flow
187 (621 years) and HK-5 & -6 the 1158 flow (852 years: refer to Table 1 for further clarification).
188 The pooled samples were concentrated using centrifugal filter devices (Millipore, Billerica,
189 MA) and standardised to a DNA concentration of 20 ng/µl prior to sequencing.

190

191 Tag-encoded FLX amplicon pyrosequencing was used to investigate the composition of the
192 fungal and bacterial communities in the soil. For fungi, a ~700 bp region spanning the
193 internal transcribed spacer 1 (ITS1), 5.8S ribosomal RNA (rRNA) and ITS2 was amplified
194 using the primers ITS1F and ITS4 (Gardes and Bruns, 1993; Lord et al., 2002; White et al.,
195 1990). For bacteria, a ~500 bp region of the 16S small subunit ribosomal RNA (SSU rRNA)
196 was amplified using primers E104-120 (Wang and Qian, 2009) and 530R (Muyzer et al.,
197 1993). Pyrosequencing was carried out at the Research and Testing Laboratory (RTL,
198 Lubbock, TX) on the Roche 454 FLX Titanium platform (454 Life Sciences, Branford, CT). All
199 analyses were performed at the Research and Testing Laboratory (454 Life Sciences,
200 Bradford, CT), based upon RTL protocols.

201

202 Bacterial 16S rRNA sequence analysis was carried out in mothur 1.31.1 (Schloss et al.,
203 2009) following the recommendations of Schloss et al. (Schloss et al., 2011). Flow, quality
204 and fasta files were extracted from the sff file. All flow files were trimmed to maximum and
205 minimum lengths of 360 and 720 flows, settings recommended by Quince et al. (2011). They
206 were denoised with shhh.flows, the mothur implementation of PyroNoise (Quince et al.,
207 2009). Primer and barcodes were removed and sequences shorter than 200 bp or with
208 homopolymers greater than 8 bp were discarded. The remaining sequences were aligned
209 using the SILVA bacterial reference alignment (Quast et al., 2013). Only sequences
210 spanning the targeted region of 16S rRNA were kept and all sequences were trimmed to the
211 same length (determined by optimising alignment end and minimum sequence length so as
212 to keep 90% of sequences). Data were further denoised by clustering together sequences
213 with 1 bp difference per 100 bp and chimeras were removed using the mothur
214 implementation of uchime (Edgar et al., 2011), with the more abundant sequences as
215 reference. Sequences were classified against the SILVA bacterial reference database using
216 the Wang method (Wang et al., 2007) with kmer size 8, 100 bootstrap iterations and a cutoff
217 value of 60% for taxonomic alignment. Those identified as organelles, Archaea or Eukarya
218 were removed. Mothur was used to cluster sequences into operational taxonomic units
219 (OTUs) at a 97% similarity level, approximately corresponding to species level.

220

221 Processing and analysis of the ITS sequences followed a pipeline similar to that of 16S
222 rRNA, with the following differences. After denoising with shhh.flows and removing the
223 primers and barcodes, fungal sequences shorter than 150 bp were removed (the lower
224 minimum length for fungi was chosen due to the wide variability that is common in the fungal
225 ITS1 region). Following this initial clean-up, the ITS1 region was extracted using the ITS
226 Extractor tool on the PlutoF Workbench (Abarenkov et al., 2010b; Nilsson et al., 2010) and
227 sequences shorter than 100 bp following this step were discarded. Chimeras were removed
228 using the mothur implementation of uchime and sequences were subsampled to the size of
229 the smallest group. Sequences were clustered into OTUs at a 93% similarity level, based on
230 the average sequence divergence between named species in GenBank (Hibbett et al.,
231 2011). For OTU clustering, a distance matrix was constructed in mothur using pairwise
232 distance values, with consecutive gaps treated as one and ignoring gaps at the end of pairs.
233 The UNITE+INSDC fungal ITS database (Abarenkov et al., 2010a) was used as a reference
234 for classification, with the following modifications. All entries without kingdom-level
235 information in the UNITE lineage column were removed, as were all entries with ambiguous
236 bases. The modified database, split into separate fasta and taxonomy files and formatted for
237 the use of mothur, contained 16279 entries. Classification against this database was carried
238 out in mothur as described above but with a cutoff value of 50% for taxonomic alignment,
239 due to the greater variability of ITS1. The following discussion of fungal varieties
240 concentrates on taxa that a) accounted for at least 1% of reads (summed across all sites)
241 and b) were resolved to a genus level or lower.

242

243 In previous environmental studies using amplicon pyrosequencing data, researchers have
244 assumed a correlation between the proportion of sequence reads for a given OTU and the
245 relative abundance of that OTU in the community sampled, allowing diversity indices to be
246 calculated (see Blaaid et al., 2012; Uroz et al., 2012 for examples of this approach).
247 Technological artefacts in the pyrosequencing process, as well as innate biological traits of
248 the organisms under consideration, may bias the relative quantification of taxa (Amend et al.,

249 2010); however, it is still possible to use pyrosequencing data in a semi-quantitative way. At
250 the very least, it should be possible to compare the proportional abundance of a given taxon
251 across samples, because the variables that bias quantification are consistent within taxa
252 (Amend et al., 2010). Consequently, we have included relative abundance figures based on
253 the pyrosequencing data as a first-order estimation of community diversity.

254

255

256 2.4.1 Accession numbers

257 Sequence data were uploaded with MIMARKS-compliant metadata to the NCBI Sequence
258 Read Archive under Bioproject number PRJNA210080.

259

260 2.5 Statistical analyses

261 The Braun-Blanquet values recorded for the vegetation were transformed to percentage
262 cover by taking the midpoints of the classes. The transformed values were then used to
263 calculate Shannon diversity indices (H) for the vegetation in each quadrat. Random selection
264 (without replacement) was used to standardise the number of sequence reads in each
265 sample, prior to the calculation of microbial diversity. Using this technique, all samples (both
266 fungal and bacterial) were reduced to the number of reads present in the smallest sample.
267 Non-metric multidimensional scaling (NMDS), a robust ordination technique, was used to
268 graphically represent vegetation and molecular data, based on OTU abundance data in each
269 case. Detrended correspondence analysis was applied to the same data sets. The
270 multivariate analyses of the molecular data were based on OTUs with $\geq 1\%$ of the sequence
271 reads in any given sample. Both the NMDS and DCA analyses were carried out using the
272 *vegan* package running in R (Oksanen et al., 2010). Percent similarity (PS), a robust index of
273 compositional similarity, was used to compare the plant species and OTU assemblages in
274 different quadrats (Faith et al., 1987). The analysis was based on abundance data (plants)
275 and presence-absence matrices (microbes). PS was calculated using the Bray-Curtis
276 dissimilarity metric for abundance data; the Sorenson dissimilarity metric (Sørensen, 1948)
277 was used with presence-absence data.

278

279

280 **3 Results**

281

282 3.1 Vegetation & soils

283 A total of 33 higher plant and cryptogam species were recorded, including 24 vascular plants
284 (7 shrubs, 9 gramminoids and 8 forbs); 3 bryophytes and 6 lichens (Table 1). The bryophyte
285 *Racomitrium lanuginosum* was overwhelmingly dominant with >75% cover in most quadrats,
286 although there were changes in vegetation structure according to terrain age. Whilst the
287 moss layer was more-or-less uninterrupted on the youngest sites, higher plants were more
288 prominent on older sites. Plant richness and Shannon diversity were relatively low on all
289 sites, but both metrics increased monotonically with terrain age as higher plants became
290 established (Fig. 2). NMDS of the vegetation data (performed using presence-absence
291 figures) highlighted compositional differences: the sites were clustered according to terrain
292 age, but distributed along axis NMDS-1 and clearly distinct from one another (Fig. 3). The
293 first axis generated by DCA was closely associated with terrain age and explained 23% of
294 the variance in the data (results not shown). The length of this axis was ~3 SD units. The *PS*
295 figures indicated high levels of similarity between transects on the same lava flow and a clear
296 difference between flows of different ages (Table 4).

297

298 Table 1: Vegetation cover data

299 Fig. 2: Plant diversity plots

300 Fig. 3: Plants NMDS

301

302 There were no significant differences in the physical properties of the soils according to
303 terrain age. All the soil samples were dark, fine-grained and had low cohesion. The fine sand
304 fraction (2 – 200 μm) accounted for 70-75% of grains by volume in all samples (Table 2). The
305 grain size distribution of the 1389 and 1158 samples was virtually identical, consisting of

306 ~95% sand-sized particles and 5% silt. Clay sized particles were absent. The 1845 samples
307 were somewhat finer and contained small amounts of clay (~0.3% by volume). All of the
308 samples were weakly acidic (pH ~6.5) and comprised around 10% SOM.

309

310 Total soil N, determined using an elemental analyzer, was low and averaged around 0.1% on
311 all three lava flows (Table 2). Total soil P, measured using ICP, exhibited a significant
312 increase with terrain age (ANOVA: $F_{2,33} = 17.9$, $p < 0.001$). Mean [K], [Mg] and [Ca] were
313 lower and did not change systematically with terrain age. There were significant correlations
314 between [K], [Mg] and [Ca] (Spearman-rank: [K], [Ca] $\rho = 0.97$, $p < 0.001$; [K], [Ca] $\rho = 0.95$,
315 $p < 0.001$). Concentrations of P and K in the organic horizon present on the 1389 and 1158
316 flows were much lower than those in the underlying mineral soils (Table 2).

317

318 Table 2: Soil characteristics

319

320 3.2 Microbial communities

321 The number of sequence reads did not vary widely between samples; the bacterial samples
322 generated more sequences than the fungal samples (ranges: 2046 – 4152 reads/transect for
323 fungi, 4069 – 5099 for bacteria). Following sequence clean-up, the median length was 270
324 base pairs (bp) for the fungi and 281 bp for the bacteria. The proportion of sequences that
325 remained unclassified at a phylum level was relatively small (2.4% of the fungal reads and
326 5.4% of the bacterial reads).

327

328 The fungal communities comprised a mixture of taxa from the Basidiomycota and
329 Ascomycota, with more reads from the Ascomycota (a ratio of 2.6:1). Other phyla were
330 present but rare: only the Zygomycota (in the form of the genus *Mortierella* sp.) had a
331 significant representation (refer to Supplementary data for details). A small number of fungal
332 taxa accounted for > 1% of reads and could be resolved to genus level or lower (Table 3).
333 Although few in number, these taxa accounted for a disproportionate number of reads
334 (36.2%). The bulk of the remaining reads were assigned to less well resolved taxa, notably

335 three unclassified Ascomycetes (one from the subphylum Pezizomycota, one from the order
336 Capnodiales and one from the order Helotiales, accounting for 26.8%, 8.1% and 5.0% of all
337 fungal reads, respectively) and an unclassified Basidiomycete from the subphylum
338 Agaricomycetes (5.0% of all fungal reads). The remaining reads were dispersed thinly across
339 a large number of taxa (refer to the Supplementary Data file for details). Large numbers of
340 reads associated with *Batcheloromyces* sp. (a genus from the order Capnodiales) were
341 recorded on the older surfaces (the 1389 and 1158 flows), but fewer were recorded on the
342 youngest terrain (Table 3). Indeed, virtually no reads from the Capnodiales were obtained
343 from the youngest lava flows, despite the general abundance of reads from this order.
344 *Hygrocybe* spp. were common on the youngest terrain but less abundant on older surfaces;
345 a similar pattern was observed with *Clavaria argillacea*. Conversely, sequence reads
346 associated with the genera *Cryptococcus* and *Epicoccum* were absent on the youngest sites,
347 but abundant on the two older flows.

348

349 Matches were obtained with 16 fungal genera linked with mycorrhizal activity. Most of the
350 matches were from the Basidiomycota. With the notable exception of *Meliniomyces bicolor*.
351 (an ericoid mycorrhizal (ERM) fungus), the overwhelming majority of these genera (14) were
352 connected with ectomycorrhizal (ECM) associations (Rinaldi et al., 2008). Two taxa of
353 arbuscular mycorrhizal (AM) fungi were identified, but they were exceptionally rare (a total of
354 five reads across all the sites) and presumably of limited ecological importance. On the
355 whole, sequences from putative mycorrhizal genera were not abundant: only reads
356 associated with the genera *Meliniomyces* and *Russula* appeared in any great number.
357 However, when the data were aggregated there was a clear relationship between the
358 frequency of putative mycorrhizal reads and terrain age (Fig. 4). Mycorrhizal fungi were
359 almost entirely absent from the youngest lava flow (HK-1 & HK-2) and much more common
360 on the oldest terrain (HK-5 & -6). Six of the putative mycorrhizal genera only occurred on the
361 oldest terrain. All of the reads associated with *Russula aeruginea* (an ECM fungus that forms
362 associations with birch trees) and 96% of those associated with *Meliniomyces bicolor*

363 (which has been demonstrated to form symbiotic associations with ericoid shrubs,
364 specifically *Vaccinium* sp. (Grelet et al., 2009)) were from the oldest lava flow.

365

366 Fig. 4: Abundance of putative mycorrhizal taxa

367

368 The analysis revealed 24 bacterial phyla. Phyla commonly associated with soil habitats,
369 notably Proteobacteria and Acidobacteria, were dominant in terms of number of reads (Table
370 3). Betaproteobacteria from the class Burkholderiales were particularly prominent, as were
371 Alphaproteobacteria from the order Rhizobiales. Actinobacteria (including *Arthrobacter* sp., a
372 common soil bacterium) were also abundant. The other bacterial phyla had many fewer
373 sequence reads. For example, the Verrucomicrobia, commonly encountered in other surveys
374 of soil bacteria (Killham, 1994), accounted for less than 2% of all the bacterial sequence
375 reads. The relative abundance of sequence reads from the Acidobacteria was lowest on the
376 oldest terrain age, even though soil pH remained constant. The abundance of Actinobacteria
377 increased somewhat with terrain age. Otherwise, there were no obvious trends in the
378 representation of other abundant bacterial phyla.

379

380 Table 3: Pyrosequencing summary

381

382 The bacterial communities sampled were richer than their fungal counterparts (Fig. 5). The
383 pyrosequencing data suggested that both communities were inequitable i.e. they were
384 characterised by a small number of dominant taxa. For fungi, top quintile of OTUs (ranked by
385 number of sequences) accounted for 85% of total reads. Dominance was less marked in the
386 bacteria, where the top quintile of OTUs accounted for 76% of total sequence reads.
387 Consequently, bacterial communities were more diverse than fungal communities. Fungal
388 richness and Shannon diversity increased with terrain age (diversity on the 1158 flow ($H =$
389 4.53) was slightly higher than that on the 1389 flow ($H = 4.47 \pm 0.02$)). Fungal equitability
390 was higher on the two oldest surfaces (HK-3 to HK-6). Similar indices of bacterial diversity
391 were much less variable and did not increase in the same fashion with terrain age.

392

393 Fig. 5: Microbial richness, diversity & equitability data

394

395 The NMDS plot of the fungal data indicated close similarities between communities on the
396 oldest terrain (HK-3 to HK-6) (Fig. 6) In contrast, the transects from the youngest surface
397 (HK-1 & HK-2) were widely separated, both from each other and the older transects. End-
398 member transects were separated by ~3.5 SD units on the first DCA axis, which accounted
399 for 36% of the variance in the data.

400

401 The NMDS plot for the bacterial data indicated similarities between transects on the same
402 lava flow. The transects were arranged along NMDS1 according to terrain age (Fig. 6).
403 Again, transects from the youngest surface (HK-1 and HK-2) appeared dissimilar. However,
404 the overall differences between transects were less for bacteria than fungi. DCA indicated
405 that end member sites were only separated by ~1.5 SD units on the first DCA axis, which
406 accounted for 45% of the variance in the data.

407

408 Fig. 6: Fungal and bacterial NMDS plots

409

410 The *PS* values for fungal communities reinforced the pattern evident on the NMDS plot i.e.
411 the communities from transects HK-3 to HK-6 were very similar and distinct from those on
412 the youngest terrain (Table 4). On the youngest lava flow, the similarity between HK-1 and
413 HK-2 was low (63%) when compared to the transects on older surfaces. HK-1 and HK-6
414 were remarkably similar in terms of fungal communities, but not plants and bacteria. The
415 pattern of similarity was different for the bacteria. A large proportion of bacterial OTUs
416 occurred across the chronosequence. Consequently, the similarities between bacterial
417 communities in different sampling locations were remarkably high and there was little
418 evidence of systematic differences according to terrain age. Transects on terrain of the same
419 age were more-or-less the same as sites on terrain of different ages in terms of community
420 composition.

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Table 4: Percent similarity data

4 Discussion

The results of this study indicated changes in plant and fungal communities with increasing terrain age. Distinct plant assemblages were identified on the three lava flows and the fungal communities on the youngest lava flow were very different from those on the older flows. Both plant and fungal communities became richer and more diverse with increasing terrain age. There was also evidence to suggest the development of mycorrhizal associations on older sites. In contrast, the composition and structure of the bacterial communities did not change markedly with terrain age and there were few changes in soil properties. SOM concentrations and pH, both of which have been demonstrated to be important to SMCs (e.g. Griffiths et al., 2011), were constant across the chronosequence. These results suggest that changes in plant community composition during the later stages of primary succession are significant for fungal communities, but less relevant for bacterial communities.

The results of this study indicated slow, progressive change in plant communities, consistent with previous studies from this location (Bjarnason, 1991; Cutler et al., 2008). Although plant species from the regional pool are gradually added during succession, the main changes to the vegetation are structural rather than functional i.e. a transition in dominance from the moss layer to shrubby vegetation (Cutler, 2010). The *Racomitrium* moss layer is a significant component of the vegetation across the chronosequence. It is probably a major contributor of organic carbon to the soil, although decomposition rates are likely to be slow, due to low prevailing temperatures and poor litter quality (Cutler, 2011). Shrubby taxa appear on Hekla's lava flows after ~100 years of primary succession, but they do not become abundant until dense birch and willow thickets form on terrain >500 years old.

449 Changes in soil properties frequently accompany primary plant succession. Typically, N and
450 organic matter accumulate, cation concentrations decrease and soils become more acidic
451 (Chapin et al., 1994; Matthews, 1992; Walker and del Moral, 2003). However, in this case
452 there was little evidence for progressive changes in soil properties: SOM concentrations, pH
453 and major cation concentrations remained constant. Total soil phosphorus did increase, a
454 pattern that has also been observed in foliar nutrient concentrations in mosses from this
455 location (Cutler, 2011).

456

457 Low levels of biotic reaction may explain the absence of progressive change in soil
458 properties. Most N fixation is probably carried out by free-living microbes (primarily
459 cyanobacteria) living in the moss layer (Henriksson et al., 1987), or in symbiosis with lichens
460 e.g. *Stereocaulon vesuvianum* and *Peltigera* spp. (Vitousek, 1994). Much of this N capital is
461 likely to remain conserved in moss tissue (DeLuca et al., 2002). Nitrogen fixing bacteria (e.g.
462 taxa from the family Bradyrhizobiaceae) were identified. However, these organisms only fix N
463 in association with a symbiont. There are no leguminous or actino-rhizal plants on the study
464 sites so soil [N] is likely to remain low as a result (Cutler, 2011).

465

466 The deposition of allocthonous material (dust and volcanic ash) by the wind is also likely to
467 play an important role in soil development (or the lack thereof) on the lava flows. Southern
468 Iceland suffers from soil erosion, resulting in high atmospheric dust loads (Arnalds et al.,
469 2012; Greipsson, 2012) and the moss layer on the sites is likely to trap some of this material.
470 Hence, the mineral component of Hekla's soils is mainly derived from aeolian deposition,
471 rather than bedrock weathering (Arnalds, 2004). Differences between nutrient cation and P
472 concentrations in the organic layer (with a low minerogenic component) and underlying
473 mineral soil (where dust accumulates) suggested that the addition of aeolian material
474 influences soil fertility (Table 2). Although decomposition rates are likely to be low,
475 accumulating SOM is continuously diluted by the addition of minerogenic material, hence
476 SOM concentrations do not vary with terrain age, even though standing biomass does.
477 Mobile cations are presumably also replenished by same route. Counter to expectations, [K],

478 [Mg] and [Ca] did not vary with terrain age, despite rainfall levels that would promote
479 leaching, suggesting continuous replenishment and cation levels in excess of plant
480 requirements. Previous work on the N:P ratios of plant tissue suggest that it is N, rather than
481 P, that is limiting, and P is also likely to be present in excess of plant requirements (Cutler,
482 2011). The continual addition of P not required by plants and the transformation of this
483 mineral to recalcitrant forms, would lead to the observed increases in total P. The
484 accumulation of aeolian dust may also buffer soil pH, preventing progressive acidification.

485

486 4.1 Changes in SMC composition [H1]

487 It was expected that changes in SMC composition would mirror changes in above-ground
488 vegetation. Plants can influence both the physical and chemical properties of the soil
489 environment in a number of different ways, notably through root growth and the production of
490 litter and root exudates (van der Heijden et al., 2008). Previous research has demonstrated
491 close links between plant community composition and SMC structure, although it is not
492 always clear whether plants structure microbial communities or *vice versa* (Read, 1994). For
493 example, Knelman et al. (2012) found that plants played a central role in structuring bacterial
494 communities in the earliest stages of primary succession and that SMCs varied according to
495 plant type. Ohtonen et al. (1999) reported similar findings over a longer timescale and
496 Mitchell et al. (2010) demonstrated the impact of vegetation change (in this case, the
497 establishment of birch trees in upland heath vegetation) on SMC structure and function. It
498 might therefore be expected that as plant communities undergo succession, SMCs respond
499 to the changing environmental conditions that result (Bardgett et al., 2005).

500

501 The microbial communities in this study exhibited differing developmental trajectories. In
502 common with plant communities, fungal assemblages appeared to change as terrain age
503 increased. In contrast, bacterial communities remained very similar in terms of composition
504 across the chronosequence. The fungal NMDS plot (Fig. 6a) suggested a change in
505 community composition between the youngest transects (HK-1 & HK-2) and the older
506 surfaces. A difference of 4 SD units in a DCA plot indicates more-or-less complete turnover,

507 so there are clearly differences between early and late successional communities. This
508 observation is suggestive of microfungal succession, which slows as the terrain age
509 increases. The increase in community similarity that occurs with terrain age (Table 4) is
510 consistent with convergent succession i.e. young sites have spatially heterogeneous
511 distributions of taxa that are homogenised as succession progresses (Lepš and Rejmánek,
512 1991; Woods, 2007). The similarity between fungal communities on the youngest and oldest
513 sites (HK-1 and HK-6: Table 4) apparently goes against this trend, but the relationship
514 between the sites is driven by the co-occurrence of many rare OTUs (over-weighted due the
515 use of presence-absence data). The widely separated position of the sites on the NMDS plot,
516 which is based on more abundant (and, presumably, more ecologically important, OTUs)
517 suggests that the similarity between these sites is an artefact of the technique used.
518 Microfungal succession has been observed before: Osana and Trofymow (2012), for
519 example, reported succession in saprotrophic fungi living in moss, but this process has not,
520 to our knowledge, been observed on a timescale of centuries.

521
522 It is likely that mycorrhizal associations are particularly important in primary successions,
523 given the stressful growth conditions (particularly low N availability) that typically prevail
524 (Walker and del Moral, 2003). ERM and ECM fungi would be expected to dominate, due to
525 the scarcity of N and the fact that most bioavailable N is likely to be tied up in organic
526 macromolecules. Previous research has suggested that AM fungi do best in mineral soils
527 where the availability of P is low relative to N (Read, 1994), so they are likely to be less
528 abundant on Hekla (where P is probably not limiting). A temporal shift in the abundance of
529 mycorrhizal taxa might also be anticipated as vascular plants colonise, spread and form
530 mycorrhizal associations over time (Bardgett et al., 2005). Jumpponen (2003) hypothesised
531 that early successional communities would be dominated by saprotrophic Ascomycetes and
532 Basidiomycetes, whereas fungal communities on older substrates would be characterised by
533 mycorrhizal fungi associated with plant roots. Read (1994) noted that whilst early plant
534 colonisers are typically non-mycorrhizal, the herbaceous species that dominate the

535 intermediate stages have a facultative requirement for AM and the trees and shrubs
536 characteristic of late succession frequently have an obligate need for ECM.

537

538 Mycorrhizal taxa were not especially abundant in this study (perhaps because plant roots
539 were not sampled directly) but there was some evidence to support the trends identified in
540 earlier studies. AM fungi were scarce and ECM and ERM fungi were found on the oldest
541 sites e.g. the occurrence of *R. aeruginea* and *M. bicolor* in association with shrub and tree
542 species on the 1158 lava flow (samples HK-5 & -6). Furthermore, it appeared that
543 mycorrhizal taxa were more abundant on the oldest terrain (Fig. 5). It was impossible to
544 establish definitively how many of the fungi observed were saprotrophs, due to the variations
545 in taxonomic resolution and uncertainties over fungal metabolism in the published literature.
546 Two of the most abundant fungal taxa may be confidently described as saprotrophs
547 (*Mortierella* sp. and *Clavaria argillacea*) and other, rarer saprotrophs were present on terrain
548 of all ages. *Clavaria argillacea* appeared to decline in abundance with increasing terrain age
549 (Table 3), consistent with predictions of Jumpponen (2003), but *Mortierella* exhibited the
550 opposite pattern. Ultimately, it was not possible to establish saprotrophic succession with
551 such limited data and a more focussed study would be required to establish whether the
552 relative proportion of saprotrophs changes systematically with successional stage.

553

554 The bacterial communities exhibited very little evidence of succession. The lava flows were
555 arranged in age order along the first NMDS axis (Fig. 6), which is consistent with succession.
556 However, the most abundant bacterial OTUs occurred on all three sites and short DCA axes
557 (~1.5 SD units) indicated low species turnover. The composition of the bacterial community
558 was not unexpected and most of the major groups commonly associated with soils were
559 present. Only the Acidobacteria and Actinobacteria exhibited a systematic change in
560 abundance with terrain age. It may be that the bacterial communities reach a long-term
561 equilibrium relatively early in the succession. Studies elsewhere have indicated that bacterial
562 communities can stabilise after a period of decades (e.g. Tscherko et al., 2003). In this study,
563 bacterial communities may have stabilised with the formation of continuous moss cover and

564 the associated accumulation of the first protosoils ~70 years after the emplacement of the
565 lava. At that point, cyanobacterial communities living on the surface of the lava would have
566 been replaced by heterotrophic bacteria as light was excluded and the moss provided a
567 reliable, if meagre, source of organic carbon. Thereafter, the progressive changes in soil
568 conditions that influence bacterial community composition and structure on other sites (e.g.
569 increasing soil pH) did not occur and the bacterial community remained relatively unchanged.

570

571 4.2 Changes in SMC structure [H2]

572 The SMCs were characterised by high levels of richness and a large number of rare OTUs
573 (the 'rare biosphere'). Pyrosequencing is a sensitive technique and these patterns are
574 common in molecular analyses of environmental samples. Because of the spatially
575 heterogeneous nature of soils, the use of small (<1 g) samples may have biased the results
576 of microbial analyses, favouring the detection of dominant species (Kirk et al., 2004). The
577 results of this study are therefore likely to underestimate true microbial diversity. For
578 example, transect HK-2 had anomalously low fungal diversity, but this was due to an
579 exceptionally large number of reads for two OTUs (both from the sub-phylum
580 Pezizomycotina but unclassified below this level).

581

582 Hypothesis H2 proposed that the taxonomic diversity of fungal and bacterial communities
583 would increase with terrain age, as ecosystem development progressed and new niche
584 space became available. Plant richness, diversity and equitability all increased with terrain
585 age. These changes represent a 'filling up' of the sites from a small regional species pool
586 and decreasing dominance of *R. lanuginosum* as vascular plants colonise and expand
587 (Cutler et al., 2008). Differences in dispersal and establishment ability probably played a role
588 in determining the rate and direction of plant succession. The relationship between SMC
589 structure and terrain age varied according to the group studied. Fungal community structure
590 paralleled changes in above ground vegetation, with monotonic increases in diversity with
591 increasing terrain age. This result supported previous research that suggests SMC diversity

592 is linked to plant species diversity (Zak et al., 2003). In contrast, bacterial community
593 structure did not change in step with vegetation succession.

594

595 In microbial ecology, it has been posited that “everything is everywhere but the environment
596 selects” (Green and Bohannan, 2006). As fungi readily disperse by spores, increasing fungal
597 richness is most likely due to the creation of new habitat niches as succession unfolds. New
598 niches created by plant succession might include the presence of recalcitrant organic
599 compounds, e.g. lignin, in litter, or the roots of new colonisers e.g. ericaceous shrubs. Some
600 mycorrhizal fungi, particularly the ECM and ERM fungi that predominant in the latter stages
601 of succession, have been shown to have high levels of host specificity (Last et al., 1987).
602 Consequently, increased plant richness is likely to be associated with increased fungal
603 diversity (Read, 1994). Compared with the bacterial communities, the fungal communities
604 had low equitability, with a few dominants and many rare species. This was particularly
605 marked on the youngest terrain. Inequitable community structure implies that a handful of
606 species have been successful in dominating resources and excluding their competitors. An
607 increase in equitability with increasing terrain age was consistent with the formation of new
608 fungal niche space as plant succession progressed.

609

610 In contrast to changes in fungal community structure, bacterial structure was invariant across
611 the chronosequence. Bacterial richness, diversity and equitability were more-or-less the
612 same on all three sites. Such invariance implies that plant succession had little impact on
613 bacterial community composition. Of course, it is possible that bacterial biomass varied whilst
614 taxonomic composition remained constant (the same point could also be made for the fungi).
615 Furthermore, PCR techniques do not discriminate between active tissue and inactive
616 spores/senesced tissue. Hence ecological interpretations of the data should be treated with
617 care. Further investigation (e.g. using quantitative PCR) would be required to establish if
618 changes in biomass not paralleled by changes in community composition have occurred on
619 Hekla’s lava flows. However, given that microbial biomass is largely determined by the
620 availability of SOM, major changes with time seem unlikely. A number of authors have

621 reported systematic increases in the ratio of fungal:bacterial biomass over time as soils
622 acidify. It is not possible to say whether or not this has occurred with the current dataset but,
623 again, it seems unlikely given the constancy of soil pH across the sites.

624

625

626 **5 Conclusions**

627 The results of this study revealed differences in the temporal dynamics of fungal and
628 bacterial communities. Fungal community composition and structure varied during
629 succession: discrete communities formed and these communities became more diverse with
630 increasing terrain age. Changes in community structure occurred without accompanying
631 changes in the soil pH and organic matter content and mirrored changes in vegetation. Our
632 study did not set out to establish a causal link between plant and microbial succession.
633 However, these data strongly suggest a connection that is worthy of further research. It is
634 likely that plant succession led to changes in the type and quality of organic carbon added to
635 soil. Thus, although SOM concentrations did not change overall, the biochemical composition
636 of the organic compounds in the soil may have done. Litter quality (usually measured in
637 terms of C:N ratios) is important to SMCs, as is carbon lability. Indeed, carbon lability, rather
638 than N content, may be the primary control over decomposition in some settings (Chapin et
639 al., 2002). In this study, increases in the availability and diversity of recalcitrant compounds,
640 e.g. lignin, are probable as shrubby species colonise and spread. An increase in the diversity
641 of these organic substrates could impact on fungal communities. In contrast, bacteria, which
642 can only metabolise more basic products, may not have experienced the same changes in
643 their environment, particularly as pH, which has been demonstrated to structure bacterial
644 communities, did not change. These communities probably became saturated early in the
645 succession and are likely to be more sensitive to changes in soil chemistry than plant
646 community structure *per se*. This work has relevance for studies of primary succession,
647 which often neglect changes in microbial communities in the soil. It is also relevant for
648 ongoing research into the biogeochemical impact of vegetation change in high-latitude
649 settings.

650

651

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660

661 **Figure Captions**

662

663 Fig. 1: Location plan. The lava flows are shaded. Contours are at 25 m intervals.

664

665 Fig. 2: Plant diversity plots (error bars indicate 1 SE).

666

667 Fig. 3: NMDS plot of data from vegetation surveys; sites (transects) are indicated by circles
668 (black = 1845 transects, grey = 1389 transects, white = 1158 transects), species by crosses.

669 Key to species: 1 = *R. lanuginosum*; 2 = *E. nigrum*; 3 = *S. vesuvianum*; 4 = *P. alpina*; 5 = *S.*

670 *herbacea*; 6 = *S. lanata*; 7 = *C. bigelowii*; 8 = *V. uliginosum*; 9 = *J. trifidus*; 10 = *F. vivipara*;

671 11 = *K. myosuroides*; 12 = *Umbilicaria* sp.; 13 = *Lecidea* sp.; 14 = *S. phyllicifolia*; 15 = *A.*

672 *vinealis*; 16 = *G. normanii*; 17 = *F. richardsonii*; 18 = *T. praecox*; 19 = *G. verum*; 20 = *P.*

673 *membranacea*; 21 = *Peltigera* sp.; 22 = *B. vivipara*; 23 = *L. multiflora*; 24 = *H. splendens*; 25

674 = *D. flexuosa*; 26 = *R. geographicum*; 27 = *A. uva-ursi*; 28 = *C. vulgaris*; 29 = *B. pubescens*,

675 30 = *Hieracium* sp.; 31 = *T. alpinum*; 32 = *B. lunaria*; 33 = *P. ciliare*.

676

677 Fig. 4: The relative abundance of putative mycorrhizal fungi on terrain of different ages
678 (expressed as a percentage of the total number of fungal sequences from each lava flow).

679 Terrain age increases from left to right.

680

681 Fig. 5: Microbial diversity statistics. The bars indicate 95% confidence intervals for samples
682 where random selection (without replacement) was used to standardise sample size. There
683 are no confidence intervals for fungal diversity on the 1158 lava flow, as this sample had the
684 lowest aggregate number of reads(2046). The units on the y-axis of 4a) refer to the number
685 of OTUs clustered by their similarity to each other (97% similarity for the bacteria, 93%
686 similarity for the fungi).

687

688 Fig. 6: Fungal and bacterial NMDS plots. Sites (transects) are indicated by circles (black =
689 1845 transects, grey = 1389 transects, white = 1158 transects), OTUs (grouped at 93%

690 similarity for fungi and 97% similarity for bacteria) by crosses. Sites HK-3 and HK-4 are so
691 similar that they overlap. Only the most abundant OTUs (i.e. those accounting for more than
692 1% of the reads in any sample) were used in the analysis.

693

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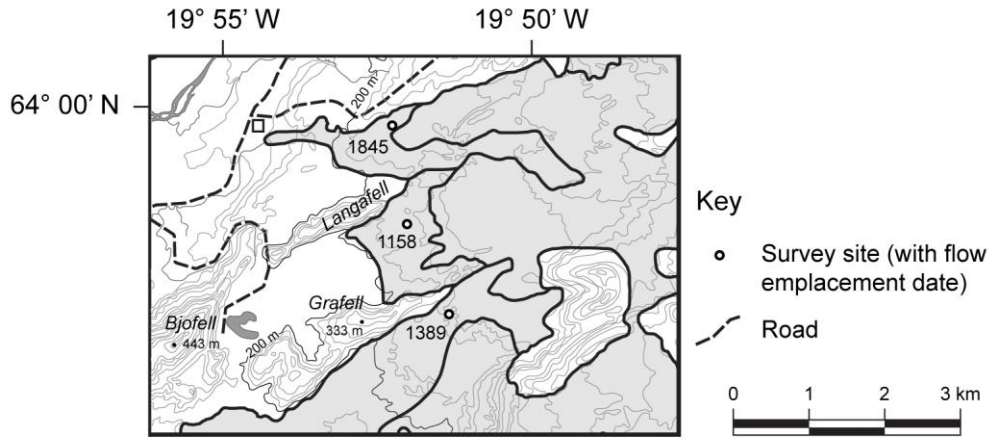
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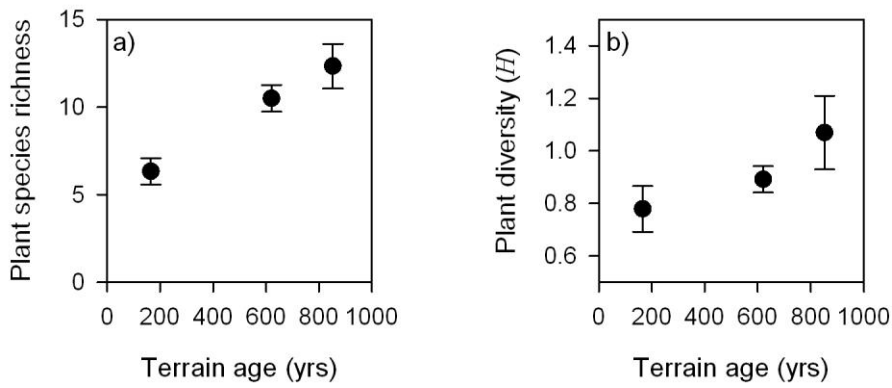
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893 Figure 1



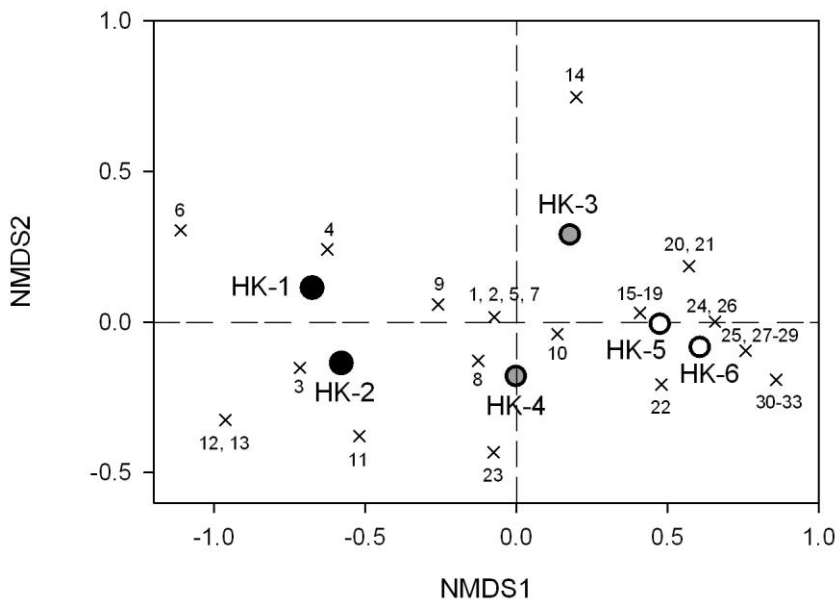
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895 Figure 2



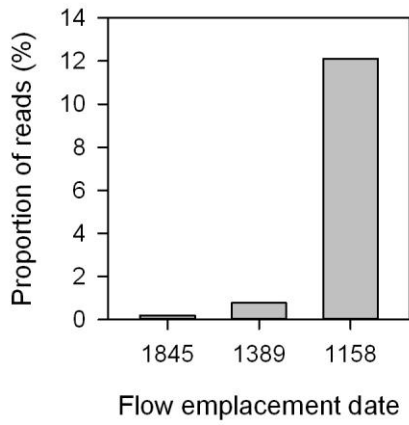
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897 Figure 3



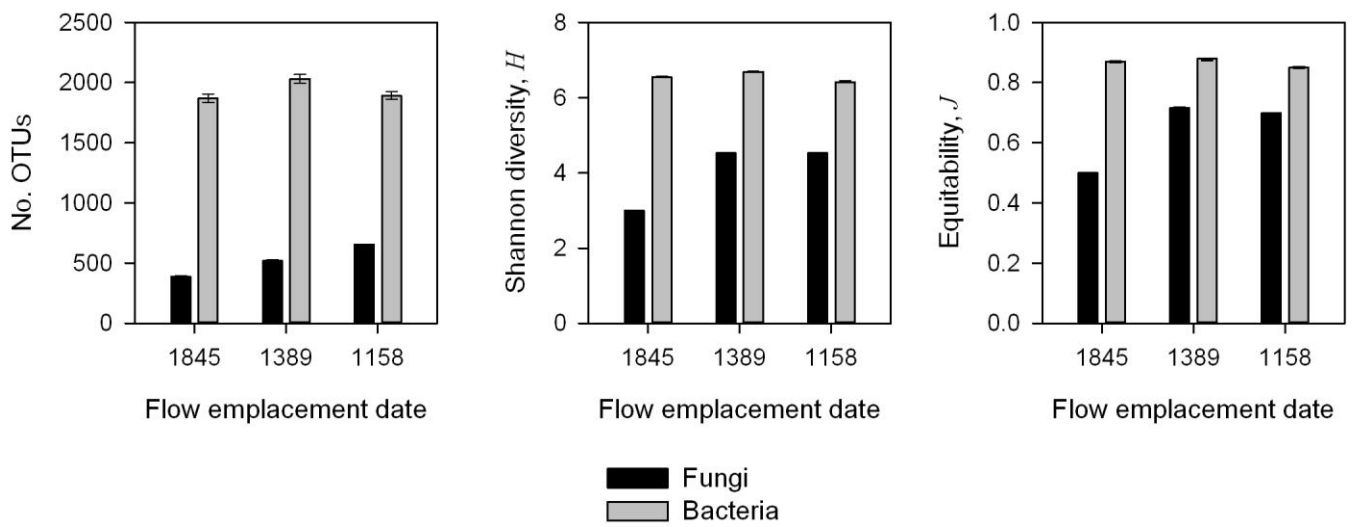
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899 Figure 4



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901 Figure 5



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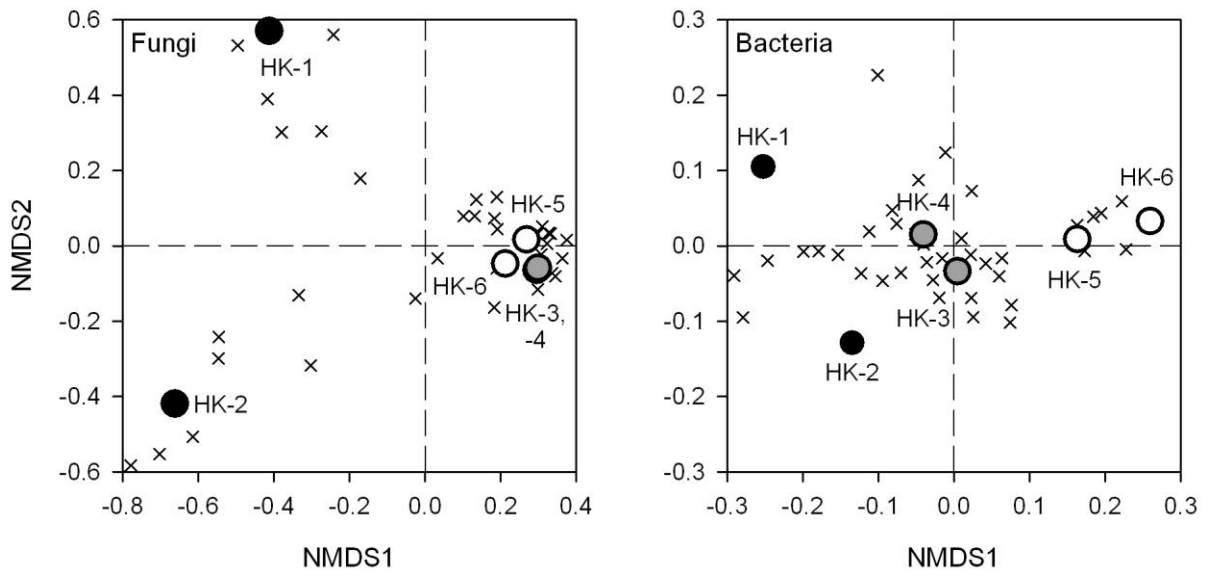
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912 Figure 6



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	HK-1			HK-2			HK-3			HK-4			HK-5			HK-6		
	1845-1-A	1845-1-B	1845-1-C	1845-2-A	1845-2-B	1845-2-C	1389-1-A	1389-1-B	1389-1-C	1389-2-A	1389-2-B	1389-2-C	1158-1-A	1158-1-B	1158-1-C	1158-2-A	1158-2-B	1158-2-C
<i>Racomitrium lanuginosum</i> (B)	5	5	5	5	5	5	5	5	5	5	5	5	3	5	5	4	5	2
<i>Empetrum nigrum</i> (S)	3	3	2	3	3	3	2	3	2	2	2	2		1	3	4	3	
<i>Salix herbacea</i> (S)	+	+	+	+	+			+	+	+	+	1		+	+		+	+
<i>Carex bigelowii</i> (G)	+		+		1		+	1	1	+		+	+	1	+	1	+	+
<i>Festuca vivipara</i> (G)				+			1	1				+	1	+	+	+	+	1
<i>Agrostis vinealis</i> (G)							1		1	1	1	1		+	+	+	+	+
<i>Galium normanii</i> (F)							+	+		1	+	+		+	+		+	+
<i>Festuca richardsonii</i> (G)							+	+	+	+	+	+		+	+	+		
<i>Stereocaulon vesuvianum</i> (L)	2	2	+	1	2	+				+		+						
<i>Juncus trifidus</i> (G)	+				+	+			+	+	+	+			+			
<i>Vaccinium uliginosum</i> (S)	+				+						1		3		1	+		3
<i>Bistorta vivipara</i> (F)											+	+		+	+	+	+	+
<i>Galium verum</i> (F)								1		+	+	+		1	+			+
<i>Thymus praecox</i> (F)							+			+	+				+		+	
<i>Peltigera membranacea</i> (L)									+				3			1		1
<i>Peltigera sp.</i> (L)									+				1			2		+
<i>Poa alpina</i> (G)	+					+	+											
<i>Kobresia myosuroides</i> (G)				+						1	+							
<i>Deschampia flexuosa</i> (G)													+			+		+
<i>Arctostaphylos uva-ursi</i> (S)															3			+
<i>Calluna vulgaris</i> (S)															1			+
<i>Betula pubescens</i> (S)															+			+
<i>Umbilicaria sp.</i> (L)					+													
<i>Lecidea sp.</i> (L)					+													
<i>Salix phylicifolia</i> (S)							2											
<i>Luzula multiflora</i> (G)												+						
<i>Hylocomium splendens</i> (B)													5					
<i>Salix lanata</i> (S)			+															
<i>Rhizocarpon geographicum</i> (L)															+			
<i>Hieracium sp.</i> (F)																		+
<i>Thalictrum alpinum</i> (F)																		+
<i>Botrychium lunaria</i> (F)																		+
<i>Ptilidium ciliare</i> (B)																		+

933

934 Table 1: Vegetation survey results based on the Braun Blanquet abundance scale (+ =
935 present, but with < 1% cover; 1 = 1-5% cover; 2 = 6-25% cover; 3 = 26-50% cover; 4 = 51-
936 75% cover; 5 = 76-100% cover). Pooled pyrosequencing samples are indicated on the top
937 line of the table; quadrat identifiers, formatted as flow emplacement date – transect number –
938 quadrat letter are in the row below (each pyrosequencing sample comprises material from
939 three quadrats). Vegetation type is indicated by the letters S (shrub), G (graminoid), F (forb),
940 B (bryophyte) and L (lichen) after the species name.

941

Soil property	Terrain age (years)		
	165	621	852
Soil pH range; mean	5.6 - 6.5; 6.0	6.0 - 6.9; 6.3	5.3 - 6.6; 6.0
SOM content (%)	11.4 ± 1.0	10.3 ± 0.9	10.8 ± 0.9
Mean grain size (% by vol.)			
Coarse sand	16.5 ± 1.6	22.7 ± 1.2	24.7 ± 1.2
Fine sand	76.9 ± 1.5	72 ± 1.1	70.8 ± 1.2
Silt	6.3 ± 0.2	5.3 ± 0.3	4.5 ± 0.2
Clay	0.3 ± 0.1	0.1 ± 0.1	0.0
Total N (%)	0.09 ± 0.01	0.11 ± 0.01	0.10 ± 0.01
Total P _M (mg l ⁻¹)	9.7 ± 0.5	12.1 ± 0.5	13.4 ± 0.3
Total P _O (mg l ⁻¹)	NA	8.7 ± 1.4	7.6 ± 1.5
Total K _M (mg l ⁻¹)	0.8 ± 0.1	0.9 ± 0.1	1.1 ± 0.1
Total K _O (mg l ⁻¹)	NA	0.4 ± 0.1	0.4 ± 0.1

942

943 Table 2: Summary of soil data for the three different terrain ages. The subscript 'M' refers to
944 the mineral soil, the subscript 'O' to the overlying organic layer identified on the 1389 and
945 1158 sites only.

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Abundance (N = no. reads, % = percent of reads)

Fungal hits	Phylum	HK-1		HK-2		HK-3		HK-4		HK-5 (852)		HK-6	
		N	%	N	%	N	%	N	%	N	%	N	%
<i>Batcheloromyces</i> sp.	Ascomycota	14	0.7	4	0.2	378	18.5	644	31.5	293	14.3	552	27.0
<i>Hygrocybe</i> spp.	Basidiomycota	874	42.7	0	0.0	169	8.3	42	2.1	24	1.2	0	0.0
<i>Epicoccum</i> sp.	Ascomycota	0	0.0	0	0.0	53	2.6	110	5.4	187	9.1	14	0.7
<i>Mortierella</i> sp.	Zygomycota	7	0.3	60	2.9	57	2.8	18	0.9	81	4.0	86	4.2
<i>Cryptococcus</i> sp.	Basidiomycota	0	0.0	0	0.0	0	0.0	1	0.0	185	9.0	44	2.2
<i>Clavaria argillacea</i>	Basidiomycota	156	7.6	30	1.5	11	0.5	7	0.3	4	0.2	19	0.9
<i>Russula</i> spp.	Ascomycota	3	0.1	0	0.0	0	0.0	0	0.0	79	3.9	118	5.8
<i>Melinomyces bicolor</i>	Basidiomycota	2	0.1	3	0.1	0	0.0	0	0.0	107	5.2	4	0.2

Bacterial hits	HK-1		HK-2		HK-3		HK-4		HK-5		HK-6	
	N	%	N	%	N	%	N	%	N	%	N	%
Acidobacteria	1235	30.4	937	23.0	972	23.9	1113	27.4	737	18.1	694	17.1
Proteobacteria (Alpha)	830	20.4	1088	26.7	866	21.3	672	16.5	858	21.1	777	19.1
Proteobacteria (Beta)	752	18.5	1118	27.5	935	23.0	877	21.6	1142	28.1	1101	27.1
Proteobacteria (Delta)	141	3.5	76	1.9	134	3.3	150	3.7	132	3.2	105	2.6
Proteobacteria (Gamma)	173	4.3	172	4.2	184	4.5	142	3.5	265	6.5	215	5.3
Actinobacteria	278	6.8	195	4.8	303	7.4	275	6.8	342	8.4	454	11.2
Verrucomicrobia	74	1.8	43	1.1	102	2.5	116	2.9	33	0.8	82	2.0

959

960 Table 3: Selected fungal and bacterial taxa at a genus and phylum level, respectively. The fungal taxa shown are varieties that account for > 1% of
961 the total number of reads (aggregated across all sites) and were resolvable to at least genus level. The relative abundance of each taxonomic group
962 is expressed as a percentage of the total number of sequence reads from each transect. The number of reads from each transect was standardised
963 to match the smallest sample by random sub-sampling (2046 reads for fungi and 4069 for the bacteria). The most abundant fungal groups from HK-2
964 were only resolved to a sub-phylum level and are not shown, hence the lack of obvious dominants.
965

Plants

	HK-1	HK-2	HK-3	HK-4	HK-5
HK-2	76				
HK-3	50	52			
HK-4	56	64	71		
HK-5	40	42	72	70	
HK-6	32	35	65	63	84

Fungi

	HK-1	HK-2	HK-3	HK-4	HK-5
HK-2	63				
HK-3	71	66			
HK-4	67	67	81		
HK-5	70	62	86	83	
HK-6	87	69	86	76	79

Bacteria

	HK-1	HK-2	HK-3	HK-4	HK-5
HK-2	96				
HK-3	96	95			
HK-4	97	96	99		
HK-5	95	93	99	94	
HK-6	91	89	95	93	96

966

967 Table 4: Percent similarity data (100% = identical community structure) calculated from OTU

968 presence-absence data. Values comparing adjacent transects on terrain of the same age are

969 indicated in bold.