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Title:

Long-term changes in soil microbial communities during primary succession

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Abstract

Soil microbial communities (SMCs) play a critical role in the cycling of carbon and nutrients in terrestrial ecosystems, as well as regulating plant productivity and diversity. However, very little is known about long-term (decades-centuries) structural changes in these communities. The development of aboveground-belowground linkages during century-scale succession is also poorly understood. Our study addressed this knowledge gap by investigating SMC and plant communities undergoing primary succession on an 850-year chronosequence of lava flows in Iceland. We hypothesised that communities of microfungi and bacteria would respond to progressive changes in vegetation and that SMC diversity would increase with terrain age. Soil samples were collected from three lava flows at different stages of primary succession (165, 621 and 852 years after lava flow emplacement). Plant community composition was surveyed as the samples were collected. The composition of the SMCs present in the soil was determined using amplicon pyrosequencing. The physical and chemical properties of the soil were also analysed. The results of the study indicated changes in plant and fungal communities with increasing terrain age. Distinct plant and fungal assemblages were identified on the three sites and both 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 systematically with terrain age. Similarly, there were few changes in soil properties: SOM concentrations and pH, both of which have been demonstrated to be important to SMCs, were constant across the chronosequence. These results suggest that plant community composition is significant for fungal communities, but less relevant for bacterial communities. This finding has implications for studies of primary succession and the biogeochemical impact of vegetation change in high-latitude ecosystems.

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

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1 Introduction

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

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

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Furthermore, Sigler & Zyer (2002) and Jumpponen (2003, studying fungal communities), could find no evidence of SMC succession; both studies stressed the importance of site-specificity and stochastic effects in SMC assembly. The central research question posed by this study is therefore: do SMCs (specifically soil bacteria and fungi) undergo predictable changes, analogous to those observed in plant communities, during long-term (multi-century) primary succession? Based on existing studies, we hypothesised that:

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

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

2 Materials and Methods

Soil samples were collected from three lava flows at different stages of primary succession. Plant community composition was surveyed as the samples were collected. The composition of the SMCs present in the soil was determined by amplicon pyrosequencing. The physical and chemical properties of the soil, both of which are important to SMCs, were also analysed.

2.1 Study sites

It is clearly difficult to observe long-term ecosystem development directly, necessitating the use of space-for-time substitution (chronosequences) to infer multi-century ecological change (Walker et al., 2010). The use of chronosequences is well-established in ecology and soil science (Matthews, 1992; Stevens and Walker, 1970; Walker et al., 2010; Wardle et al.,

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

2.2 Sampling

Soil samples were collected from lava flows emplaced in 1845, 1389 and 1158 CE (i.e. surfaces that were 165, 621 and 852 years old in August 2010) (Fig. 1). Two transect lines

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

Fig. 1: Site plan

2.3 Soil analysis

Grain size distribution was analysed using a laser particle sizer in accordance with the manufacturer's instructions (Malvern Mastersizer 2000, Malvern Instruments Ltd, Malvern, UK). Soil organic matter (SOM) content was determined by loss on ignition (LOI) measurements. Samples were dried at 105°C overnight to drive off moisture, then heated to 550°C for four hours to determine the organic content (Heiri et al., 2001). Total concentrations of soil phosphorus, potassium, magnesium and calcium (hereafter, the format [nutrient] is used to indicate nutrient concentrations) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES), using an Optima 2100 DV (Perkin Elmer, Waltham, MA), following digestion in aqua regia (6 ml 70% HCl and 2 ml 70% HNO_3) heated

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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 H_2O 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).

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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

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the Wang method (Wang et al., 2007) with kmer size 8, 100 bootstrap iterations and a cutoff value of 60% for taxonomic alignment. Those identified as organelles, Archaea or Eukarya were removed. Mothur was used to cluster sequences into operational taxonomic units (OTUs) at a 97% similarity level, approximately corresponding to species level.

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

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In previous environmental studies using amplicon pyrosequencing data, researchers have assumed a correlation between the proportion of sequence reads for a given OTU and the relative abundance of that OTU in the community sampled, allowing diversity indices to be calculated (see Blaalid et al., 2012; Uroz et al., 2012 for examples of this approach). Technological artefacts in the pyrosequencing process, as well as innate biological traits of the organisms under consideration, may bias the relative quantification of taxa (Amend et al., 2010); however, it is still possible to use pyrosequencing data in a semi-quantitative way. At the very least, it should be possible to compare the proportional abundance of a given taxon across samples, because the variables that bias quantification are consistent within taxa (Amend et al., 2010). Consequently, we have included relative abundance figures based on the pyrosequencing data as a first-order estimation of community diversity.

2.4.1 Accession numbers

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

2.5 Statistical analyses

The Braun-Blanquet values recorded for the vegetation were transformed to percentage cover by taking the midpoints of the classes. The transformed values were then used to calculate Shannon diversity indices (H) for the vegetation in each quadrat. Random selection (without replacement) was used to standardise the number of sequence reads in each sample, prior to the calculation of microbial diversity. Using this technique, all samples (both fungal and bacterial) were reduced to the number of reads present in the smallest sample. Non-metric multidimensional scaling (NMDS), a robust ordination technique, was used to graphically represent vegetation and molecular data, based on OTU abundance data in each case. Detrended correspondence analysis was applied to the same data sets. The multivariate analyses of the molecular data were based on OTUs with ≥1% of the sequence

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reads in any given sample. Both the NMDS and DCA analyses were carried out using the *vegan* package running in R (Oksanen et al., 2010). Percent similarity (PS), a robust index of compositional similarity, was used to compare the plant species and OTU assemblages in different quadrats (Faith et al., 1987). The analysis was based on abundance data (plants) and presence-absence matrices (microbes). PS was calculated using the Bray-Curtis dissimilarity metric for abundance data; the Sorenson dissimilarity metric (Sørensen, 1948) was used with presence-absence data.

3 Results

3.1 Vegetation & soils

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

Table 1: Vegetation cover data

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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).

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The fungal communities comprised a mixture of taxa from the Basidiomycota and Ascomycota, with more reads from the Ascomycota (a ratio of 2.6:1). Other phyla were present but rare: only the Zygomycota (in the form of the genus *Mortierella* sp.) had a significant representation (refer to Supplementary data for details). A small number of fungal taxa accounted for > 1% of reads and could be resolved to genus level or lower (Table 3). Although few in number, these taxa accounted for a disproportionate number of reads (36.2%). The bulk of the remaining reads were assigned to less well resolved taxa, notably three unclassified Ascomycetes (one from the subphylum Pezizomycota, one from the order Capnodiales and one from the order Helotiales, accounting for 26.8%, 8.1% and 5.0% of all fungal reads, respectively) and an unclassified Basidiomycete from the subphylum Agaricomycetes (5.0% of all fungal reads). The remaining reads were dispersed thinly across a large number of taxa (refer to the Supplementary Data file for details). Large numbers of reads associated with *Batcheloromyces* sp. (a genus from the order Capnodiales) were recorded on the older surfaces (the 1389 and 1158 flows), but fewer were recorded on the youngest terrain (Table 3). Indeed, virtually no reads from the Capnodiales were obtained from the youngest lava flows, despite the general abundance of reads from this order. *Hygrocybe* spp. were common on the youngest terrain but less abundant on older surfaces; a similar pattern was observed with *Clavaria argillacea*. Conversely, sequence reads associated with the genera *Cryptococcus* and *Epicoccum* were absent on the youngest sites, but abundant on the two older flows.

Matches were obtained with 16 fungal genera linked with mycorrhizal activity. Most of the matches were from the Basidiomycota. With the notable exception of *Meliniomyces bicolor*. (an ericoid mycorrhizal (ERM) fungus), the overwhelming majority of these genera (14) were connected with ectomycorrhizal (ECM) associations (Rinaldi et al., 2008). Two taxa of arbuscular mycorrhizal (AM) fungi were identified, but they were exceptionally rare (a total of five reads across all the sites) and presumably of limited ecological importance. On the

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whole, sequences from putative mycorrhizal genera were not abundant: only reads associated with the genera *Meliniomyces* and *Russula* appeared in any great number. However, when the data were aggregated there was a clear relationship between the frequency of putative mycorrhizal reads and terrain age (Fig. 4). Mycorrhizal fungi were almost entirely absent from the youngest lava flow (HK-1 & HK-2) and much more common on the oldest terrain (HK-5 & -6). Six of the putative mycorrhizal genera only occurred on the oldest terrain. All of the reads associated with *Russula aeruginea* (an ECM fungus that forms associations with birch trees) and 96% of those associated with *Melinionmyces bicolour* (which has been demonstrated to form symbiotic associations with ericoid shrubs, specifically *Vaccinium* sp. (Grelet et al., 2009)) were from the oldest lava flow.

Fig. 4: Abundance of putative mycorrhizal taxa

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

Table 3: Pyrosequencing summary

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

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.

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438 The results of this study indicated slow, progressive change in plant communities, consistent
439 with previous studies from this location (Bjarnason, 1991; Cutler et al., 2008). Although plant
440 species from the regional pool are gradually added during succession, the main changes to
441 the vegetation are structural rather than functional i.e. a transition in dominance from the
442 moss layer to shrubby vegetation (Cutler, 2010). The *Racomitrium* moss layer is a significant
443 component of the vegetation across the chronosequence. It is probably a major contributor of
444 organic carbon to the soil, although decomposition rates are likely to be slow, due to low
445 prevailing temperatures and poor litter quality (Cutler, 2011). Shrubby taxa appear on Hekla's
446 lava flows after ~100 years of primary succession, but they do not become abundant until
447 dense birch and willow thickets form on terrain >500 years old.
448
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).
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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

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communities in the earliest stages of primary succession and that SMCs varied according to plant type. Ohtonen et al. (1999) reported similar findings over a longer timescale and Mitchell et al. (2010) demonstrated the impact of vegetation change (in this case, the establishment of birch trees in upland heath vegetation) on SMC structure and function. It might therefore be expected that as plant communities undergo succession, SMCs respond to the changing environmental conditions that result (Bardgett et al., 2005).

The microbial communities in this study exhibited differing developmental trajectories. In common with plant communities, fungal assemblages appeared to change as terrain age increased. In contrast, bacterial communities remained very similar in terms of composition across the chronosequence. The fungal NMDS plot (Fig. 6a) suggested a change in community composition between the youngest transects (HK-1 & HK-2) and the older surfaces. A difference of 4 SD units in a DCA plot indicates more-or-less complete turnover, so there are clearly differences between early and late successional communities. This observation is suggestive of microfungal succession, which slows as the terrain age increases. The increase in community similarity that occurs with terrain age (Table 4) is consistent with convergent succession i.e. young sites have spatially heterogeneous distributions of taxa that are homogenised as succession progresses (Lepš and Rejmánek, 1991; Woods, 2007). The similarity between fungal communities on the youngest and oldest sites (HK-1 and HK-6: Table 4) apparently goes against this trend, but the relationship between the sites is driven by the co-occurrence of many rare OTUs (over-weighted due the use of presence-absence data). The widely separated position of the sites on the NMDS plot, which is based on more abundant (and, presumably, more ecologically important, OTUs) suggests that the similarity between these sites is an artefact of the technique used. Microfungal succession has been observed before: Osana and Trofymow (2012), for example, reported succession in saprotrophic fungi living in moss, but this process has not, to our knowledge, been observed on a timescale of centuries.

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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. bicolour* 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

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opposite pattern. Ultimately, it was not possible to establish saprotrophic succession with such limited data and a more focussed study would be required to establish whether the relative proportion of saprotrophs changes systematically with successional stage.

The bacterial communities exhibited very little evidence of succession. The lava flows were arranged in age order along the first NMDS axis (Fig. 6), which is consistent with succession. However, the most abundant bacterial OTUs occurred on all three sites and short DCA axes (~1.5 SD units) indicated low species turnover. The composition of the bacterial community was not unexpected and most of the major groups commonly associated with soils were present. Only the Acidobacteria and Actinobacteria exhibited a systematic change in abundance with terrain age. It may be that the bacterial communities reach a long-term equilibrium relatively early in the succession. Studies elsewhere have indicated that bacterial communities can stabilise after a period of decades (e.g. Tscherko et al., 2003). In this study, bacterial communities may have stabilised with the formation of continuous moss cover and the associated accumulation of the first protosoils ~70 years after the emplacement of the lava. At that point, cyanobacterial communities living on the surface of the lava would have been replaced by heterotrophic bacteria as light was excluded and the moss provided a reliable, if meagre, source of organic carbon. Thereafter, the progressive changes in soil conditions that influence bacterial community composition and structure on other sites (e.g. increasing soil pH) did not occur and the bacterial community remained relatively unchanged.

4.2 Changes in SMC structure [H2]

The SMCs were characterised by high levels of richness and a large number of rare OTUs (the ‘rare biosphere’). Pyrosequencing is a sensitive technique and these patterns are common in molecular analyses of environmental samples. Because of the spatially heterogeneous nature of soils, the use of small (<1 g) samples may have biased the results of microbial analyses, favouring the detection of dominant species (Kirk et al., 2004). The results of this study are therefore likely to underestimate true microbial diversity. For

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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

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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

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likely that plant succession led to changes in the type and quality of organic carbon added to soil. Thus, although SOM concentrations did not change overall, the biochemical composition of the organic compounds in the soil may have done. Litter quality (usually measured in terms of C:N ratios) is important to SMCs, as is carbon lability. Indeed, carbon lability, rather than N content, may be the primary control over decomposition in some settings (Chapin et al., 2002). In this study, increases in the availability and diversity of recalcitrant compounds, e.g. lignin, are probable as shrubby species colonise and spread. An increase in the diversity of these organic substrates could impact on fungal communities. In contrast, bacteria, which can only metabolise more basic products, may not have experienced the same changes in their environment, particularly as pH, which has been demonstrated to structure bacterial communities, did not change. These communities probably became saturated early in the succession and are likely to be more sensitive to changes in soil chemistry than plant community structure *per se*. This work has relevance for studies of primary succession, which often neglect changes in microbial communities in the soil. It is also relevant for ongoing research into the biogeochemical impact of vegetation change in high-latitude settings.

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Figure Captions

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

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

Fig. 3: NMDS plot of data from vegetation surveys; sites (transects) are indicated by circles (black = 1845 transects, grey = 1389 transects, white = 1158 transects), species by crosses. Key to species: 1 = *R. lanuginosum*; 2 = *E. nigrum*; 3 = *S. vesuvianum*; 4 = *P. alpina*; 5 = *S. herbacea*; 6 = *S. lanata*; 7 = *C. bigelowii*; 8 = *V. uliginosum*; 9 = *J. trifidus*; 10 = *F. vivipara*; 11 = *K. myosuroides*; 12 = *Umbilicaria* sp.; 13 = *Lecidea* sp.; 14 = *S. phylicifolia*; 15 = *A. vinealis*; 16 = *G. normanii*; 17 = *F. richardsonii*; 18 = *T. praecox*; 19 = *G. verum*; 20 = *P. membranacea*; 21 = *Peltigera* sp.; 22 = *B. vivipara*; 23 = *L. multiflora*; 24 = *H. splendens*; 25 = *D. flexuosa*; 26 = *R. geographicum*; 27 = *A. uva-ursi*; 28 = *C. vulgaris*; 29 = *B. pubescens*, 30 = *Hieracium* sp.; 31 = *T. alpinum*; 32 = *B. lunaria*; 33 = *P. ciliare*.

Fig. 4: The relative abundance of putative mycorrhizal fungi on terrain of different ages (expressed as a percentage of the total number of fungal sequences from each lava flow). Terrain age increases from left to right.

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

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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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Tables

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

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

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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

884

885 Table 2: Summary of soil data for the three different terrain ages. The subscript ‘M’ refers to
886 the mineral soil, the subscript ‘O’ to the overlying organic layer identified on the 1389 and
887 1158 sites only.

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		Abundance (N = no. reads, % = percent of reads)											
		HK-1		HK-2		HK-3		HK-4		HK-5 (852)		HK-6	
Fungal hits	Phylum	N	%	N	%	N	%	N	%	N	%	N	%
Batcheloromyces sp.	Ascomycota	14	**0.7**	4	**0.2**	378	**18.5**	644	**31.5**	293	**14.3**	552	**27.0**
Hygrocybe spp.	Basidiomycota	874	**42.7**	0	**0.0**	169	**8.3**	42	**2.1**	24	**1.2**	0	**0.0**
Epicoccum sp.	Ascomycota	0	**0.0**	0	**0.0**	53	**2.6**	110	**5.4**	187	**9.1**	14	**0.7**
Mortierella sp.	Zygomycota	7	**0.3**	60	**2.9**	57	**2.8**	18	**0.9**	81	**4.0**	86	**4.2**
Cryptococcus sp.	Basidiomycota	0	**0.0**	0	**0.0**	0	**0.0**	1	**0.0**	185	**9.0**	44	**2.2**
Clavaria argillacea	Basidiomycota	156	**7.6**	30	**1.5**	11	**0.5**	7	**0.3**	4	**0.2**	19	**0.9**
Russula spp.	Ascomycota	3	**0.1**	0	**0.0**	0	**0.0**	0	**0.0**	79	**3.9**	118	**5.8**
Meliniomyces bicolor	Basidiomycota	2	**0.1**	3	**0.1**	0	**0.0**	0	**0.0**	107	**5.2**	4	**0.2**

	HK-1		HK-2		HK-3		HK-4		HK-5		HK-6	
Bacterial hits	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**

889

890 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
891 the total number of reads (aggregated across all sites) and were resolvable to at least genus level. The relative abundance of each taxonomic group
892 is expressed as a percentage of the total number of sequence reads from each transect. The number of reads from each transect was standardised

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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 were only resolved to a sub-phylum level and are not shown, hence the lack of obvious dominants.

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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**

Table 4: Percent similarity data (100% = identical community structure) calculated from OTU presence-absence data. Values comparing adjacent transects on terrain of the same age are indicated in bold.

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902 **Figures**

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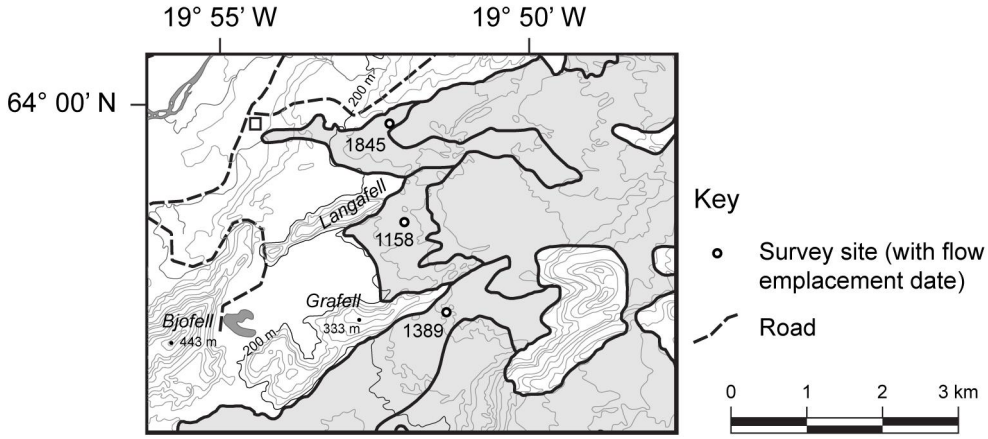

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905 Fig. 1

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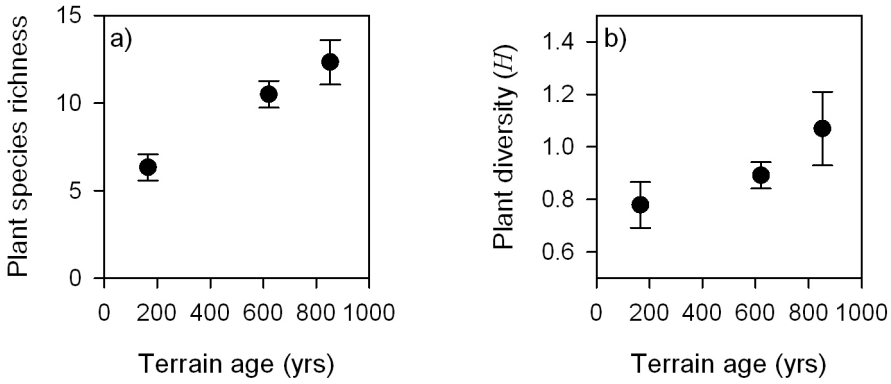

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909 Fig. 2

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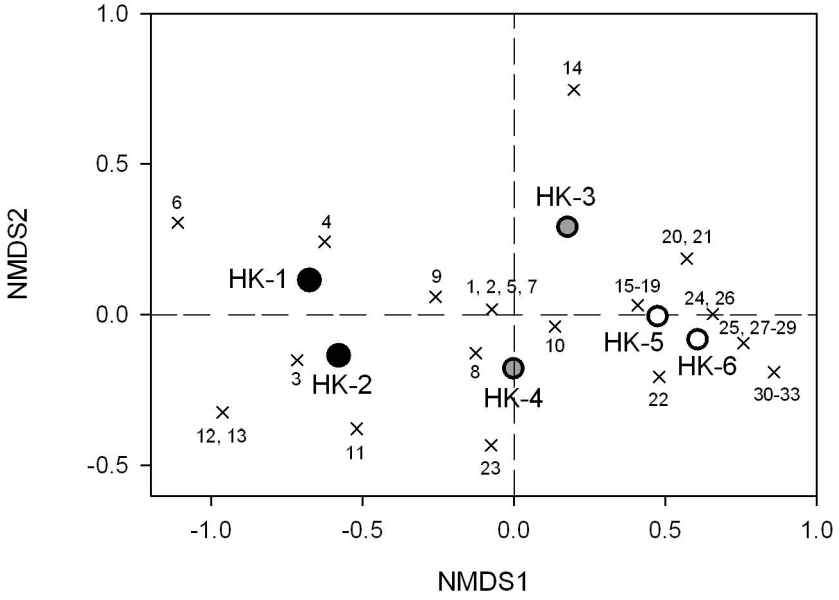

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913 Fig. 3

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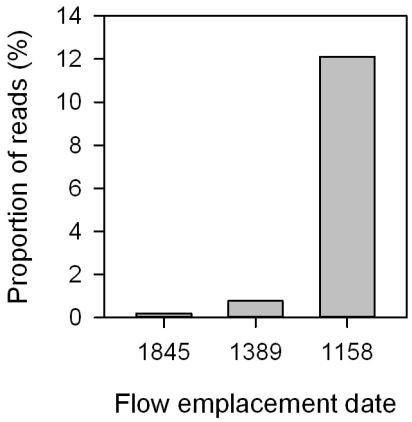

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917 Fig. 4

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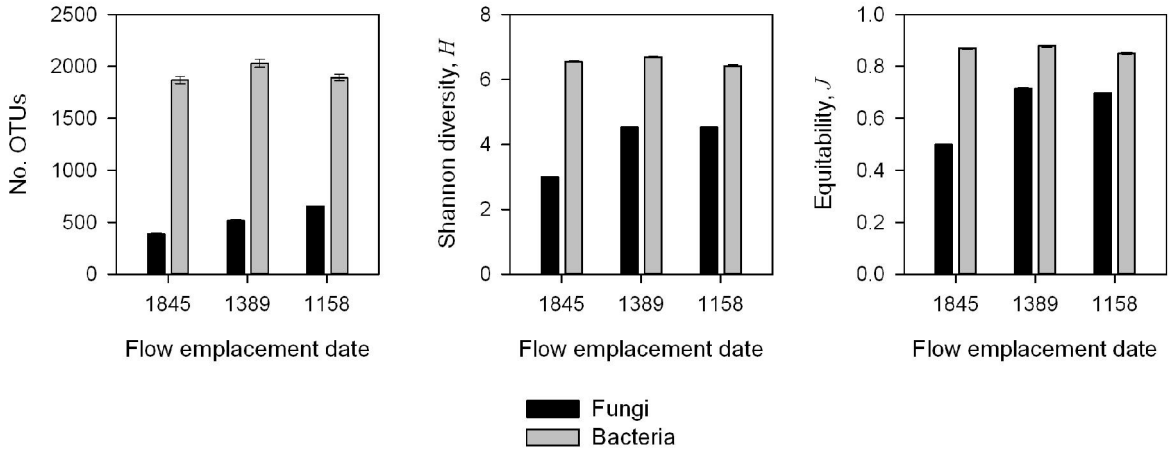

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921 Fig. 5

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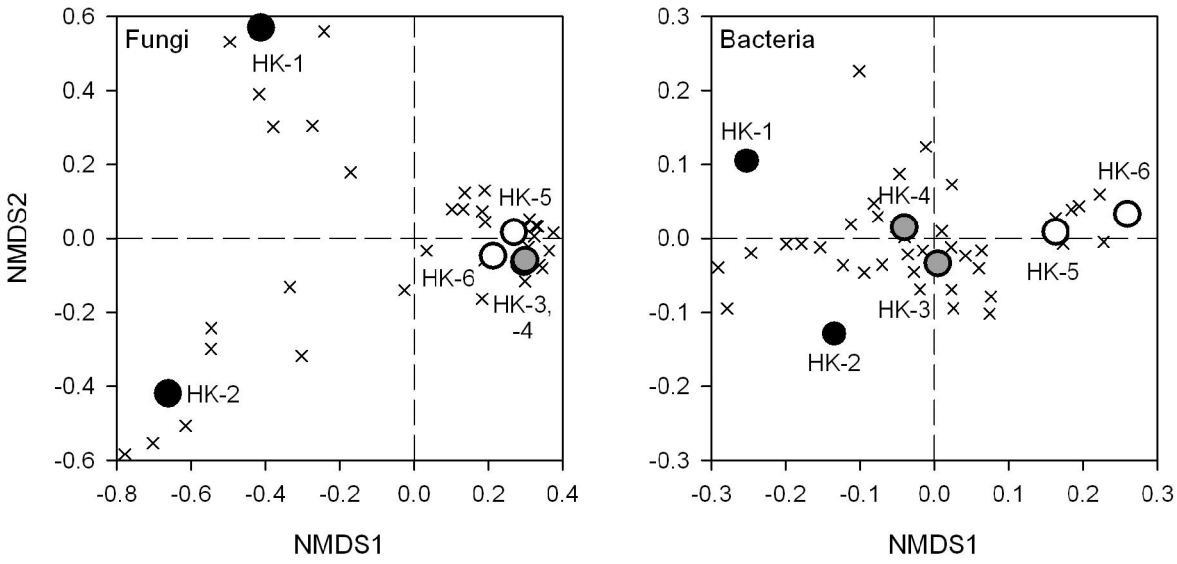

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925 Fig. 6

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