ORIGINAL ARTICLE

Sources of edaphic cyanobacterial diversity in the Dry Valleys of Eastern Antarctica

Susanna A Wood^{1,2}, Andreas Rueckert², Donald A Cowan³ and S Craig Cary²

¹Coastal and Freshwater Group, Cawthron Institute, Nelson, New Zealand; ²Thermophile Research Unit, Department of Biological Sciences, University of Waikato, Hamilton, New Zealand and ³Department of Biotechnology, University of the Western Cape, Cape Town, South Africa

Cyanobacteria are major components of Antarctic Dry Valley ecosystems. Their occurrence in lakes and ponds is well documented, however, less is known about their distribution in edaphic environments. There has been considerable debate about the contribution of aquatic organic matter derived largely from cyanobacteria to terrestrial ecosystems. In this study, automated rRNA intergenic spacer analysis (ARISA) and 16S rRNA gene clone libraries were used to investigate cyanobacterial diversity in a range of soil environments within the Miers and Beacon Valleys. These data were used to elucidate the input of aquatic cyanobacteria to soil communities. Thirty-eight samples were collected from a variety of soil environments including dry and moist soils, hypoliths and lake and hydroterrestrial microbial mats. The results from the ARISA and 16S rRNA clone library analysis demonstrated that diverse cyanobacterial communities exist within the mineral soils of the Miers Valley. The soil samples from Beacon Valley were depauperate in cyanobacterial signals. Within Miers Valley, significant portions (29%–58%) of ARISA fragment lengths found in aquatic cyanobacterial mats were also present in soil and hypolith samples, indicating that lacustrine and hydroterrestrial cyanobacteria play a significant role in structuring soil communities. The influence of abiotic variables on the community structure of soil samples was assessed using BEST analysis. The results of BEST analysis of samples from within Miers Valley showed that total percentage of carbon content was the most important variable in explaining differences in cyanobacterial community structure. The BEST analyses indicated that four elements contributed significantly to species compositional differences between valleys. We suggest that the complete absence of lakes or ponds from Beacon Valley is a contributing factor to the low cyanobacterial component of these soils.

The ISME Journal (2008) 2, 308–320; doi:10.1038/ismej.2007.104; published online 31 January 2008

Subject Category: microbial ecology and functional diversity of natural habitats

Keywords: cyanobacteria; Antarctica; ARISA; 16S rRNA gene; intergenic transcribed spacer (ITS)

Introduction

Cyanobacteria are an ancient group of photosynthetic prokaryotic organisms that are integral parts of many terrestrial and aquatic ecosystems. They are found worldwide in diverse and extreme environments including oceans, freshwater, hot springs, bare rock and desert soils (Whitton and Potts, 2000). In Antarctica, one of the harshest, coldest locations on Earth, cyanobacteria are considered the most important and dominant phototrophs in freshwater and terrestrial ecosystems (Taton *et al.*, 2003).

Cyanobacteria have been identified in the majority of ice-free habitats on the continent (Broady, 1996, 2005; Adams *et al.*, 2006). However, it is their presence in Antarctic lakes and ponds that have attracted the most interest and led to detailed research into their taxonomy (morphological and molecular), community structure, biogeographical distribution and physiology (for example, Vincent *et al.*, 1993; Taton *et al.*, 2003, 2006a,b; de los Ríos *et al.*, 2004; Jungblut *et al.*, 2005; Novis and Smitten, 2006). Recent molecular-based studies on these systems have revealed a higher diversity than that documented by morphological methods and identified potentially endemic Antarctic species (Taton *et al.*, 2003, 2006a). In contrast, the edaphic cyanobacterial communities of Antarctica have received less attention (Broady, 1996; Cavacini, 2001). The few detailed studies undertaken relied primarily on microscopic identification, and, in

Correspondence: SC Cary, Thermophile Research Unit, Department of Biological Sciences, University of Waikato, Hamilton, New Zealand 2001, New Zealand.

E-mail: c.cary@waikato.ac.nz

Received 22 August 2007; revised 18 October 2007; accepted 22 October 2007; published online 31 January 2008

most instances, cyanobacterial and algal abundance was insufficient for direct microscopic detection, and culturing was required (Cavacini, 2001). There is also a paucity of research characterizing Antarctic soil microbiology and probing the environmental parameters, which might influence cyanobacterial community structure.

The McMurdo Dry Valleys are the largest ice-free region of Antarctica. They are characterized by extremely low temperatures and precipitation, the latter occurring only as snow, which is largely lost by sublimation (Doran *et al.*, 2002). These valleys are considered to be the coldest, most arid deserts on Earth (Cowan *et al.*, 2002; Doran *et al.*, 2002). In general, most of the mineral soils of the valley floors lack any visible cyanobacterial or algal growth, whereas biomass is more apparent in glacial streams and lake margins (Taton *et al.*, 2006a). There has been considerable debate on assembly and viability of biotic communities in Dry Valley soils. It has been suggested that algae and cyanobacteria present in these mineral soils are not actively growing, but are derived from more suitable environments (for example, lakes) and are transported to the valley floors by wind (Horowitz *et al.*, 1972; Broady, 1996; Cavacini, 2001; Aislabie *et al.*, 2006). Aeolian distribution of freeze-dried mats from lakes and ponds has been documented (for example, Parker *et al.*, 1982; Moorhead *et al.*, 2003). In these extremely low carbon environments, microbial mats have been shown to be an important source of present-day carbon (Adams *et al.*, 2006), and thus may play an important role in soil ecosystem functioning. Recent molecular-based studies investigating entire prokaryote communities of Dry Valley soils have shown contradicting results regarding cyanobacterial diversity. A recent study in Luther Vale (TD Niederberger, IR McDonald, AL Hacker, RM Soo, JE Barrett, DH Wall, SC Cary, unpublished) found that cyanobacterial signals were confined to samples with significant soil water content, whereas Smith *et al.* (2006) identified multiple cyanobacterial species in a low moisture (0.6 weight % H₂O) soil sample. Additionally, the latter study indicated that cyanobacterial phylotypes were dominating members of 'universal' bacterial 16S rRNA libraries from high- and low-altitude Dry Valley sites, but absent from mid-altitude sites, suggesting that gravimetric water content may be a poor indicator of cyanobacterial distribution.

Large-scale, community-level studies of cyanobacteria have been limited by the time-consuming nature of culturing and microscopic identification and the corresponding high level of taxonomic expertise required (especially for *Oscillatoriales*). Polyphasic approaches, including the use of 16S rRNA clone libraries, have provided detailed phylogenetic information and have added greatly to the knowledge of Antarctic cyanobacterial biodiversity (for example, Taton *et al.*, 2003, 2006a,b; Jungblut *et al.*, 2005). However, these methods are extremely

protracted and therefore not applicable for analysis of large sample numbers. Recently, a sensitive and high-throughput fingerprinting method known as automated rRNA intergenic spacer analysis (ARISA) has been developed (Fisher and Triplett, 1999). This PCR-based method exploits the length heterogeneity of the intergenic transcribed spacer (ITS) region between the 16S and 23S ribosomal genes. Total community DNA is amplified with a fluorescently labelled forward oligonucleotide, allowing the electrophoretic step to be performed with an automated system in which a laser detects the fluorescent DNA fragments. Molecular culture-independent methods are subject to the well-documented pitfalls of PCR-based techniques (von Wintzingerode *et al.*, 1997), and this is also true for ARISA. Despite such limitations, ARISA provides a valuable tool that can be used to characterize and compare microbial communities. This technique has proven to be very robust and has been used in multiple studies of microbial populations in a variety of habitats including freshwater bodies (Newton *et al.*, 2006), coastal and oceanic marine environments (Brown *et al.*, 2005; Hewson *et al.*, 2006), and differing soil types (Ranjard *et al.*, 2001; Schloss *et al.*, 2003).

The objectives of the present study were to investigate cyanobacterial diversity in a range of soil environments within the Miers and Beacon Valleys using ARISA and 16S rRNA gene clone libraries and to investigate plausible physicochemical parameters that may be essential in driving the cyanobacterial multiplicity. Miers Valley is a coastal valley with a lake at its centre. Beacon Valley is a high-altitude valley (1200 m) with no lake system. We were particularly interested in assessing the contribution of aquatic and hydroterrestrial cyanobacterial mats to edaphic cyanobacterial communities.

Materials and methods

Site descriptions

Samples for this study were collected from two valleys: the Miers and Beacon Valleys. The 25 km long Miers Valley is a coastal valley (78°6'S, 164°0'E) at the southern end of the McMurdo Dry Valley region. No long-term air temperature data exist for Miers Valley; however, nearby Taylor's Valley has a mean annual air temperature of 17.6 °C (Foley *et al.*, 2007). Two glaciers are positioned at the upper end of the valley (Miers Glacier on the northern side and Adams Glacier on the southern side), both of which sustain the lake volume via glacial melt-water streams. Lake Miers (elevation, 170 m) is approximately 1.5 km long and 0.7 km wide (Figure 1), approximately 20 m deep and permanently covered with about 6 m of surface ice (Baker, 1967; Voytek *et al.*, 1999).

Beacon Valley is one of the higher elevation ice-free valleys in the region and is in close proximity to

Figure 1 Location of the McMurdo Dry Valleys and sampling locations within Miers Valley.

the Polar Plateau (77°48'S, 160°48'E). The valley consists of a flat floor approximately 18 km long with an elevation of 1200 m at the northeastern end and 1350 m at the southwestern end (Ugolini *et al.*, 1973). As a consequence of this higher altitude, the valley experiences lower temperatures and less precipitation and glacial melt than Miers Valley. The mean annual temperature is approximately −35 °C (Foley *et al.*, 2007).

Sample collection

Surface soil samples were collected in Miers Valley at 50-m altitudinal intervals along two vertical transects on the southern (S171–S634) and northern (N171–N626) sides of the valley. Each transect started at the edge of Lake Miers at an altitude of 171 m and finished on the ridge above the valley at an approximate altitude of 630 m (Figure 1). Cyanobacterial mats were collected from three locations on the southern side of Lake Miers. Samples LMM1 and -2 were collected from lake mats close to the shoreline. Sample LMM3 was a dried mat collected 10 m from the lake's edge. Microbial mat material was also collected from a stream flowing into the lake (LMM4). Two samples were collected from hydroterrestrial environments; one at the edge of a stream in front of Adams Glacier (MVAG1) and the second from a moist soil area in front of Miers Glacier (MVMG1) (Figure 1). Samples were collected using a stainless steel spatula (swabbed with alcohol between collections) and placed in sterile 50-ml

Falcon tubes. Seven hypoliths (microbial communities found under translucent rocks) were collected from a 50-m radius site on a sloping gravel field approximately 200 m west of Lake Miers (Figure 1). These were carefully removed from the soil and placed in sterile polyethylene Whirl-Pak bags. Samples were collected between 6 and 11 December 2006.

Five surface soil samples were collected from the upper northern side of Beacon Valley. Samples BA and BD were collected at the terminal end of 50 m long transects that each radiated out from a central sampling point (BX). Samples were collected as described for Miers Valley soil samples. They were collected on 13 December 2006 at an altitude of 1380 m.

All samples were stored in the dark and below 0 °C while in the field and at −80 °C in the laboratory until needed for further analysis.

Soil chemistry analysis

Gravimetric soil moisture was determined for all soil samples from the mass loss of a 5-g subsample at 105 °C after 48 h. Elemental concentrations in the soil samples were determined following aqua regia digestion on an inductively coupled plasma mass spectrophotometer (ICP/MS) (ELAN DRC II; Perkin Elmer, Auckland, New Zealand) by the ICP Laboratory at the Waikato University School of Science. The total percentage of carbon (organic carbon and carbonates) and nitrogen was determined using a

TruSpec Carbon/Nitrogen determinator (LECO Corp., St Joseph, MI, USA) at the Waikato University Stable Isotope Laboratory. Analysis of variance followed by a Tukey's honestly significant difference (HSD) *post hoc* test (Zar, 1974) was used to determine if there were significant differences in physicochemical variables between sample types (that is, North and South transects and Beacon Valley) at a 95% confidence interval.

DNA extraction

DNA was extracted from all samples using the modified methods of Coyne *et al.* (2001), described in Barrett *et al.* (2006a).

Automated rRNA intergenic spacer analysis

PCRs were performed in duplicate in 50- μ l volumes containing between 20 and 100 ng of DNA, 300 nM of the cyanobacteria-specific primers CY-ARISA-F (5'-FAM-GYCAYRCCCCGAAGTCRTTAC-3'; SA Wood, A Rueckert, SC Cary, unpublished) and 23S30R (5'-CHTCGCCTCTGTGTGCCWAGGT-3'; modified from Taton *et al.*, 2003) (Invitrogen, Auckland, New Zealand), 200 μ M dNTPs (Roche Diagnostics, Auckland, New Zealand), 1 $\times$ Taq PCR buffer (Invitrogen), 1 U of Platinum Taq DNA polymerase (Invitrogen), 4 mM MgCl₂ (Invitrogen) and 0.6 μ g non-acetylated bovine serum albumin (Sigma, Auckland, New Zealand). Thermal cycling conditions were 94 °C for 2 min followed by 94 °C for 45 s, 50 °C for 30 s, 72 °C for 2 min, repeated for 35 cycles with a final extension of 72 °C for 7 min. PCRs were run on a PTC-200 Peltier Thermal Cycler (MJ Research Inc., Waltham, MA, USA). Duplicate PCR products were combined and visualized on a 1.5% agarose gel, then purified using a High Pure PCR product purification kit (Roche Diagnostics) and eluted in 30 μ l.

Amplicons were diluted 1 in 10 with sterile water and 2 μ l of product mixed with 0.25 μ l of ROX-labelled genotyping internal size standard ETR900R (GE Healthcare, Auckland, New Zealand). Each sample was made to 10 μ l with 0.2 v/v Tween 20 in sterile water. Intergenic spacer lengths were determined by electrophoresis using the MegaBACE system (Amersham Pharmacia Biotech, Auckland, New Zealand). Run conditions were 44 °C separation temperature, 10 kV voltage and 120-min separation time. Analysis was undertaken by the University of Waikato Sequencing Facility (Hamilton, New Zealand).

Statistical analysis of ARISA fingerprints

Automated rRNA intergenic spacer analysis fragment lengths (AFLs) were analysed by Genetic Profiler V.2. (GE Healthcare) and the data transferred to Microsoft Excel for further processing. AFL and fluorescence for each sample were aligned using an

Excel Macro. Previous research undertaken on unialgal cyanobacterial cultures has shown that there are occasionally small shifts in the AFLs between ARISA analyses (SA Wood, A Rueckert, SC Cary, unpublished). To account for this variability and ensure that species diversity was not overestimated, AFLs that differed by less than or equal to 2 bp were considered identical. If multiple AFLs fell within this range, then only the AFL with the highest fluorescence was maintained. AFLs falling below a threshold of 250 Fluorescence Units were considered 'background noise' and discarded. AFLs of less than 300 bp were considered to be too short to be true ITSs and were removed from further analysis. All AFL information was transposed to presence/absence data for statistical analysis.

Statistical analyses were undertaken using the PRIMER 6 software package (PRIMER-E Ltd, Ivy-bridge, UK). Multivariate analysis of the ARISA fingerprints from all sites was performed using multidimensional scaling (MDS) based on Bray-Curtis similarities. MDS analysis provides a graphical ordination of the samples based on the relative values of similarity to each other. Ordination of the Bray-Curtis similarities was undertaken using non-metric MDS, with 100 random restarts and the results plotted in two dimensions. Agglomerative, hierarchical clustering of the Bray-Curtis similarities was performed using the CLUSTER method of the PRIMER software, and this information superimposed onto the two-dimensional MDS plot at similarity levels of 20% and 40%.

Analysis of similarities (ANOSIM) provides a method to assess statistical differences between groups in multivariate data sets. ANOSIM was used to compare the following defined groups: South transect—Miers Valley (MV), North transect—MV, lake cyanobacterial mats—MV, hydroterrestrial cyanobacterial mats—MV, hypoliths—MV and Beacon Valley. ANOSIM produces a sample statistic, *R*, which represents the degree of separation between test groups (Clarke, 1993). A value close to 1 indicates the community composition is totally different, whereas a value of 0 indicates no difference. A Monte Carlo randomization was used to test the statistical significance of *R*.

The influence of abiotic variables on the community structure of the soil samples (that is, South and North transect—Miers Valley and Beacon Valley samples) was assessed using the BEST analysis in PRIMER 6 (Clarke and Gorley, 2006). No abiotic information was available for the other samples, and these were not included in the analysis. The BEST analysis selects the environmental variables that may explain biotic patterns. The BEST procedure achieves this by using every possible combination of abiotic and biotic variables until it finds the 'best' fit. This is done by finding the combination of predictor variables whose Euclidean distance matrix produces the highest Spearman's rho (ρ). The BEST analysis was run twice on our data set, once

comparing only the North and South transect samples and the second time comparing all soil samples.

16S rRNA and internal transcribed spacer clone libraries

Three samples, LMM1, S334 and N184, were selected for the construction of cyanobacterial clone libraries. The samples were selected as they contained a high diversity of AFLs. The 16S rRNA gene and ITS were amplified from genomic DNA using primers 338F 5'-ACTCCTACGGGAGGCAGC-3' (Muyzer *et al.*, 1993), and a modification of primer 23S30R (Taton *et al.*, 2003) 5'-CHTCGCCTCTGTGTGCCWAGGT-3'. PCR was performed in 25- μ l reaction volume containing 20 ng of DNA, 600 nM of each primer, 200 μ M dNTPs (Roche Diagnostics), 1 $\times$ AmpliTaq PCR buffer (Roche Diagnostics), 0.75 U of AmpliTaq (Roche Diagnostics), 4 mM MgCl₂ (Roche Diagnostics) and 0.8 μ g non-acetylated bovine serum albumin (Sigma). Thermal cycling conditions were 94 °C for 2 min followed by 19 cycles of 94 °C for 1 min, 65 °C for 1 min and 72 °C for 2 min with annealing temperature being gradually decreased by 0.5 °C per cycle, followed by 14 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min. Final extension was at 72 °C for 7 min. PCRs were run on a PTC-200 Peltier Thermal Cycler (MJ Research Inc.).

Cloning of the PCR product was performed with a TOPO TA cloning kit (Invitrogen) following the manufacturer's instructions. Twenty-four clones with an insert of the correct size from sites LMM1 and S334 and 10 from N184 were randomly selected for sequencing. Plasmids were extracted following the protocol described in Kotchoni *et al.* (2003) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Auckland, New Zealand). Sequencing employed primers T3, T7 (TOPO TA cloning kit; Invitrogen) and 1392R (5'-ACGGGCGGTGTGTRC-3') (Lane *et al.*, 1985).

Phylogenetic analysis of 16S rRNA

The phylogenetic relatedness of the 16S rRNA gene sequences obtained in this study was established using 16S rRNA gene sequences (1200–1600 bp) from the Ribosomal Database Project (RDP; <http://rdp.cme.msu.edu/>). Sequences obtained in this study were deposited in NCBI GenBank database under accession nos. EU032357–032396.

Results

Soil chemistry

Soil physical and chemical properties varied significantly among sample types. The mean water content was highest in samples collected from Beacon Valley (2.8–3.6% g/g) relative to the samples from either the North (0.2–3.9% g/g) or South

(0.3–4.7% g/g, $F = 4.3$, $P < 0.02$) Miers Valley transect samples (Table 4). For both the North and South transects, the samples collected at lowest altitude near the lake's shore (S/N171) contained higher water contents than all other samples from the respective transects. The total percentage of nitrogen and carbon contents of soil samples did not differ significantly ($F = 3.12$, $P < 0.06$ and $F = 2.99$, $P < 0.07$, respectively; Table 4). In contrast, the elemental analysis showed some significant differences in composition among the sample types. These differences were most significant between the Miers Valley and Beacon Valley sites (Table 4).

Community structure

Analysis of the ARISA data for all samples identified a total of 108 distinct ALF (that is, peaks). When AFLs were totalled across each sample type, the highest diversity and average AFLs per sample was observed in the South transect samples (63 and 19.2, respectively) (Table 1). This was closely followed by the hypolith samples with a total AFL number of 51 and an average AFL number of 12.1 per sample (Table 1).

Beacon Valley samples BA and BB contained no AFL, and these samples were removed from all further analysis. Samples BC, BD and BX contained only one identical length AFL. Triplicate DNA extractions and ARISA PCRs were undertaken on these samples, and this result was reproducible. In addition, DNA from these samples was amplified using the universal bacterial ARISA primer set 1406F/23SR (Fisher and Triplett, 1999), and diverse AFL profiles were observed (data not shown). Thus, we believe this result reflects the true cyanobacterial diversity and is not due to the presence of substances inhibiting PCRs. The AFL found in the Beacon Valley samples was not unique to these samples and was also found in soil and hypolith samples from the Miers Valley.

All AFLs found in all cyanobacterial mats (that is, lake and hydroterrestrial) were compared to all other samples to assess similarities. The highest similarity was found in the North transect samples, in which 58% of the AFLs in the soil samples were also found in the cyanobacterial mats. This similarity was markedly higher than the 29% for the South transect and 32% for hypolith samples (Table 1).

Multivariate analyses revealed that the community structure differed among sampling sites (ANO-SIM $R = 0.722$, $P < 0.001$). Pair-wise comparisons of lake and hydroterrestrial mats, and hypolith and hydroterrestrial mats revealed no significant differences (Table 2). The Beacon Valley and hydroterrestrial mats did not differ significantly, but this result was probably due to the low number of samples from each location.

There were several prominent patterns in the two-dimensional MDS ordination of the 38 samples (Figure 2). With the exception of S171 and S184, the

Table 1 Mean soil physical and chemical properties for the three soil sample types: South transect (Miers Valley), North transect (Miers Valley) and Beacon Valley

	North transect	South transect	Beacon valley	$F_{2,22}$	P	Tukey test
Moisture	1.1	0.8	3.1	4.37	0.025	<u>N S B</u>
% total nitrogen	0.04	0.1	0.1	3.12	0.064	—
% total carbon	0.7	0.3	0.1	2.99	0.071	—
Altitude (m)	387	384	1376	55.0	<0.001	<u>N S B</u>
Ag	40.3	27.9	3.7	17.71	<0.001	<u>N S B</u>
Al	8323.3	8713.2	5728.5	5.17	0.014	<u>N S B</u>
As	0.3	0.2	0.6	1.03	0.375	—
B	2.0	2.5	4.4	1.90	0.174	—
Ba	62.3	53.7	11.9	27.07	<0.001	<u>N S B</u>
Bi	0.1	0.1	0.0	6.68	0.005	<u>N S B</u>
Ca	17195.1	8352.6	4509.3	1.78	0.192	—
Cd	0.2	0.1	0.1	5.10	0.015	<u>N B S</u>
Co	8.7	9.5	8.8	0.85	0.440	—
Cr	5.4	13.8	0.6	6.28	0.007	<u>N B S</u>
Cu	11.3	10.8	67.7	1323.01	<0.001	<u>N S B</u>
Fe	12893.7	13967.8	14237.7	0.89	0.426	—
In	0.1	0.1	0.0	27.97	<0.001	<u>N S B</u>
K	2740.6	2646.7	655.8	3.86	0.036	<u>N S B</u>
Li	6.3	5.9	4.6	5.70	0.010	<u>N S B</u>
Mg	8840.0	9705.1	3642.4	18.58	<0.001	<u>N S B</u>
Mn	258.5	285.9	157.8	8.24	0.002	<u>N S B</u>
Na	3257.9	3321.9	1356.0	19.08	<0.001	<u>N S B</u>
Ni	35.3	38.8	11.6	22.70	<0.001	<u>N S B</u>
Pb	2.1	1.7	3.9	15.49	<0.001	<u>N S B</u>
Se	0.2	0.2	0.5	6.63	0.006	<u>N S B</u>
Sr	178.8	113.0	17.9	2.91	0.076	—
Tl	0.2	0.1	0.0	8.71	0.002	<u>N S B</u>
V	29.2	31.8	19.9	5.63	0.011	<u>N S B</u>
Zn	24.9	25.2	28.5	1.59	0.227	—

Abbreviations: B, Beacon Valley; N, North transect; S, South transect.

The underline shows significant differences between sites, i.e., a line under two sites indicates no difference between them.

All given element concentrations are in mg kg^{-1} of dried weight. Results of a one-way analysis of variance followed by a Tukey test ($\alpha = 0.05$).**Table 2** Summary of AFLs for each sample type

Sample location	Total no. of different AFL in sample type	Average AFL per sample (range)	No. of AFL in common with cyanobacterial mats
Lake mats	24	7.1 (5–14)	—
Hydroterrestrial mats	23	8.4 (8–19)	—
North transect	38	12.4 (4–19)	22
South transect	63	19.2 (11–27)	18
Hypolith	51	12.1 (5–19)	16
Beacon Valley	1	1.0	0

Abbreviations: AFL, ARISA fragment length; ARISA, automated rRNA intergenic spacer analysis.

South transect samples formed a homogeneous cluster that was clearly separate from the other samples. Samples S171 and S184 clustered more closely with samples from the North transect, which formed a loose cluster, with some distinctly different samples, for example, N484 and N534. Interestingly, there was no definitive clustering of the hypolith samples, indicating that each is a unique community. A similar pattern was observed for the hydroterrestrial mats. The Beacon Valley samples

formed a separate cluster, reflecting the extremely low diversity in these samples compared to the Miers Valley samples.

16S rRNA sequence and internal transcribed spacer analyses

The analysis of the 16S rRNA sequences showed a similar diversity to that observed in the ARISA profiles. From a total of 24 clones, 10 unique

Figure 2 Two-dimensional, non-metric MDS ordination (stress = 0.19) based on Bray–Curtis similarities of ARISA fingerprints of cyanobacterial communities from various locations in the Miers Valley and Bezcon Valley. Points enclosed by solid line cluster at 20% similarity, points enclosed by dashed line cluster at 40% similarity. ■ North transect, ◆ South transect, ▼ Lake cyanobacterial mat, ✕ Hydroterrestrial cyanobacterial mat, ▲ Hypolith, + Beacon Valley. Only labels of samples referred to in text included.

Table 3 ANOSIM statistics for tests involving a comparison of all six sampling locations

Comparison		R-statistic	P-value
Location 1	Location 2		
Beacon Valley	Lake mats (MV)	1	0.029
Beacon Valley	Hydroterrestrial mats (MV)	1	0.100
Beacon Valley	South transect (MV)	1	0.003
Hydroterrestrial mats (MV)	South transect (MV)	0.987	0.013
Beacon Valley	North transect (MV)	0.976	0.003
Hydroterrestrial mats (MV)	North transect (MV)	0.955	0.013
Beacon Valley	Hypolith (MV)	0.905	0.008
Lake mats (MV)	South transect (MV)	0.821	0.002
Hypolith (MV)	North transect (MV)	0.676	0.001
North transect (MV)	South transect (MV)	0.657	0.001
Hypolith (MV)	South transect (MV)	0.615	0.002
Lake mats (MV)	North transect (MV)	0.612	0.002
Lake mats (MV)	Hydroterrestrial mats (MV)	0.429	0.133
Hypolith (MV)	Hydroterrestrial mats (MV)	0.256	0.111
Hypolith (MV)	Lake mats (MV)	0.149	0.145

Abbreviations: ANOSIM, analysis of similarities; MV, Miers Valley.

genotypes were identified for LMM1 and 16 for S334. Although only 10 clones were sequenced for site N184, six unique genotypes were identified (Table 3). Of the 32 clones, there were only seven matches where 16S rDNA sequence similarity was >97% to database entries in the RDP. The phylogenetic analysis identified species belonging to the orders *Nostocales*, *Chroococcales* and *Oscillatoriales*. ITS sequence and length information was obtained for 30 of the clones (Table 3). 16S rRNA sequences of some clones (for example, S334–S336 and S334–S3319) identified them as the same

phylotype, but with distinctive ITS lengths. Some of the phylotypes present in the LMM1 clone library were also present in the S334 and/or N184 clone libraries (for example, *Leptolyngbya antarctica*). The clone libraries constructed from the altitudinal transect soil samples (S334 and N184) contained additional species that were not observed in the LMM1 clone libraries (for example, *Chroococcidiopsis* sp. and *Acaryochloris* sp.). Identification of the length of the ITS region enabled the identification of some species in the ARISA profiles. For example, *Nostoc edaphicum*, with an ITS length of 477, was

observed in AFL profiles from nine of the South transect samples, one of the North transect sites and one of the hypolith samples. An AFL of 767 was found to correspond to an uncultured cyanobacterium (DQ181681), which had the closest sequence similarity to *Leptolyngbya frigida* (96.5%; Table 3), and this peak was apparent in many of the samples from all Miers Valley soil environments.

BEST analysis

Due to the differences in soil chemical and physical properties between Beacon Valley and Miers Valley, two separate BEST analyses were undertaken. To elucidate potential abiotic parameters responsible for differences in community structure within the Miers Valley soil samples, the first BEST analysis used only the North and South transects samples. The results showed that the highest rank correlation ($\rho = 0.531$, $P < 0.002$) was due solely to the total percentage of carbon content (log transformed). A second BEST analysis compared all soil samples. In this instance, the highest correlation ($\rho = 0.954$, $P < 0.001$) involved five elements (Mg, Mn, Sr, Ba and Ti).

Discussion

The McMurdo Dry Valleys comprise the majority of the less than 0.3% ice-free regions of Antarctica. Organisms that survive in these valleys are subjected to extreme conditions including low temperatures and precipitation, severe katabatic winds and dramatic physical and chemical gradients such as high salt concentrations and low carbon supply (Cowan and Tow, 2004; Barrett *et al.*, 2006a). In the absence of vascular plants, the mineral soils of the Dry Valleys are dominated by microbial communities. Contrary to earlier assumptions, which predicted low community complexity and spatial heterogeneity, recent molecular-based research (for example, Cowan *et al.*, 2002; Aislabie *et al.*, 2006; Barrett *et al.*, 2006b; Smith *et al.*, 2006; TD Niederberger, IR McDonald, AL Hacker, RM Soo, JE Barrett, DH Wall, SC Cary unpublished) has shown that the Dry Valley soils contain a much more diverse community assemblage than would have been expected given the harsh environmental conditions.

Cyanobacteria are major components of Dry Valley ecosystems. Their dominance, particularly in lakes, ponds and on moist soils, has resulted in research on numerous aspects of their taxonomy, physiology and ecology (Vincent *et al.*, 1993; Taton *et al.*, 2003; de los Ríos *et al.*, 2004). However, reports on cyanobacteria in Antarctic mineral soils are scarce and variable. Additionally, the source of the cyanobacterial component and their contribution to the organic matter of dry soils is the subject of debate (Broady, 1996; Cavacini, 2001; Aislabie *et al.*, 2006).

Comparison of the AFLs among samples has enabled us to make wide-scale spatial comparisons of cyanobacterial communities in a variety of soil environments across a broad range of climate and edaphic conditions. Cloning of the 16S rRNA gene and ITS region has allowed phylogenetic information to be attached to ARISA profiles, enabling tracking of specific species throughout samples. Interoperonic differences in spacer length occur within the genomes of microorganisms (Nagpal *et al.*, 1998), such that a single species may contribute more than one peak to an ARISA profile. Previous studies (for example, Gugger *et al.*, 2002; SA Wood, A Rueckert, SC Cary, unpublished) indicate that species of the order *Nostocales* commonly have two types of intergenic spacer regions (that is, two AFLs), whereas *Chroococcales* and *Oscillatoriales* have only one. This was also apparent in our results when 16S rRNA analysis identified clones LMM1–3 and LMM1–10 as the same phylotype despite two different (sequence and length) ITSs being obtained. This information can be used to make predictions on the number of species that might be present in a sample from an ARISA profile. ARISA can underestimate species diversity because ITSs of different sequences, but identical length, can exist and be represented as only one peak in the ARISA profile (Fisher and Triplett, 1999; SA Wood, A Rueckert, SC Cary, unpublished).

Our results show that multiple cyanobacterial species exist within the mineral soils of the Miers Valley. Four to twenty-seven AFLs were seen in the ARISA profiles from the Miers Valley soils. Previous studies (for example, Cavacini, 2001; Broady, 2005) and our data show that *Chroococcales*, *Nostocales* and *Oscillatoriales* are present in soil samples. Interestingly, many of the sequences obtained from the clone libraries had low similarity scores to any 16S rDNA database entries, suggesting the presence of as yet uncharacterized cyanobacteria. Further polyphasic studies within Miers Valley should be undertaken to explore these potentially unique species. On the basis of the known number of ITSs (one or two) in species within these orders, it is likely that there are > 15 species present in a single sample. While the clone libraries used in this study were not exhaustive, they clearly confirm the ARISA profile data and show multiple cyanobacterial genotypes in the soil samples. Smith *et al.* (2006) also noted a high cyanobacterial miscellany in a soil sample from the Miers Valley. This study used 16S rRNA gene clone libraries to assess soil microbial biodiversity and identified eight different cyanobacterial species in a high-altitude sample from a ridge above Miers Valley. Interestingly, the authors suggested that the cyanobacterial signals identified may constitute free-living cyanobacteria.

Elucidating the contribution of aquatic (lake and hydroterrestrial) cyanobacteria to soil cyanobacterial communities is important for many reasons. It

provides valuable information on disbursal mechanisms and assists in prediction of potential community change with increased moisture content (that is, due to regional warming). Additionally, cyanobacteria are a rich source of organic matter and thus could play an important role in the contribution of nutrients to nutrient-deficient valley soils. During favourable conditions, soil cyanobacteria may make a significant contribution to *in situ* net primary productivity (Barrett *et al.*, 2006a). Several researchers have indicated that cyanobacteria found in Dry Valley soils are sourced from lakes. For example, in an assessment of Victoria Land soils, Aislabie *et al.* (2006) found only one cyanobacterial ribotype (*Leptolyngbya* sp.). This species is dominant in all benthic mats of Victoria Land and was postulated to have been wind dispersed from nearby Lake Vanda. In a comparison of the AFLs from cyanobacterial mats and soil communities, we have shown that a significant number of the mat AFLs correlated to identical length AFLs in the Miers Valley soil samples. These results were further confirmed by the 16S rRNA clone libraries in which species present in the cyanobacterial lake mat sample (LMM1) were also found in clone libraries from two soil samples (S334 and N184). Our data indicate that at least within the confines of Miers Valley, the lake and hydroterrestrial cyanobacterial mats play a significant role in structuring the soil cyanobacterial communities. Extensive 16S rRNA gene libraries were constructed in a recent study (TD Niederberger, IR McDonald, AL Hacker, RM Soo, JE Barrett, DH Wall, SC Cary, unpublished) of dry soils collected within close proximity to Luther Lake in Luther Vale, North Victoria Land. The complete absence of cyanobacteria in their low moisture content soils is in stark contrast to our results and requires further investigation.

Genetic tools provide new facets to study microbial diversity. However, one caveat (when making assumptions on species diversity based on molecular techniques) is that these methods detect the presence of genes (or gene fragments), and this does not necessarily indicate the viability or intactness of the parent organism (Adams *et al.*, 2006). In the inherently arid and cold habitat of the Antarctic Dry Valleys, the degradation of DNA may be very slow (Adams *et al.*, 2006). This is possibly reflected in our study results. The high number of similar AFLs between soil and cyanobacterial mat samples indicates that at least some of the cyanobacteria in the soil is probably wind-borne dried mat material of lake origin. An extension of our work, which may shed light on the viable components of the cyanobacterial soil communities, could involve attempts to cultivate cyanobacteria from the soil samples, as was recently undertaken by Cavacini (2001). However, as with other bacteria, the culturing of multiple cyanobacterial species (that is, to gauge true diversity) is difficult and requires different media types, nutrient concentration and matrices (that is, liquid

or solid). The need for multiple techniques when isolating Antarctic cyanobacteria was recently illustrated by the recent study of Taton *et al.* (2006a, b), who used a variety of procedures to culture cyanobacteria from lake mats. A second alternative that is currently being explored by our research group involves using molecular techniques based on environmental RNA (for example, direct analysis of specific genetic transcripts), which is a better indicator of current bioactivity.

Cyanobacteria are well-known for their ability to survive repeated desiccation and hydration (Vincent, 1988; Cavacini, 2001). The presence of multiple cyanobacteria in the soils may have significant implications in terms of climate warming. A rise in temperature in ice-free areas is expected to increase the availability of liquid water (from glacial and permafrost melting; Cowan and Tow, 2004). Such changes could dramatically increase water availability within Miers Valley soil systems, possibly facilitating cyanobacterial outgrowth. With 'dormant' cyanobacteria already present in the soils, it seems plausible that extensive macroscopic growth could occur within a relatively short time frame. Further evidence for this has been provided by experiments using cloches positioned on arid Dry Valley soils, which showed rapid growth of moss and cyanobacterial biomass (DD Wynn-Williams, personal communication, as cited in Cowan and Tow, 2004).

Water availability has often been suggested as a major factor controlling biomass and diversity of Antarctic vegetation (Kennedy, 1993). There is little doubt that a significant difference in gross water content affects cyanobacterial biomass. This is macroscopically apparent when comparing the dense mats from the lakes and hydroterrestrial environments (where water is not limited) to the dry soils of Miers Valley (where there is no macroscopic algal growth). However, the ARISA profiles do not show that increasing gravimetric water content increases diversity within soil environments in the Miers Valley. For example, the average number of AFLs per lake cyanobacterial mat was only 7.1 compared to 19.2 for the South transect soil samples. The comparison between the Beacon Valley and Miers Valley soil samples also sheds some light on this suggestion. The soil samples from the Beacon Valley have significantly higher moisture contents than those collected from the Miers Valley (Table 4), and a simple correlation would suggest that a higher diversity and/or biomass would be expected in the former. However, few cyanobacterial AFLs were detected in the five soil samples from the Beacon Valley. This would suggest that variables other than gravimetric moisture content control habitat suitability for cyanobacteria in Antarctic soils. Our samples were collected only at one point in time and do not take into consideration potential seasonal changes in moisture content of the soils, which would be required before drawing

Table 4 16S rRNA clones identified in South transect site S334 (Miers Valley), North transect site 184 (Miers Valley) and in the Lake Miers cyanobacterial mat LMM1

Sample site/clone	Accession no.	ID of nearest match (accession no.)	% ID	ITS length (bp)
LMM1-3	EU032357	<i>Nostoc</i> sp.; EF174219	0.944	678
LMM1-4	EU032358	Uncultured cyanobacterium; EF111085	0.788	638
LMM1-9	EU032360	<i>Leptolyngbya antarctica</i> ; AY493572	0.898	790
LMM1-10	EU032361	<i>Nostoc</i> sp.; AY493593	0.984	529
LMM1-11	EU032362	Uncultured cyanobacterium; DQ181681	0.980	767
LMM1-18	EU032363	<i>Phormidium pristleyi</i> ; AY493578	0.987	706
LMM1-19	EU032364	Uncultured cyanobacterium DQ181681	0.987	640
LMM1-20	EU032365	<i>L. antarctica</i> ; AY493572	0.958	763
LMM1-21	EU032366	Uncultured cyanobacterium DQ181674	0.869	847
LMM1-24	EU032368	Uncultured Antarctic cyanobacterium; AF076164	0.886	NA
S334-1	EU032369	Uncultured cyanobacterium; DQ514216	0.915	640
S334-3	EU032370	<i>Phormidium murrayi</i> ; DQ493872	0.758	452
S334-4	EU032371	<i>Nostoc</i> sp.; EF174217	0.840	NA
S334-6	EU032372	Uncultured bacterium; DQ532167	0.796	627
S334-7	EU032373	<i>Chroococcidiopsis</i> sp.; DQ914863	0.878	641
S334-8	EU032374	<i>Leptolyngbya</i> sp.; AJ639894	0.944	675
S334-9	EU032375	<i>Chroococcidiopsis</i> sp.; DQ914865	0.695	423
S334-10	EU032376	<i>Nostoc calcicola</i> VI; AJ630448	0.994	477
S334-11	EU032377	Uncultured cyanobacterium; DQ451473	0.801	719
S334-12	EU032378	<i>Nostoc edaphicum</i> X; AJ630449	0.990	477
S334-13	EU032379	<i>Nostoc calcicola</i> III; AJ630447	0.814	598
S334-14	EU032380	<i>L. antarctica</i> ; AY493590	0.756	463
S334-15	EU032381	<i>Nostoc</i> sp. ANT.LH34.1; AY493591	0.977	451
S334-18	EU032384	<i>Chroococcidiopsis</i> sp.; DQ914863	0.882	642
S334-19	EU032385	Uncultured cyanobacterium; DQ532167	0.802	452
S334-21	EU032387	<i>Chroococcidiopsis</i> sp. CC3; DQ914865	0.786	459
N184-3	EU032390	Uncultured bacterium; DQ532167	0.888	638
N184-5	EU032392	<i>L. antarctica</i> ANT AY493590	0.811	467
N184-6	EU032393	Uncultured cyanobacterium; DQ181682	0.786	647
N184-7	EU032394	Uncultured bacterium; DQ532167	0.884	629
N184-8	EU032395	<i>Acaryochloris</i> sp.; AY790243	0.818	638
N184-9	EU032396	Uncultured bacterium; DQ532167	0.822	625

Abbreviation: NA, not available.

further conclusions from these results. The results of the BEST analysis indicated that differences in soil elemental composition may be a contributing factor. Research on a variety of cyanobacterial species has shown that they have metal ion requirements often absent from other bacteria (Cavet *et al.*, 2003) and that depauperate concentrations of some elements limit cyanobacterial growth. For example, oceanic cyanobacterial growth has been shown to be limited by iron, possibly due to its requirement in nitrogenase and hence nitrogen fixation (Chisholm *et al.*, 2001). The BEST analyses indicated that four elements contributed significantly to species compositional differences. The concentrations of two of these, magnesium and manganese, were significantly lower in the Beacon Valley than in the Miers Valley samples. Both of these elements are involved in key cellular processes in cyanobacteria, magnesium in chlorophyll production (Cavet *et al.*, 2003) and manganese in the thylakoidal water-splitting oxygen-evolving complex (Bartsevich and Pakrasi, 1996). Some metals have also been shown to have a toxic effect on cyanobacteria. Suroszl and Palinska (2004) showed that increasing copper dramatically inhibited the growth and caused damage to the ultrastructure of *Anabaena* sp. The major mechan-

ism of copper toxicity is via disruption of plasma membrane integrity, which inevitably leads to loss of cell viability (Avery *et al.*, 1996). Notably, copper concentrations were significantly higher in the Beacon Valley soil and this may be a contributing factor to the low cyanobacterial abundance in the Beacon Valley samples. *In situ* spiking experiments or dose–response studies using Antarctic cyanobacterial cultures could be undertaken to investigate this hypothesis. Further investigation into the effect of elemental concentrations on cyanobacterial communities throughout the Dry Valleys are required and were beyond the scope of this study. Furthermore, we suggest that the complete absence of lakes or ponds from Beacon Valley is likely to be a contributing factor to the low cyanobacterial component of the soils. Beacon Valley is relatively isolated from the other valleys with the nearest major lakes being Bonny and Vanda, both located more than 35 km distant. The high altitude of the Beacon Valley results in colder year-round temperatures and less glacial melt, resulting in few suitable sites for hydroterrestrial cyanobacterial mats.

Wind has been suggested to be the most important dispersal agent for biomass in Antarctica (Broady, 1996; Nkem *et al.*, 2005). Our results add further

evidence to support this contention. However, studies using longer scale transects on a spatial scale of kilometres from Lake Miers would be of interest to establish the extent of the lacustrine influence in this region.

The MDS analysis and ANOSIM indicated that there were significant differences in cyanobacterial community structure encountered over fine scales between two transects located in the same valley. This difference was surprising given the short distance (less than 2 km) between transects, but correlates with other Dry Valley studies, suggesting that prokaryotes are not ubiquitously distributed (Smith *et al.*, 2006; Barrett *et al.*, 2006a). The BEST analysis indicated that total percentage of carbon was a possible variable accounting for this difference, with South transect soil samples showing slightly lower total percentage of carbon values than North transect soil samples. In general, the South transect ARISA profiles had a higher number of AFLs than the North (average 19.2 and 12.4, respectively), indicating greater species diversity in the former. An important consideration when interpreting ARISA data is that without an in-depth understanding of the species in a system and extensive experiments to clarify PCR bias, the data provide little or no quantitative information on the community members. In this study, we have therefore only used the AFL data to indicate the presence of a species (ITS region) in a sample. Although there were, on average, fewer AFLs in the North transect, this does not necessarily correlate to a lower cyanobacterial biomass. As a higher percentage of the AFLs in the North transect samples correlated to AFLs in the cyanobacterial mat samples, it is plausible that the influence of the lake is not homogeneous, possibly due to dominant wind direction/circulation patterns.

Carbon dynamics are one of the most useful measures for delimitating soil biota and ecosystem functioning (Barrett *et al.*, 2006b). Harsh environmental conditions limit carbon fixation by primary producers (Barrett *et al.*, 2006a,b), resulting in the extremely low organic carbon concentrations in Antarctic soils (Burkins *et al.*, 2001). Microbial mats are known to be a source of present-day carbon (Adams *et al.*, 2006), and our data suggest that the hydroterrestrial and lacustrine cyanobacterial mats of the Miers Valleys have a significant effect on the biological functioning of mineral soils, that is, the more cyanobacterial mat AFLs in a soil sample, the higher the corresponding carbon content of the soil. Further studies are underway to ascertain the effect of differences in carbon content on total prokaryote community composition within Miers Valley.

Hypolith communities are found beneath translucent stones (Cowan and Tow, 2004). Their distribution in the Dry Valleys is probably limited to areas where suitable diaphanous stones exist. Algae and cyanobacteria are often dominant members of hypolith communities, and it has been observed

that the salinity, nutrient and water availability has an effect on determining the dominance of each species within a hypolithon (Broady, 1996; Thomas, 2005). The hypolith ARISA profiles from this study suggest multiple cyanobacteria species exist (5–19 AFLs) within these tightly confined communities. The MDS analysis shows clear differences in the hypolithic cyanobacterial community structures. Hypoliths sampled in this study generally had >20% AFL similarities. However, ANOSIM showed that hypolith cyanobacterial community diversity was not significantly different to that of the lake or hydroterrestrial cyanobacterial mats. The most likely explanation for this is that protection provided by the rock prevents wind evaporation and trapped moisture increases the available water (Cockell and Stokes, 2004), thus creating an environment suitable for species with higher water requirements.

The ARISA and 16S rRNA clone libraries revealed that the soils of Miers Valley contain multiple cyanobacterial phylotypes. A significant portion of the phylotypes present in the soil samples were also present in the lake and hydroterrestrial cyanobacterial mats, indicating that these play a significant role in structuring edaphic cyanobacterial communities. In contrast, very low cyanobacterial diversity was apparent in soil samples collected from Beacon Valley. We postulate that this is partly due to the absence of a nearby lake and may also be linked to the more extreme environmental parameters and colder temperatures of this remote, high-altitude valley. Previous research has shown that cyanobacterial mats are an important present-day source of carbon (Adams *et al.*, 2006). Our data strengthen these findings and suggest that within the Miers Valley, cyanobacterial mats may play an important role in soil ecosystem functioning either as an active biological component or as a nutrient source.

Acknowledgements

We acknowledge Antarctica New Zealand for logistic support to SCC and DAC. The research was also partially supported through a grant from the National Science Foundation (OPP-0229836 and EPSCoR-0447610) to SCC. We especially thank Steve Cameron and Anjana Rajendram (Waikato University) for assistance with soil chemistry analysis. We are indebted to Lisa Robson (Waikato University) and Stephanie Burton (University of Cape Town) for field assistance. SAW thanks the New Zealand Foundation for Research, Science and Technology for post-doctoral fellowship funding (CAWX0501). Dean Olsen (Cawthron Institute) is gratefully acknowledged for assistance with statistical analysis.

References

- Adams BJ, Bardgett RD, Ayres E, Wall DH, Aislabie J, Bamforth S *et al.* (2006). Diversity and distribution of Victoria Land biota. *Soil Biol Biochem* **38**: 3003–3018.

- Aislabie JM, Chhour K, Saul DJ, Miyauchi S, Ayton J, Paetzold RF *et al.* (2006). Dominant bacteria in soils of marble point and Wright Valley, Victoria Land, Antarctica. *Soil Biol Biochem* **38**: 3041–3056.
- Avery SA, Howlett NG, Radice S. (1996). Copper toxicity towards *Saccharomyces cerevisiae*: dependence on plasma membrane fatty acid composition. *Appl Environ Microbiol* **62**: 3960–3966.
- Baker AN. (1967). Algae from lake Miers, a solar heated Antarctica lake. *NZ J Bot* **5**: 453–468.
- Barrett JE, Virginia RA, Hopkins DW, Aislabie J, Bargagli R, Bockheim JG *et al.* (2006a). Terrestrial ecosystem processes of Victoria Land, Antarctica. *Soil Biol Biochem* **38**: 3019–3034.
- Barrett JE, Virginia RA, Wall DH, Cary SC, Adams BJ, Hacker AL *et al.* (2006b). Co-variation in soil biodiversity and biogeochemistry in northern and southern Victoria Land, Antarctica. *Antarct Sci* **18**: 535–548.
- Bartsevich VV, Pakrasi HB. (1996). Manganese transport in the cyanobacterium *Synechocystis* sp. PCC 6803. *J Biol Chem* **271**: 26057–26061.
- Broady PA. (1996). Diversity, distribution and dispersal of Antarctic terrestrial algae. *Biodivers Conserv* **5**: 1307–1335.
- Broady PA. (2005). The distribution of terrestrial and hydro-terrestrial algal associations at three contrasting locations in southern Victoria Land, Antarctica. *Arch Hydrobiol Suppl Algal Stud* **118**: 95–112.
- Brown MV, Schwalbach MS, Hewson I, Fuhrman JA. (2005). Coupling 16S-ITS rDNA clone libraries and automated ribosomal intergenic spacer analysis to show marine microbial diversity: development and application to a time series. *Environ Microbiol* **7**: 1466–1479.
- Burkins MB, Virginia RA, Wall DH. (2001). Organic carbon cycling in Taylor Valley, Antarctica: quantifying soil reservoirs and soil respiration. *Glob Chang Biol* **7**: 113–125.
- Cavacini P. (2001). Soil algae from northern Victoria Land (Antarctica). *Polar Biosci* **14**: 45–60.
- Cavet JS, Borrelly GPM, Robinson NJ. (2003). Zn, Cu and Co in cyanobacteria: selective control of metal availability. *FEMS Microbiol Rev* **27**: 165–181.
- Chisholm SW, Falkowski PG, Cullen JJ. (2001). Discrediting ocean fertilization. *Science* **294**: 309–310.
- Clarke KR. (1993). Non-parametric multivariate analysis of changes in community structure. *Aust J Ecol* **18**: 117–143.
- Clarke KR, Gorley RN. (2006). *PRIMER v6: User Manual*. Plymouth Marine Laboratory: Plymouth, UK.
- Cockell CS, Stokes MD. (2004). Widespread colonization by polar hypoliths. *Nature* **431**: 414.
- Cowan DA, Ah Tow L. (2004). Endangered antarctic environments. *Annu Rev Microbiol* **58**: 649–690.
- Cowan DA, Russell NJ, Mamais A, Sheppard DM. (2002). Antarctic Dry Valley mineral soils contain unexpectedly high levels of microbial biomass. *Extremophiles* **6**: 431–436.
- Coyne KJ, Hutchins DA, Hare CE, Cary SC. (2001). Assessing temporal and spatial variability in *Pfiesteria piscicida* distributions using molecular probing techniques. *Aquat Microb Ecol* **24**: 275–285.
- de los Ríos A, Ascaso C, Wierzbos J, Fernández-Valiente E, Quesada A. (2004). Microstructural characterization of cyanobacterial mats from the McMurdo Ice Shelf, Antarctica. *Appl Environ Microbiol* **70**: 569–580.
- Doran PT, McKay CP, Clow G, Dana G, Fountain A, Nylen T *et al.* (2002). Valley floor climate observations from the McMurdo Dry Valleys, Antarctica 1986–2000. *J Geophys Res* **21**: 4772.
- Fisher MM, Triplett EW. (1999). Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Appl Environ Microbiol* **65**: 4630–4636.
- Foley KK, Lyons WB, Barrett JE, Virginia RA. (2007). Pedogenic carbonate distribution with glacial till in Taylor Valley, Southern Victoria Land, Antarctica. In: Alonso-Zarza AM, Tanner LH (eds). *Paleoenvironmental Record and Applications of Calcretes and Palustrine Carbonates*. GSA Special Papers. Geological Society of America: Boulder, CO. pp 89–104.
- Gugger M, Lyra C, Henriksen P, Coute A, Humbert JF, Sivonen K. (2002). Phylogenetic comparison of the cyanobacterial genera *Anabaena* and *Aphanizomenon*. *Int J Syst Evol Microbiol* **52**: 1867–1880.
- Hewson I, Steele JA, Capone DG, Fuhrman JA. (2006). Remarkable heterogeneity in meso- and bathypelagic bacterioplankton assemblage composition. *Limnol Oceanogr* **51**: 1274–1283.
- Horowitz NH, Cameron RE, Hubbard JS. (1972). Microbiology of the dry valleys of Antarctica. *Science* **176**: 242–245.
- Jungblut A, Hawes I, Mountfort D, Hitzfeld B, Dietrich DR, Burns BP *et al.* (2005). Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. *Environ Microbiol* **7**: 519–529.
- Kennedy AD. (1993). Water as a limiting factor in the Antarctic terrestrial environment: a biogeographical synthesis. *Arct Alp Res* **25**: 308–315.
- Kotchoni SO, Gachomo EW, Betiku EB, Shonukan OO. (2003). A home made kit for plasmid DNA mini-preparation. *Afr J Biotechnol* **4**: 86–87.
- Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc Natl Acad Sci USA* **82**: 6955–6959.
- Moorhead DL, Barrett JE, Virginia RA, Wall DH, Porazinska D. (2003). Organic matter and soil biota of upland wetlands in Taylor Valley, Antarctica. *Polar Biol* **26**: 567–576.
- Muyzer G, de Waal EC, Uitterlinden AG. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59**: 695–700.
- Nagpal ML, Fox KF, Fox A. (1998). Utility of 16S–23S rRNA spacer region methodology: how similar are interspace regions within a genome and between strains for closely related organisms? *J Microbiol Methods* **33**: 211–219.
- Newton RJ, Kent AD, Triplett EW, McMahon KD. (2006). Microbial community dynamics in a humic lake: differential persistence of common freshwater phylotypes. *Environ Microbiol* **8**: 956–970.
- Nkem JN, Wall DH, Virginia RA, Broos EJ, Barrett JE, Porazinska DL *et al.* (2005). Wind dispersal of soil invertebrates in the McMurdo Dry Valleys, Antarctica. *Polar Biol* **29**: 346–352.
- Novis PM, Smissen RD. (2006). Two genetic and ecological groups of *Nostoc commune* in Victoria Land, Antarctica, revealed by AFLP analysis. *Antarct Sci* **18**: 573–581.

- Parker BC, Simmons GM, Wharton RA, Seaburg KG, Love FG. (1982). Removal of organic and inorganic matter from Antarctic lakes by aerial escape of blue-green algal mats. *J Phycol* **18**: 72–78.
- Ranjard L, Poly F, Lata JC, Mougél C, Thioulouse J, Nazaret S. (2001). Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. *Appl Environ Microbiol* **67**: 4479–4487.
- Schloss PD, Hay AG, Wilson DB, Walker LP. (2003). Tracking temporal changes of bacterial community fingerprints during the initial stages of composting. *FEMS Microbiol Ecol* **46**: 1–9.
- Smith JJ, Ah Tow L, Stafford W, Cary SC, Cowan DA. (2006). Bacterial diversity in three different Antarctic cold desert mineral soils. *Microb Ecol* **51**: 413–421.
- Suroszl W, Palinska KA. (2004). Effects of Heavy-metal stress on cyanobacterium *Anabaena flos-aqua*. *Arch Environ Contam Toxicol* **48**: 40–48.
- Taton A, Grubisic S, Balthasart P, Hodgson DA, Laybourn-Parry J, Wilmotte A. (2006a). Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. *FEMS Microbiol Ecol* **57**: 272–289.
- Taton A, Grubisic S, Brambilla E, De Wit R, Wilmotte A. (2003). Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. *Appl Environ Microbiol* **69**: 5157–5169.
- Taton A, Grubisic S, Ertz D, Hodgson DA, Piccardi R, Biondi N *et al.* (2006b). Polyphasic study of Antarctica cyanobacterial strains. *J Phycol* **42**: 1257–1270.
- Thomas DN. (2005). Photosynthetic microbes in freezing deserts. *Trends Microbiol* **13**: 87–88.
- Ugolini FC, Bockheim JG, Anderson DM. (1973). Soil development and patterned ground evolution in Beacon Valley, Antarctica. In: *North American Contribution, Permafrost*, Second International Conference, 13–28 July, 1973. Yakutsk, USSR, National Academy of Sciences: Washington, DC. pp 246–254.
- Vincent WF. (1988). *Microbial Ecosystems in Antarctica*. University Press: Cambridge, UK.
- Vincent WF, Downes MT, Castenholz RW, Howard-Williams C. (1993). Community structure and pigment organisation of cyanobacteria-dominated microbial mats in Antarctica. *Eur J Phycol* **28**: 213–221.
- von Wintzingerode FV, Goebel UB, Stackebrandt E. (1997). Determinations of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev* **21**: 213–229.
- Voytek MA, Priscu JC, Ward B. (1999). The distribution and relative abundance of ammonia-oxidizing bacteria in lakes of the McMurdo Dry Valley, Antarctica. *Hydrobiologia* **401**: 113–130.
- Whitton BA, Potts M. (2000). *The Ecology of Cyanobacteria, Their Diversity in Time and Space*, 1st edn. Kluwer Academic Publishers: Dordrecht, The Netherlands.
- Zar JH. (1974). *Biostatistical Analysis*. Prentice-Hall Inc: Englewood Cliffs, NJ, USA.