

RESEARCH ARTICLE

Diverse and highly active diazotrophic assemblages inhabit ephemeraally wetted soils of the Antarctic Dry Valleys

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Abstract

Eolian transport of biomass from ephemeraally wetted soils, associated with summer glacial meltwater runoffs and lake edges, to low-productivity areas of the Antarctic Dry Valleys (DV) has been postulated to be an important source of organic matter (fixed nitrogen and fixed carbon) to the entire DV ecosystem. However, descriptions and identification of the microbial members responsible for N₂ fixation within these wetted sites are limited. In this study, N₂ fixers from wetted soils were identified by direct *nifH* gene sequencing and their *in situ* N₂ fixation activities documented via acetylene reduction and RNA-based quantitative PCR assays. Shannon-index *nifH* diversity levels ranged between 1.8 and 2.6 and included the expected cyanobacterial signatures and a large number of phylotypes related to the gamma-, beta-, alpha-, and delta-proteobacteria. N₂ fixation rates ranged between approximately 0.5 and 6 nmol N cm⁻³ h⁻¹ with measurements indicating that approximately 50% of this activity was linked with sulfate reduction at some sites. Comparisons with proximal dry soils also suggested that these communities are not ubiquitously distributed, and conditions unrelated to moisture content may define the composition, diversity, or habitat suitability of the microbial communities within wetted soils of the DVs.

Introduction

The McMurdo Dry Valleys (DV) of Antarctica comprise the largest (approximately 15 000 km²) perpetually ice-free regions in the Antarctic continent (Hopkins *et al.*, 2006b; McKnight *et al.*, 1999). A combination of prevailing katabatic winds and extremely low precipitation rates renders the DV arguably one of the coldest, driest deserts on Earth (Barrett *et al.*, 2002; Hopkins *et al.*, 2006b; Cary *et al.*, 2010). The valleys were originally formed by successive glaciation events with soils derived from glacial tills and weathered bedrock (Cary *et al.*, 2010). DV soils are characterized by a complete lack of vascular plants, high pH, low organic matter, high salt levels, and mass water content typically below 2% (Virginia & Wall, 1999; Hopkins *et al.*, 2006b; Barrett *et al.*, 2007; Cary *et al.*, 2010).

Biogeochemical cycling of nitrogen (N) and primary sources of N in the bulk arid, severely oligotrophic soils of the DV are still poorly understood (Barrett *et al.*,

2007; Hopkins *et al.*, 2008; Cary *et al.*, 2010). It has been hypothesized that the eolian transportation of microbial biomass from high-productivity zones to low-productivity bulk arid soils may be a major dispersal mechanism of organic matter and therefore an important source of fixed N to the entire DV ecosystem (Gordon *et al.*, 1998; Hopkins *et al.*, 2006a, b; Barrett *et al.*, 2007; Novis *et al.*, 2007; Wood *et al.*, 2008; Pointing *et al.*, 2009; Cary *et al.*, 2010). High-productivity sites in the DV soils are characterized as biological crust communities that form in ephemeraally wet soils associated with summer glacial meltwater streams and lake edges. During the short Antarctic summer, temperatures become warm enough for melting to occur on the exposed surfaces of glaciers, resulting in the formation of short-lived (approximately 4–10 weeks) meltwater streams. These streams are the hydrological link between glaciers and lakes and represent the main source of liquid water, ions, and nutrients to the DV lakes and surrounding soils (McKnight *et al.*,

2004). Microbial communities that form in the wet soils adjacent to the source (lake/stream) are typically cyanobacterial and/or moss dominated, exist as a thin crust-like layer binding the top 1–5 cm of soil together and have a patchy distribution (McKnight *et al.*, 2004; Adams *et al.*, 2006; Takacs-Vesbach *et al.*, 2010). High flow rates and/or the steep reaches of stream runoffs may limit the development of these communities owing to the associated turbulences and abrasions of the hydrological flow and are therefore restricted to the zones adjacent to stream banks and lake edges (McKnight *et al.*, 1999, 2007). These communities survive the winter months in a desiccated state and in some cases are re-activated through hydration by summer meltwaters (Takacs-Vesbach *et al.*, 2010), and as a result, they can form large concentrations of biomass even under the extreme *in situ* environmental conditions (Vincent & Howard-Williams, 1986; McKnight *et al.*, 1999).

Ephemerally wet soils of the DV are documented as hotspots of microbial activity; however, previous studies have focused on measurements of bulk geochemical processes as opposed to identifying the microbial members involved in these processes (Vincent, 1988; Runkel *et al.*, 1998; McKnight *et al.*, 1999, 2004, 2007; Virginia & Wall, 1999; Maurice *et al.*, 2002; Gooseff *et al.*, 2004; Hopkins *et al.*, 2006a; Takacs-Vesbach *et al.*, 2010). From classical morphological-based observations, cyanobacteria have been postulated to be the dominant primary producer and N₂ fixer, with black biomass dominated by *Nostoc* sp., green-colored by *Prasiola* sp. and orange/red by *Oscillatoria* and *Phormidium* (Vincent & Howard-Williams, 1986; Vincent *et al.*, 1993; McKnight *et al.*, 1999; Vincent, 2000; Takacs-Vesbach *et al.*, 2010).

Although cyanobacteria in wetted soils of the DV may represent keystone species, indirectly supplying fixed N to the entire DV ecosystem, comprehensive characterization via modern molecular methods and their contribution to total N budgets remain unresolved. The presence and N inputs by nonphototrophic (i.e. heterotrophic) N fixers in DV soils are also currently unknown. Heterotrophic-associated N₂ fixation has been shown to account for a large contribution (approximately 10–30%) of N₂ fixed in Lake Bonney ice aggregates in the DV (Olson *et al.*, 1998; Paerl & Priscu, 1998), and nonphototrophic diazotrophs have been detected in soils associated with Canada stream in the DV (Olson *et al.*, 1998). Therefore, to address these knowledge gaps and through the complementation of classical activity-based analyses (e.g. acetylene reduction assays) with molecular-based genetic methodology, we strived to resolve the identity, diversity, and activity of both photo- and heterotrophic N fixers in wetted soils of the DV. The *nifH* gene, encoding a subunit of the iron protein of the nitrogenase reductase complex was used as

a molecular marker for N₂ fixation as it yields tree topologies similar to 16S rRNA gene phylogeny (Young, 1992; Zehr *et al.*, 2003a, b). This work represents the first comprehensive study of N₂ fixation in DV soils and the microbial communities associated with these activities.

Materials and methods

Sampling and soil characteristics

Samples for this study were collected from the Miers Valley region of Antarctica (Fig. 1). Miers Valley is located at the southern region of the McMurdo Dry Valleys (DV) in Victoria Land and has two glaciers located at the upper (west) end of the valley: Miers Glacier is situated at the northern side and Adams Glacier on the southern side of the Valley. During the summer months, meltwater streams from both glaciers flow into the permanently ice-covered Lake Miers (Fig. 1). A number of hyporheic zones and wetted lake boundaries as presented in Fig. 1 were sampled along transects (as described later) during a 2-week period of January 2009. Four transects are described in detail in this study, including: (1) MS1, Miers Stream 1 (S78°05.748', E163°44.822'), a site located at the edge of the runoff stream flowing from Miers Glacier into Miers Lake in Miers Valley; (2) ML1, Miers Lake 1 (S78°05.615', E163°49.912') located on the north side of Miers Lake; and (3) BL, Baby Buddha Lake (S78°03.621', E163°46.463'), and (4) NP, Nostoc Pond (S78°03.920', E163°46.497') adjacent to lake systems located over the north ridge of Miers Valley. Transects consisted of 4 sampling sites originating from site 1 defined as a 'wet' zone (sampling within the lake/stream edge) extending through moist soil zones containing crust communities (sites 2 and 3) to the final site 4 located in a typical Dry valley desert mineral soil. Soil samples were collected aseptically at each site into sterile Whirl-Pak bags using a sterile metal spatula and kept frozen during transport to the laboratory. A second sampling season (November–December 2009) was also carried out, encompassing nitrogenase activity assays with some transects from the January 2009 expedition being resampled. Soil pH was determined on slurries composed of 2 g of soil and 5 mL of de-ionized H₂O. Percentage of moisture content of soils was calculated following drying for 24 h at 90 °C. Chlorophyll *a* was measured via the acidification method of Holm-Hansen *et al.* (1965). Nitrate, silicate, and orthophosphate were analyzed using the Bran and Luebbe AutoAnalyzer II according to standard Bran and Luebbe AutoAnalyzer Applications procedures, G-172-96, G177-96, and G-175-96. Cell counts were determined by the addition of 9 mL of 2% formalin in 0.2-µm filtered de-ionized water to 1 cm³ of soil. The sample was vortexed for 20 s, placed

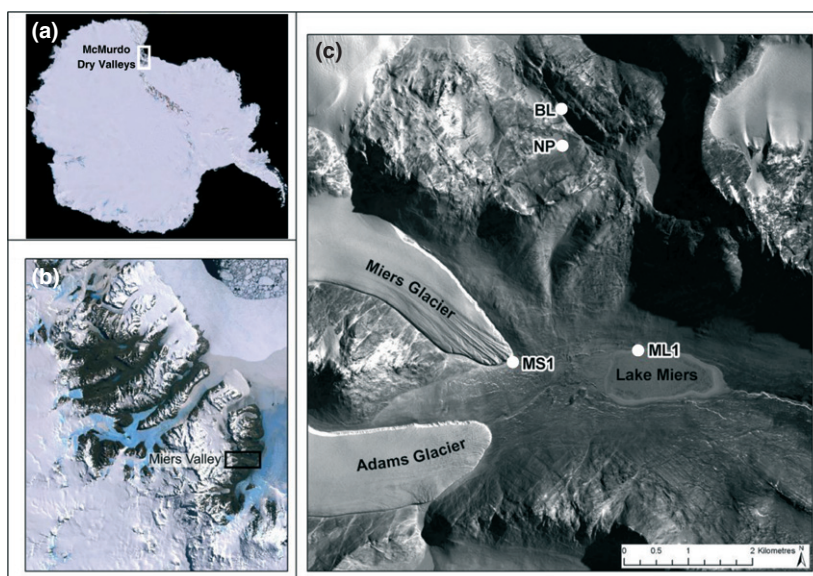


Fig. 1. The McMurdo Dry Valleys and the location of the transect sites in the Miers Valley region. (a) The Antarctic continent with the location of the McMurdo Dry Valleys indicated by the inset box. (b) An enlargement of the inset box showing the McMurdo Dry Valley region including the Miers Valley study region. (c) An enlargement of Miers Valley and presents the four sampling sites included in the study. Images are reproduced and sourced from the NASA Earth Observatory, the Landsat Image Mosaic of Antarctica (LIMA) Project, and the Advanced Land Observing Satellite (ALOS). This paper incorporates copyright data (JAXA, 2007, Australian Center of Remote Sensing, ACRES) under the permission of the Commonwealth of Australia (Geoscience Australia; 'the Commonwealth') which have not evaluated the data as altered and incorporated within the paper and therefore give no warranty regarding its accuracy, completeness, currency, or suitability for any particular purpose.

in ice, and sonicated for six 20-s 'on' and 20-s 'off' intervals using a Branson 250 sonicator (output set at 5) within a 15-mL polypropylene tube. The sample was allowed to settle for 20 min, and 50 μL of a DAPI solution (5 mg mL^{-1}) was added to 5 mL of the supernatant and incubated for 20 min at room temperature prior to filtering onto a Nuclepore filter (0.2 μm pore size; 25 mm diameter). A drop of nonfluorescing immersion oil was added to the filter and a coverslip placed. DAPI fluorescing bacteria were counted at 1000 \times under oil immersion using a 0.01-mm² grid in the ocular of the microscope.

Nitrogenase activity

Nitrogenase activity was measured in the field in triplicate using the acetylene (C_2H_2) reduction method (Capone, 1993; McKnight *et al.*, 2007). Samples were collected with a plastic syringe with the tip cut off; 41-cm-depth plugs were placed into 27-mL serum vials and sealed. 2 mL of C_2H_2 was added to each bottle, and the increase in ethylene (C_2H_4) was monitored in the field for the next 6–10 h on a Shimadzu mini-2 gas chromatograph with a flame ionization detector. Samples were incubated outside in full sunlight. The rate of increase in C_2H_4 is the nitrogenase activity, and this was converted into N_2 fixed with

a conversion factor of $3\text{C}_2\text{H}_2/1\text{N}_2$ and then multiplied by 2 to account for the two N atoms released per molecule of N_2 fixed. In the second field season (November–December 2009) of this project, we carried out experiments to measure the contribution of N_2 fixation by sulfate reducers; sulfate reduction can be inhibited by MoO_4 when added in concentrations similar to sulfate concentrations found in the surrounding water (Oremland & Capone, 1988). Samples were collected for N_2 fixation as described previously, but a second set of triplicates was inoculated with NaMoO_4 to a final concentration of approximately 1 nM and incubated for 2 h before initiating measurement of N_2 fixation, as described previously.

Nucleic acid extractions and cDNA synthesis

DNA was extracted from samples at the University of Waikato (New Zealand) using the PowerSoilTM DNA Isolation kit (MO BIO), as per manufacturer's instructions, and yields quantified using a NanoDrop system (Thermo Fisher Scientific). Total RNA was isolated in the field (a semi-permanent hut in Miers Valley) and at the Crary Laboratory, McMurdo Station, Antarctica, from preserved (LifeGuardTM, MO BIO) samples using the RNA PowerSoilTM Total RNA Isolation kit (MO BIO). cDNA was prepared at the Crary Laboratory, McMurdo Station,

Antarctica. A total of 16 µL of the total RNA extract was added to 2 µL of deoxyribonuclease I (1 U/µL; Invitrogen) and 2 µL of 10× DNase I reaction buffer (Invitrogen). The reaction was well mixed, incubated at room temperature for 15 min and 2 µL of 25 mM EDTA added and incubated at 65 °C for 10 min. cDNA was subsequently constructed from the DNase-treated RNA using the SuperScriptTM III First-Strand Synthesis SuperMix (Invitrogen) with random hexamers, as per manufacturer's instructions. cDNA was stored at −80 °C, and the presence of remnant DNA in RNA extracts and DNase-treated extracts checked via 16S rRNA gene PCR as described later.

Gene amplification via the polymerase chain reaction (PCR)

All PCR primers utilized in the study are listed in Table 1. Terminal restriction fragment length polymorphism (T-RFLP) PCR was undertaken as described by Danovaro *et al.* (2006). Following PCR, a total of three 25-µL reactions for each DNA sample were combined, and approximately 500–600 ng of purified amplicons digested in duplicate as per manufacturer's instructions using 10 U of *Alu* I (Roche). Duplicate digests were combined and purified as described previously and terminal fragment sizes determined [MegaBACE system (Amersham); Waikato DNA Sequencing Facility, University of Waikato, Hamilton, New Zealand]. Cyanobacterial-specific automated rRNA intergenic spacer analysis (ARISA) PCR was undertaken as described by Wood *et al.* (2008).

Various commonly used *nifH* PCR primer sequences were aligned to multiple *nifH* and alternative dinitrogenase

(*anfH* and *vnfH*) gene sequences to check for PCR primer coverage and specificity (results not shown). The most universal degenerated PCR primer pairs were chosen (Table 1), capable of targeting *nifH*, *anfH*, and *vnfH* alternative dinitrogenases using a nested approach based on the protocols of Yeager *et al.* (2004) and Hewson & Fuhrman (2006). Appropriate negative controls were utilized throughout the molecular analyses, and previously reported contamination problems were not encountered (Zehr *et al.*, 2003a, b). An initial PCR was undertaken utilizing primer pair 19F and *nifH*3 (Table 1) followed by a nested PCR of the resulting amplicons with primer pair *nifH*1 and *nifH*2 (Table 1) resulting in a approximately 370-bp product. The initial PCR comprised of 3 mM MgCl₂, 1× PCR buffer without MgCl₂ (Invitrogen) 0.2 mM each deoxynucleotide triphosphates, 0.8 µM each primer, 0.5 U Platinum[®] *Taq* DNA polymerase (Invitrogen), and approximately 100–200 ng of template DNA or 1 µL DNase-treated RNA extract/cDNA (neat and diluted 1 : 10 in pure water) in a final reaction volume of 25 µL. Thermocycling conditions consisted of 95 °C for 5 min followed by 21 cycles of 94 °C for 45 s, 48 °C for 1 min, and 72 °C for 1 min with a final extension step at 72 °C for 10 min. The subsequent nested PCR consisted of 2.5 mM MgCl₂, 1× PCR buffer without MgCl₂ (100 mM Tris-HCl pH 8.3, 500 mM KCl, SIGMA) 0.2 mM each deoxynucleotide triphosphates, 0.5 µM each primer, 20 µg bovine serum albumin, 1.25 U JumpStartTM *Taq* polymerase (SIGMA), and 1 µL of template (PCR product from the initial PCR) in a final reaction volume of 50 µL. The thermocycling conditions consisted of 94 °C for 5 min followed by 20 cycles of 94 °C for 45 s, 65 °C (−0.5 °C each cycle) for 1 min, and 72 °C for 1 min and then 12 cycles of 94 °C for 45 s, 55 °C for 1 min, and

Table 1. Oligonucleotides utilized in this study

Primer	Purpose	Target (amplicon size)	Sequence (5' -3')	Reference
27F	T-RFLP	Bacteria	FAM-AGA GTT TGA TCC TGG CTC AG	Lane (1991)
1492R			GGT TAC CTT GTT ACG ACT T	
CY-ARISA-F	ARISA	Cyanobacteria	FAM-GYC AYR CCC GAA GTC RTT AC	Wood <i>et al.</i> (2008)
23S30-R			CHT CGC CTC TGT GTG CCW AGG T	
19F	<i>nifH</i> initial amplification	<i>nifH</i> gene	GCI WTY TAY GGI AAR GGI GG	Ueda <i>et al.</i> (1995)
<i>nifH</i> 3			ATR TTR TTN GCN GCR TA	Zani <i>et al.</i> (2000)
<i>nifH</i> 1	<i>nifH</i> nested		*TGY GAY CCN AAR GCN GA	Zehr & McReynolds (1989)
<i>nifH</i> 2			ADN GGC ATC ATY TCN CC	
deltaG1F	<i>Delta-proteobacterial-specific nifH</i> gene qPCR	Group 1 OTUs: 2, 7, 13 and 47 ^A (260 bp)	AGG CGG ATT CGA CTC GGC TAT	This study
deltaG1R			GCC GCA TAC CAC GTC ACC C	
deltaG2F	<i>Delta-proteobacterial-specific nifH</i> gene qPCR	Group 2: OTUs: 11 and 27 ^A (322 bp)	CAT GCC AAG GCG CAG AAY ACG	This study
deltaG2R			CCA TCA TYT CGC CGG AGC AG	
cyanoUF	<i>Cyanobacterial-specific nifH</i> gene qPCR	All cyanobacterial OTUs ^A (152 bp)	TAT YAT CAC CGC YAT YAA CT	This study
cyanoUR			TAG ATT TCT TGB GCY TTR CC	
betaF	<i>Beta-proteobacterial-specific nifH</i> gene qPCR	OTUs: 1, 10, 31, 33 ^A (182 bp)	GTC ATG MRD ATY GGC TAC AAR	This study
betaR			GCC TTG TTK TCR CGG ATS GG	

72 °C for 1 min with a final extension step at 72 °C for 10 min.

DNA fingerprinting comparisons

Fluorescent peak data from both the T-RFLP and ARISA assays were uploaded and aligned in the *T-REX* online platform (Hewson & Fuhrman, 2006). Peaks below 300 bp were manually removed for ARISA as outlined by Wood *et al.* (2008). Aligned data were imported into PRIMER6 (Primer-E Ltd., Plymouth, UK), and comparison of community fingerprints was undertaken by two-dimensional principle component analyses (PCA) with overlaid resemblance matrix similarities, and BEST analysis was used to identify abiotic factors that best explain the observed community fingerprints (Clarke & Gorley, 2006). For BEST analyses, samples ML1-4 and NP4, and cell count data were excluded from the analyses owing to missing data (Table 2). All abiotic variables were log transformed with the expectation of chlorophyll *a* concentrations (as decided by draftsman plots), normalized, and comparisons made between community structure and abiotic factor similarity matrices (Euclidean distance) using Spearmans rank correlation with 999 permutations.

Partial length *nifH* gene cloning

Duplicate *nifH*1- and *nifH*2-primed PCR amplicons (as described previously) were combined, purified (GenElute™ PCR clean-up kit; SIGMA) and subsequently ligated into the pCR4-TOPO vector (Invitrogen), and transformed into One Shot TOP10 chemically competent *Escherichia coli* cells (Invitrogen) using the TOPO TA cloning kit for Sequencing (Invitrogen). Transformants containing the vector were selected on Luria Broth (LB) with kanamycin (50 µg mL⁻¹)-selective solid media, and the resulting plasmids with inserts were Sanger sequenced at the Genome Center at Washington University (St. Louis, MO).

Sequence analysis

nifH sequences were aligned using default parameters within CLUSTALW (Thompson *et al.*, 1994) and distances calculated using the Jukes–Cantor correction for multiple substitutions using the DNADIST program of PHYLIP (www.phylip.com). Rarefaction analyses were undertaken using the DOTUR program (Schloss & Handelsma, 2005), and representative *nifH* sequences (cutoff of 95% sequence similarity) were aligned and translated within the GENEIOUS software environment (www.geneious.com) with respect to translated *nifH* genes obtained from the NCBI GenBank database. The alignment was manually checked and a phylogenetic tree constructed using

Jukes–Cantor corrected distances and the Neighbor-joining method with 1000 bootstrap re-samplings. *nifH* sequences (cutoff of 95% sequence similarity) have been deposited in the NCBI GenBank database as accession numbers HM140726 to HM140774.

Quantitative PCR (qPCR)

qPCR primer pairs specific to the major *nifH* phylogenies (delta-proteobacteria group 1 and 2, cyanobacteria, and beta-proteobacteria, Fig. 4) were identified using the Oligo software package (Version 6; Molecular Biology Insights, Inc., Cascade, CO). Owing to the high dissimilarity between *nifH* sequences within each *nifH* phylogenetic grouping, minor OTUs were removed to deliver primer pairs specific to the major *nifH* clades. Table 1 provides the primer sequences, amplicon size, and the target OTUs. Specificity, including optimization of primer concentrations and annealing temperatures of each primer pair, was determined by screening representative *nifH* clones from each phylogenetic grouping.

The Minimum Information for Publication of Quantitative Real-Time Experiments (MIQE; Bustin *et al.*, 2009) has been followed. qPCR assays were undertaken in 96 well-plate format with an ABI7500 Real-Time PCR system, and results were analyzed using the associated Sequence Detection Software (version 1.2.; Applied Biosystems, ABI, Carlsbad, CA). Triplicate 10-µL assays were undertaken using 5 µL of SYBR® Green PCR master mix (ABI), 1 µL of each primer (9 µM), 10 or 20 ng of template DNA or 1 µL of cDNA diluted 1 : 10 and 1 : 50 in PCR grade H₂O (MO BIO), and the volume brought to 10 µL with PCR grade H₂O (MO BIO). Thermocycling conditions consisted of 50 °C for 2 min, initial denaturing at 95 °C for 10 min followed by 50 cycles of 95 °C for 15 s, and an annealing and extension step for 1 min at 68 °C for the delta-proteobacterial-specific primer pairs and 60 °C for the cyanobacterial- and beta-proteobacterial-specific primer pairs. All qPCR assays included a final dissociation stage consisting of 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. Standard curves used to estimate gene copy numbers were determined by the utilization of pCR4-TOPO vectors with inserted *nifH* as described previously. *r*² values of standard curves were above > 0.98 with slopes ranging between –3.2 and –3.6 equating to efficiencies of 105.35–89.57%. Quantification cycles (*C*_q) of the DNA samples ranged between approximately 18–33 and 26–31 for the cDNA samples with *C*_q values being below detection limits or below 40 for nontemplate controls. Assays were repeated for consistency, and PCR inhibition (i.e. change in *C*_q values) of DNA and cDNA amplification was not witnessed when spiked with positive control plasmid solutions.

Table 2. Transect descriptions and associated data. All data collected from initial field season (January 2009)

Location	Transect label	Dist. from Pt 1.	Macroscopic Appearance	N ₂ fix. (\pm S.D.)*	pH	Moisture content (%)	DNA g ⁻¹ (μ g)	Chl. a cc ⁻¹ soil (μ g)	Cells in 0–2 cm top soil (cm ⁻³)	Cells in porewater (ml ⁻¹)	Nitrate (NO ₃ +NO ₂) (μ M)	Silicate (Si(OH) ₄) (μ M)	Ortho-phosphate (PO ₄) (μ M)
Baby Buddha Lake	BL1	na	Lake edge (saturated black mat)	1.53 (0.75)	7.48	34.14	4.709	0.0526	1.37 \times 10 ⁷	3.09 \times 10 ⁴	0.54	62.39	0.17
	BL2	1 m	Yellow-colored mat	2.80 (1.41)	7.66	22.75	2.054	0.1636	3.65 \times 10 ⁸	2.28 \times 10 ⁴	0.43	154.24	0.68
	BL3	2 m	Black patchy mat	0.04 (0.07)	7.92	17.92	1.945	0.2176	2.76 \times 10 ⁷	1.23 \times 10 ⁴	0.44	162.32	0.24
	BL4	6 m	Dry gravel soil	0	9.20	0.35	0.036	0.2324	3.00 \times 10 ⁷	3.66 \times 10 ⁴	0.52	100.17	0.17
Miers Lake 1	ML1-1	na	Sediment of Lake	0.70 (0.28)	8.59	nd [†]	1.09	0.4857	1.09 \times 10 ⁸	8.30 \times 10 ³	25.73	63.71	0.79
	ML1-2	3 m	Black-colored mat	2.96 (0.39)	7.70	22.73	4.927	0.1020	9.87 \times 10 ⁷	3.89 \times 10 ³	54.1	130.87	1.87
	ML1-3	3.65 m	Patchy biofilm	0.22 (0.096)	9.52	14.54	1.509	0.2018	1.21 \times 10 ⁸	2.69 \times 10 ³	17.81	184.78	2.17
	ML1-4	8.3 m	Dry gravel soil	0	9.02	2.1	1.218	0.0104	2.04 \times 10 ⁸	nd	nd	nd	nd
Miers Stream 1	MS1-1	na	Stream bed, loose gravel	0	7.01	nd [†]	bdl	0.0129	nd	9.21 \times 10 ³	44.16	90.07	0.35
	MS1-2	9.6 m	Edge of stream bank	1.00 (0.66)	9.26	4.5	2.909	5.0438	3.57 \times 10 ⁸	4.46 \times 10 ⁴	39.42	76.56	0.29
	MS1-3	13.5 m	Extensive dry mat	3.38 (0.88)	8.58	19.2	3.963	0.0598	8.43 \times 10 ⁷	3.75 \times 10 ⁴	36.86	52.41	0.51
	MS1-4	20 m	Dry gravel soil	0.53 (0.33)	9.94	3.2	0.981	0.3284	5.54 \times 10 ⁷	3.11 \times 10 ⁴	22.88	151.35	2.47
Nostoc Pond	NP1	na	Lake edge (red/green mats)	2.03 (0.97)	7.63	32.74	2.627	0.0339	1.41 \times 10 ⁹	3.68 \times 10 ⁴	19.20	170.52	0.42
	NP2	30 cm	Green-black extensive mat	5.83 (2.11)	7.19	31.35	2.363	0.0608	9.56 \times 10 ⁷	1.62 \times 10 ⁴	25.54	213.82	0.87
	NP3	1.8 m	Black moss-like mat/gravel	3.04 (0.17)	8.35	11.43	1.363	0.2586	1.15 \times 10 ⁷	5.54 \times 10 ⁴	29.99	256.34	0.56
	NP4	3.7 m	Dry gravel soil	0	8.86	3.29	0.581	nd	nd	nd	nd	nd	nd

*units = nmol N cm⁻³ h⁻¹, with standard deviations.

†Saturated (sample collected below lake water level).

na, not applicable; nd, not determined; bdl, below detection limits.

Results

Transects and associated geochemistry

Locations of meltwater-to-dry soil transects sampled are indicated in Fig. 1, and associated geochemical data are presented in Table 2. Microbial communities sampled in the wetted soils existed as a thin (1–2 cm) crust layer and exhibited a patchy distribution. The lengths of the transects varied from 3.7 to 20 m ensuring that a typical arid DV desert soil was always sampled at position 4 in each transect (Table 2). Dry soils (site 4 of each transect) were considerably drier (0.35–3.3%) than that of the remainder of the wetted soils (sites 2, 3; 4.5–31% moisture) and the fully saturated (site 1) locations. Noticeable trends within the transects included a pH shift from neutral (approximately 7.0–8.6) to alkaline (approximately 8.8–9.9) from wet to dry points, and highest concentrations of DNA (approximately 2–5 $\mu\text{g g}^{-1}$ soil) and N_2 fixation rates (approximately 2.0–5.8 $\text{nmol N cm}^{-3} \text{ h}^{-1}$) observed in the wettest soils. Cell counts were higher for wet and dry topsoil (approximately 10^7 – 10^9 cells cm^{-3}) than that of the associated porewater (approximately 10^4 cells mL^{-1}). Ranges of nitrate, silicate, and orthophosphate concentrations were also similar between sites from each transect with the exception of lower nitrate concentrations at Baby Buddha Lake (Table 2).

Microbial community comparisons

Comparison of bacterial (T-RFLP) and cyanobacterial (ARISA) community fingerprints by principle component analysis is presented in Fig. 2. Distinct clusters at 40%

similarity were apparent for the bacterial fingerprints. Generally, wet sources (site 1) of NP and BL; arid soils of NP, ML1, and MS1; and hyporheic soils (sites 2 and 3) from MS1 and ML1 were grouped together at 40% similarity in the bacterial plot. A discernible distinction in clustering was not observed for the cyanobacterial ARISA-based plot (Fig. 2).

The total number of T-RFs within the T-REX analysis framework for both bacterial T-RFLP and cyanobacterial ARISA analyses were similar between transects and sites within a single transect (Supporting information, Table S1). For example, the average number of T-RFLP and ARISA fragments for the four dry (site 4) soils (61 and 64, respectively, standard deviation approximately 7.0 for both) were similar to the average number of fragments for the wet and moist (sites 1, 2, and 3) transect sites (approximately 56 and 63, standard deviations approximately 13 and 16, respectively). The highest correlation between abiotic measurements (Table 2) and microbial community structure via BEST analyses included distance, N_2 fixation rates, and nitrate concentrations ($\rho = 0.513$) for bacterial communities, and distance, N_2 fixation rates, pH, and nitrate concentrations ($\rho = 0.535$) for cyanobacterial communities.

Nitrogenase activities

In situ N_2 fixation rates measured in the first season varied from undetectable to a maximum of approximately 5.8 $\text{nmol N cm}^{-3} \text{ h}^{-1}$ (Table 2), and as expected, rates were highest from the hyporheic sites (sites 2 and 3) ranging from 0.04 to 5.8 $\text{nmol N cm}^{-3} \text{ h}^{-1}$ and low or undetectable activities (0–0.5 $\text{nmol N cm}^{-3} \text{ h}^{-1}$) in arid

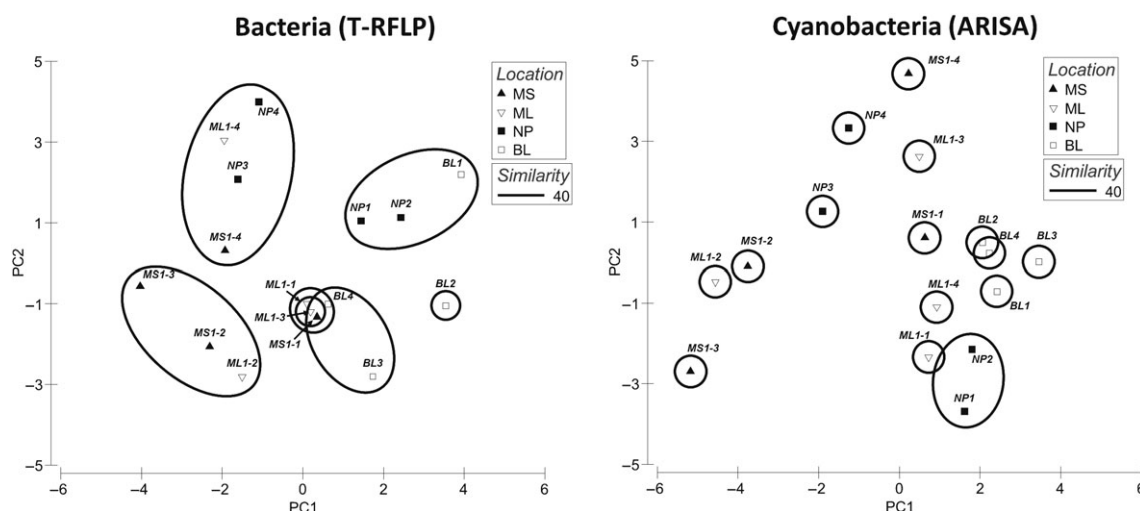


Fig. 2. Principle component analyses of bacterial T-RFLP and cyanobacterial ARISA fingerprints. Similarity (%) between fingerprints, as determined by presence/absence of T-RFs, has been overlaid within the PRIMER6 framework.

soils (site 4). The 'wet' source samples (site 1) were also positive for N₂ fixation (0.7–2.0 nmol N cm⁻³ h⁻¹) with the exception of site 1 of the MS transect, which was collected in the center of the stream where high flow rates presumably inhibited the formation of a stable microbial community. Owing to the discovery of sulfate-reducing N₂ fixers from samples collected during the initial January 2009 sampling season, wetted DV soils were measured for N₂ fixation activities that were linked with sulfate reduction during the second, December 2009 sampling expedition. Through the inhibition of sulfate reduction by the addition of NaMoO₄, it was found that sulfate-dependent N₂ fixation activity accounted for a large proportion of the total N₂ fixation activities detected within wetted DV sites (Fig. 3). For example, approximately 50% of total N₂ fixation activities for both wetted transect sites, ML1-2 and MS1-3, were linked with sulfate reduction with rates decreasing from approximately 2.7 to 1.4 nmol N cm⁻² h⁻¹.

nifH gene analyses

The *nifH* gene was not initially detected by PCR in the 'dry' (site 4) soil samples with the exception of BL4, suggesting low gene copy numbers. However, an increase from 21 to 30 PCR cycles during the initial amplification resulted in detectable *nifH* amplicons. The *nifH* gene could not be detected by PCR in samples from the high flow rate Miers Stream (MS1-1) site. *nifH*-based T-RFLP analyses (method by Hewson & Fuhrman, 2006) were undertaken on all transect sites; however, owing to both

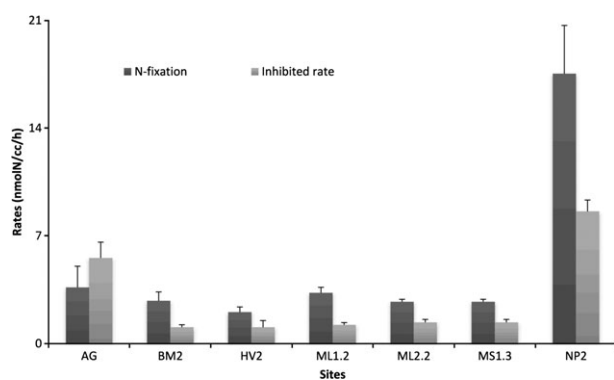


Fig. 3. N₂ fixation rates measured at various wetted Antarctic soils by the acetylene reduction assay during the second field season (November–December 2009). Activities are presented as both with and without the inhibition of sulfate reduction by the addition of NaMoO₄. Sites: AG, base of the Adams Glacier; BM, Black Mat from a flooded region of the Miers Valley; HV, site at the shore of the Hidden Valley lake; ML, Miers Lake transects; MS, Miers Stream; NP, Nostoc Pond.

low numbers of *nifH* TRFs (between 2 to 10) and single T-RFs for MS1-4 and ML1-3 (results not shown), results were not analyzed further. *nifH* clone libraries were constructed from the sampling sites with the highest detected N₂ fixation rates from each transect, that is, BL2, NP2, MS1-3, and ML1-2 (Table 3), and the cDNA from ML1-2, as this was the only cDNA sample that provided positive *nifH* PCR amplification (Table 3). Rarefaction curves of *nifH* libraries (Fig. S1) indicate that ML1-2 cDNA has reached its diversity plateau with slopes beginning to plateau for MS1-3 and ML1-2 and steep rarefaction curves for NP2 and BL2, signifying that the diversity of *nifH* in these sites are under-represented. Shannon diversity indices (at 95% sequence similarity) ranged between 1.8 and 2.6 for each individual *nifH* library (Table 3).

Four major *nifH* clusters were observed (Fig. 4) including two clades (group 1 and 2) within the delta-proteobacteria (one of which grouped closely with *Geobacter* sp.), one cluster within the cyanobacteria, and a group most closely related to the beta-proteobacteria. *nifH* phylotypes were also represented within the alpha- and gamma-proteobacteria. Table 3 includes the percentages of each phylotype detected within the samples. The MS1-3 and BL2 clone libraries were dominated by delta-proteobacterial-related signatures. Cyanobacterial phylotypes were most well represented in ML1-2, but not detected in BL2. ML1-2 cDNA contained a total of 6 OTUs that were dominated by cyanobacterial-related signatures and a single delta-proteobacterial OTU (OTU23) as opposed to 16 OTUs in DNA from the same site (at 95% sequence similarity). In total, only 3 of the 6 OTUs of the ML1-2 cDNA library were detected in the ML1-2 DNA library. Similarities of *nifH* phylotypes between sites were typically below approximately 56% with the exception of MS1-3 and ML1-2 sharing approximately 78% of their collective phylotypes (Table S2).

The majority of the *nifH* sequences showed low sequence similarity to *nifH* sequences within the NCBI GenBank database with sequence homologies typically not exceeding 90%. Higher sequence similarities (> 90%) were typically only obtained for *nifH* sequences related to cyanobacterial signatures previously recovered from the ice cover of Lake Bonney, Antarctica (Olson *et al.*, 1998), and those most closely related to clones (OraP15 and OraP17) were recovered from microbial mat communities of a meltwater pool on the McMurdo ice shelf (Jungblut & Neilan, 2010).

Quantitative PCR (qPCR) assays (oligonucleotides listed in Table 1) were developed to target the four major *nifH* phylotypes (delta-proteobacteria group 1 and 2, cyanobacteria and the beta-proteobacteria; Fig. 4) detected in the collective *nifH* clone libraries (Table 4). qPCR targeting these phylotypes were only undertaken on

Table 3. Percentage of *nifH* gene phylotypes (as designated in Fig. 4) detected in each clone library. Numbers in parentheses represent the number of clones

A. % <i>nifH</i> clone phylotypes	BL2	NP2	MS1-3	ML1-2	ML1-2 cDNA
Shannon diversity index	2.6	2.1	1.9	2.1	1.8
<i>deltaproteobacteria</i>	75.7 (53)	33.3 (24)	64.3 (45)	43.2 (32)	18.4 (14)
<i>cyanobacteria</i>	nd	13.9 (10)	10 (7)	35.1 (26)	81.6 (62)
<i>gammaproteobacteria</i>	8.6 (6)	4.2 (3)	5.7 (4)	6.8 (5)	nd
<i>alphaproteobacteria</i>	5.7 (4)	nd	nd	12.2 (9)	nd
<i>betaproteobacteria</i>	10 (7)	48.6 (35)	20 (14)	2.7 (2)	nd
Total	100 (70)	100 (72)	100 (70)	100 (74)	100 (76)

the hyporheic soils and indicated that cyanobacterial *nifH* genes were the most abundant (approximately 10^5 – 10^7 copies g^{-1} soil), with the exception of BL2 (approximately 10^3 copies g^{-1} soil) (Table 4). Delta-proteobacterial phylotypes dominated the BL2 library, approximately 10^5 and 10^4 copies g^{-1} soil for groups 1 and 2, respectively (Table 4). Nonphototrophic *nifH* gene copy numbers ranged from approximately 10^1 to 10^5 for all samples (Table 4). Cyanobacterial *nifH* genes were the only signatures detected in the cDNA fractions (Table 4) from MS1-3 and ML1-2 (approximately 10^6 and 10^7 copies g^{-1} soil) with *nifH* being below detection levels for cDNA from both BL2 and NP2.

Discussion

The bulk mineral soils of the Antarctic Dry Valleys (DV) are extremely dry (approximately < 2% mass water content), making water availability a particularly important driver of microbial activity (Zeglin *et al.*, 2009; Cary *et al.*, 2010) as is evident from the formation of extensive microbial crust communities associated with or adjacent to perpetually wet areas of the valleys (Kennedy, 1993). While water availability is critical for biomass formation, evidence is accumulating to suggest that other environmental variables may constrain the presence, distribution, and diversity of certain organisms within DV soil habitats. For example, Wood *et al.* (2008) concluded that moisture content of DV soils was a poor indicator of cyanobacteria distribution and that other variables such as soil elemental composition may play a role in influencing edaphic cyanobacterial habitat suitability. Likewise, Smith *et al.* (2006) observed a similar trend of cyanobacterial distribution within DV soils. From principal component analysis (PCA) of ARISA-based fingerprints (Fig. 2), our results also suggest that cyanobacterial communities in the DV are not ubiquitously distributed and local conditions unrelated to moisture content may define their composition or habitat suitability. Although cyanobacterial communities differed between biotopes, community structures of both bacteria and cyanobacteria were correlated with distance from the hydrological source, N_2

fixation rates, pH levels, and nitrate concentrations indicating that, while communities may differ (i.e. no distinction in clustering of ARISA plots in Fig. 2), community structure may be defined by these previously mentioned local factors. Similarly, a recent study by Smith *et al.* (2010) found high spatial variability of bacterial communities in soils from Taylor Valley in the DV and an ice-free coastal region (Cape Hallett) in northern Victoria land that were correlated with soil pH and moisture content ($\rho = 0.564$). In our study, differences in *nifH* phylotypes between the sampled hydrological sources also suggest a nonubiquitous distribution of microbial communities with wetted DV soils, that is, approximately 80% of *nifH* phylotypes shared between the hydrologically linked Miers Valley lake (ML1-2) and stream (MS1-3) systems and approximately 50% shared between the lake systems of Nostoc Pond (NP) and Baby Buddha Lake (BL), which were located at higher elevation and in an adjacent valley (Table S2).

As discussed previously, moisture has been hypothesized not to be an important driver of microbial distribution. Studies also suggest that microbial diversity is not influenced by moisture within DV environments (Wood *et al.*, 2008). This has been evidenced by reports of similar Shannon diversity indexes of 16S rRNA gene clone libraries of Antarctic soils of both high (approximately 9% soil moisture) and low (approximately 2–4% soil moisture) primary productivity potential (Niederberger *et al.*, 2008) and similar number (approximately 5–27, depending on biotope) of analysis fragment lengths of cyanobacterial-specific community ARISA profiles between DV lake and soil samples (Wood *et al.*, 2008). We also obtained similar numbers of bacterial and cyanobacterial TRFs from transect sites of contrasting water content (Table S1). This trend has also been noted in the temperate Chihuahuan desert (Liu *et al.*, 2000) and therefore may hold true for wide-ranging desert soil biotopes.

Cell counts in the top 0.2 cm layer of the soil samples were also similar between all soil types tested, that is, approximately 1.2×10^7 to 1.4×10^9 cm^{-3} . These cell density values fall within the cell count ranges previously reported for various DV soil biotopes as estimated by

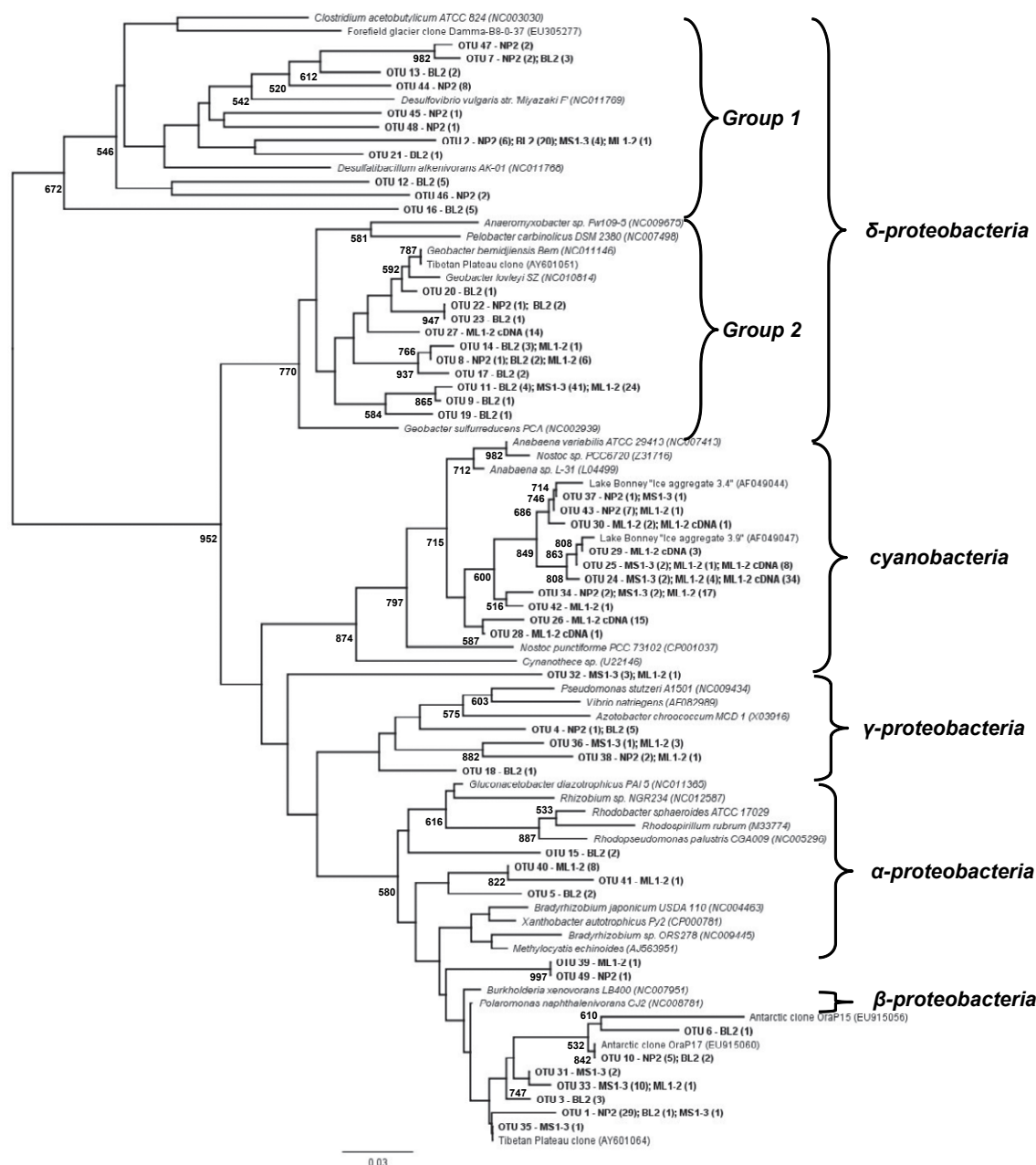


Fig. 4. Phylogenetic tree based on amino acid sequences translated from *nifH* OTUs grouped at 95% DNA sequence similarity for Miers Lake site 2 (ML1-2) DNA and cDNA, Miers Stream site 3 (MS1-3), Nostoc Pond site 2 (NP2), and Buddha Lake site 2 (BL2). The numbers of clones in each group are indicated in parentheses. The tree was constructed from a total of 115 amino acid residues using the Neighbor-joining method with 1000 bootstrap re-samplings. Bootstraps above 50% (i.e. 500 of 1000) are presented. *Methanocaldococcus jannaschii* DSM 2661 (L177117) and *Methanococcus voltae* (X03777) were used as an out-group and have been removed from the tree. The scale bar represents the number of amino acid substitutions per site. Groups 1 and 2 represent the two delta-proteobacterial lineages targeted by quantitative PCR (qPCR).

ATP analyses (Cowan *et al.*, 2002), including, rich/moist soils (1×10^7 to 4×10^9 g⁻¹ wet weight) and bulk desert soils (5×10^5 to 4×10^8 g⁻¹ wet weight⁻¹).

Assuming that the majority of the measured N₂-fixation rates were in the mats themselves, measured rates (approximately 0.5–6 nmol N cm⁻³ h⁻¹) can be consid-

ered areal estimates are comparable with areal-based rates detected in stream (0.3 nmol N cm⁻² h⁻¹), soil (4.7 nmol N cm⁻² h⁻¹), and pond (1.9 nmol N cm⁻² h⁻¹) microbial mats of the sub-Antarctic Islands, Livingston Island, and South Shetland Islands (Fernández-Valiente *et al.*, 2007). Similar rates were also previously recorded

Table 4. *nifH* gene copy number per gram of soil as determined by qPCR. Numbers in parentheses represent standard deviations of triplicate assays

<i>B. nifH</i> copies g ⁻¹ soil	BL2	NP2	MS1-3	ML1-2	MS1-3 cDNA	ML1-2 cDNA
<i>delta-proteobacteria</i> G1	1.20 (0.09) × 10 ⁵	3.49 (2.82) × 10 ¹	1.56 (0.56) × 10 ²	bd	bd	bd
<i>delta-proteobacteria</i> G2	1.29 (0.03) × 10 ⁴	3.43 (1.35) × 10 ²	9.74 (0.19) × 10 ³	2.88 (1.38) × 10 ²	bd	bd
<i>cyanobacteria</i>	3.64 (1.36) × 10 ³	2.98 (1.02) × 10 ⁶	2.92 (0.23) × 10 ⁵	1.90 (0.24) × 10 ⁷	2.07 (0.04) × 10 ⁶	2.04 (0.004) × 10 ⁷
<i>beta-proteobacteria</i>	5.00 (1.72) × 10 ³	4.09 (0.73) × 10 ⁴	3.88 (0.97) × 10 ⁴	9.13 (0.92) × 10 ³	bd	bd
<i>nifH</i> copies cm ⁻³ soil						
<i>delta-proteobacteria</i> G1	1.74 × 10 ⁵	4.85 × 10 ¹	2.81 × 10 ²			
<i>delta-proteobacteria</i> G2	1.87 × 10 ⁴	4.77 × 10 ²	1.75 × 10 ⁴	4.58 × 10 ²		
<i>cyanobacteria</i>	4.93 × 10 ³	4.14 × 10 ⁶	5.26 × 10 ⁵	3.02 × 10 ⁷	3.73 × 10 ⁶	324 × 10 ⁷
<i>beta-proteobacteria</i>	2.25 × 10 ³	5.69 × 10 ⁴	6.98 × 10 ⁴	1.45 × 10 ⁴		

nd, Not detected; bdl, below detection limits.

from Antarctic pond-/lake-associated sites: McMurdo Ice shelf cyanobacterial mats (5.1 nmol N cm⁻² h⁻¹; Fernández-Valiente *et al.*, 2001) and lake ice aggregates, for example, 9.4–91.3 nmol N mg⁻¹ chl *a* h⁻¹ (Priscu *et al.*, 1998) and approximately 0.08 nmol N g⁻¹ (dry weight) h⁻¹ (Olson *et al.*, 1998; Paerl & Priscu, 1998). Interestingly, these rates are of the same magnitude as seen in cyanobacterial mats found in temperate, subtropical, and tropical shallow marine systems such as coral reefs (Charpy *et al.*, 2010) and intertidal lagoons (Steppe & Paerl, 2005). While cyanobacterial mats from warmer climates are composed of different species, the similarity of these rates indicates that temperature may be a driving factor in defining species composition, but not setting the areal N₂ fixation rates possible in these mats.

As typically found in other terrestrial habitats (Izquierdo & Nüsslein, 2006; Zehr *et al.*, 2003a, b), a large diversity of *nifH* sequences was observed. Shannon diversity index values ranged between 1.8 and 2.6 (Table 3), comparable with soils of the Arctic tundra (1.97), glacier fore-field (2.16–2.46) and tropical forest soil (2.41; Izquierdo & Nüsslein, 2006; Duc *et al.*, 2009). Unexpectedly, cyanobacterial *nifH* phylotypes did not dominate the gDNA clone libraries; phylotypes from within the cyanobacteria, but also the alpha-, gamma-, delta-proteobacteria, and a clade most closely related to the beta-proteobacteria were all represented. These results mirror results from in marine-associated microbial mats and rice roots, whereby a large diversity of heterotrophic *nifH* genes have also been found to dominate *nifH* clone libraries (Ueda *et al.*, 1995; Zehr *et al.*, 1995; Steppe & Paerl, 2002). Low sequence homology (approximately < 90%) of the heterotrophic *nifH* sequences to their closest NCBI GenBank matches also indicates that they currently remain uncharacterized. Although cyanobacteria did not dominate the gDNA libraries, they were the most abundant phylotype (approximately 10⁵–10⁷ gene copies g⁻¹ soil) in the wetted soils and were the only phylotypes detected by qPCR in the cDNA fractions for both the Miers Valley transects: ML1-2 and MS1-3. However, it is important to note that the presence of *nifH* mRNA transcripts may not represent definitive N₂ fixation activity as nitrogenase is subject to posttranslational control (Steppe & Paerl, 2002). Also, some cDNA sequences were not retrieved from gDNA clone libraries and may indicate biases in the PCR assays or low copy genes that are highly expressed, whereas gDNA clones not present in cDNA represent genes that were not transcribed at the time of sampling. Likewise, differences between qPCR and cDNA libraries may indicate biases owing to the contrasting PCR conditions, for example, primers and annealing temperatures.

Olson *et al.* (1998) also documented *nifH* sequences from Antarctic mat, soil, and ice samples, including Lake Bonney, Dry Pond (located on the hillside above Lake Bonney), and Canada stream that flows into Lake Fryxell (16 km from Lake Bonney). A limited number of *nifH* sequences were obtained (3 soil and 12 ice aggregate-associated *nifH* sequences) containing similar cyanobacterial clades as detected in our study (Fig. 4). Jungblut & Neilan (2010) also sequenced *nifH* genes from a cyanobacterial mat within a meltwater pond of the McMurdo Ice Shelf; however, in contrast to our study, all *nifH* transcript phylotypes were related to the *Nostoc* genus within the cyanobacteria (Jungblut & Neilan, 2010).

Delta-proteobacterial-related phylotypes dominated the gDNA *nifH* gene clone libraries (Table 3). *nifH* sequences of cultured delta-proteobacteria are typically from anaerobic sulfate reducers that group within cluster III of the four phylogenetic *nifH* clusters as proposed by Chien & Zinder (1996) and updated by Zehr *et al.* (2003a, b). These organisms also provide similar phylogenies when grouped according to 16S rRNA gene sequences (Zehr *et al.*, 2003a, b). A large number of our delta-proteobacterial *nifH* sequences were related to *Geobacter* sp. (*nifH* subcluster IA; Duc *et al.*, 2009). *Geobacter* species are able to fix N₂ under oxic conditions (Holmes *et al.*, 2004), with some species capable of utilizing oxygen as an alternative electron acceptor (Methe *et al.*, 2003; Lin *et al.*, 2004). Therefore, cycles of aerobic and anaerobic conditions, as characterized by dry and wet periods of hyporheic zones and lake margins, may have a selective advantage for *Geobacter* species. This has also recently been suggested by Duc *et al.* (2009), who reported that 21% of *nifH* clones detected in the fore-field soils of a receding alpine glacier in Central Switzerland exposed to fluctuating periods of snowmelt and periods of drought were also related to *Geobacter* species. Nonphototrophic-associated N₂ fixation rates in Antarctic habitats have also been detected in lake ice aggregates of the DV and mats of the McMurdo ice shelf under dark incubation conditions and incorporation of a photosystem II inhibitor (3-[3,4-dichlorophenyl]-1, 1-dimethyl urea; Olson *et al.*, 1998; Paerl & Priscu, 1998; Fernández-Valiente *et al.*, 2001; Jungblut & Neilan, 2010). Heterotrophic N₂ fixation activities were not detected in the ice shelf samples (Fernández-Valiente *et al.*, 2001; Jungblut & Neilan, 2010), and heterotrophs accounted for approximately 10–30% of N₂ fixation activities detected within ice aggregate collected from a depth of 1.5 m in Lake Bonney of the DV (Olson *et al.*, 1998; Paerl & Priscu, 1998). Many sulfate-reducing bacteria are known to contain *nifH* (Zehr *et al.*, 1998) and have been shown to carry out a substantial portion of N₂ fixation in benthic marine environments (Capone, 1982; Steppe & Paerl, 2002; Bertics *et al.*,

2010). Similarly, in this study, sulfate reduction was linked with a large proportion (approximately 50%) of the N₂ fixation activities in the ML1-2 and MS1-3 transect sites (rates dropped from approximately 2.7 to 1.4 nmol N cm⁻² h⁻¹) with high levels of sulfate reduction-linked N₂ fixation also detected at other wetted DV soil sites (Fig. 3). Steppe & Paerl (2002) have also employed inhibition of sulfate reduction by the addition of MoO₄ in a marine intertidal microbial mat and found that nighttime nitrogenase activity was inhibited by up to 64% with daytime rates remaining unaffected. The detection of sulfate-dependent N₂ fixation and the identification of *nifH* associated with sulfate reducers (i.e. delta-proteobacterial group 2, Fig. 4) in the gDNA and the cDNA for ML1-2 may also indicate a similar link between the sulfur and nitrogen cycles in wetted Antarctic soils, suggesting heterotrophic diazotrophs are important contributors to N₂ fixation.

Although the nitrogen cycle is poorly understood in soils of the DV (Barrett *et al.*, 2002), it is assumed that cyanobacteria are the major contributors to N₂ fixation owing to their conspicuous presence within microbial communities of wetted soils (Takacs-Vesbach *et al.*, 2010). For the first time, we report rates of N₂ fixation and identify *nifH* phylotypes and diversity within high-productivity soils of the DV. Collectively, our results suggest that both cyanobacteria and a diverse range of heterotrophic diazotrophs contribute to total N₂ fixation rates in wetted DV soils. However, further work is needed to define the dominance and niche differentiation between the autotrophic and heterotrophic N₂-fixing populations within these most extreme environments.

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Supporting Information

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

Fig. S1. Rarefaction curves (95% cut-off) for *nifH* gene clone libraries of BL2, NP2, MS1-3, ML1-2 and ML1-2 cDNA.

Table S1. Terminal-restriction fragments (TRFs) from each sample site utilized for PCA-based analyses.

Table S2. Percentage (total number of clones in parentheses) of *nifH* gene sequences in common between sites (95% sequence similarity).

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