RESEARCH ARTICLE

Abiotic factors influence microbial diversity in permanently cold soil horizons of a maritime-associated Antarctic Dry Valley

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Abstract

The McMurdo Dry Valleys collectively comprise the most extensive ice-free region in Antarctica and are considered one of the coldest arid environments on Earth. In low-altitude maritime-associated valleys, mineral soil profiles show distinct horizontal structuring, with a surface arid zone overlying a moist and biologically active zone generated by seasonally melted permafrost. In this study, long-term microenvironmental monitoring data show that temperature and soil humidity regimes vary in the soil horizons of north- and south-facing slopes within the Miers Valley, a maritime valley in the McMurdo Dry Valleys. We found that soil bacterial communities varied from the north to the south. The microbial assemblages at the surface and shallow subsurface depths displayed higher metabolic activity and diversity compared to the permafrost soil interface. Multivariate analysis indicated that K, C, Ca and moisture influenced the distribution and structure of microbial populations. Furthermore, because of the large % RH gradient between the frozen subsurface and the soil surface we propose that water transported to the surface as water vapour is available to microbial populations, either as a result of condensation processes or by direct adsorption from the vapour phase.

Introduction

The Antarctic continent is widely acknowledged as harbouring some of the most extreme climatic conditions on Earth (Hansom & Gordon, 1998). While the bulk of the continent is ice-covered, 0.4% of the terrestrial surface is essentially ice-free, of which the McMurdo Dry Valleys in South Victoria Land comprise the largest coherent region. The extensive mineral soils of the Dry Valleys are characterized by multiple environmental characteristics considered to be biologically extreme, including large seasonal and diurnal variations in temperature, low precipitation and atmospheric humidity (leading to extremely low surface soil water content), low nutrient availability, high levels of salinity and solar radiation (UVB) and strong katabatic winds (Boyd *et al.*, 1966; Cameron *et al.*, 1970; Claridge & Campbell, 1977; Smith *et al.*, 1992; Vishniac, 1993). Microorganisms surviving these extreme conditions are thought to have adopted a wide range of different physiological and adaptive strategies in response to these impacts (Zeglin *et al.*, 2009; Casanueva *et al.*, 2010).

While earlier culture-dependent microbiological surveys of Dry Valley soils suggested that both the biomass levels and microbial diversity were, at best, low (Horowitz *et al.*, 1972; Friedmann, 1993), more recent phylogenetic surveys have indicated otherwise. ATP titres suggested that cell numbers in mineral soils in Miers Valley (a low-altitude coastal valley in the southern McMurdo region) were in the order of 10^6–10^8 per gram (Cowan *et al.*, 2002; Cowan & Casanueva, 2007). Phylogenetic analyses from a wide range of different sites have shown that at least 14 different phyla are present, including psychrophilic and psychrotolerant heterotrophs of the *Actinobacteria*, *Acidobacteria*, *Proteobacteria* and *Bacteroidetes* groups (Elberling *et al.*, 2006; Smith *et al.*, 2006; Aislabie

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et al., 2008) and numerous genera of the photoautotrophic *Cyanobacteria* (Jungblut *et al.*, 2005; Wood *et al.*, 2008). Dry Valley soils have been shown to support relatively low levels of eukaryotic microorganisms (Cowan, 2009; Pointing *et al.*, 2009), and so bacteria are thought to represent the major biotic component in these systems.

While biomass levels, bacterial diversity and community structure in Antarctic habitats are all influenced by multiple environmental parameters (Pointing *et al.*, 2009; Yergeau *et al.*, 2009; Zeglin *et al.*, 2009; Newsham *et al.*, 2010), temperature and water availability are thought to be the most important drivers (Noy-Meir, 1973; Kennedy, 1993; Parsons *et al.*, 2004; Warren-Rhodes *et al.*, 2006; Zeglin *et al.*, 2009; Cary *et al.*, 2010). In Antarctic Dry Valley soils, water availability can vary in both time and space (Noy-Meir, 1973; Hopkins *et al.*, 2006a) as a result of temperature fluctuations and the presence of the permafrost layer. Permafrost, described as permanently frozen sediment (Gilichinsky *et al.*, 2007), is ubiquitous in all but high altitude valleys in the McMurdo region and is present as ground ice or buried ice, ice-cemented permafrost and dry-frozen permafrost (Bockheim, 2008). The Dry Valley permafrost is generally dry and overlain by the active layer, defined as the layer of ground subjected to seasonally freezing and thawing cycles (Adlam, 2010). The active layer extends from the surface to a depth of approximately 10–25 cm (Gilichinsky *et al.*, 2007) depending on the seasonal cycle, aspect, slope (Guglielmin *et al.*, 2008) and location (Campbell *et al.*, 1998) and shows a climate-dependent variation (Campbell & Claridge, 1987). The upper few centimetres of this horizon is susceptible to mobilization by storms and katabatic winds (Gilichinsky *et al.*, 2007) but is partially stabilized by the desert pavement.

Because of the predominance of sublimation processes water is present in the permafrost zone but never in liquid form (Gilichinsky *et al.*, 2007). Nevertheless, permafrost contains diverse populations of both aerobic (gram-positive and gram-negative) and anaerobic bacteria (methanogens, sulphate reducers, etc.) (Gilichinsky *et al.*, 1995; Ponder *et al.*, 2004). These communities are thought to have retained viability for very long periods (from hundreds to millions of years) (Kastovska *et al.*, 2005) and are believed to be at least as old as the permafrost and thus may represent the oldest microorganisms discovered on Earth (Gilichinsky *et al.*, 2007). Observations that microbial activity extends to well below 0 °C (Shi *et al.*, 1997; Vorobyova *et al.*, 1997; Rivkina *et al.*, 2000) suggest that permafrost communities may retain low but significant levels of metabolic activity (Willerslev *et al.*, 2004; Steven *et al.*, 2006; Gilichinsky *et al.*, 2007).

In the active zone, liquid water is present on a seasonal basis. Moist mineral soils are exposed to temperatures above 0 °C for approximately 3 months of each annual cycle, and it is assumed that the conditions necessary for metabolic activity are met. Little is known of either the microbial populations of this zone or of their metabolic activity. Furthermore, moisture movement through the active layer is affected by soil particle size which regulates soil porosity and hydraulic properties (Sauer & Logsdon, 2002). It has been recently shown that water content generally increases with active layer depth and is regulated by temperature and atmospheric relative humidity (RH) and less influenced by latitude (Seybold *et al.*, 2010).

Occasional light snow falls temporarily wet the upper few centimetres of the Dry Valley soils (Hopkins *et al.*, 2006b), but surface liquid water is very rapidly lost because of evaporative and ablation processes driven by the very low atmospheric humidity. Furthermore, in the Dry Valleys sublimation rates exceed precipitation, thus limiting the amount of water able to penetrate the ground (Fountain *et al.*, 2009). Typically, gravimetric analyses of water content in surface soils show extremely low moisture content values (Campbell *et al.*, 1994; Bockheim, 1995; Bockheim & Tarnocai, 1998; Campbell *et al.*, 1998). Recently, Newsham *et al.* (2010) reported that soil water content had only a minor effect on the bacterial community composition on maritime Antarctic soils. This conclusion is in stark contrast to hot desert soils where water availability is the dominant driver of both macro- and microbiological systems (McKay *et al.*, 2003; Warren-Rhodes *et al.*, 2006; Pointing *et al.*, 2007; Warren-Rhodes *et al.*, 2007). Other important variables influencing the diversity and composition of soil bacterial communities include soil pH (Fierer & Jackson, 2006; Männistö *et al.*, 2007; Soo *et al.*, 2009; Yergeau *et al.*, 2009; Zeglin *et al.*, 2009), salinity (Zeglin *et al.*, 2009), latitude (Yergeau *et al.*, 2009), UV impact (for surface microbial community development) (Tosi *et al.*, 2005), soluble salts, K and C (Pointing *et al.*, 2009). An altitude-related effect on development of lithic communities in close association with soil has also been observed (Cowan *et al.*, 2010).

Fine-scale geographic positioning could also affect microbial distribution and diversity. It has been reported that this aspect influences the conditions for microbial development (Wynn-Williams, 1990). Because of higher solar gain, north-facing slopes generally have a larger active layer, a longer seasonal period of thaw and fewer freeze–thaw cycles (Legget *et al.*, 1961; Rieger, 1974). Thus, the north facing slopes are typically less extreme than the south-facing slopes (Keys, 1980) and are characterized by environmental conditions that are biotically more favourable (Wise & Gressit, 1965; Wynn-Williams, 1990).

Together, these findings suggest that microbial diversity and function in Antarctic Dry Valley soils is dictated by a wide range of abiotic factors, almost certainly in combination. To understand the abiotic 'drivers' of the biotic system, one valuable approach has been to select model communities where it is possible to isolate one or more of these drivers (Cary *et al.*, 2010). A study of soils in shallow-depth transects offers one such system, where clearly defined horizons are subject to quantifiable and distinct regimes of temperature, water availability and chemical properties. Here we have used a range of molecular techniques to analyze the microbial diversity of soil samples collected from shallow-soil-depth transects in a coastal Dry Valley. The use of samples from two sites of different aspect (south and north facing slope) potentially allows us to observe the effects of fine-scale variables. Furthermore, to gain insight into the abiotic drivers influencing and controlling community diversity, structure and composition, microbial profiles have been linked to medium-term microenvironmental variables and soil chemical properties.

Materials and methods

Sample collection

Soil samples (approximately 50 g) were collected from shallow trenches excavated from a north- and a south-facing-site on the lower slopes of Miers Valley during 2009 austral summer (see Table 1 for details of the sampling points). Single soil core samples were recovered aseptically from the surface (0–2 cm) and at depths of 2–5, 5–10, 10–15 and 15–20 cm (south-facing) and from the surface (0–2 cm) and at depths of 2–5, 5–10, 10–15, 15–20, 20–25 and 25–30 cm (north-facing). The permafrost interface level was recorded at approximately 20 and 30 cm, respectively. All samples were stored at < 0 °C in the field and during transport and at -80 °C in the laboratory prior to further analysis.

In situ temperature and humidity measurements

At the surface *in situ* air and soil temperatures (T, °C) were measured using a JENWAY 230 temperature meter. Air and soil relative humidity (% RH) values were measured with a Digitron 2020R meter. Temperature and RH were logged using Thermochron/Hygrochron iButtons (model DS1921G, Embedded Data Systems) embedded at each depth at 10-min intervals for 12 days during November/December 2009 (from 28 November 2009 to 11 December 2009). In addition, soil surface (0–2 cm) temperature and % RH data were logged at 4-h intervals over a 10-month period during 2008. We note, for clarification, that this period does not include the two warmest months of the McMurdo Dry Valley calendar and that mean and maximum temperature and % RH values will be underestimated. Kruskal–Wallis followed by a Wilcoxon–Mann–Whitney *post hoc* test was used to assess T and % RH differences between samples.

ATP assays

ATP measurements were obtained within 90 min of sampling using the commercially available luminometric assay system designed to operate with the SystemSURE Y2 K portable monitor (Celsis Instruments, Cambridge, UK). Data are expressed as relative luminosity units (RLU). All assays were performed in duplicate with appropriate controls.

Soil physicochemical analysis

Soil physicochemical variables including moisture content, pH, organic carbon, total nitrogen, phosphorous, potassium, exchangeable cation and base saturation determinations were performed at BemLab (SANAS Accredited Testing Laboratory, Somerset West, South Africa), according to standard quality control procedures (SSSA, 1996).

Metagenomic DNA extraction and amplification

Metagenomic DNA was extracted in triplicate from soil samples using the PowerSoil™ DNA Isolation Kit (MoBio, West Carlsbad, CA) according to the manufacturer's instructions. Triplicates were pooled and used as template for PCR amplification. General bacterial primers E9F (5′-GAGTTTGATCCTGGCTCAG-3′; Hansen *et al.*, 1998) and U1510R (5′-GGTTACCTTGTTACGACTT-3′; Reysenbach & Pace, 1995) were used for amplification of 16S rRNA genes. All polymerase chain reactions (PCR) were carried out in a Perkin Elmer Thermocycler (Gene Amp PCR system 6700) in a 50 μL reaction volume containing 1× PCR buffer, 200 μM of each dNTP, 0.5 μM of each primer, 0.2 U of Dream-*Taq*™ polymerase (Fermentas) and 10 ng of template. Thermal cycling conditions were 4 min denaturation at 94 °C; 30 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 105 s; final elongation step at 72 °C for 10 min. All PCR reactions were carried out in triplicate and DNA concentrations were measured with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Montchanin, DE).

Table 1. Samples description, soil T (°C),% RH, ATP values, and diversity indices obtained from the T-RFLP analysis

Sample	S-0	S-5	S-10	S-15	S-20	N-0	N-5	N-10	N-15	N-20	N-25	N30
Slope*,†	South	South	South	South	South	North	North	North	North	North	North	North
Depth (cm)	0–1	2–5	5–10	10–15	15–20	0–1	2–5	5–10	10–15	15–20	20–25	25–30
Zone	Surface	Active layer	Active layer	Active layer	Permafrost boundary	Surface	Active layer	Active layer	Active layer	Active layer	Active layer	Permafrost boundary
ATP‡ (RLU × 10^3)	33 ± 4	7 ± 1	4 ± 0	4 ± 1	2 ± 1	15 ± 4	8 ± 1	5 ± 1	4 ± 0	3 ± 1	1 ± 0	ND
DNA‡ (ng g^{-1})	2033 ± 29	2016 ± 6	1586 ± 30	1523 ± 21	1420 ± 20	1760 ± 56	1753 ± 45	1747 ± 40	1607 ± 179	1660 ± 80	1577 ± 23	1490 ± 30
Min T (°C)§	−4.6	−1.7	−0.4	0.1	−0.2	−3.1	−0.7	−0.2	−0.9	−2.7	−4.2	−4.9
Max T (°C)§	25.4	14.2	10.2	7.9	11.6	25.5	20.4	24.3	23.0	20.4	19.6	26.5
Mean T (°C)§	6.9	5.2	3.8	2.6	1.6	7.9	3.9	2.3	1.31	−0.2	−1.5	−2.1
Min% RH§	23.5	91.6	92.8	85.7	97.3	26.1	76.4	92.4	93.4	92.4	89.0	88.9
Max% RH§	87.5	111.9	109.7	108.7	108.0	86.6	106.9	110.5	107.8	113.8	106.4	108.0
Mean% RH§	50	105.1	105.2	105.0	105.2	39.6	101.7	106.4	104.9	108.6	102.4	104.3
Pielou's Eveness (J′)	0.9	0.9	0.7	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.8
Shannon's Index (H′)	1.8	2.5	1.7	3.1	3	1.7	2.8	2.2	1.9	1.9	1.1	1.9
Simpson Index (1 − λ′)	0.8	0.9	0.7	0.9	0.9	0.7	0.9	0.9	0.8	0.8	0.6	0.8

*South facing slope GPS coordinates: S 78° 05.590′, E 163° 48.270′ (slope angle approximately 5°, altitude 177 m).

†North-facing slope GPS coordinates: S 78° 06.144′, E 163° 48.468′ (slope angle approximately 5°, altitude 177 m).

‡Mean (±SD).

§T (°C) minimum, maximum and mean values for South- and North-facing slope samples data logged at 10-min. intervals over a 12-day period. Measurements (South and North T) = 1747; Measurements (South and North % RH) = 1570. ND = Not determined.

Denaturing gradient gel electrophoresis (DGGE)

Amplicons obtained with the 16S rRNA gene primer set (E9F-U1510R) were used as template for a nested PCR for subsequent DGGE. Template DNA (1 μL) was PCR-amplified using 341F-GC (5′-CCTACGGGAGGCAGCAG-3′, with a GC clamp, CGCCCGCCGCGCGCGGCGG GCGGG GCGGGGGCACGGGGGG, added to the 5′ end) and 534R (5′- ATTACCGCGGCTGCTG-3′) (Muyzer *et al.*, 1993) in a 50-μL reaction containing 1× PCR buffer, 200 μM of each dNTP, 0.5 μM of each primer and 0.2 U of Dream*Taq*™ polymerase (Fermentas). Thermal cycling conditions for DGGE amplification were 4 min at 94 °C; 20 cycles of 94 °C for 45 s, 65 °C for 45 s and 72 °C for 60 s; 20 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s; final elongation step at 72 °C for 10 min. DGGE was performed essentially as described by Muyzer *et al.* (1993) using the DCode DGGE system (Biorad) at 100 V for 16 h at 60 °C in 1× TAE buffer. After EtBr (0.5 μg mL^{-1}) staining, gels were visualized with an AlphaImager 3400 imaging system. DGGE profiles were analyzed using GelCompar® II, version 5.0 (Applied Maths).

Terminal restriction fragment length polymorphism (T-RFLP) analysis

For T-RFLP analysis, PCRs were conducted using the primer set E9F-FAM (5′-labelled with tetrahydrochloro-6-carboxyfluorescein) and U1510R. PCR products were purified with an Illustra GFX™ PCR DNA and gel Band Purification kit (GE Healthcare, UK) and similar amounts of DNA (200 ng) digested with HaeIII (Fermentas). The digested products were purified as above and separated by capillary electrophoresis using an ABI3130XL (Applied Biosystems). ROX 1.1 (Slabert *et al.*, 2010) was used as a size standard. T-RFLP profiles were analyzed using Peak Scanner 1.0 (Applied Biosystems, https://products.appliedbiosystems.com) and the web-based programme T-REX (Culman *et al.*, 2009) (trex.biohpc.org). T-REX software uses the methodology described by Abdo *et al.* (2006) and Smith *et al.* (2005) to identify and align true peaks, respectively. We used one standard deviation in peak area as the limit to identify true peaks and 1 bp as the cluster threshold for the alignment. All T-RFLP analyses were performed in triplicate. An OTU was considered present if it appeared in at least two of the three PCR replicates. Fingerprint profiles were standardized by dividing each individual peak area by the total area of peaks in a sample profile. The Phylogenetic Assignment Tool (PAT) (Kent *et al.*, 2003) (https://secure.limnology.wisc.edu/trflp/) was used to putatively assign T-RFLP peaks. Programme options were set to allow a sizing error of 1 bp only for the smallest T-RFs and up to 4 bp with the longest T-RFs.

Statistical analysis and diversity index calculations of T-RFLP data were undertaken with PRIMER 6 (PRIMER-E Ltd, Ivy-bridge, UK). Analysis of similarities (ANOSIM) was performed to test for significant differences between *a posteriori* sample groupings. BEST analysis (Clarke & Gorley, 2006) was used to rank the influence of abiotic variables on the community structure and to select the combination of variables that better explain biotic patterns. The significance of the correlation between DGGE and T-RFLP matrices was calculated using Mantel test with 999 matrix permutations. Mantel test and other statistical analyses were performed using R (http://www.R-project.org).

16S rRNA gene clone library construction

16S rRNA gene fragments, PCR-amplified with primers E9F/U1510R from sample S20 (south-facing slope sample collected at a depth of 20 cm) and N30 (north-facing slope sample collected at a depth of 30 cm), were used for clone library construction (96 clones for each clone library). The PCR products were purified with an Illustra GFX™ PCR DNA and Gel Band Purification kit (GE Healthcare), ligated into pGEM®-T Easy vector System (Promega) and transformed into competent *Escherichia coli* GeneHogs® (Invitrogen). Transformants were selected by blue/white screening. The presence of correctly sized inserts was confirmed by colony PCR and de-replicated by Amplified Ribosomal DNA Restriction Analysis (ARDRA) with restriction enzyme HaeIII (Fermentas). Clones with unique ARDRA patterns were considered as phylotypes and purified with a PeqGOLD Plasmid Miniprep Kit I (Biotechnologie GmbH) according to the manufacturer's instructions and sequenced with a Hitachi 3730xl DNA Analyzer (Applied Biosystems). Chromatograms were edited using Chromas (Technelysium), checked for chimeras using CCODE (Gonzalez *et al.*, 2005) and screened with the GenBank database through a standard BLASTN search (Altschul *et al.*, 1990). Phylotypes were further delineated according to sequence similarities of $\leq$ 97%. CLUSTALW alignments were carried out using Bio Edit (Hall, 1999). Phylogenetic trees were constructed in MEGA4 (Tamura *et al.*, 2007).

A virtual HaeIII digest of sequences obtained from the clone libraries was carried out to confirm the phylogenetic identities of individual peaks (T-DistinctiEnz in silico T-RFLP tool; http://www.bioinformatics.org/~docreza/cgi-bin/restriction/t_DistinctEnz.pl).

Sequences obtained in this study were submitted to GenBank under accession numbers HQ616027–

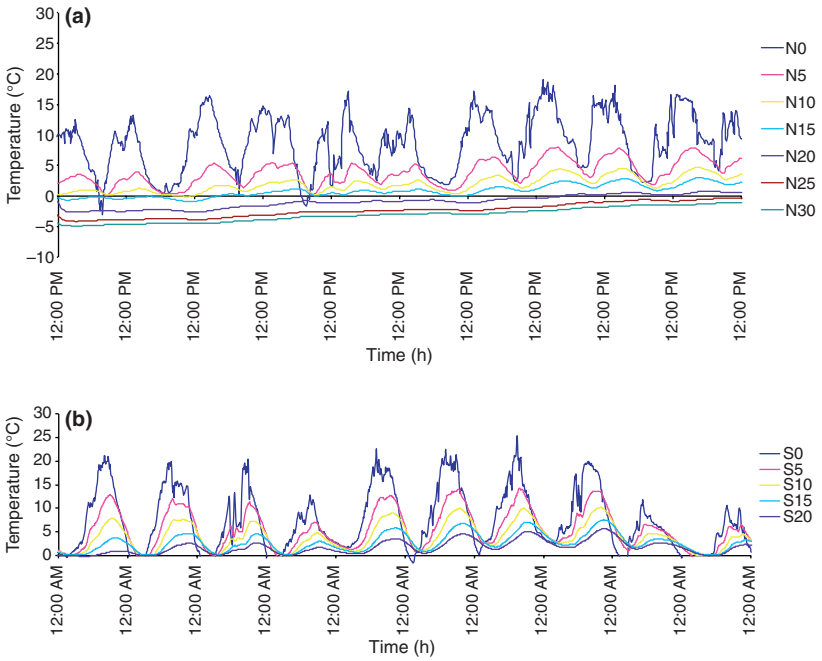

Fig. 1. Temperature (°C) logged at 10-min intervals over 12 days in samples collected from the south (a) and north (b) facing side of the McMurdo Dry Valley during the 2009 expedition. Samples have been collected from the surface and at 5, 10, 15 and 20 cm in depth.

HQ616073. Chao1 estimates were calculated using the web interface provided by Kemp & Aller (2004) (http://www.aslo.org/lomethods/free/2004/0114a.html).

Results

Data sets were acquired from shallow vertical transects at two sites on the lower slopes of the upper Miers Valley. Analysis performed included temperature, humidity, microbial biomass, total DNA, mineral soil physicochemical properties and phylogenetic fingerprinting.

In situ temperature and humidity measurements

Data from the 12-day *in situ* monitoring showed strong diurnal fluctuations in both temperature and % RH for the surface samples with increasing buffering with depth (Figs 1 and 2). Differences in temperature and % RH between the surface and the deepest soil samples from both south- and north-facing aspects were significant ($P < 0.05$). Mean temperature values for south (S)- and north (N)-facing samples ranged from 1.6 (S20) to 6.9 °C (S0) and from −0.2 (N20) to 7.9 °C (N0), respectively. % RH values ranged from 50.0 (S0) to 105.2 (S20) and from 39.6 (N0) to 108.6 (N20), respectively (Table 1). Over the 10-month *in situ* monitoring period (Fig. 3), the minimum temperature and % RH recorded were −41.1 °C and 69.6% and the maximum were 6.7 °C and 106.1%, respectively, while the mean annual temperature was −24.5 °C and the mean annual % RH was 91.8.

Microbial biomass

ATP titres decreased dramatically with depth in both facing slopes [3.3×10^4–2×10^3 RLU (S0-S20) and 1.5×10^4–1×10^3 RLU (N0-N25)] (Table 1). Using the conversion values of Cowan & Casanueva (2007), we estimate the surface cell titres to be 2.3×10^9 cells g^{-1} and 1×10^9 cells g^{-1} in the S- slope and N- slope sites, respectively. Calculated biomass levels for the deepest soil samples were 1.4×10^8 and 7×10^7 cells g^{-1} for south and north, respectively. DNA concentrations decreased significantly from surface samples (2033 ng g^{-1} in sample S0 and 1760 ng g^{-1} in sample N0) to samples collected at depth (1420 ng g^{-1} in sample S20 and 1490 ng g^{-1} in sample N30) from both sides (Table 1).

Community structure

DGGE analysis showed that surface and shallow subsurface soil samples from both sites (except for sample S5) clustered together (71% similarity). Intermediate depth samples from both sites formed a second coherent cluster (81.3% similarity) together with the south permafrost interface sample. The two deepest samples, recovered from the north permafrost interface, clustered together with a 93.1% similarity (Fig. 4).

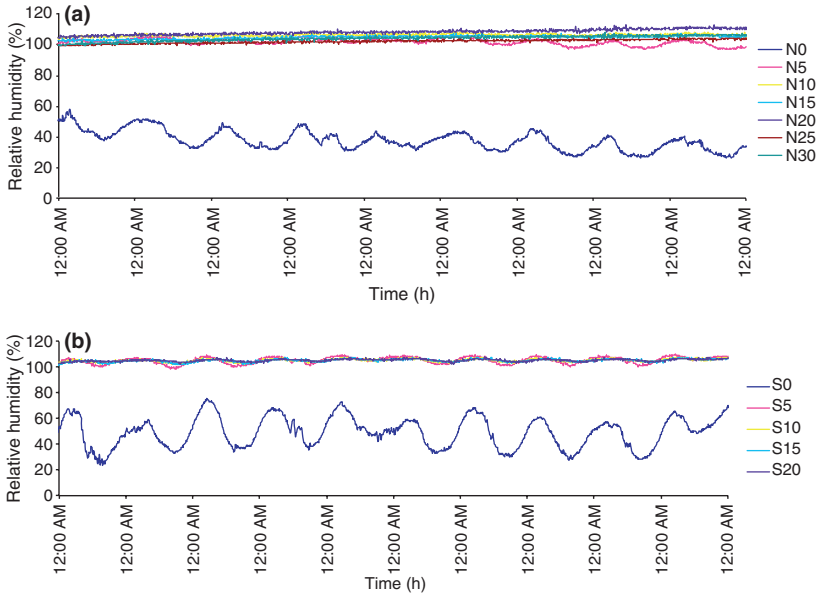

Fig. 2. Relative humidity (%) logged at 10-min intervals over 12 days in samples collected from the south (a) and north (b) facing side of the McMurdo Dry Valley during the 2009 expedition. Samples have been collected from the surface and at 0, 5, 10, 15, 20, 25 and 30 cm in depth.

A cluster analysis of T-RFLP data showed a similar trend to DGGE. This was confirmed by a significant correlation between the two matrices (Mantel R 0.4, $P < 0.05$). However, after ANOSIM analysis no differences were found between samples collected at different depths and samples collected from the two sites (ANOSIM $P > 0.05$). As T-RFLP can be linked to the phylogenetic information (clone libraries) all the data presented here refer to this molecular tool. A total of 76 T-RFs were found, of which 17% were unique to the north and 54% unique to the south (29% overlap). The highest number of OTUs was found in sample S20 (28) and the lowest in sample N25 (4). A few of the TRFs were dominant (e.g., TRFs 77, 92, 96, 121) occurring in over 50% of the samples. OTU richness and number of occurrence of each OTU are shown in Supporting Information, Fig. S1.

Using the Phylogenetic Assignment Tool (PAT) (Kent *et al.*, 2003) putative identities were assigned to a relatively high percentage (70%) of the T-RFLP peaks (Fig. S2). Samples were dominated by *Alphaproteobacteria* (72 bp), *Betaproteobacteria* (77, 91, 199, 205 bp), *Gammaproteobacteria* (210 bp), *Deltaproteobacteria* (234 bp), *Actinobacteria* (94, 121, 183 bp), *Firmicutes* (327 bp), *Bacteroidetes* (254, 259 bp), *Acidobacteria* (267 bp), *Spirochetes* (79 bp), *Cyanobacteria* (127 bp), *Chlorobi* (165 bp), *Planctomycetes* (182 bp) and *Deinococcus/Thermus* (63 bp). Furthermore, a total of 11 TR-Fs could be matched with 16S rRNA gene sequences and their respective taxonomic group (Table S2).

The possible relationships between abiotic parameters and microbial community structures were assessed by BEST analysis (Clarke & Gorley, 2006). C and K were the environmental variables that most strongly influenced the structure of microbial populations ($pw = 0.275$ and $pw = 0.255$, respectively), followed by the combination of K + Ca, K + C and K + C + Ca + moisture ($pw = 0.259$, 0.253 and 0.246, respectively). Including the 12-day mean % RH and temperature values of all the samples (data from Table 1) in the analysis did not alter these patterns. However, as most abiotic factors were highly correlated [except moisture and C (Table 2)], it may be difficult to determine their role in shaping the structure of the microbial communities analyzed.

To elucidate the phylogenetic diversity of the deepest samples (S20 and N30) two 16S rRNA gene clone libraries were generated, as surface soils microbial diversity has been extensively characterized (De la Torre *et al.*, 2003; Smith *et al.*, 2006; Yergeau *et al.*, 2007; Aislabie *et al.*, 2008; Niederberger *et al.*, 2008; Khan *et al.*, 2011). A total of 69 phylotypes were inferred from ARDRA analysis, 39 from sample S20 and 30 from sample N30. Sequence analysis identified 46 OTUs (at 97% identity). *Proteobacteria* and *Actinobacteria* were dominant in the south permafrost interface while *Bacteroidetes*, *Acidobacteria*, *Firmicutes* and *Actinobacteria* were the predominant phyla in sample N30 (Fig. S3). Moreover, the clone library constructed from sample S20 showed higher OTU richness and revealed the presence of other bacterial phyla that were not detected in sample N30 (i.e. *Gemmatinomonadates*, *Verrucomicrobia*,

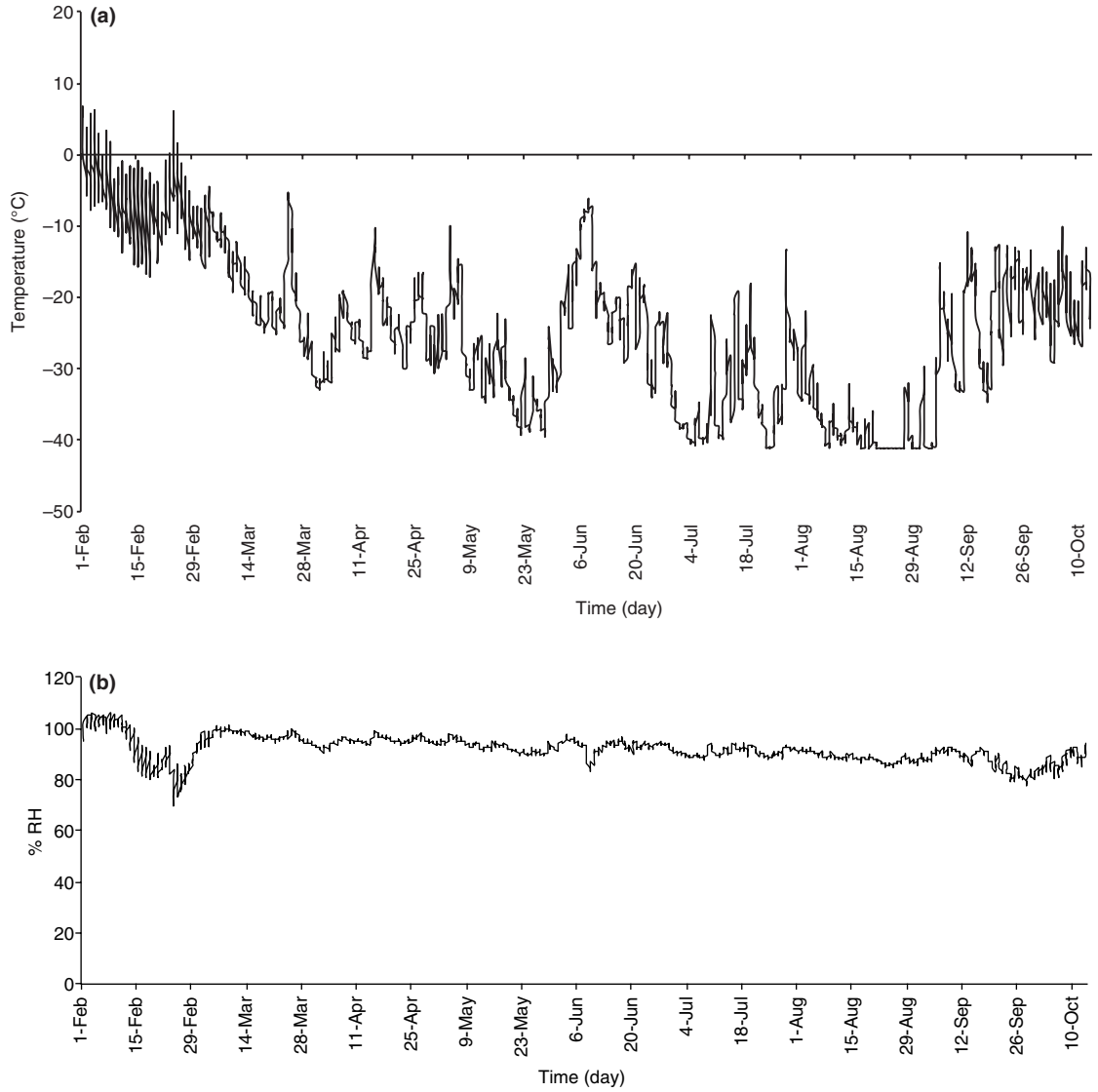

Fig. 3. Soil surface T (°C) (a) and % RH (b) conditions logged at 4-h intervals over a 10-month period during the 2008 expedition in the McMurdo Dry Valley (n = 2044).

Chlorobi and *Cyanobacteria*) (Fig. S3). This result was in agreement with the Chao1 index, which predicted that only 25% and 52% of the total diversity in samples S20 and N30 was observed, respectively.

The majority of these sequences showed close affinity to uncultivated bacteria (Fig. S4) previously reported from Antarctica (Dry Valleys, Alexander Island) and other cold and/or dry environments (Table S2). Virtual digests of the 16S rRNA gene clone libraries showed 11 OTUs corresponding to those of the dominant T-RFs (±4 bp) in the T-RFLP profiles (Table S2). Although too numerous to list, examples include T-RF 199 affiliated to *Gammaproteobacteria*, T-RF 234 to *Actinobacteria* and T-RF 210 to two different *Gammaproteobacteria* sequences.

Discussion

Recent studies on a wide range of different Antarctic desert soils have demonstrated an unexpectedly rich microbial diversity (Cowan *et al.*, 2002; Aislabie *et al.*, 2006; Smith *et al.*, 2006; Niederberger *et al.*, 2008; Pointing *et al.*, 2009). However, despite extensive phylogenetic analysis, comparatively little is known of the gross functionality of these 'communities', the key functional members and the influence the microenvironmental drivers pose on their development. Here we aimed to establish whether abiotic factors influence microbial profiles in permanent cold soil horizons. Although the number of samples analyzed in this study prevents extrapolation of

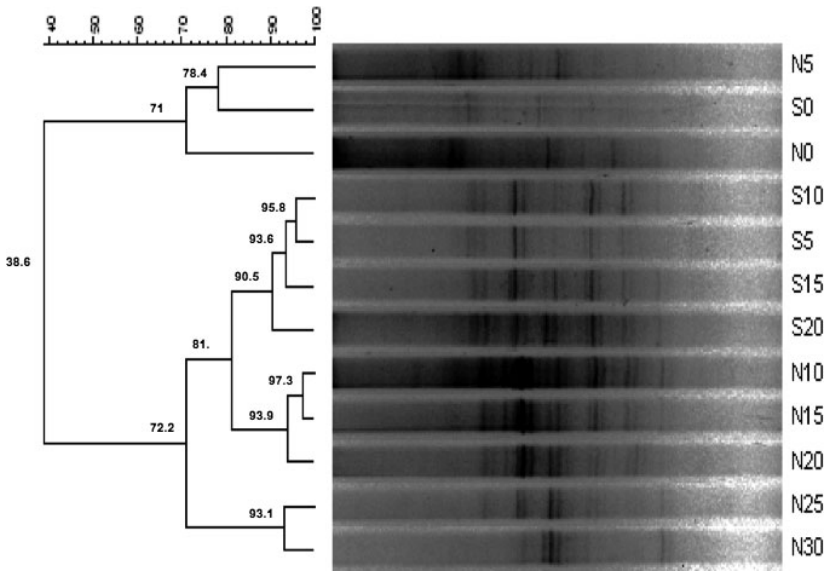

Fig. 4. Cluster analysis (UPGMA algorithm) from DGGE profiles of soil bacterial communities (North- and South-facing slopes of McMurdo Dry Valley).

the results to the wider Antarctic environs, the data does provide evidence that bacterial communities in the Dry Valleys are not homogenous and their development is abiotically driven.

Salinity (Zeglin *et al.*, 2009), pH (Männistö *et al.*, 2007), UV (Tosi *et al.*, 2005), latitude (Yergeau *et al.*, 2009), altitude (Cowan *et al.*, 2010), K, C, soluble salts (Pointing *et al.*, 2009) and other factors have been shown to influence microbial diversity, and temperature and moisture are considered to be the most critical variables (Kennedy, 1993; Noy-Meir, 1973; Parsons *et al.*, 2004; Barrett *et al.*, 2007).

It is widely accepted that water availability is a critical 'driver' of microbial activity in cold desert systems (Barrett *et al.*, 2007). It is also assumed that Antarctic desert soil communities are largely, if not completely, dependent on periodic precipitation in the form of light and intermittent snow falls (Claridge & Campbell, 1977; Hopkins *et al.*, 2006b; Fountain *et al.*, 2009). However, our study and others (e.g. Newsham *et al.*, 2010) have failed to demonstrate a significant relationship between soil water content and biological parameters. Antarctic soil surface microbial communities are in close proximity to a potential source of water in the form of a permafrost horizon (Friedmann, 1993; Bockheim, 1997; Hopkins *et al.*, 2006b). At least 25% of Antarctic desert regions contain permafrost in the form of ground ice soil (Bockheim, 1995). In the maritime Dry Valleys, the permafrost interface during the austral summer season is typically within 30 cm of the soil surface and is commonly overlaid by a moist active layer, where liquid water is entrained upward from the melting interface by capillary action (Bockheim, 2002; Barrett *et al.*, 2007). Active-layer thickness and depth to ice-cemented permafrost are seasonally controlled and related to local climate, solar gain (a product of aspect and slope), proximity to glaciers and albedo of surface rocks (Bockheim, 2002). The active zone represents an intermediate and dynamic continuum between the permanently cemented 'permafrost' and the surface.

Seybold *et al.* (2010) noted an increase in water content with depth. In this study, although soil moisture content did not show a linear increase along the vertical gradient, it did show a clearly increasing trend with depth. A possible explanation for the lack of a linear change in water content with depth would be, as suggested by Seybold *et al.* (2010), that soil water content is influenced both by capillary action (upward entrainment) and recharge from intermittent precipitation events (downward entrainment). However, we did record a very large % RH gradient between surface and depth, suggesting upward water vapour mobility as a strong thermodynamic driver. This gradient occurs within the top 5 cm and stabilizes with depth. We suggest that the transport of water vapour from melted permafrost to atmosphere provides a mechanism for a continuous supply of water to soil microbial populations either as the result of condensation processes or by direct adsorption from the vapour phase (e.g., via hydroscopic exopolymer secretions layers) (Potts, 1994; Azúa-Bustos *et al.*, 2011).

Table 2. Pearson correlation matrix of physicochemical soil parameters

Variables	pH	Moisture	C	N	Na†	K†	Ca†	Mg†	TValue‡	Na§	K§	Ca§	Mg§	P
Moisture	0.29													
C	−0.01	0.56												
N	−0.59*	−0.32	−0.27											
Na†	0.70*	0.28	0.32	−0.56										
K†	−0.13	−0.2	−0.49	0.34	−0.39									
Ca†	0.69*	0.17	0.25	−0.62*	0.93***	−0.51								
Mg†	−0.34	−0.26	−0.51	0.44	−0.45	0.91***	−0.62*							
TValue‡	0.70*	0.17	0.23	−0.62*	0.95***	−0.46	1.00***	−0.58						
Na§	0.52	0.4	0.36	−0.27	0.65*	0.02	0.35	0.06	0.39					
K§	−0.54	−0.2	−0.4	0.63*	−0.81**	0.66*	−0.95***	0.79**	−0.93***	−0.17				
Ca§	0.52	0.19	0.4	−0.62*	0.75**	−0.68*	0.92***	−0.82***	0.89***	0.08	−0.99***			
Mg§	−0.55	−0.23	−0.43	0.63*	−0.78**	0.67*	−0.92***	0.83***	−0.90***	−0.13	0.99***	−1.00***		
P	−0.29	0.22	0.18	0.36	−0.48	0.42	−0.58*	0.39	−0.57	−0.02	0.59*	−0.57	0.55	
K	−0.14	−0.19	−0.5	0.35	−0.4	1.00***	−0.53	0.92***	−0.48	0.04	0.68*	−0.70*	0.70*	0.43

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.
†Exchangeable cations.
‡Expressed as the sum of exchangeable cations.
§Base saturation.

Biomass levels (as indicated by ATP titres and DNA yields) consistently decreased with depth in shallow vertical soil transects. This observation reinforces the argument for the importance of metabolic capacity (expected to be highest at the surface because of the presence of active phototrophy and because of the higher surface temperatures) and indicates that soil water content is not the most critical factor driving the development and activity of microbial communities in cold desert soils. Although functional microbial ecosystems are thought to exist in permafrost (Vorobyova *et al.*, 1997; Steven *et al.*, 2006) and given that metabolically active microorganisms have been found below −10 °C (Gilichinsky *et al.*, 1995; Shi *et al.*, 1997; Rivkina *et al.*, 2000) most permafrost microorganisms are considered to be in a state of dormancy (Friedmann *et al.*, 1994; Gilichinsky *et al.*, 2007). It is highly likely that viable microorganisms in the intermediate active zone, with consistently low ambient temperatures, exhibit only limited metabolic activity.

Soil organic carbon levels are not inconsistent with this view where organic C levels generally decreased with depth (Table S1). Carbon is usually a limiting factor in the Dry Valley soils (Barrett *et al.*, 2005; Steven *et al.*, 2006; Pointing *et al.*, 2009); however, the origins of organic carbon both in surface soils and at depth are complex. While surface photoautotrophic processes are the obvious renewable source of fixed carbon (Wood *et al.*, 2008), in Dry Valleys harbouring lake systems (such as the Miers Valley) surface soil carbon stocks may be supplemented by the aeolian transport of lacustrine cyanobacterial biomass (Parker *et al.*, 1982; Moorhead *et al.*, 2003). Subsurface carbon is influenced to an unknown extent by transport from the surface and by microbial mineralization processes and may be further complicated by a background of legacy carbon (Moorhead *et al.*, 1999; Burkins *et al.*, 2000). Recent Dry Valley estimates of soil organic C turnover (20–150 years) (Burkins *et al.*, 2000; Elberling *et al.*, 2006) suggest a small contribution of past organic matter to current C cycling. The data presented here and those from other studies (Hopkins *et al.*, 2006a, b; Elberling *et al.*, 2006; Wood *et al.*, 2008) indicate that current soil food webs, marine detritus and endolithic microorganisms subsidize Dry Valley soil organic matter. Surface phototrophic activity and/or the deposition of lacustrine biomass might quantitatively be the more significant processes, resulting in higher organic carbon levels in surface samples.

Bacterial diversity was assessed using three culture-independent techniques: DGGE, T-RFLP and 16S rRNA gene clone libraries. Sediment depth explained changes in community structure, highlighting that surface and shallow subsurface samples cluster separately from the permafrost and active layer samples (Fig. 4). Sample S20

showed a higher level of diversity, both in clone library and T-RFLP analyses, than sample N30. Whether this is attributed to a north/south influence can only be determined through surveying of additional sampling points.

Furthermore, members of the *Proteobacteria*, *Actinobacteria* and *Firmicutes* were found to be the dominant phyla in all horizons. In contrast the *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Gammaproteobacteria* appeared to be more abundant at the permafrost interface. These phyla are typically all well represented in Antarctica (e.g., Aislabie *et al.*, 2006; Smith *et al.*, 2006; Khan, 2008) and other soils (Fierer & Jackson, 2006) and have been shown to contain members well adapted to harsh environmental conditions (Niederberger *et al.*, 2008).

Signatures for photoautotrophic cyanobacteria were recovered from subsurface soil samples. Cyanobacteria usually comprise a relatively small or undetectable fraction of Dry Valley open soil (Cowan, 2009; Pointing *et al.*, 2009; Wood *et al.*, 2008) and permafrost (Gilichinsky *et al.*, 2007). While it is reasonably assumed that these signals do not represent metabolically active organisms, it is unclear whether they represent legacy signals (Cary *et al.*, 2010; Moorhead *et al.*, 1999; Burkins *et al.*, 2000) or the result of soil turnover processes. Furthermore, cyanobacteria are often dominant members of Antarctic hypolithic communities where nitrogen fixation takes place (Cowan *et al.* 2011). Therefore, our data suggest cyanobacteria are cosmopolitan taxa in Antarctic soils and strengthen the view that cyanobacteria are important players in soil ecosystem functioning, either as an active biological component or as a nutrient source (Wood *et al.*, 2008).

A significant portion of the 16S rRNA gene sequences affiliated closely with other uncultured bacteria from cold environments, showing little if any evidence of high-altitude particulate transport and depositional processes, which would be expected to contribute phylotypes with tropical and temperate affiliations (Vincent, 2000) and in general supports the concept of a endogenous origin of Antarctic terrestrial microbial populations. Indeed, this may be an interesting line of enquiry given that hypolithic cyanobacteria from Dry Valley soils have been shown to be genetically isolated from other arid soil populations as before the last glacial maximum (Bahl *et al.*, 2011).

Some of the divisions (i.e. *Spirochaete*, *Planctomycetes*, *Deinococcus*/*Thermus*) identified by T-RFLP analysis were not detected using clone library analysis or did not show the same abundance (i.e. *Acidobacteria*). Indeed, the Chao1 indexes calculated from both clone libraries revealed the presence of a much richer microbial diversity than estimated, highlighting the importance of employing polyphasic approaches to more accurately evaluate the microbial diversity in environmental samples.

In direct contrast to the results of this study, early culture-dependent analyses indicated that the surface soils of the Antarctic Dry Valleys contained fewer microorganisms than the underlying layers (Cameron *et al.*, 1970; Horowitz *et al.*, 1972) and that microbial diversity was higher in subsurface samples (Friedmann, 1982; Meyer *et al.*, 1988; Nienow & Friedmann, 1993). At least two possible explanations have been suggested: the greater stability and longevity of both vegetative and resting cells at depth, probably as a result of the stable low-temperature environment and the tendency for culture-dependent studies to recover only the fast-growing spore-formers. However, given the possibility of long-term preservation of DNA in Antarctic soils (Ah Tow & Cowan, 2005) and permafrost (Willerslev *et al.*, 2004; Gilichinsky *et al.*, 2007; Johnson *et al.*, 2007) and that culture-independent techniques do not discriminate between extracellular DNA or DNA from live or dead cells, the inference of functionality from such analyses is inappropriate. This is, to an extent, reflected in our data as the decrease in metabolic activity with depth is not directly proportional to the decrease in recoverable DNA (Table 1). The phylogenetic signatures obtained, therefore, reflect both present and historical microbial existence. A transcript-based analysis would more accurately reflect the metabolically active diversity; however, the technical challenges of extracting usable amounts of mRNA from Antarctic desert soils have, so far, prevented the parallel analysis of the functional microbial fraction.

As Antarctica is characterized by an extremely delicate biological equilibrium, the relative simplicity of trophic structures will allow for the examination of future perturbations (i.e. climate changes) and their impact on microbial diversity and ecosystem sustainability.

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Supporting Information

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

Fig. S1. OTU Richness (left) and T-RFLP fragments occurrences (right).
Fig. S2. (a) Putative phylogenetic assignment of the T-RFLP peaks using the Phylogenetic Assignment Tool. (b) Relative peak height of the T-RFs.
Fig. S3. Distribution of phyla detected in 16S rRNA gene libraries from soil at the permafrost boundary of Miers Valley.
Fig. S4. 16S rRNA gene neighbor joining tree (Not necessary. This reference is provided in Supporting Information).
Table S1. Soil chemical analysis of the samples.
Table S2. 16S rRNA gene sequences from clone libraries obtained in this study.

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