FEMS MICROBIOLOGY ECOLOGY

RESEARCH ARTICLE

Influence of soil properties on archaeal diversity and distribution in the McMurdo Dry Valleys, Antarctica

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Received 10 December 2013; revised 27 February 2014; accepted 5 March 2014.

DOI: 10.1111/1574-6941.12322

Editor: John Priscu

Keywords
Antarctic soils; *Archaea*; *Euryarchaeota*; *Thaumarchaeota*; pyrosequencing.

Abstract

Archaea are the least understood members of the microbial community in Antarctic mineral soils. Although their occurrence in Antarctic coastal soils has been previously documented, little is known about their distribution in soils across the McMurdo Dry Valleys, Victoria Land. In this study, terminal-restriction fragment length polymorphism (t-RFLP) analysis and 454 pyrosequencing were coupled with a detailed analysis of soil physicochemical properties to characterize archaeal diversity and identify environmental factors that might shape and maintain archaeal communities in soils of the three southern most McMurdo Dry Valleys (Garwood, Marshall, and Miers Valley). *Archaea* were successfully detected in all inland and coastal mineral soils tested, revealing a low overall richness (mean of six operational taxonomic units [OTUs] per sample site). However, OTU richness was higher in some soils and this higher richness was positively correlated with soil water content, indicating water as a main driver of archaeal community richness. In total, 18 archaeal OTUs were detected, predominately *Thaumarchaeota* affiliated with Marine Group 1.1b (> 80% of all archaeal sequences recovered). Less abundant OTUs (2% of all archaeal sequences) were loosely related to members of the phylum *Euryarchaeota*. This is the first comprehensive study showing a widespread presence and distribution of *Archaea* in inland Antarctic soils.

Introduction

The McMurdo Dry Valleys of Antarctica encompass the largest ice-free area (4500 km^2) on the Antarctic continent (Levy, 2012). These typically U-shaped glacial valleys were primarily formed by a series of major glacial advances of ice sheets located between the Polar Plateau and the western coast of the Ross Sea, Southern Victoria Land (Denton *et al.*, 2004). Each of these westward flowing ice fluctuations was successively less extensive than the former (Brook *et al.*, 1995; Denton *et al.*, 2004) resulting in different types of deposits and landforms within each valley, that is, different types of glacial push moraines, calcareous sandy eolian and fluvial sediments, and bedrock dominated by granite and metamorphosed rocks (Elberling *et al.*, 2006). Classified as a hyperarid desert, Dry Valley soils are thought to be the driest, coldest ecosystem on Earth (Stonehouse, 2002). Due to the dry atmosphere, snowfall is typically ablated almost immediately, resulting in minimal liquid–water input to soils (Wynn-Williams, 1990; Doran *et al.*, 2002; Gooseff *et al.*, 2012; Eveland *et al.*, 2013). Dry Valley soils are also characterized by large daily temperature variations during the Austral summer (−12 to 5 °C) (Doran *et al.*, 2002; Aislabie *et al.*, 2006), high salinity (Bockheim, 1997; Poage *et al.*, 2008), and low nutrient availability (Vishniac, 1993). The effects of such severe physiochemical soil conditions combine to create one of the harshest environments known to support life (Bockheim, 2002; Barrett *et al.*, 2007). Consequently, vascular plants and vertebrates are absent, while a number of invertebrate species and bacteria are widely distributed and dominate the entire Dry Valley soil

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food web (Adams *et al.*, 2006). Physicochemical soil parameters such as availability of water, soil organic carbon and nitrogen, salinity, and temperature are the determining factors of habitat suitability for soil communities (Freckman & Virginia, 1997; Barrett *et al.*, 2004; Gregorich *et al.*, 2006).

Bacterial diversity is well documented within Dry Valley soil microbial communities (Wynn-Williams, 1990; Barrett *et al.*, 2006; Smith *et al.*, 2006; Niederberger *et al.*, 2008; Pointing *et al.*, 2009; Lee *et al.*, 2012). Although surprisingly high levels of bacterial diversity have been reported (Smith *et al.*, 2006; Cary *et al.*, 2010; Lee *et al.*, 2012), little is known about the presence, diversity, and community composition of *Archaea* within Dry Valley soils (Pointing *et al.*, 2009; Yergeau *et al.*, 2009). Only coastal soils, adjacent to the Ross Sea, have been reported to harbor archaeal communities (Ayton *et al.*, 2010). This is in stark contrast to temperate soils, where molecular studies have revealed a widespread distribution of both *Thaumarchaeota* and *Euryarchaeota* (Bintrim *et al.*, 1997; Jurgens *et al.*, 1997; Buckley *et al.*, 1998; Ochsenreiter *et al.*, 2003; Nicol & Schleper, 2006). *Thaumarchaeota* may account for up to 5% of the total temperate soil prokaryotic community (Buckley *et al.*, 1998; Ochsenreiter *et al.*, 2003) but are highly restricted to a few lineages, namely Marine groups 1.1b and 1.1c (Jurgens *et al.*, 1997; Ochsenreiter *et al.*, 2003; Nicol *et al.*, 2006). Although a worldwide distribution has been assumed due to their detection in diverse soil environments (Ochsenreiter *et al.*, 2003; Nicol *et al.*, 2005, 2006), molecular studies failed to detect *Archaea* in many Antarctic soils, including soils from the western Antarctic Peninsula (Yergeau *et al.*, 2009) and from McKelvey Valley in the McMurdo Dry Valleys (Pointing *et al.*, 2009).

It has been proposed that Dry Valley soil is an example of an environment where abiotic factors (e.g., moisture, pH, conductivity) have a stronger influence on the diversity and structure of communities than biotic factors (e.g., competition, herbivory, predation) (Convey, 1996; Hogg *et al.*, 2006; Pointing *et al.*, 2009; Zeglin *et al.*, 2011; Lee *et al.*, 2012). This makes Dry Valley soils a perfect model system for investigating the direct effects of physicochemistry on microbial biodiversity and ecosystem function, which is one of the main questions being addressed by the New Zealand Terrestrial Antarctic Biocomplexity Survey (nzTABS, http://nztabs.ictar.aq). As part of nzTABS, this study aims to characterize the composition and structure of archaeal communities across a wide range of Antarctic soils and to identify environmental factors influencing their distribution within three smaller valleys (Miers, Garwood, and Marshall Valleys) in the southern part of the McMurdo Dry Valley system.

Materials and methods

Site location, sampling, and physicochemical analysis

Mineral soil samples were collected from four areas in the McMurdo Dry Valleys (Fig. 1), located between the Royal Society Range and the McMurdo Sounds at the lobe of the Koettlitz Glacier: Miers Valley (78°6′S 164°0′E), Marshall Valley (78°4′S 164°10′E), Garwood Valley (78°2′S 164°10′E), and Shangri-La, an elevated plain west of Marshall Valley. Each valley is characterized by three different types of glacial push moraines: (i) Moraine 1, located on ridges up to 550 m high, is characterized by the relatively old ‘McMurdo Glaciation’ deposits such as red porphyritic rocks and wind-planed granite boulders (Pewe, 1958, 1960); (ii) Moraine 2, located inland on the valley floor and along the coastal foothills, is characterized by older glacial deposits (‘Marshall Drift’); and (iii) Moraine 3, located around the coastal margins, is the result of the youngest glaciation (‘Ross Sea Drift’) and is composed of till and stratified sediments made up of clasts of dark, volcanic rocks derived from the McMurdo Volcanic Group (Brook *et al.*, 1995; Denton *et al.*, 2004). Samples were collected along four transects (Fig. 1). A single transect was laid across each valley floor running from the coast up to the inland glaciers at the end of each valley (three total). The fourth transect crossed the granite ridges of all three valleys (hereinafter ‘ridge transect’, Fig. 1). Additional alluvial soil samples were taken from the shores of Lake Miers (Miers Valley), Lake Colleen (Garwood Valley), Lake Buddha (Shangri-La) and a pond in the upper Marshall Valley.

A total of 36 samples (400 g each) were collected from the top 2 cm of soil in 42 oz Whirlpak bags (Nasco, Fort Atkinson, WI) with a sterile spatula. Large stones and pebbles (> 2 cm diameter) were removed; the samples homogenized and then split into two portions. One portion was used immediately for electrical conductivity, pH, and ATP measurements, and the remainder stored at −20 °C for later analyses.

Electrical conductivity and pH were measured in the field using a CyberScan PC 510 Bench Meter (Eutech Instruments Pte Ltd., Singapore) using the slurry technique (Edmeades *et al.*, 1985). ATP content was determined in the field using the 3M™ Clean-Trace™ Beverage Test Kit (3M Centre, St. Paul, MN) following the manufacturer’s recommendations. Moisture content of soils was measured gravimetrically in the laboratory by drying 40 g of each soil sample at 105 °C until samples reached a consistent weight (Barrett *et al.*, 2004). To determine total soil organic carbon and nitrogen, each soil sample

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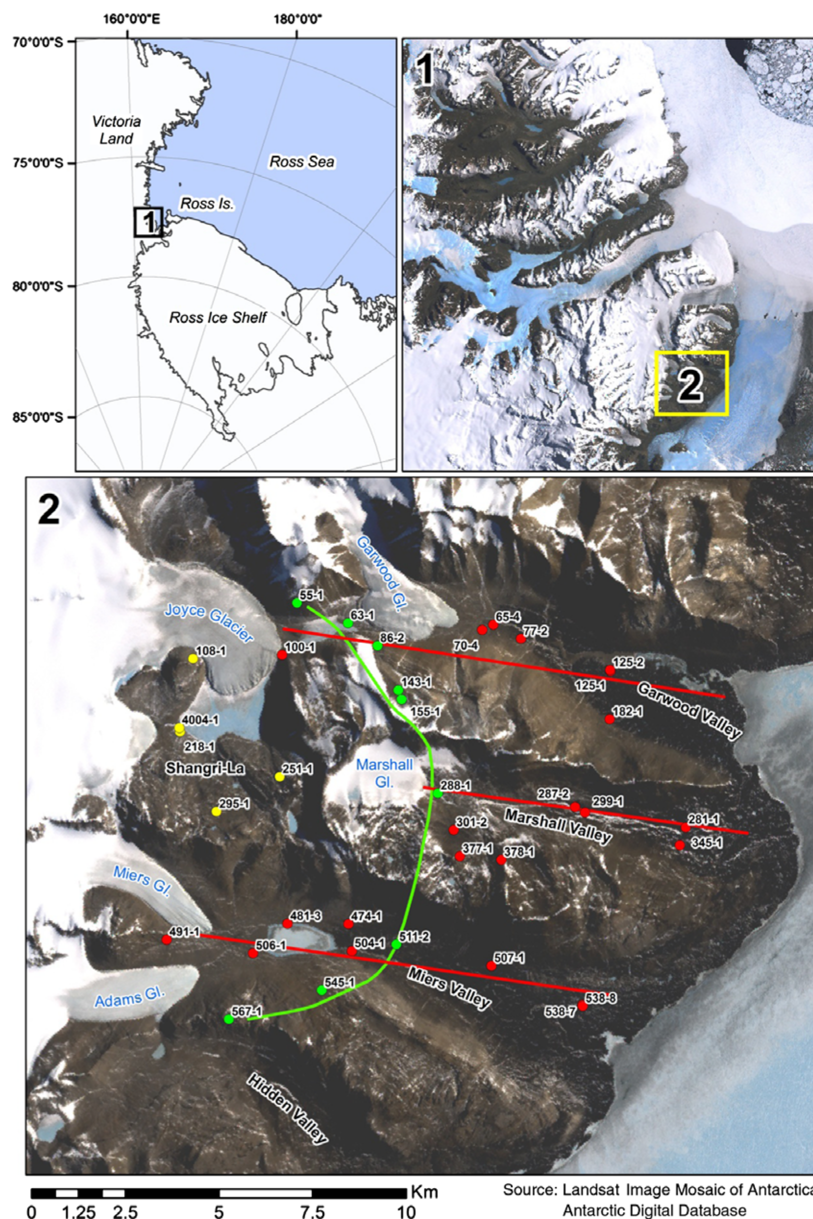

Fig. 1. Map of the Ross Sea Region, Antarctica showing the McMurdo Dry Valleys (black rectangle) and the study site (yellow rectangle). 2. Locations of sampling points in Miers Valley, Marshall Valley, Garwood Valley (red), and Shangri-La (yellow). Red lines denote moraine transects for each valley, and the green line shows the ridge transect across all three valleys.

was air-dried and ground in a ball mill. Dry soil (300 mg) was analyzed on a Flass EA ElanTech elemental analyzer in Virginia Tech Ecosystem Analysis Laboratory (Barrett *et al.*, 2009).

Archaeal t-RFLP

DNA was extracted from the soil (600 mg) using a CTAB/bead-beating extraction method (Niederberger *et al.*, 2008). Primers were designed to be as inclusive of *Archaea* as possible, but in particular to provide coverage of archaeal sequences previously detected in the Antarctic, although as with all primers as new sequences become available, their coverage changes and primer bias is always a potential problem. *Archaea*-specific PCR of the 16S rRNA gene was performed in 25 μL reactions containing 200 nM of primer A92F (5′–FAM-ACGGCTCAGTAAC RC–3′) (Buckley *et al.*, 1998), 100 nM of primer A836R (5′–GTGCTCCCCCGCCAATTCCT–3′) (Stahl & Amann, 1991), 3 mM $MgCl_2$, 1× Platinum *Taq* PCR buffer, 0.4 U of Platinum *Taq* DNA polymerase (Invitrogen Ltd., New Zealand), 32 μg mL^{-1} bovine serum albumin, 200 μM of each dNTP (Roche Diagnostics, New Zealand), 10 ng of extracted template DNA, and UltraPure™ distilled water (Invitrogen Ltd.). The master mix, containing all reagents except the fluorescently labeled forward primer, was treated with 0.1 μg $μL^{-1}$ ethidium monoazide bromide (Biotium Inc., Hayward, CA) by incubation in the dark

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for 1 min followed by exposure to high wattage light for 1 min (Rueckert & Morgan, 2007). Amplification was performed using an initial denaturation step at 94 °C for 2 min followed by 35 cycles of 94 °C, 20 s; 56 °C, 10 s; 72 °C, 40 s; and a final extension step of 72 °C for 5 min. Triplicate PCRs were pooled together to minimize stochastic PCR bias, purified (UltraClean™ 15 DNA Purification Kit; MO BIO Laboratories Inc., Carlsbad, CA), resuspended in MilliQ water, and quantified using the Qubit® fluorometer (Quant-iT™ dsDNA HS Assay Kit; Invitrogen Ltd.).

Purified amplicons (100 ng) were digested at 37 °C for 3 h in 20 μL reactions containing 10–20 U of MspI, 1× Buffer 4 (Thermo Fisher Scientific Inc.) and UltraPure™ distilled water followed by heat inactivation at 65 °C for 20 min. All reactions were stored at −20 °C until further analysis.

Aliquots (2 μL) of the diluted (1 in 10 in UltraPure™ distilled water) restriction digest were denatured in the presence of 17.75 μL Hi-Di™ Formamide at 95 °C for 4 min and then chilled to 4 °C. The samples were loaded onto an ABI 3130 xI sequencer (PE Applied Biosystems, Foster City) and run under GeneScan mode at 15 kV for 45 min according to the manufacturer's instruction. Each sample contained 0.25 μL of the internal GS1200LIZ Zy-Standard (PE Applied Biosystems) to determine the size of fluorescently labeled fragments during analysis.

Data analysis

T-RFLP electropherograms were processed using PEAKSCANNER™ v1.0 (PE Applied Biosystems). Peaks were denoised and binned using an in-house pipeline modified from Abdo *et al.* (2006) written using PYTHON 2.7.1 (Python Software Foundation) and R (http://www.r-project.org). Abdo's method was modified to model parameters for a log-normal distribution. Iteratively, peaks with an area exceeding the 99.9% cumulative distribution of the calculated log-normal distribution for noise were accepted as true peaks. Peaks were binned into t-RFs with width = 1 nt, and total peak area of each bin was used to calculate relative abundance of each t-RF in a given dataset.

The influence of physicochemical variables on the community structure of the soil samples was assessed using BEST (Bio-Environment Stepwise) analysis with the PRIMER 6 software package (PRIMER-E Ltd., UK). The BEST procedure calculates the value of Spearman's rank correlation coefficient (ρ) using every possible combination of variables until it finds the 'best' fit (i.e., the combination of parameters whose Euclidean distance matrix gives the highest ρ). The combination of geochemical variables yielding the highest ρ are the most correlated with archaeal richness. Physicochemical variables (water, pH, conductivity, and ATP) showing no linear relationship or a heavily skewed distribution were normalized using square root transformation prior to BEST analysis. Alpha-diversity (species richness) was analyzed using multiple regression, Pearson's product-moment correlation, and Spearman's rank correlation implemented in R.

Pyrosequencing and noise removal

Ten PCRs were run for each of the four samples (299-1, 377-1, 77-2, and 567-1, Table 1) following the t-RFLP amplification protocol described earlier. Individual PCRs were subsequently pooled together and gel-purified using the UltraClean™ 15 DNA Purification Kit. Cleaned amplicons were used as template (125 ng) in a second PCR to attach 454-specific sequence tags to the 794-bp PCR product. Amplification was performed using 454-specific archaeal primers Ar14A-A92F and ArB-A836R under conditions described for the t-RFLP PCR. Both primers contained an adapter sequence at the 5′ end, followed by a key sequence and a unique tag sequence (MID).

The final amplicon was gel-extracted using the UltraClean™ 15 DNA Purification Kit, cleaned using the Agencourt AMPure XP Bead Cleanup Kit (Beckman Coulter Inc.) and quantified (Quant-iT™ dsDNA HS Assay Kit). Sequencing was performed at the University of Waikato DNA Sequencing Facility (http://sci.waikato.ac.nz/research/facilities/dna) using the GS Junior Titanium emPCR Kit (Lib-L), the GS Junior Titanium Sequencing Kit, PicoTiterPlate Kit, and GS Junior System sequencer (Roche 454 Life Sciences, Branford, CT) yielding a mean read length of 324 bp.

AMPLICONNOISE v1.22 (Quince *et al.*, 2011) was used to process raw flowgrams from each sample separately. Reads matching sample-specific barcodes exactly were required to extend for at least 360 cycles before the first noisy signal (i.e., 0.5–0.7 or no signal in all four nucleotides). Flowgrams were truncated at 360 cycles and processed using PYRONOISE and SEQNOISE (Quince *et al.*, 2011). PCR chimeras were removed using PERSEUS, which is included within the AMPLICONNOISE package. Sequence predictions were first required to perfectly match primer sequences and then both barcode and primer sequences were removed.

Identification of archaeal phylotypes

To separate archaeal from bacterial sequences, taxonomy was assigned to each sequence using RDP Classifier (Wang *et al.*, 2007). All sequences that were classified as either bacterial or archaeal at a high confidence level

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Table 1. Properties of soil samples analyzed and the number of t-RFLP phylotypes identified

Samples	GM*	Aspect	Height†	Slope	MC‡	pH	EC§	ATP¶	Total Organic C (%)	Total *N* (%)	C/N Ratio	t-RFLP Phylotypes**
Miers Valley transect												
538-8	M3	South	100	5	0.75	8.6	670	13 319	0.0239	0.0028	8.4	3
538-7	M3	South	98	6	0.75	8.6	670	13 319	0.3466	0.0522	6.6	5
507-1	M3	South	216	17	0.52	8.3	5130	36 519	0.0436	0.0068	6.4	13 (2)
504-1	M2	Flat	161	6	1	9.2	130	16 872	0.0391	0.0048	8.1	13 (3)
506-1	M1	North	178	5	0.82	9.7	924	7457	0.0526	0.0055	9.4	8 (2)
491-1	M1	South	393	16	0.99	9.2	140	30 964	0.0825	0.0102	8.0	7 (1)
474-1	Scr	South	233	15	0.7	9.4	42	22 617	0.0479	0.0047	10.0	10
481-3	All	Flat	164	5	1	9.8	370	99 864	0.2566	0.0223	11.5	9 (2)
Mean					0.82	9.1	1009	30 116	0.1116	0.0136	8.5	8.5
Std					0.16	0.5	1584	27 874	0.1130	0.0156	1.6	
Marshall Valley transect												
281-1	M3	South	172	9	0.59	7.5	789	53	0.0751	0.0072	10.4	3
287-2	M2	South	237	12	0.99	9.8	336	21 674	0.0455	0.0062	7.3	3
345-1	M2	North	185	8	1.01	8.4	130	41 447	0.5322	0.0768	6.9	8 (1)
299-1††	M1	North	285	9	1.01	8.9	310	75 331	0.1278	0.0161	7.9	7 (2)
377-1††	Gra	East	756	23	1	9.3	70	38 739	0.0338	0.0031	10.7	9
378-1	Sch	West	782	15	1	9.2	90	28 453	0.0474	0.0050	9.3	6
301-2	All	Flat	555	Flat	1	8.3	60	32 991	0.0346	0.0035	9.8	11
Mean					0.94	8.7	255	34 098	0.1280	0.0168	8.9	6.7
Std					0.1	0.7	242	21 119	0.1678	0.0248	1.4	
Garwood Valley transect												
182-1	M3	North	104	10	0.99	8.6	136	10 817	0.0316	0.0021	15.0	10 (1)
125-2	M3	North	26	7	0.98	7.8	66	5980	0.1297	0.0138	9.3	3
125-1	M3	North	30	8	0.9	8.2	46	17 240	0.0283	0.0028	9.9	7 (1)
100-1	M3	East	8	6	0.99	7.9	868	10 270	0.0486	0.0049	9.9	8
77-2††	M2	North	98	5	1	8.9	57	6665	0.0744	0.0093	7.9	10 (1)
70-4	M2	North	134	4	0.99	9	114	11 123	0.0502	0.0048	10.2	2
65-4	M1	North	114	4	0.99	9.5	90	10 725	0.0724	0.0025	27.9	3
Mean					0.97	8.5	196	10 402	0.0621	0.0057	12.9	4
Std					0	0.6	276	3404	0.0321	0.0040	6.5	
Ridge transect												
567-1††	Gra	East	647	16	1	7.8	90	7735	0.0446	0.0068	6.5	10
545-1	Gne	North	374	12	0.45	8.4	29	40 034	0.0986	0.0139	7.0	1
511-2	M2	South	203	6	0.51	9.7	73	5057	0.0209	0.0031	6.6	4
288-1	M1	East	540	7	1.01	9.7	110	38 069	0.0336	0.0031	10.6	15 (2)
155-1	Gra	North	910	22	0.99	8.3	33	2466	N/D	N/D	N/D	9 (1)
143-1	Gra	North	866	21	0.97	8.3	29	1155	0.0477	0.0050	9.5	5
86-2	Gra	North	462	26	0.99	9	48	3870	0.1142	0.0095	11.9	8
63-1	All	Flat	357	2	0.99	8.5	84	3739	0.0540	0.0053	10.1	2
55-1	M1	South	678	36	0.99	9	56	20 209	0.0378	0.0048	7.7	4
Mean					0.87	8.7	61	13 592	0.0564	0.0064	8.7	6.4
Std					0.2	0.6	28	14 603	0.0306	0.0034	1.9	
Shangri-La												
4004-1	All	North	393	3	0.41	9	58	861	0.1671	0.0041	39.9	2 (1)
108-1	Gra	North	564	30	0.98	8.6	61	45 384	0.0747	0.0096	7.7	15 (1)
251-1	Gra	North	645	13	0.99	9.1	50	39 488	0.0800	0.0095	8.3	9
295-1	Gra	East	565	9	0.99	9	101	24 164	0.0487	0.0061	7.9	1
218-1	M1	North	400	6	0.98	8	754	10 286	0.0425	0.0065	6.4	4
Mean					0.87	8.7	205	24 036	0.0826	0.0071	14	6.2
Std					0.2	0.4	275	16 856	0.0446	0.0021	12.9	

*Geomorphology: M1, Moraine 1; M2, Moraine 2; M3, Moraine 3; Gra, Granite; Sch, Schist; Scr, Scree; All, Alluvia; Gne, Gneiss.
†As meters above sea level.
‡Total soil moisture content as percentage.
§Soil electrical conductivity in $\mu S\ cm^{-1}$.
¶As relative fluorescent units.
**Total number of phylotypes per sample based on t-RFLP results (unique phylotypes are given in parenthesis).
††These samples were used for 454 pyrosequencing.

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(> 80%) were either removed (*Bacteria*) or retained (*Archaea*) for further analysis. Sequences that were classified as *Archaea* and *Bacteria* at a low confidence level (< 80%) were matched to genome sequences in GenBank (http://www.ncbi.nlm.nih.gov/) using BLAST at default settings. Sequences that were confidently identified as bacterial (> 99% max identity) were removed, and sequences that could feasibly have come from *Archaea* (> 70% max identity) were retained. All archaeal sequences were then manually checked for chimeras by splitting the sequence in half, and using BLAST to find best hits within the NCBI genome database. If one half of the sequence could be confidently identified as bacterial (> 99% max. identity) and the other half as archaeal, the sequence was regarded as chimeric and subsequently removed.

Phylogenetic analysis

Sequence alignment and all phylogenetic analyses were performed using MEGA 4 (Molecular Evolutionary Genetics Analysis software version 4.0, Tamura *et al.* 2007). Archaeal sequences and the bacterial isolate *Thermoanaerobacter methranii* (outgroup) were aligned using CLUSTALW (Thompson *et al.*, 1994) and end-trimmed down to an overlapping region present in all sequences: the final length (in bases) of the overlapping region was 220 bp. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were a total of 177 positions in the final alignment. The unrooted phylogeny was inferred using the neighbor-joining algorithm (Saitou & Nei, 1987) with Maximum Composite Likelihood Distances (Tamura *et al.*, 2004) and tested using 1000 bootstraps. The resulting tree was rooted on *T. methranii* (outgroup) to define clades. Archaeal sequences generated in this study are deposited in the Sequence Read Archive (SRA) under accession numbers ERX141792-95 (experiments accessions) and ERR165915-18 (runs accessions). Original pyrosequencing flowgram files are also available from the SRA database (http://www.ebi.ac.uk/ena/data/view/ERP001744).

Richness estimation

Pairwise distances between archaeal sequences were calculated using ESPRIT (Sun *et al.*, 2009), and sequences were clustered into OTUs using mean neighbor clustering at a distance of 0.03 in MOTHUR (Schloss *et al.*, 2009). MOTHUR was also used to generate Venn diagrams and rarefaction curves based on the number of observed OTUs and the Chao1 richness estimator (Chao, 1987).

Results

Soil characteristics

Physicochemical properties of soil samples from four valleys studied are shown in Table 1. Cluster analysis based on Euclidean distance and multidimensional scaling (MDS) ordination revealed no significant differences in physicochemical properties between and within valleys (data not shown). All mineral soils were alkaline ranging from pH 7.5 to 9.8. Mean soil moisture content was low in all samples, ranging from 0.82% (Miers Valley transect) to 0.97% (Garwood Valley transect). Electrical conductivity, a representation of salinity, was lowest in the ridge transect (ranging from 29 to 110 μS cm^{-1}), while the highest value was recorded in Miers Valley transect (5130 μS cm^{-1}). ATP content, a proxy for biological activity, was low in Garwood Valley and ridge transects (5980–17 240 RFU and 1150–40 034 RFU, respectively), whereas samples from Miers Valley and Marshall Valley transects showed a high ATP content (7457–99 864 RFU and 21 764–75 331 RFU, respectively) with one exception (53 RFU in Marshall Valley transect). Organic carbon and total nitrogen contents were very low in all samples (mean 0.09 ± 0.01% and 0.03 ± 0.005%, respectively).

Diversity and distribution of soil *Archaea*

Archaeal signals were detected using t-RFLP analysis in all 36 soil samples, yielding a total of 21 unique t-RFLP phylotypes with a mean of six phylotypes in each sample (Table 1). The total number of phylotypes observed in each transect was estimated by pooling the phylotypes from all samples within each transect *in silico*. Species richness was highest in the Miers Valley transect (nine phylotypes per sample), followed by Marshall Valley (seven phylotypes), Garwood Valley (six phylotypes), and ridge transects (six phylotypes, Table 1).

The four samples selected for in-depth community analysis, using 454 pyrosequencing, were chosen based on (i) high t-RFLP species richness compared to the mean number of phylotypes per sample (> six phylotypes) and (ii) their geographical location within each valley, covering both the ridge transect (Miers Valley ridge and Miers Marshall Valley ridge) and different types of moraines (Marshall Valley moraine 1 and Garwood Valley moraine 2; Table 1). Sequencing yielded a total of 33 707 reads, with a mean of 8427 reads per sample (Table 2). Flowgram clustering and denoising was performed, followed by the removal of low-quality sequencing tags, bacterial sequences, and chimeras (see Materials and methods), yielding a total of 20 395 archaeal reads with a mean of

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Table 2. Summary of total sequences, total OTUs, and diversity estimates (calculated using mean neighbor at 3% distance) for four archaeal assemblages examined

Samples	Number of tags					Number of OTUs		Chao1	Effort
	A	B	C	D	E	F	G	H	I
Garwood Valley moraine 2	7740	5217 (25)	80	4931	22	10	10	10.5	2564
Marshall Valley moraine 1	10 349	7068 (14)	181	6830	15	6	7	6	3226
Miers Marshall Valley ridge	12 423	8496 (25)	44	7012	5	5	9	11	1706
Miers Valley ridge	3195	2108 (8)	64	1622	10	5	10	11	1543
Mean	8427	5722 (18)	92	5099	12.75	6.5	9	9.6	2260

A: Total number of tags matching MID without the preclustering step (see Materials and methods Pyrosequencing and noise removal). B: Total number of tags passing AMPLICONNOISE (see Materials and methods Pyrosequencing and noise removal). Tags removed as Chimera in parenthesis. C: Number of unique tags passing AMPLICONNOISE. D: Number of archaeal tags after removal of Bacterial sequences and manually identified chimeras. E: Number of unique archaeal tags after removal of Bacterial sequences and manually identified chimeras. F: Number of OTUs based on 454 pyrosequencing clustered using mean neighbor at the 3% difference level. G: Number of OTUs based on t-RFLP results. H: Estimation of OTU richness in samples. I: Number of sequencing reads required for discovering a new OTU calculated using terminus of rarefaction curves in reads/OTU.

5099 tags per sample. The total number of unique reads was low (52, Table 2), and all unique reads were clustered into 18 distinct OTUs using average neighbor at 3% distance.

Overall, the number of OTUs per sample was similar for both pyrosequencing (5–10 OTUs) and t-RFLP (7–10 OTUs, Table 2). Richness estimates (Chao1) based on the number of species observed using pyrosequencing agreed well with the number of OTUs detected using t-RFLP richness estimates (Table 2). The number of sequencing reads (effort) required to discover a new OTU was calculated by taking the inverse of the terminal slope of the rarefaction curves (Supporting information, Fig. S1) over the last 500 reads (Table 2). On average, 2895 reads would have been required to find a new OTU in moraine samples, whereas 1624 reads would have been required in ridge samples. These estimates suggest that the natural communities were very well sampled by the sequencing effort.

Archaeal community composition and phylogenetic analysis

Of the 18 unique archaeal OTUs obtained from 454 analysis, all but three were represented by sequences closely affiliated with members of the phylum *Thaumarchaeota*. The exceptions were OTUs 16–18, which belonged to the *Euryarchaeota* (Table S1). *Thaumarchaeota* were present in all soils, whereas *Euryarchaeota* (OTUs 16–18) were not detected in ridge samples (Table S1). Among known *Thaumarchaea* species, *Nitrososphaera gargensis* (accession number GU797786) was the closest relative of all observed thaumarchaeal sequences (87–98% maximum identity). All thaumarchaeal sequences showed a high level of identity to existing environmental sequences in the database (93–100% identity over the 780 nucleotides examined). Euryarchaeal sequences exhibited a similar pattern of similarity, showing a maximum identity of 80–85% to isolates and 92–98% identity to environmental sequences.

Thaumarchaeota

Placement of OTUs within the *Thaumarchaeota* was supported by phylogenetic analysis (Fig. 2a). All sequences clustered inside *Thaumarchaeota* Marine Group 1.1b (also called *Nitrososphaera* cluster) (Fig. 2a). OTU 7 was the most abundant thaumarchaeal OTU, representing 80% of the recovered archaeal communities. Although this highly abundant OTU was common to all samples, and 99% of the ridge communities were comprised of OTU 7, OTU 7 only constituted 38% of moraine communities (Table S1). The remaining 62% were comprised of OTUs 1–5, and 8 (0.01–17% of all sequences), which were found almost exclusively in moraines. The second most abundant OTU (OTU 1) represented 17% of the estimated archaeal community and was closely related to *N. gargensis* (GU797786, 93% maximum identity) (Hatzenpichler *et al.*, 2008) (Fig. 2a).

Euryarchaeota

Euryarchaeota accounted for 0.005–0.01% of all archaeal sequences and included three OTUs (OTU 16–18) recovered from inland moraines near the valley floor. OTU 16 contained three sequences found only in Marshall Valley moraine 1 (Table S1), while OTU 17, represented by a singleton, and OTU 18, represented by three sequences, were only present in soil from Garwood Valley moraine 2 (Table S1). Phylogenetic analysis confirmed the placement of these OTUs within the *Euryarchaeota* (Fig. 2b).

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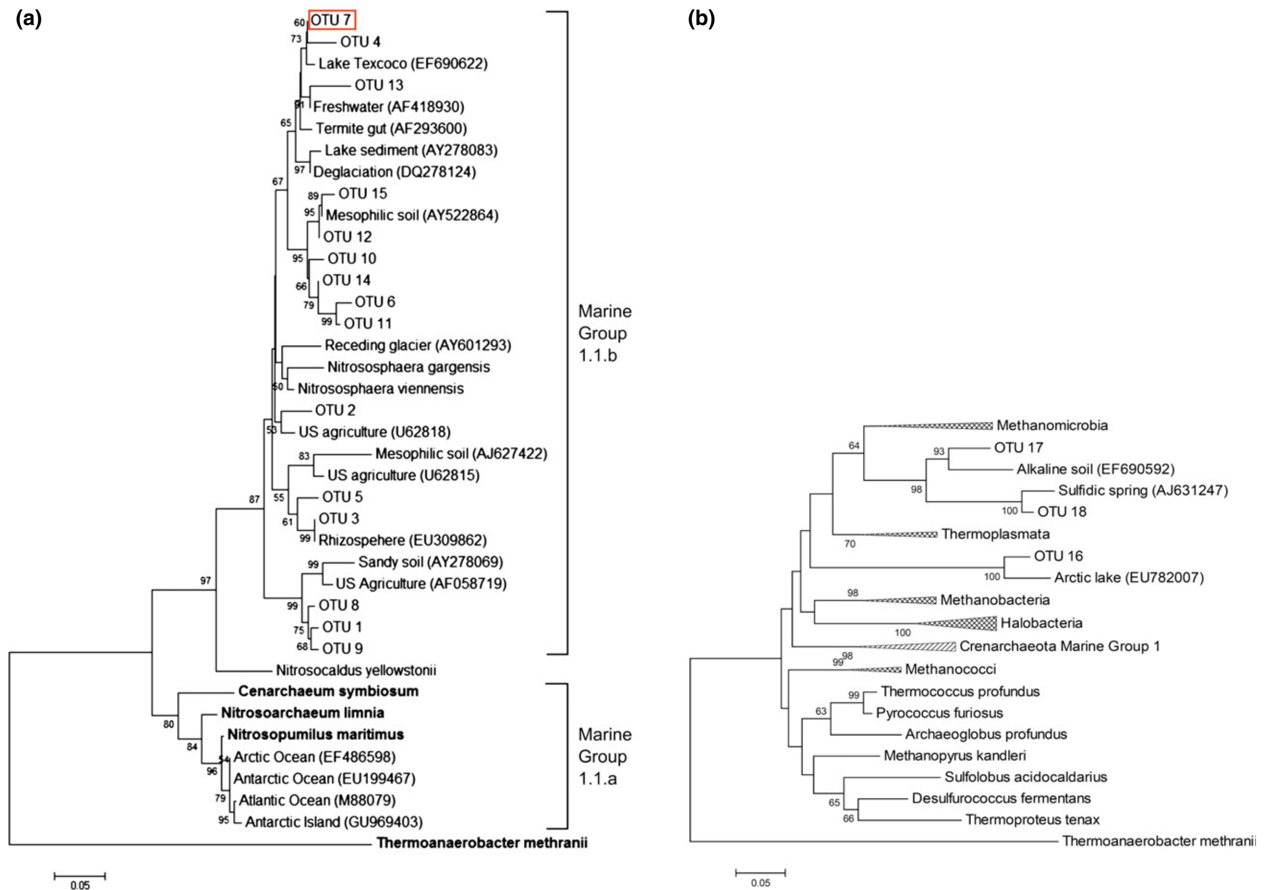

Fig. 2. Neighbor-joining tree of thaumarchaeal (a) and euryarchaeal (b) OTUs retrieved from Dry Valley soil. Reference sequences of Marine Group 1 *Thaumarchaeota* and sequences of isolates representing the main phylogenetic lineages of *Euryarchaeota* were retrieved from GenBank. Bootstrap values are calculated from 1000 replicates and are shown next to the branches. The scale bar shown represents a 5% difference in nucleotide composition.

Environmental drivers of archaeal diversity

Multiple regression and BEST analyses were used to investigate the influence of biogeochemical soil properties (i.e., aspect, elevation, slope, soil moisture content, soil pH, soil electrical conductivity, total organic carbon, and total nitrogen) on archaeal community structure. A significant correlation between archaeal diversity and water content was observed (Table 3, $P < 0.01$), but BEST analysis did not detect significant correlations between archaeal community structure and environmental parameters (data not shown).

Discussion

Distribution and diversity of *Archaea*

We used t-RFLP fingerprinting and 454 pyrosequencing to characterize archaeal richness in multiple Dry Valley areas located in the subxerous, coastal climate zone, in the McMurdo Dry Valleys (Campbell & Claridge, 1987).

In contrast to limited success by earlier attempts (Pointing *et al.*, 2009; Yergeau *et al.*, 2009; Ayton *et al.*, 2010), we detected *Archaea* in all of the Antarctic soils sampled in this study. Additionally, the extensive t-RFLP analysis in this study extended the known range from coastal areas (Ayton *et al.*, 2010) to a variety of inland landscapes such as meltwater stream deltas, coastal, and terminal moraines, lake edges, hill slopes, ridges, and polygons, including soils exceeding electrical conductivity of 5000 μS cm^{-1} and pH greater than 9.5 (Table 1). These highly alkaline and saline soils were previously thought not to be colonized by *Archaea* (Ayton *et al.*, 2010).

Following t-RFLP analysis, four archaeal communities (Miers Valley ridge, Miers Marshall Valley ridge, Marshall Valley moraine 1, and Garwood Valley moraine 2) were analyzed with greater scrutiny by 454 pyrosequencing of

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Table 3. Multiple regression of environmental parameters and archaeal diversity

Residuals	Min	1Q	Median	3Q	Max
	−15.5143	−8.0158	−0.2628	6.9429	15.9906

Coefficients	Estimate	Std error	*t*-value	Pr(> *t*)
(Intercept)	8.44892	6.96204	1.214	0.23505
Elevation	−0.20180	0.23442	−0.861	0.39664
Slope	0.34755	0.24314	1.429	0.16395
Aspect	0.06231	0.17446	0.357	0.72365
Water	0.49287	0.17452	2.824	0.00864**
pH	0.05825	0.18036	0.323	0.74911
Conductivity	−0.17474	0.18742	−0.932	0.35913
ATP	−0.04114	0.19574	−0.210	0.83504

Data were rank-transformed prior to analysis. Significance codes are as follows: $P < 0.001$ (**).

16S rRNA gene PCR amplicons. The low number of archaeal phylotypes from our study (mean of six OTUs per sample) is comparable to low diversity estimates reported for soils of the Ross Sea region (Ayton *et al.*, 2010), McKelvey Dry Valley (Chan *et al.*, 2013) and similar environments such as the Canadian High Arctic (Steven *et al.*, 2008).

Small discrepancies between 454 pyrosequencing and t-RFLP diversity estimates (Table 2) may be due to overestimation of the actual community richness by t-RFLP analysis due to bacterial contamination (Dunbar *et al.*, 2001). This is supported by the relative high proportion of bacterial sequences that were identified through pyrosequencing (Table 2).

Thaumarchaeota marine group 1.1b

More than 99% of sequences retrieved from the Dry Valley soils belonged to the newly described phylum *Thaumarchaeota* (Brochier-Armanet *et al.*, 2008; Spang *et al.*, 2010). The most abundant thaumarchaeal OTU (OTU 7) exhibited a close phylogenetic relationship with other Marine Group 1.1b *Thaumarchaeota* from moderate environments (Friedrich *et al.*, 2001; Stein *et al.*, 2002; Ochsenreiter *et al.*, 2003; Nicol *et al.*, 2006; Valenzuela-Encinas *et al.*, 2008) (Fig. 2a). Thaumarchaeal species belonging to group 1.1b are highly abundant and ubiquitous in terrestrial environments, where they can make up to 5% of prokaryotic 16S rRNA genes (Ochsenreiter *et al.*, 2003; Bates *et al.*, 2011; Pester *et al.*, 2012; Stahl & de la Torre, 2012). Recently, members of this lineage have also been reported for coastal soils of the Ross Sea region: Scott Base, Marble Point, Granite Harbour, and Victoria Valley (Ayton *et al.*, 2010) as well as in Antarctic cryoconite holes (Cameron *et al.*, 2012). Pesaro *et al.* (2003) reported a higher resistance to freeze-thaw cycles for members of group 1.1b than for members of group 1.1a (*Nitrosopumilus* cluster), which may explain their ability to successfully colonize Antarctic soils, where daily temperature fluctuations greater than 20 °C can cause multiple freeze-thaw cycles within a single day (Aislabie *et al.*, 2006).

Thaumarchaeota are key players within the global nitrogen cycle due to their involvement in nitrification (Venter *et al.*, 2004; Koenneke *et al.*, 2005; Hatzenpichler *et al.*, 2008). During the first step of nitrification, autotrophic *Thaumarchaeota* oxidize ammonia to nitrite ($NH_3 \rightarrow NH_2OH \rightarrow NO_2$), mediated by ammonia monooxygenase (*amoA*). In one study, the abundance of *amoA* indicated that ammonia-oxidizing *Thaumarchaeota* outnumber their bacterial counterparts in soil, particularly at depth (Leininger *et al.*, 2006). It has been shown that *Thaumarchaeota* affiliated with group 1.1b contribute to oxidation of ammonia across a wide range of habitats (Treusch *et al.*, 2005; Leininger *et al.*, 2006; Hatzenpichler *et al.*, 2008). Antarctic soils are characterized by high levels of salinity (Bockheim, 1997; Barrett *et al.*, 2007), with ammonia comprising a small fraction of total soil nitrogen in continental Antarctica (Barrett *et al.*, 2009; Magalhaes *et al.*, 2012). Interestingly, *N. gargensis* has been shown to be inhibited by total ammonium concentrations in the lower mM-range (Hatzenpichler *et al.*, 2008) giving physiological support for a preference of low substrate concentrations (Leininger *et al.*, 2006). Based on our findings, we propose that this Antarctic soil *Thaumarchaeota* may also be an ammonia oxidizer as it clustered within the *Thaumarchaeota,* which included the 16S rRNA gene from a *Thaumarchaeota* fosmid 54d9 (AJ627422, Fig. 2a), containing *amoA* (Treusch *et al.*, 2005). This is supported by the presence of ammonia-oxidizing *Archaea* in Antarctic soils from coastal areas, which were shown to be well adapted to cold temperatures (Jung *et al.*, 2011; Han *et al.*, 2013) as well as the recent discovery of *amoA* in soils and lithic environments from the McKelvey Dry Valley (Chan *et al.*, 2013). However, to strengthen the phylogenetic observations described in this study, future investigations are needed to identify the role of *Thaumarchaeota* in Antarctic soils and their potential involvement in ammonia oxidation.

Euryarchaeota

Putative *Euryarchaeota* were also present in soils from inland moraines located near the valley floor, although apparently at very low abundances (< 0.02%). Most of the euryarchaeal OTUs detected in Dry Valley soils were very loosely related to *Methanosarcinales* and environmental sequences retrieved from cold sulfidic springs (Rudolph *et al.*, 2004) and the former Lake Texcoco,

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Mexico (Valenzuela-Encinas *et al.*, 2008). Although species belonging to the *Methanosarcinales* are present in subglacial Antarctic environments (Stibal *et al.*, 2012), Antarctic lake sediments (Purdy *et al.*, 2003; Karr *et al.*, 2006), and surface waters (Brambilla *et al.*, 2001; Taton *et al.*, 2003), *Methanosarcinales* have not previously been detected in Dry Valley soils, and further investigations are required to confidentially place these archaeal phylotypes within the *Euryarchaeota* and assign them to specific lineages.

Physicochemical drivers of archaeal diversity

Species richness at local sites (alpha-diversity) is most likely influenced by soil water content (Table 3), although BEST analysis failed to detect correlations between differences in archaeal community structure and differences in environmental parameters (data not shown). Water availability has been shown to crucially influence the composition of soil communities (Barrett *et al.*, 2004; Aislabie *et al.*, 2006) and is considered the primary limiting resource for microbial life in terrestrial regions of Antarctica (Kennedy, 1993; Gregorich *et al.*, 2006). Water availability is locally high in elevated areas due to condensation, snowfalls, presence of snow patches, and subsequent snowmelt (Schroeter *et al.*, 2010; Green *et al.*, 2011; Eveland *et al.*, 2013; Van Horn *et al.*, 2013). The importance of snowmelt as a source of moisture has been reported for the crustose lichen *Buellia frigid* in southern continental Antarctica (Kappen *et al.*, 1998), which can only be found on slopes higher than 600 m but not in the valley floor (Kappen *et al.*, 1981; Sancho *et al.*, 2007).

Failure to detect environmental influences using BEST analysis may be explained by high levels of variation in physical and chemical soil properties within a single sample. The severity of the Dry Valley environment and extreme desiccation has led to highly heterogeneous soils creating protected environments for microbial colonization (Cockell & Stokes, 2004). The most substantial of these are lithic environments, where well-developed microbial communities have colonized cracks in rocks (endolithic communities), the undersides of translucent rocks (hypolithic communities), and surface rock ‘flakes’ produced by laminar weathering (chasmolithic communities). These microniches potentially provide physical stability, desiccation buffering, increased water availability, and protection from UV fluxes for residing microorgnisms (Cary *et al.*, 2010). For example, hypolithic communities were shown to represent local ‘hotspots’ of microbial diversity (Smith *et al.*, 2000) and productivity (Pointing & Belnap, 2012) compared to soils. The mean productivity of these communities is equivalent to those of lichens and bryophytes (Cockell & Stokes, 2004), possibly exceeding productivity levels in open Dry Valley soils. Moreover, large regional differences in edaphic soil characteristics such as pH, soil organic carbon, and conductivity are likely to generate differences in bacterial communities in Dry Valley soils (Van Horn *et al.*, 2013).

Summary

For the first time, *Archaea* were successfully detected and characterized in all inland and coastal mineral soils in the three southern most McMurdo Dry Valleys. This systematic, landscape-scale study revealed an extensive distribution of archaeal communities in Antarctic mineral soils. Archaeal communities are not limited to coastal margins as previously thought and are instead present in inland glacial push moraines, high elevation ridge areas, slopes, and inland plains. T-RFLP fingerprinting analysis and 454 high-throughput sequencing revealed low archaeal richness in all samples analyzed. However, archaeal richness was higher in some soils, and this higher richness was significantly correlated with soil water content revealed by multiple regressions. Dry Valley soil archaeal communities examined were dominated by a potential ammonia oxidizer affiliated with the globally abundant *Thaumarchaeota* Marine Group 1.1b, and a number of potential *Euryarchaeota* were found exclusively associated with inland glacial push moraine. Future studies should focus on detection and functional characterization of ammonia-oxidizing *Thaumarchaeota* and *Euryarchaeota* to understand their role and potential involvement in the nitrogen and carbon cycles in Antarctic soils.

Acknowledgements

nzTABS was supported by funding from the Foundation for Research, Science and Technology and the International Centre for Terrestrial Antarctic Research. We acknowledge Antarctica New Zealand for logistics and their overriding support for the project. We especially thank Eric Bottos and Sarah Kelly (Waikato University) for assistance with DNA extraction and sample preparation for 454 pyrosequencing that was carried out at the Waikato DNA Sequencing Facility, Hamilton, New Zealand. We thank Steve Pointing for help with the manuscript. This research was also partially supported by a National Science Foundation grant to SCC and JEB (ANT-0229836). The New Zealand Marsden Fund provided financial support for CWH, IRM, and SCC (UOW0802) and CKL (UOW1003).

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Supporting Information

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

Table S1. OTU abundances of archaeal sequences recovered from Antarctic Dry Valley soil.

Fig. S1. Rarefaction curves for pyrosequencing effort. OTUs were defined by mean neighbor d = 0.03.

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