RESEARCH ARTICLE

Land coverage influences the bacterial community composition in the critical zone of a sub-Arctic basaltic environment

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Abstract

Silicate weathering improves soils by releasing bioessential nutrients from the bedrock to the soil ecosystem. However, whether bacteria are capable of inhabiting subsurface critical zones (zone of active rock weathering), and their role therein, are unknown. Next-generation sequencing and community fingerprinting permitted us to characterize communities from an Icelandic critical zone environment. Communities were compared with respect to physico-chemical properties of the environment to determine the factors influencing bacterial diversity. We showed that land coverage influenced critical zone communities. Analysis of tree-covered site (TCS) soils exhibited high cell densities (TCS = 2.25×10^7 g^{-1}), whereas lichen- and moss-covered sites (LMS) had lower cell densities (LMS = 1.06×10^7 cells g^{-1}), thought to be a result of the organic carbon produced by the trees. Differences in the bacterial community were observed from the abundance of 16S rRNA gene sequences affiliated with *Acidobacteria* and *Proteobacteria*, with TCS possessing higher abundances of *Proteobacteria* [no of sequences: LMS = 1526 (±497); TCS = 2214 (±531)], specifically *Alpha-* and *Betaproteobacteria*, and lower *Acidobacteria* numbers [no of sequences: LMS = 1244 (±338); TCS = 598 (±140)]. Diversity indices and 16S rRNA gene rarefaction showed that communities from TCS soils had lower α-diversity than sites without, indicative of specialized communities at sites with root-forming plants.

Introduction

Weathering-induced, long-term sequestration of atmospheric CO_2 as carbonates is an important Earth system process (Kump *et al.*, 2000). Basaltic substrates have received significant study, due to their rapid rates of weathering (Suchet & Probst, 1995). In particular, silicate weathering has been reported as responsible for 1.5–3.3 $\times$ 10^8 tons $year^{-1}$ of CO_2 sequestered from the atmosphere (Hilley & Porder, 2008). The physico-chemical mechanisms of the weathering of basaltic substrates are well documented (see Dessert *et al.*, 2003 for a review), and the overall rates of weathering were quantified (Navarre-Sitchler & Brantley, 2007). However, previous studies (Ellis, 1988; Gislason *et al.*, 1996; Moulton & Berner, 1998; Navarre-Sitchler & Brantley, 2007) of the solute flux of dissolved ions in rivers and streams are predominately focused on only chemical and physical weathering, with many potential biotic influences amalgamated into a 'black box'. A better understanding of weathering at the subsurface critical zone (the boundary between rock and soil, in which weathering can occur) requires that the organisms present there are identified so that their potential role can be better assessed.

The role of biota in weathering has received considerable study, with plants being reported to enhance rates of silicate weathering (Drever, 1994; Cochran & Berner, 1996; Banfield *et al.*, 1999; Adamo & Violante, 2000; Augusto *et al.*, 2000; Bonneville *et al.*, 2009), potentially through root formation and chemical exudations. Root-forming plants play a key role in the weathering of silicate substrates (Moulton & Berner, 1998), particularly

mafic materials (Drever, 1994), breaking down minerals such as biotite, apatite and plagioclase into their constituent elements, which are essential nutrients for the ecosystem. The effect is more pronounced in areas inhabited by fine-root-forming plants as the presence of root structures can increase denudation rates by an order of magnitude compared with similar sites lacking root structures (Cochran & Berner, 1996). In addition, there is a well-known relationship between plants and fungi in that the presence of plant roots increases mycorrhizal hyphae abundance in soils (Rambelli, 1973). This relationship is symbiotic and is beneficial to both organisms; while plants provide organics for fungi, there is also evidence suggesting that fungi are capable of weathering minerals and liberating insoluble nutrients previously unavailable to the root system of the plant (Kim *et al.*, 1997; Barea *et al.*, 2002). Although fungal weathering of silicates is an important area of research, there is already a large body of evidence in circulation (Hoffland *et al.*, 2004; Bjelland & Ekman, 2005; Uroz *et al.*, 2007; Bonneville *et al.*, 2009; Suzuki *et al.*, 2009; Karlsson *et al.*, 2012). Therefore, in this study, we have concentrated on the bacterial communities associated with silicate environments.

The relationships between plants and soil bacterial communities have been investigated previously (see Kent & Triplett, 2002 for a review) and through processes such as the mycorrhizosphere effect (Rambelli, 1973; Barea *et al.*, 2002) and rhizodeposition (Jones *et al.*, 2004; Hartmann *et al.*, 2009) nutrients are released into the soil matrix that can influence the microbial population. Wardle *et al.* (1999) further reported that the bacterial communities from soil inhabited by plants were more active, when activity was measured via soil respiration. However, the influence of plants on bacterial communities has not addressed the potential for plants to select the bacterial communities, which have been implicated in actively weathering silicate structures (Thorseth *et al.*, 1995; Herrera *et al.*, 2008; Cockell *et al.*, 2009a, b), particularly those which inhabit the subsurface critical zone. It is also unclear as to the extent of bacterial weathering and whether this is sufficient to impact on soil nutrient reservoirs. Therefore, the interaction between soils, plants and the bacterial communities at the subsurface critical zone requires further investigation.

Identifying which bacteria are present at the critical zone is necessary prior to identifying the particular taxa responsible for weathering. The bacterial communities present in the saprolitic soils surrounding a lake in western Iceland were examined using 16S rRNA gene sequence identification and community profiling. We studied this site because the weathering of volcanic rocks in this area has previously been used as a model for understanding chemical and potential macrobiotic contributions to basaltic rock weathering (Moulton & Berner, 1998; Moulton *et al.*, 2000). In addition to revealing new insights into the communities that inhabit the critical zone specifically, the study sought to more generally widen our understanding of which taxa live in sub-Arctic volcanic environments. Understanding which organisms live in basaltic habitats yields new data on the distribution of microbial life in Earth's crust.

We believe this study to be the first characterization of the bacterial communities located at the subsurface critical zone of a cold basaltic environment. Using 16S rRNA gene sequence identifications and community fingerprinting, comparisons of bacterial community compositions with soil chemistry were made, to (1) determine whether soil chemistry influences bacterial communities at the critical zone and (2) determine whether the soil surface floral coverage influences the soil critical zone bacterial community structure and diversity.

Materials and methods

Sample locations

To characterize the bacterial community from the Icelandic critical zone, soil samples ($n = 63$) were collected from four sites surrounding Skorradalur lake, Iceland (TCS1 = 64°30.577N, 21°23.497W; LMS1 = 64°29.279N, 21°31.660W; TCS2 = 64°30.676N, 21°27.191W and LMS2 = 64°29.408N, 21°31.109W). Previous studies have investigated the observed weathering rates of each site and shown that they differed in denudation rates and vegetation coverage, yet shared broadly similar basaltic lithology (Moulton & Berner, 1998; Moulton *et al.*, 2000). Vegetation present at sample sites consisted of root-forming plants such as birch and conifers at tree-covered sites (TCS), whereas sites partially covered by lichens and mosses (LMS) showed no evidence of root formation in the soils. Soil samples were collected from the critical zone at a depth of *c.* 30 cm below any visible root systems and in the weathered critical zone that contained partially unweathered rocks and mineral fragments. Samples were collected aseptically during June 2009, June 2010 and May 2011 and stored in sterile bags (Whirlpak, Fisher Scientific, Loughborough, UK) at ambient outdoor temperature (*c.* 11 °C) and frozen at −20 °C on return to the laboratory, which was within 7 days.

Elemental analysis

The major elements within soil samples were determined by X-ray fluorescence (XRF) spectrometry. Samples were prepared by fusing one part powdered soil sample with five parts FluXana flux (20% lithium tetraborate w/w,

80% lithium metaborate w/w) at 1000 °C. Samples were then poured into a mould and cooled to room temperature, forming a glass disc. These glass discs were analysed using an ARL 8420+ dual goniometer wavelength dispersive XRF spectrometer. Data analysis was conducted as previously described (Ramsey *et al.*, 1995; Watson, 1996). Soil organic carbon was measured by oven-drying all samples at 100 °C overnight to obtain dry weight. Soil organic carbon–free weight was obtained following 1 h at 500 °C as described by Heiri *et al.* (2001). Soil organic carbon was calculated by subtracting soil organic carbon–free weight from the air-dried weight. Samples analysed were from sites LMS1 and TCS1 collected in June 2010.

Soil pH

Given that pH is known to be a major factor determining bacterial diversity (Fierer & Jackson, 2006), it was important to determine the pH of the soils being examined. Measurements of soil pH were conducted on 18 samples from sites LMS1 and TCS1. These were made into a 1 : 2.5 (soil/H_2O) slurry according to Suzuki *et al.* (2009). pH was measured using a Hydrus 300 digital pH meter (Fisher Scientific) equipped with a HI-1230B electrode (Hanna Instruments, UK).

Cell enumerations

Total cell numbers from samples were determined by adding 0.55 g wet weight of soil sample to 5 mL sterile distilled water. Five hundred microlitres of the nucleic acid-binding dye, SYBR® Green I DNA (0.1% w/v stock; Invitrogen, Paisley, UK), was added to the sample and incubated at 20 °C for 1 h in the dark before analysis. To assist detachment of cells from the soil matrix, the solution was briefly sonicated for 10 s at 20 °C. To capture cells, 100 μL of solution was passed through a 0.2-μm black polycarbonate filter and then washed with 100 μL of sterile distilled water. Cells were enumerated using a Leica DMRP microscope equipped with epifluorescence (Leica Microsystems, Bensheim, Germany), with an excitation wavelength of 450–490 nm and a long-band cut-off filter of > 515 nm. All enumerations were conducted with 50 fields of view counted per sample. This procedure was repeated for nine further samples, resulting in a total of five samples from site LMS1 and five samples from site TCS1 collected in June 2009.

454 next-generation sequencing (NGS)

Amplicons from six samples collected in June 2010, three samples from TCS1 site and three samples from LMS1 site were sequenced using bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) directly using a Roche 454 FLX Genome Sequencer at the Research and Testing Laboratory (Lubbock, TX, USA). Briefly, the bTEFAP was carried out using a barcode tag attached to a universal bacterial primer 63f (Marchesi *et al.*, 1998) and a nonbarcoded primer 530r (Lane, 1991) during amplification of 16S rRNA gene, prior to sequencing. Analysis of all sequences was conducted using the QIIME pipeline as previously described by Caporaso *et al.* (2010). Briefly, sequences were filtered using user-defined size criteria (≥ 300 nucleotides) and grouped based on the unique barcode attached to each sample, and chimeric sequences were checked for using ChimeraSlayer (Haas *et al.*, 2011). Phylogenetic tree generation was conducted using the FASTTREE version 2 package (Price *et al.*, 2009) with representative sequences of each operational taxonomic unit (OTU; in this study defined as 97% sequence similarity); bootstrap analyses were performed with 1000 repetitions, and only values higher than 50% are shown in the phylogenetic tree. Sequences were aligned against the Silva sequence database (Pruesse *et al.*, 2007) and taxonomy generated using the Naïve Bayesian rRNA Classifier version 1.0 analysis tool within Ribosomal Database Project (RDP) classifier (Wang *et al.*, 2007). Trees were visualized using ITOL software (Letunic & Bork, 2011). Subsequent analysis of nonchimeric sequences was conducted using MOTHUR software version 1.24 (Schloss *et al.*, 2009) to generate rarefaction curves.

All sequences obtained in this study have been submitted to MG-RAST (Meyer *et al.*, 2008) under accession numbers: LMS – 4505426.3, 4505427.3 and 4505428.3; TCS – 4505422.3, 4505423.3 and 4505424.3.

Terminal restriction fragment length polymorphism (tRFLP)

Soil bacteria communities were fingerprinted by tRFLP analysis of 16S rRNA genes. Analyses were conducted on 63 samples taken between June 2009–May 2011 from sites LMS1 + 2 and TCS1 + 2. Nucleic acids were extracted from 0.5 g (wet weight) of soil using the hexadecyltrimethylammonium bromide (CTAB) bead-beating method as described by Griffiths *et al.* (2000). The 16S rRNA gene was partially amplified using PCR with the following primers, labelled (6FAM) 63f (Marchesi *et al.*, 1998) and 530r (Lane, 1991). Amplification was carried out using 1 μL of template DNA in a 50-μL reaction mixture containing 250 nM of each primer, 5 μL of 10× Taq buffer, 2 mM $MgCl_2$, 0.1 mM of each dNTP, 5 μg bovine serum albumin (BSA; New England Biolabs, MA) and 1.75 U of *Taq* (New England Biolabs, MA). PCR conditions were as follows: initial denaturation for 90 s at 94 °C followed by

35 cycles of 45 s at 94 °C; annealing for 60 s at 55 °C; elongation for 3 min at 72 °C; and a final elongation step for 10 min at 72 °C. All PCR products were verified on a 1.25% (w/v) agarose gel by electrophoresis using ethidium bromide staining.

PCR products were purified using an Illustra™ GFX™ PCR clean-up kit (GE Healthcare, Buckinghamshire, UK) according to manufacturer's guidelines. Purified PCR products were quantified using a Nanodrop spectrophotometer (NanoDrop technologies, Montchanin, DE) according to manufacturer's instructions. Fifty nanograms of PCR products was digested in a 10-μL reaction mixture containing 10 U MspI restriction endonuclease (New England Biolabs, MA), 1 μg BSA and 1 μL of enzyme buffer. Digestion was carried out for 4 h at 37 °C. The digested product (2 μL) was added to 9 μL of Hi-Di formamide (highly deionized formamide; Applied Biosystems, CA) and 0.35 μL of Liz 600 size standard (Applied Biosystems, CA) and analysed on a 3730 DNA sequencer (Applied Biosystems, CA). Resulting electropherograms were analysed using GENEMARKER software (SoftGenetics, PA), using manually created bins. Individual peaks (tRFs) presenting < 50 nucleotides. in length and having an intensity of < 50 units were not included in subsequent analysis.

In silico digestion of 454 NGS sequences

Individual tRFs were assigned an approximate taxonomic identity through *in silico* digestion of 454 NGS sequences. All sequences were analysed using TRFLPMAP software (http://nebc.nerc.ac.uk/cgi-bin/trflp0_2.cgi) using restriction endonuclease MspI cleavage site. Each tRF was assigned a taxon where at least 75% of high-throughput sequences of the same taxonomic identity matched the same cleavage site (Table S1).

The individual samples analysed by 454 NGS were chosen due to a high number of tRF peaks being observed; this offered the highest confidence in tRF taxonomic affiliations through *in silico* analysis.

Statistical methods

All statistical analyses were carried out using the Primer (version 5) package (Clarke & Warwick, 2001) or the open source R stats version 2.15.1 (R Development Core Team, 2010) using the Vegan package (Oksanen *et al.*, 2011). A visual evaluation of the chemical composition of samples at each site was conducted using canonical correspondence analysis (CCA) ordination. The Envfit function in Vegan was used to observe individual variables related to differences in ordinations. Confirmation of observed differences was identified using a *t*-test.

A rarefaction curve was generated to visualize OTU richness of each sample. Differences in OTU richness evident from rarefaction were confirmed by a Mann–Whitney *U*-test of difference.

Visualization of tRF dissimilarity was conducted using principle component analysis (PCA) of arcsine-transformed relative abundance data, using the Bray–Curtis measure of dissimilarity. The dissimilarity of tRFs between land coverage was tested using an analysis of similarity (ANOSIM) test (Clarke, 1993). Measures of tRF diversity were obtained by calculating the Shannon–Wiener (Shannon–Weaver) diversity index (H'), although attributing diversity indices for community fingerprints provides an inherent error (Blackwood *et al.*, 2007). Nevertheless, this method was appropriate for comparisons between sample sites, but comparisons between these results to other studies may be less powerful. To determine which tRFs had the greatest influence on differences between bacterial communities, a SIMPER analysis (Clarke & Warwick, 2001) was carried out.

A *t*-test was used to test for differences between sample sites for pH and soil organic carbon. Nonparametric data, which did not meet the assumptions required for parametric analysis and were resistant to normalization by data transformation, were analysed using the Mann–Whitney *U*-test; these included cell enumerations and differences in H' diversity indices.

Results

Soils' chemical and physical properties

Individual samples were shown to separate according to land coverage across the primary axis using CCA ordination (Fig. 1). Using the Envfit test of correlation, individual elements that exhibited the lowest *P* value were Al, Si, P and Na, and there was no correlation apparent between CaO or K_2O and sample sites. Elemental analysis of critical zone soils showed that samples from LMS were significantly correlated with elevated concentrations of Al_2O_3 and MgO (Table 1), whereas significantly higher concentrations of Fe_2O_3, TiO_3, P_2O_5, MnO, Na_2O and SiO_2 were measured in samples from TCS. A test of overall similarity showed that the soils at the TCS were significantly different from soils at the LMS (ANOSIM, $R = 0.759$, $P = 0.001$).

Although some elements were significantly correlated between land coverage sites, individual examination of the different concentrations showed that only 6 elemental oxides were significantly different between land coverage sites: Al, Fe, Mn, Na, P, Si and Ti. Only Mg was recorded as significantly correlated with the overall differences in samples yet having no significant difference in oxide concentration.

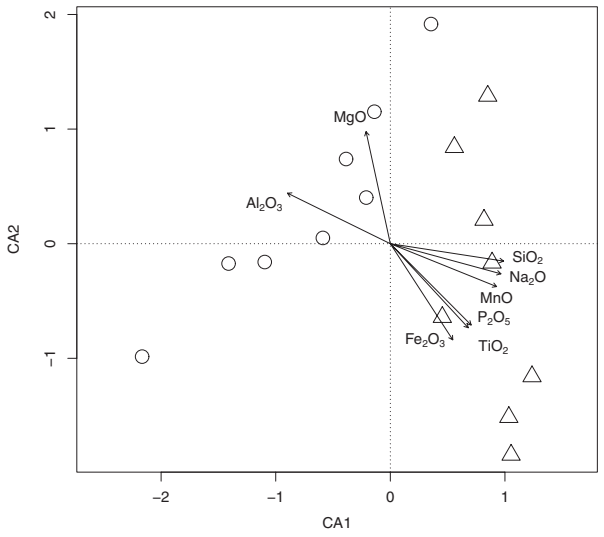


Fig. 1. A CCA ordination showing the distribution of the elemental oxide composition between soils from TCS (Triangles) and LMS (circles). The vectors indicate individual elements that contribute significantly to the overall differences in soil types from either site.

Concentrations of soil organic carbon present in soils were higher in samples from the TCS, with mean soil organic carbon (with standard deviations) for the TCS measured as 45.0 g C kg^{-1} ($\pm$14.1) and 14.6 g C kg^{-1} ($\pm$8.6) for the LMS (*t*-test, $P < 0.001$). Mean soil pH (with standard deviations) was significantly lower in the TCS (6.56 ± 0.11) compared with the LMS (6.94 ± 0.08) (*t*-test, $P < 0.001$).

Cell enumerations

Total cell numbers from the TCS and LMS were 2.25×10^7 ($\pm 2.03 \times 10^7$) cells g^{-1} and 1.06×10^7 ($\pm 0.96 \times 10^7$) cells g^{-1}, respectively. The cell numbers were significantly higher at the TCS (Mann–Whitney *U*-test, $U = 54924.5$, $P < 0.001$).

454 next-generation sequencing (NGS)

The partial 16S rRNA gene sequences obtained using 454 NGS from the two sample sites (TCS, $n = 3$; and LMS, $n = 3$) revealed the contribution of the abundant classes of the bacterial communities to each sample site. The data from the 454 NGS resulted in a total of 28 074 raw partial 16S rRNA gene sequences (*c.* 4600 sequences per sample). Following quality filtering (as described in the methods section), 15 315 16S rRNA gene sequences (*c.* 2500 sequences per sample) with a mean read length of > 350 bases were used for further analysis.

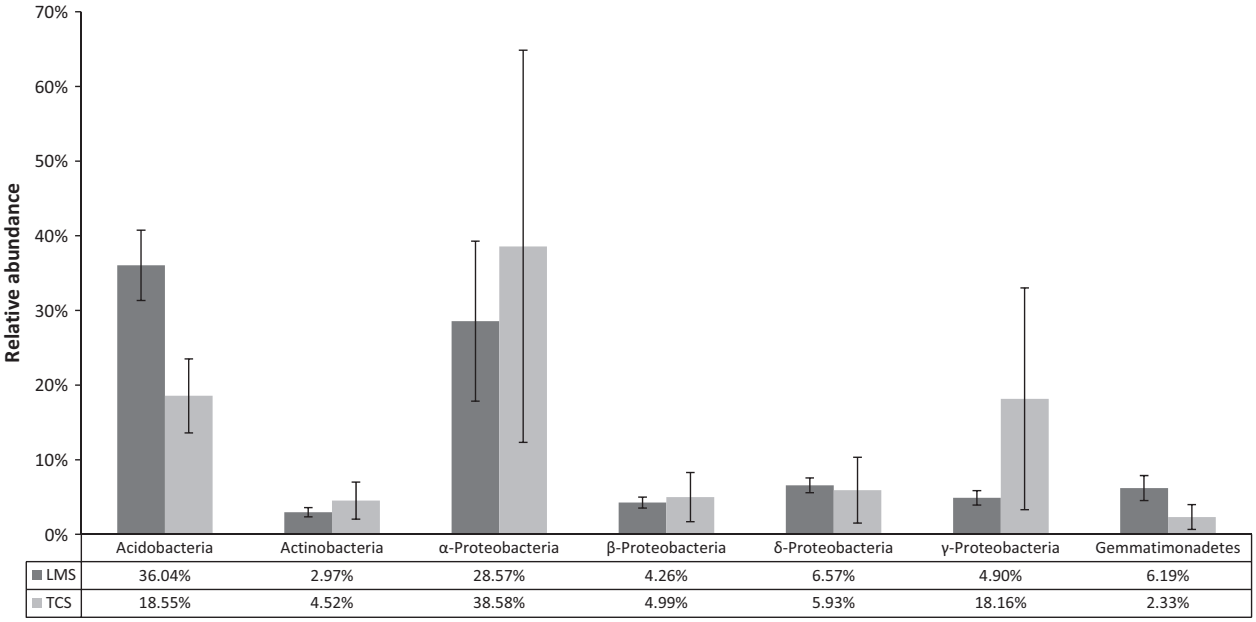
Bacterial communities at the critical zone were dominated by seven classes of bacteria in both sites, with *c.* 90% of all bacteria identified as *Alpha-, Beta-, Delta- and Gammaproteobacteria*, *Actinobacteria*, *Gemmatimonadetes* and *Acidobacteria*. Across both sites, *Alphaproteobacteria* were the most abundant (*c.* 34%) followed by *Acidobacteria* GP6 (*c.* 17%), *Gammaproteobacteria* (*c.* 11%), *Acidobacteria* GP1 (*c.* 6%), *Deltaproteobacteria* (*c.* 6%), *Betaproteobacteria* (*c.* 5%), *Gemmatimonadetes* (*c.* 4%) and *Actinobacteria* (*c.* 3.5). However, the relative abundances of these bacterial phylogenetic groups differed between TCS and LMS (Fig. 2).

There was no observable difference in relative abundance for three common taxa (*Deltaproteobacteria, Betaproteobacteria and Actinobacteria*). However, there were observed differences in the relative abundance of other taxa. *Alphaproteobacteria* made up of 38% of sequences at the TCS yet only 28% of sequences at the LMS. This was also observed for *Gammaproteobacteria* although variability was high at TCS offering less confidence. Conversely, *Acidobacteria* and *Gemmatimonadetes* were less abundant in the TCS samples.

Table 1. Percentage composition of elemental oxides (with standard deviations; $n = 8$ at each land usage site)

	Al_2O_3	Fe_2O_3	MgO	MnO	Na_2O	P_2O_5	SiO_2	TiO_2
LMS mean (wt%) (SD)	15.23 (0.24)	14.8 (0.52)	6.11 (0.18)	0.21 (0.009)	1.93 (0.12)	0.23 (0.01)	44.52 (1.08)	2.67 (0.09)
TCS mean (wt%) (SD)	14.15 (0.34)	15.7 (0.31)	5.92 (0.25)	0.23 (0.007)	2.09 (0.17)	0.29 (0.04)	46.9 (0.85)	2.95 (0.11)
T-test of difference between land coverage								
T-test statistic	7.17	−4.12	1.71	−4.67	−2.21	−4.47	−4.94	−5.06
P value	**< 0.001**	**0.002**	0.111	**< 0.001**	**0.045**	**0.001**	**< 0.001**	**< 0.001**
Correlation with CCA Ordination (Fig. 1)								
R^2	0.8836	0.5121	0.594	0.5808	0.7022	0.8283	0.9635	0.5963
P value	**0.001**	**0.012**	**0.005**	**0.006**	**0.002**	**0.001**	**0.001**	**0.006**

Results of a *t*-test between sites, giving statistical significance. Envfit outputs indicating any correlations of elemental oxide abundance with the overall differences between samples.
Statistically significant values are given in bold.



	Acidobacteria	Actinobacteria	α-Proteobacteria	β-Proteobacteria	δ-Proteobacteria	γ-Proteobacteria	Gemmatimonadetes
LMS	36.04%	2.97%	28.57%	4.26%	6.57%	4.90%	6.19%
TCS	18.55%	4.52%	38.58%	4.99%	5.93%	18.16%	2.33%

Fig. 2. Differences in relative abundance for the six most common taxa identified from the critical zone by 454 NGS analysis. LMS in dark grey and TCS in light grey. Error bars indicate standard deviations (TCS, $n = 3$; and LMS, $n = 3$).

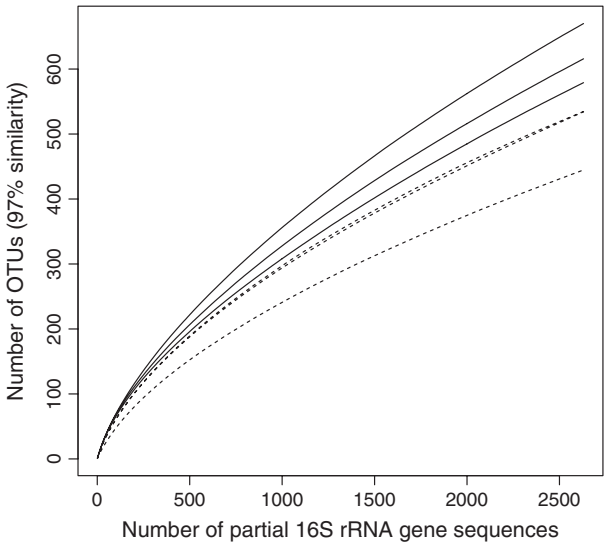


Fig. 3. Rarefaction analyses, indicating the α-diversity of bacterial communities at TCS and LMS. Solid lines indicate LMS, and broken lines indicate TCS.

The overall qualitative operational taxonomic unit richness (OTUs; 97% sequence similarity) from the soil samples appears to be high as rarefaction curves did not reach asymptote (Fig. 3). Comparisons between 16S rRNA gene sequences using rarefaction curves showed that the mean OTU richness at each sample site was higher at LMS (621.5 ± 45.7) than at the TCS (504.5 ± 51.8; Mann–Whitney *U*-test, $U = 36$, $P = 0.004$).

The number of *Acidobacteria* OTUs was fewer at TCS (9%) than at LMS (14.5%), whereas the number of OTUs identified as *Betaproteobacteria* and *Actinobacteria* was higher at the TCS (16.8–10.2% and 11.1–7.0%, respectively; Fig. 4). There was no obvious difference in *Alpha-* or *Gammaproteobacteria* and *Gemmatimonadetes* OTU abundance between LMS and TCS.

Terminal restriction fragment length polymorphism analysis

The bacterial communities from both TCS1 and TCS2, and LMS1 and LMS2 sites were examined using community fingerprinting. This also provided a measure of the relative abundances of individual terminal restriction fragments (tRFs).

Bacterial communities from both sample sites showed clusters in PCA ordination (Fig. 5), with overlap in the groupings indicating similarity between communities. However, the bacterial communities differed significantly between sites (ANOSIM, $R = 0.284$, $P = 0.001$). The data variability appeared to have been described by the primary PCA axis as samples from both sites exhibited diffuse clusters that crossed the primary axis. However, differences in land coverage were apparent as the samples sites diverged across the secondary axis. SIMPER analysis of tRF relative abundances revealed that 37.65% of the observed dissimilarity between sample sites could be attributed to 16% of the tRFs (Table 2). These tRFs represented *Proteobacteria* (*Alpha*, *Beta* and *Gamma* classes)

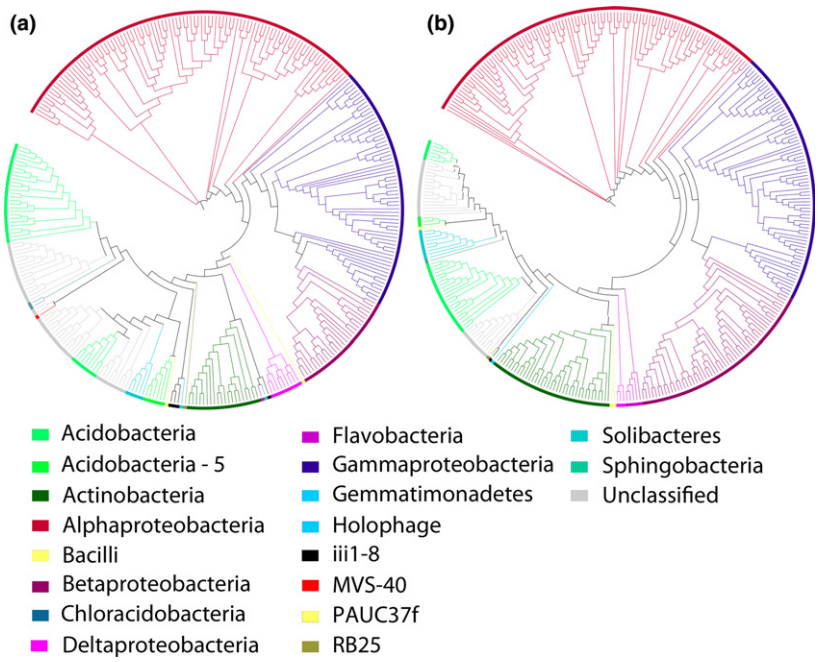


Fig. 4. Approximate maximum-likelihood phylogenetic tree of representative bacterial OTUs inhabiting soils from (a) LMS and (b) TCS. All OTUs are identified at class level and represent the proportional distribution of each class to the bacterial community. Listed here are all classes of bacteria identified in addition to those common classes discussed in the text. All sequences obtained through 454 NGS.

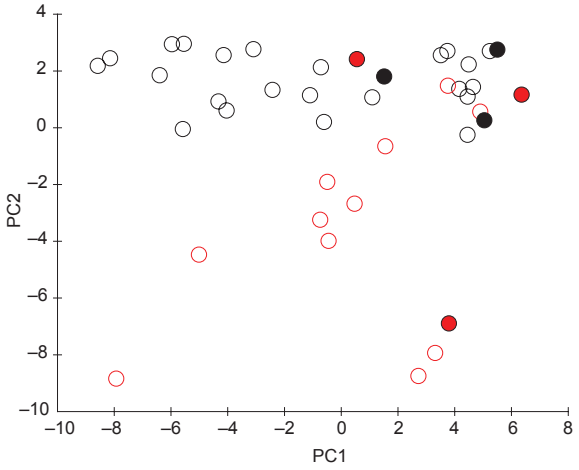


Fig. 5. PCA ordination presenting the differences in bacterial community structure between TCS (red circles) and LMS (black circles) using tRFLP analysis. Relative abundance plots were transformed to indicate the variability and similarity of samples and sample sites. Filled circles indicate samples that were sequenced and compared with tRFs using *in silico* digestion. The primary axis represents 13.0 % of the variability in the data set while the secondary axis represents 7.3 % of the variability.

and *Acidobacteria* predominately. *Gemmatimonadetes* and *Bacilli* accounted for 1.95% and 1.45% of community differences observed, respectively. Only 3.4% of the observed dissimilarity was accounted for by other taxa.

The tRFs identified as *Alphaproteobacteria* were entirely represented by the order *Rhizobiales*; those identified as *Gammaproteobacteria* were represented by *Pseudomonadales* with only one unclassified tRF. Of the *Proteobacteria*, the *Betaproteobacteria* had the least influence (2.96%) on the difference between sample sites, with all representatives from the order *Burkholderiales*. Individual tRFs identified as *Acidobacteria* had representatives from three groups: GP1, GP6 and GP18, of which GP1 had the largest influence (2.4%) on dissimilarity between sites. The remaining 60% of differences between bacterial communities of each sample site were accounted for by 127 individual tRFs, each tRF contributing $< 1.1\%$ of observed difference (data not shown). The α-diversity of tRFs, given as Shannon–Wiener diversity indices (H'), was significantly higher at LMS (H'; 3.14 ± 0.41, $n = 26$) than at the TCS (H'; 2.86 ± 0.33, $n = 14$) (Mann–Whitney U-test, $U = 607$, $P < 0.04$).

For the six samples that were subject to 454 NGS, the H' values were also calculated and compared with the corresponding six tRF H' values. In general, the six tRF data points maintained the differences observed in the study tRF data overall (LMS = 3.06 ± 0.53; TCS = 2.63 ± 0.19). However, the 454 16S rRNA gene sequences H' values showed very high similarity in diversity indices between the two land coverages (LMS = 3.38 ± 0.14; TCS = 3.36 ± 0.11). Neither of these examples was statistically significantly different.

Table 2. SIMPER analysis showing the cumulative effect of individual tRFs on bacterial community dissimilarity between TCS and LMS. Individual tRFs responsible for < 1% of the differences discussed were not included

tRF length	Taxon (Class)	Taxon (Order)	Individual contribution (%)	Cumulative contribution (%)
107	*Alphaproteobacteria*	*Rhizobiales*	2.9	2.9
104	*Alphaproteobacteria*	*Rhizobiales*	2.63	5.53
52	*Acidobacteria* GP1	Unclassified	2.4	7.93
106	*Alphaproteobacteria*	*Rhizobiales*	2.18	10.11
112	*Alphaproteobacteria*	*Rhizobiales*	1.98	12.09
98	*Gemmatimonadetes*	*Gemmatimonadetes*	1.95	14.04
455	*Gammaproteobacteria*	*Pseudomonadales*	1.9	15.94
105	*Alphaproteobacteria*	*Rhizobiales*	1.89	17.83
93	*Gammaproteobacteria*	*Incertae sedis*	1.85	19.68
400	*Alphaproteobacteria*	*Rhizobiales*	1.8	21.48
239	*Acidobacteria* GP1:GP18	Unclassified	1.79	23.27
102	*Betaproteobacteria*	*Burkholderiales*	1.59	24.86
250	*Acidobacteria* GP6	Unclassified	1.56	26.42
434	*Bacilli*	*Bacillales*	1.45	27.87
116	*Alphaproteobacteria*	*Rhizobiales*	1.45	29.32
121	*Alphaproteobacteria*	*Rhizobiales*	1.4	30.72
97	*Betaproteobacteria*	*Burkholderiales*	1.37	32.1
87	*Alphaproteobacteria*	*Rhizobiales*	1.36	33.46
242	*Acidobacteria* GP1:GP6:GP18	Unclassified	1.32	34.78
447	*Gammaproteobacteria*	*Pseudomonadales*	1.29	36.07
450	*Gammaproteobacteria*	*Pseudomonadales*	1.28	37.35
416	*Alphaproteobacteria*	*Rhizobiales*	1.26	38.62
395	*Alphaproteobacteria*	*Rhizobiales*	1.24	39.85
252	*Acidobacteria* GP6	Unclassified	1.2	41.05

The comparison of soil elemental compositions with tRF relative abundances indicated that *Acidobacteria* (tRF 52 nucleotides), *Gemmatimonadetes* (tRF 98 nucleotides) and *Alphaproteobacteria* (tRFs 112 and 110 nucleotides) were significantly correlated with soil spatial ordinations; each of the above tRFs was positively correlated with the soil chemistry from the LMS. Samples from the TCS showed a correlation with tRF peak 85 nucleotides in length, which was most closely aligned with *Nitrospira* and *Acidobacteria* taxa (Fig. 6).

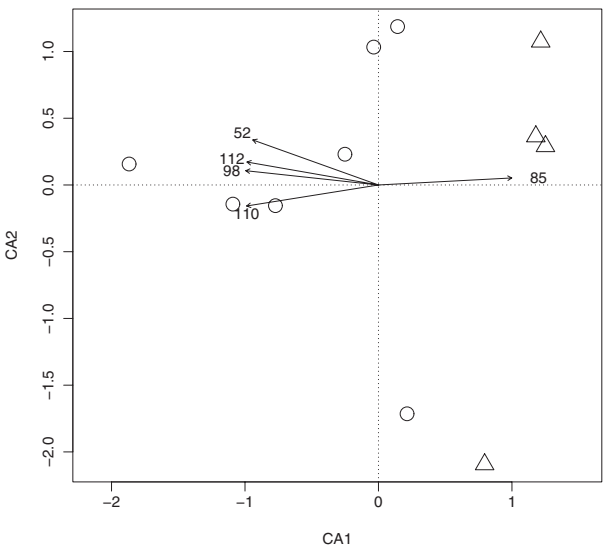


Fig. 6. CCA presenting elemental composition of soils from XRF data with statistically significant tRFs overlain. Significant correlations between soil samples and tRF length relative abundances presented as vectors ($P \leq 0.05$).

Discussion

This study characterizes the soil and its bacterial communities in the subsurface critical zone of two Icelandic field sites that differ in vegetation and weathering rates (Moulton & Berner, 1998; Moulton *et al.*, 2000).

Bacterial communities at the critical zone were found to be numerically dominated by *Alphaproteobacteria, Acidobacteria* and *Gammaproteobacteria.* These three taxa are important components of soil bacterial diversity (see Janssen, 2006 for a review). *Proteobacteria* have previously been shown to be abundant in Arctic soils (Neufeld & Mohn, 2005), with undisturbed tundra being dominated by this group. *Alphaproteobacteria* have specifically been reported as a dominant member of Arctic soils (Zhou *et al.*, 1997; Neufeld & Mohn, 2005). The importance of *Acidobacteria* in microbial diversity measures has also been reported for Arctic soil ecosystems (Chu *et al.*, 2010) and together with *Alphaproteobacteria* in temperate environments (Griffiths *et al.*, 2011).

In temperate soils, the ratio of *Alphaproteobacteria* to *Acidobacteria* has been proposed to be an indicator of soil nutrient status (Fierer *et al.*, 2007; Eilers, 2011; Griffiths *et al.*, 2011). Furthermore, the ratio of *Alphaproteobacteria* to *Acidobacteria* has been shown to be linked to the type of vegetation present at the soil surface (Thomson *et al.*, 2010; Griffiths *et al.*, 2011). The ratio of *Alphaproteobacteria* to *Acidobacteria* in previous studies has been calculated as 2.25 and 1.44 for vegetated and bare soils, respectively (Thomson *et al.*, 2010). For this study, based upon relative abundance of tRFs matching *Alphaproteobacteria* and *Acidobacteria*, resulting ratios of 3.09 and 1.74 were recorded here for TCS and LMS, respectively. We hypothesize that the ratios of *Alphaproteobacteria* to *Acidobacteria* are based upon nutrient status between the sites and reflect the differential nutrient inputs from TCS and LMS. For example, *Acidobacteria* are thought to be oligotrophic (Fierer *et al.*, 2007), whereas *Proteobacteria* encompass a large copiotrophic contingent (Simonato *et al.*, 2010). The lower soil organic carbon observed in the LMS may permit *Acidobacteria* to outcompete *Alphaproteobacteria*, as the former carbon requirements are best suited to the low concentrations of soil organic carbon in the LMS. This hypothesis to explain the community structure differences observed at our sites further expands the presumption that overarching ecological rules govern the presence of key microbial taxa such as observed in other studies (Fierer *et al.*, 2007) and may also extend to cold Arctic environments.

Previous work in the Arctic and in temperate soils has shown that pH influences microbial diversity, with low pH values coinciding with high *Acidobacteria* numbers (Griffiths *et al.*, 2011). However, Fujimura *et al.* (2012) showed that *Proteobacteria* dominated low pH (pH 3.0–2.6) volcanic soils from the island of Miyake, Japan, while *Acidobacteria* were more abundant in surface soils with a pH of *c.* 6 in Beech (*Fagus sp.*) stands, suggesting that plants may confound the influence of pH on the ratio of these two taxa. Our study corroborates the findings of Fujimura *et al.* (2012), as the TCS had significantly lower pH, yet harboured a larger proportion of *Proteobacteria*, specifically *Alphaproteobacteria.* Although pH is a major factor in determining microbial diversity, the small yet significant differences in pH recorded in this study may be overridden by the presence of plants and associated nutrient deposition. The influence of vegetation coverage on bacterial diversity was also observed by Griffiths *et al.* (2011). However, the effects of the vegetation were superseded by the highly significant effects of pH on bacterial diversity.

The higher numbers of cells observed in the TCS may be linked to higher concentration of soil organic carbon present, with the higher concentration of carbon acting as a nutrient source for the bacterial community, which is considered to be predominately heterotrophic (Chorover *et al.*, 2007; Akob & Küsel, 2011). The interaction between root-forming plants and microorganisms within the rhizosphere offers a mutually beneficial environment, stimulating floral and microbial growth (Radutoiu *et al.*, 2003). Moreover, the higher abundance of *Rhizobiales* associated with the root-forming plants in the TCS may be responsible for N_2 fixation (Deslippe *et al.*, 2006). This potential for diazotrophic behaviour combined with the higher concentrations of soil organic carbon and root exudates from the TCS can potentially enhance bacterial cell numbers at the cost of other taxa such as Archaea (Karlsson *et al.*, 2012). We did not, however, investigate Archaea in our study.

Although the bacterial communities as measured by tRF analysis at each sample site are significantly different, the 16S rRNA gene sequences indicated that *c.* 50% of OTUs recorded at either site were common between sites. Of these common OTUs, > 99% were represented by the phyla *Acidobacteria* and *Proteobacteria*. These similarities between bacterial communities at each sampling site were visualized by PCA (Fig. 6). This showed clustering of bacterial communities from the LMS running parallel with the first axis, indicating high variability in LMS samples, whereas the bacterial communities from the TCS were less clustered and did not parallel either axis. However, samples from the LMS did not cross the secondary axis, clearly separating the two land coverage types based on the secondary axis. The overlap of communities demonstrates that in a few particular samples, bacterial communities were more similar between sites compared with within sites. One explanation for this may be spatial limitations on the radius of influence that plant species have on the soil microbial communities, resulting in community similarity decreasing with distance from the plants. Special limits to the influence of plant rhizodeposition can be between 20 and 40 cm (Miniaci *et al.*, 2007). As these samples were collected at depths of *c.* 30 cm from areas without visible evidence of root systems, they may be at the limits of this influence.

A higher abundance of *Actinobacteria* species obtained from the TCS was apparent from the 16S rRNA gene sequences, yet was not corroborated by tRF SIMPER examination; this was due to the lower resolution on tRFLP affiliation that was apparent from the partial 16S rRNA gene sequences. The lower taxonomic resolution of tRFs is expected as several taxa can possess similar cleavage sites for particular digestion enzymes, resulting in tRFs of equal length. Furthermore, the numbers of representative OTUs identified as *Actinobacteria* were lower in samples from TCS; this indicates a higher abundance yet lower diversity of this taxon. *Actinobacteria* are found in terrestrial lithic environments studied for min-

eral weathering (Abdulla, 2009; Cockell *et al.*, 2009a, b; Kelly *et al.*, 2010) and are believed to enhance weathering (Cockell *et al.*, 2009a, b). A higher proportion of *Actinobacteria* would be expected given the higher soil organic carbon concentrations at TCS, as *Actinobacteria* are known to be important organisms in carbon cycling (Goodfellow & Williams, 1983). Furthermore, Hamdali *et al.* (2012) have shown that siderophores alone may not permit growth in phosphorus-limited oligotrophic environments, such as those studied here, potentially limiting *Actinobacteria* growth at the LMS. To maximize mineral leaching from the soil matrix, some plants, through the provision of root and mycorrhizal exudates, have been shown to actively encourage populations of mineral weathering microorganisms (Calvaruso *et al.*, 2006; Uroz *et al.*, 2007, 2011). The fungal and bacterial populations associated with the root systems from our TCS sample site may be altering the mineral weathering potential of the mycorrhizosphere. However, as differing species of root-forming plants are known to differentially influence the mineral weathering efficacy of the bacterial communities from forest soils (Collignon *et al.*, 2011), further investigation of the influence of individual tree species on critical zone soil bacterial communities would be required to confirm these effects for this study.

In our study, it is not possible to directly link the elemental abundances observed in the soil to bacterial weathering. Many elements covary with several environmental and chemical factors. For example, phosphorus (P) is bound by aluminium (Al) species (Lukito *et al.*, 1998; Kimble *et al.*, 2000). The lower concentration of P in the LMS might be caused by the higher concentrations of Al, rather than a lower rate of P liberation from rock apatites by chemical or biological processes. However, other elements are more likely to reflect weathering rates. The use of Si as an indicator of weathering basaltic rock has been described previously (Navarre-Sitchler & Brantley, 2007), and the higher concentration of Si measured for the TCS suggests that the lower pH associated with the site causes a higher release of Si into the soil and/or the biota is causing an enhanced release of Si from the rocks. Other elements such as Na and Ti, which are also elevated in the TCS, are likely to reflect rock weathering (Fig. 1).

Although the dominant tRF peaks corresponded with *Proteobacteria* and *Actinobacteria* taxa and showed significant correlations with the elemental ordination, the analysis of the data was not able to show any relationship between individual bacterial taxa and a chemical species.

In conclusion, we have carried out a study of the bacterial community present at the subsurface critical zone of a cold basaltic environment in two sites with different plant covers. The critical zone was dominated by *Proteobacteria* and *Acidobacteria*. Despite differences in chemical composition between the two sites, which may be correlated with the different rates of weathering caused, among other factors, by the presence of plants and differences in pH, this study shows that the soil critical zone at both sites shared 50% of bacterial OTUs (97% sequence similarities). These data suggest that a core of closely related taxa may inhabit the nutrient-poor environment of the cold volcanic critical zone, but that overall composition is influenced by other factors such as pH, organic availability and the rate of weathering itself. For example, the LMS were more bacterially diverse; this may reflect communities at TCS being more specialized in the processing of available nutrients afforded to them by plants through competitive exclusion processes. Future studies must determine whether the organisms that inhabit the critical zone are optimally adapted to this environment, whether they are capable of contributing actively to weathering or whether they are just passive inhabitants of the environment.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Comparison of tRF data and *in silico* digestions of 454 NGS provided an approximate taxon for each tRF below.