Characterization of bacterial communities in lithobionts and soil niches from Victoria Valley, Antarctica

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Abstract

Here we provide the first exploration of microbial diversity from three distinct Victoria Valley edaphic habitats, namely lithobionts (hypoliths, endoliths) and surface soils. Using a combination of terminal restriction fragment length polymorphism (T-RFLP) analysis and 16S rRNA gene amplicon pyrosequencing we assess community structure and diversity patterns, respectively. Our analysis revealed that habitat type (endolithic vs hypolithic vs surface soils) significantly influenced bacterial community composition, even though dominant phyla such as *Actinobacteria* (41% of total reads) were common to all samples. Consistent with previous surveys in other Dry Valley ecosystems, we found that lithobionts were colonised by a few highly-dominant phylotypes (such as *Gemmatimonas* and *Leptolyngbya*). Our analyses also show that soil bacteria were more diverse and evenly distributed than initially expected based on previous evidence. In contrast to total bacteria, the distribution of *Cyanobacteria* was not strongly influenced by habitat type, although soil- and endolith-specific cyanobacterial lineages were found. The detection of cyanobacterial lineages in these habitats appears to be influenced by the dispersal of aquatic inocula from lacustrine

communities or benthic mats which are abundant in Victoria Valley. Together, our results provide insights into the phylogenetic variation and community structure across niche habitats in Victoria Valley.

Key words

Antarctica, bacteria, *Cyanobacteria*, endolith, hypolith, soil

Introduction

Despite the recent advances in molecular analysis, we know comparatively little regarding the effect of environmental stressors on microbial diversity and function. The McMurdo Dry Valleys of Antarctica are an ideal model system for assessing how adverse conditions may shape microbial diversity and functional processes (Cowan et al., 2014). Extremely low moisture availability, below-freezing temperatures, katabatic wind episodes, frequent freeze-thaw cycles and very low levels of precipitation all combine to render the Dry Valleys inhospitable to most higher organisms (Cowan and Ah Tow, 2004), resulting in a simple ecosystem dominated by microbial communities (Cary et al., 2010). There is evidence suggesting that abiotic factors are strong drivers of microbial community composition, which include the scarcity of water (Fell et al., 2006), local soil geochemistry (Lee et al., 2012) and temperature (Hopkins et al., 2006). We now know that the controls on microbial community structure extend from local (Pointing et al., 2009) to regional scale differences (Van Horn et al., 2013, Bottos et al., 2014). Whilst the growing body of knowledge has suggested significant differences exist between lithobiontic and soil communities, very little is known regarding community dynamics across various habitats especially in understudied Dry Valleys such as the Victoria Valley.

The Antarctic Dry Valleys are largely colonised by a variety of cold-adapted microorganisms (Cowan et al., 2014, Williams et al., 2014, Makhalanyane et al., 2015b) that survive in an array of habitats such as hypolithic and endolithic environments (communities that inhabit undersides and interstices of rocks, respectively), moranic soils, permafrost and ice-covered lakes. Recent phylogenetic surveys have shown significant differences in hypolithic and surface soil community compositions (Pointing et al., 2009, Makhalanyane et al., 2013a), which are broadly consistent with the differences in community structure found between these habitats in hot hyperarid deserts (Azua-Bustos et al., 2011, Makhalanyane et al., 2013b). In addition, it is known that Dry Valleys are highly heterogenous in terms of physicochemical conditions, although the effect of these differences in shaping microbial diversity is not yet fully understood (Lee et al., 2012, Van Horn et al., 2013).

Lithic-associated communities are often dominated by *Cyanobacteria*, reviewed by Chan et al. (2012), which are almost certainly the major drivers of photosynthetic carbon fixation and nitrogen cycling in desert ecosystems (Warren-Rhodes et al., 2006). Moreover, lithons comprise simple trophic systems of primary producers involved in ecosystem processes (almost exclusively *Cyanobacteria*) and heterotrophic consumers and degraders (Pointing et al., 2009, Robinson et al., 2013). Furthermore, lithons harbour variable microbial compositions (Makhalanyane et al., 2013a, Makhalanyane et al., 2015b), with some endoliths dominated by

lichens rather than *Cyanobacteria* (De Los Ríos et al., 2014b), and experience unique levels of environmental stress resulting from lithic buffering (Warren-Rhodes et al., 2006). *Cyanobacteria* are also generally ubiquitous and widespread in Antarctic aquatic environments including lakes and ponds (Cowan and Ah Tow, 2004). Lacustrine and benthic habitats are reported to serve as important sources of novel cyanobacterial (and to a lesser extent heterotrophic bacterial) diversity, which may alter terrestrial community structures after introduction and colonisation events occur (Wood et al., 2008).

Exposed Dry Valley soils generally support fewer cyanobacterial lineages than lithobiontic consortia and are dominated by heterotrophic species (Lee et al., 2012). Soil bacterial diversity frequently exceeds that of lithic communities, in spite of lower microbial biomass (Cary et al., 2010). Interestingly, some Dry Valley soil communities are surprisingly rich in *Cyanobacteria*, such as the low-altitude maritime mineral soils of Miers Valley (Wood et al., 2008).

Here we provide data on the phylogeny of microbial communities associated with lithobiontic and soils habitats from the Victoria Valley, in the northern region of the McMurdo Dry Valleys of Antarctica. We also focus on the distribution of *Cyanobacteria* in the different terrestrial communities, and suggest that cyanobacterial populations may be influenced by the transport of lineages from surrounding aquatic systems on the basis of previous findings (Wood et al., 2008). To enhance our understanding of community structure and diversity we performed microbial fingerprinting analysis, using terminal restriction fragment length polymorphisms (T-RFLP), and 16S rRNA gene amplicon sequencing of bacterial communities.

Materials and Methods

Study site and sampling

All samples were retrieved from the Victoria Valley region of Eastern Antarctica (77°20' S, 161°39' E) during the austral summer season of January 2013 (Fig. 1). A total of 15 samples, five each of surface soils, endoliths and hypoliths were collected. Ventrally-colonised quartz rocks (hypoliths) and internally colonized sandstone rocks (endoliths) were randomly retrieved and stored in sterile Whirl-Pak sample bags (Nasco, WI, USA). The endolithic samples included both fungal-dominated and *Cyanobacteria*-dominated communities. Soil samples were transferred into sterile 50 ml tubes. Samples were maintained at < 0°C in the field and during transit to the laboratory. Sample material was stored at -80°C upon arrival until required.

Environmental DNA isolation and T-RFLP analysis

DNA was isolated from duplicate 1 g samples using a modified phenol/chloroform extraction method (Miller et al., 1999). Hypolithic and endolithic sample material was removed from rock substrates with sterile razor blades. DNA was eluted in 20 µl nuclease-free water and quantified using the NanoDrop 2000 spectrophotometer (NanoDrop Products, Wilmington, DE, USA). For T-RFLP analysis, 6' carboxyfluorescein-labelled forward (341F) and unlabelled reverse (908R) primers amplified universal bacterial 16S rRNA genes (Lane et al., 1985) under cycling conditions described previously (Makhalanyane et al., 2013a). Following purification and digestion with *Msp*I endonuclease, electrophoresis on an ABI3500xl genetic analyser (Applied Biosystems®, CA, USA) was performed to separate restriction fragments. Fragments were analysed at SeqServe (Bioinformatics and Computational Biology, University of Pretoria, South Africa; http://seqserve.bi.up.ac.za/). R scripts were used to bin T-RF profiles into operational taxonomic units (OTUs) using increments previously prescribed (Abdo et al., 2006). A nested PCR protocol was used to evaluate cyanobacterial communities with the *Cyanobacteria*-specific primers 6' FAM-359F and 781R (Nübel et al., 1997). The amplification of 442 bp products was achieved using the following thermal cycling conditions: 5 min denaturation at 95°C, followed by 30 cycles of the following three steps; 95°C denaturation for 30 sec, 64°C annealing for 30 sec and 72°C elongation for 90 sec; with a final elongation step at 72°C for 10 min. All downstream analyses were performed as described above.

16S rRNA gene amplicon pyrosequencing and phylogenetic analysis

Roche 454 GS FLX+ titanium platform was used for tagged-amplicon pyrosequencing using DNA samples (0.5 ng) from each habitat. We randomly selected single soil (S1) and hypolith (H3) samples, while we chose to sequence two morphologically-distinct endolithic communities, E2 and E5.1, which appeared to be visually dominated by fungal and cyanobacterial morphotypes, respectively. PCR amplification was carried out under a modified bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP®) protocol (Dowd et al., 2008) for hyper-variable regions V1 – V3 of the 16S rRNA gene. An Agencourt® AMPure® XP PCR Purification system (Agencourt Bioscience Corporation, MA, USA) was used to purify equal concentrations of pooled amplicons. Samples were sequenced at the Molecular Research LP next-generation sequencing

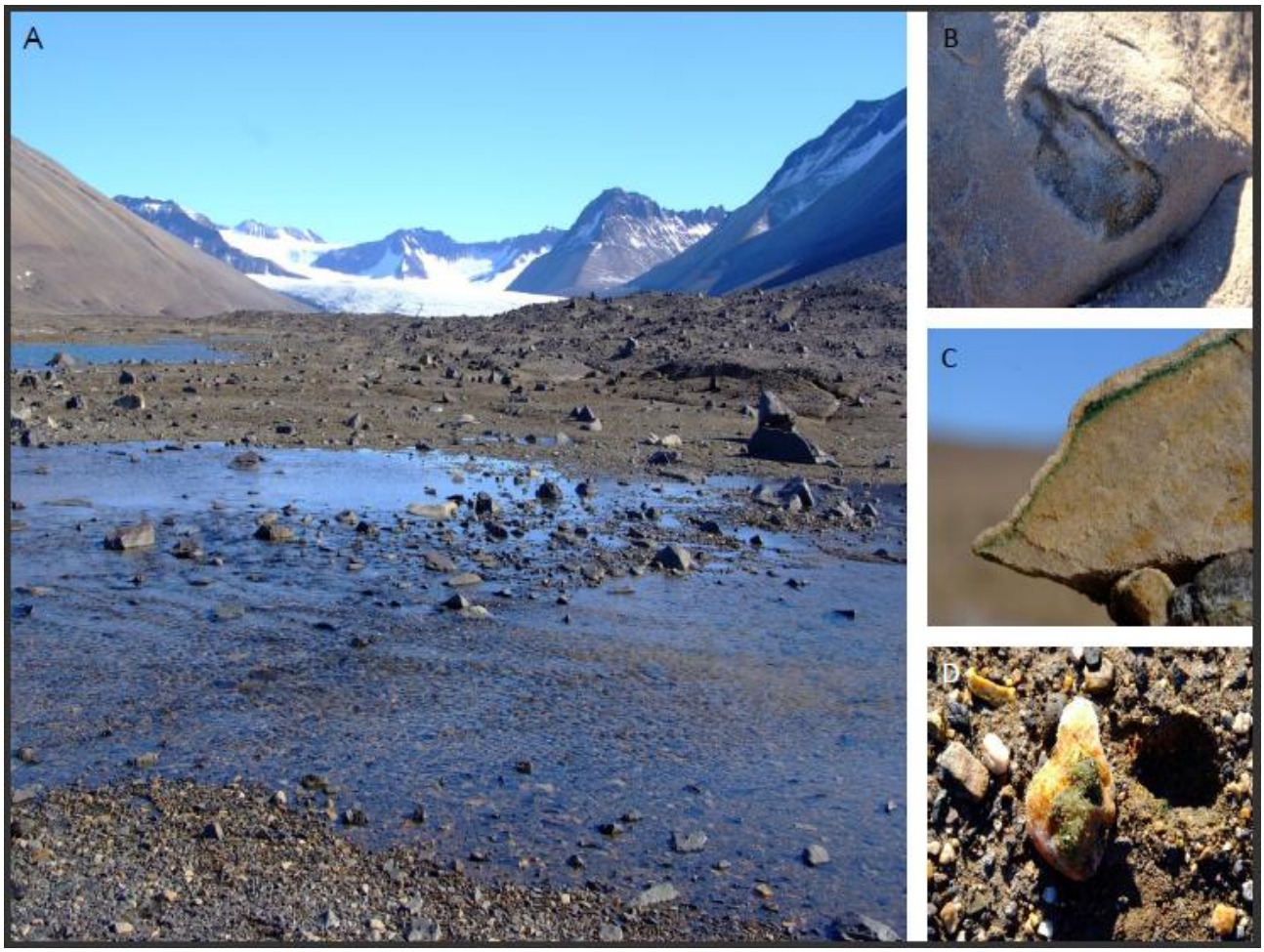

Figure 1. The sampling site in Victoria Valley (A) as well as the lithic habitats characterized; fungal-dominated endolith (B), cyanobacterial-dominated endolith (C) and hypolith (D). (Photographs courtesy of DAC).

service (http://www.mrdnalab.com/). Purification of the final amplicon products preceded unidirectional 454-pyrosequencing with GS FLX+ Titanium chemistry using standard protocols (Roche Diagnostics, CT, USA).

MOTHUR v.1.33.1 (Schloss et al. (2009); http://www.mothur.org/wiki/454_SOP/) was used to analyse the raw pyrosequencing data following an established pipeline (Schloss et al., 2011). Low-quality reads were removed from datasets using the shhh.flows command, while sequence lengths below 200 bp and reads containing homopolymers longer than 8 bp or ambiguous bases (N) were also removed. Dataset simplification was achieved with the unique.seqs command. The UCHIME algorithm (v4.2; Edgar et al. (2011); http://drive5.com/uchime/) was used in MOTHUR to remove chimeras. Alignments to the latest version of the SILVA 16S bacterial ribosomal reference database (Pruesse et al. (2007); http://www.arb-silva.de/download/arb-files/) was performed using align.seqs. A distance matrix was constructed in MOTHUR using sequences defined as OTUs at a 97% cut-off. The naïve Bayesian rRNA classifier (Wang et al., 2007) was used to assign taxonomic affiliations to the OTUs at 80% confidence.

Sequences were imported into ARB (Ludwig et al., 2004) and aligned using manual curation in addition to automated alignment. Reference sequences were selected from the GenBank database for tree reconstruction. Multiple alignments were exported from ARB and imported into the software package MEGA v6.06 for tree annotation and visualisation. The sequence data have been made available on the NCBI Sequence Read Archive under the accession number SRR2420889.

Data analysis

A Bray-Curtis dissimilarity matrix of Hellinger-transformed T-RFLP data (Bray and Curtis, 1957, Legendre and Gallagher, 2001, Abdo et al., 2006) was used to visualise differences in community composition; depicted using non-metric multidimensional scaling (nMDS) ordination plots generated in R. Differences in the total bacterial and cyanobacterial compositions between habitats (hypolith vs soil vs endolith) was tested using ANOSIM (Analysis of Similarity; Clarke (1993)), after 9999 iterations. Intra-habitat variation was tested using *betadisper* (permutation dispersion) (Anderson et al., 2006). We permitted singletons in the datasets as no effect on beta-diversity patterns were found after their exclusion. The effect of habitat on microbial abundance and diversity was assessed using Kruskal-Wallis (KW) tests which were considered significant ($P < 0.05$) after *post-hoc* Wilcoxon-Mann Whitney comparisons were made. We used Fisher's exact test to detect significant taxonomic differences between the communities after Bonferroni corrections using STAMP software (Statistical Analysis of Metagenomic Profiles; v2.1.3; Parks and Beiko (2010)).

Results and Discussion

Recently, a number of studies have focused on characterizing the lithic and soil-based microbial communities across a range of McMurdo Dry Valley niche habitats (Yung et al., 2014, de los Ríos et al., 2014a). However, the majority of these studies have focused on the low-altitude maritime Miers Valley (Khan et al., 2011, Makhalanyane et al., 2013a), with few studies exploring other valleys (De Los Ríos et al.,

2007, Lee et al., 2012, Lacelle et al., 2013). Based on habitat heterogeneity these edaphic systems are hypothesised to be highly variable, but how this heterogeneity may alter microbial diversity remains unknown. Here we provide the first characterization of microbial diversity of Victoria Valley lithobionts and soil niches. We explore bacterial diversity and focus on cyanobacteria, which are the likely drivers of major functional processes.

Community structure analysis via T-RFLP

We observed a total of 66 bacterial OTUs across all samples (n=15) of which 33 (50%) were cosmopolitan (so-called habitat generalists) to the three habitats. In contrast, only 20 OTUs (30%) were specific to a particular habitat, which is visualised as a low level of community dissimilarity between the three habitats (Fig. 2). The number of bacterial OTUs per individual sample (α-diversity) ranged from 19 to 48 [29 (mean) ± 7 (SD)]. Consistent with previous studies conducted in the Dry Valleys and in other hyperarid deserts (Pointing et al., 2007, Azua-Bustos et al., 2011, Makhalanyane et al., 2013a), lithobionts had higher average OTU numbers ($\tilde{\alpha}$) than soils (Table 1). Both the Shannon diversity index (H') and the Gini-Simpson index (1-λ') remained relatively constant between habitat types suggesting similar diversity levels between lithobionts and soils (Kruskal-Wallis test, $P > 0.05$; Table 1). Exposed surface soils had the highest levels of γ-diversity; 20% higher than the lithobionts on average.

nMDS ordination plots showed that bacterial communities grouped according to habitat type (Fig. 3; ANOSIM, Global $R = 0.38$, $P < 0.05$). Furthermore, we found significant pairwise differences between soils and endolithic communities ($R = 0.42$, $P < 0.01$) and between soils and hypolithons ($R = 0.492$, $P < 0.02$). Lithic communities did not differ significantly from one another ($P > 0.05$). These results support previous Dry Valley surveys showing significant differences between soil and hypolithic bacterial community structures (Pointing et al., 2009, Khan et al., 2011, Makhalanyane et al., 2013a). Habitat type also plays an important role in driving maritime Antarctic microbial community structure (Yergeau et al., 2007), overall assembly in hyperarid deserts (Makhalanyane et al., 2013b) as well as functional potential (Chan et al., 2013). To further explore community diversity patterns, we tested the homogeneity of group dispersions (Anderson et al., 2006). The lithobiontic communities showed higher beta diversity trends, although these were not significantly different when compared to soil.

While some (14%) of the general bacterial community variation could be explained by habitat type alone, our analysis suggest that this is not the case for cyanobacterial populations. T-RFLP analysis showed 27 cyanobacterial OTUs across all samples, which ranged from 3 to 16 OTUs per individual sample [8.2 (mean) ± 3.9 (SD)], and represented approximately 41% of the total bacterial diversity. Ten OTUs (37% of the cyanobacterial OTUs) were found in all samples, while 11 OTUs (41%) were habitat-specific. In contrast to the general bacterial diversity, soils supported more diverse populations of *Cyanobacteria* than lithobionts (data not shown). The lack of distinction between cyanobacterial populations from distinct habitat types is proposed to be the result of introduced aquatic *Cyanobacteria* derived from proximal lacustrine and benthic communities. The mechanism of aeolian redistribution is thought to play a significant role in shaping

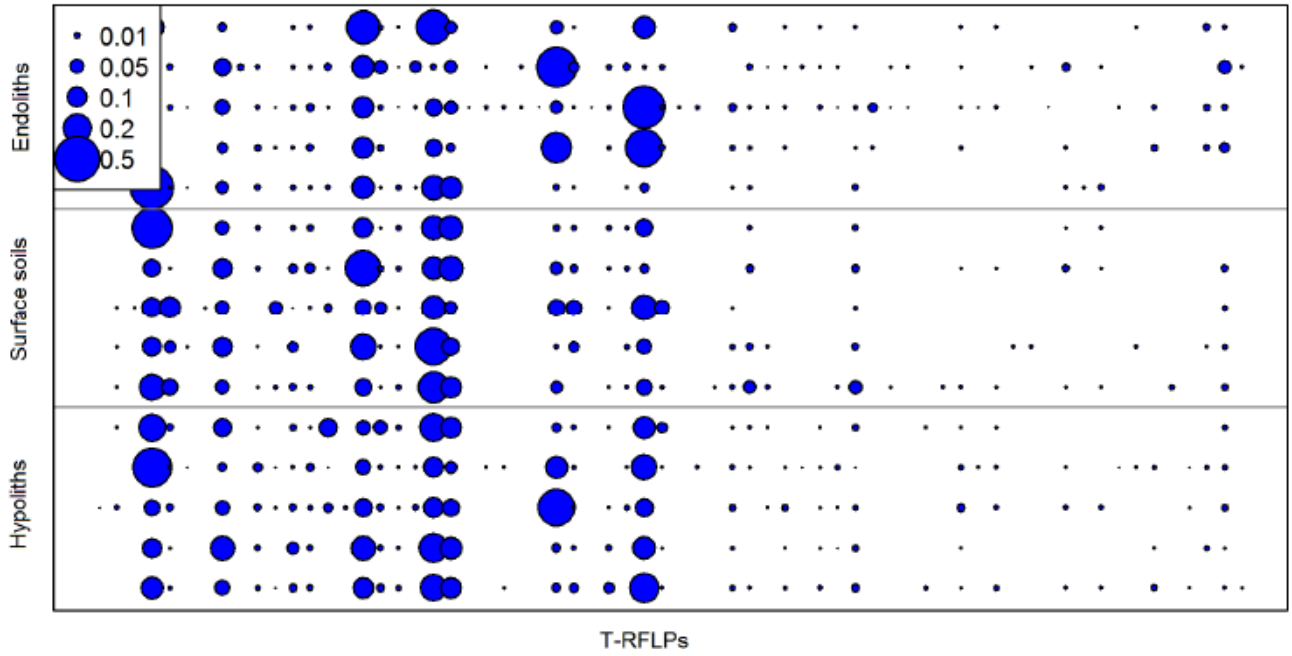

Figure 2. Bubble plot representing individual T-RFs (columns) per sample (rows). Bubbles are sized by the relative abundance at which the T-RF was observed.

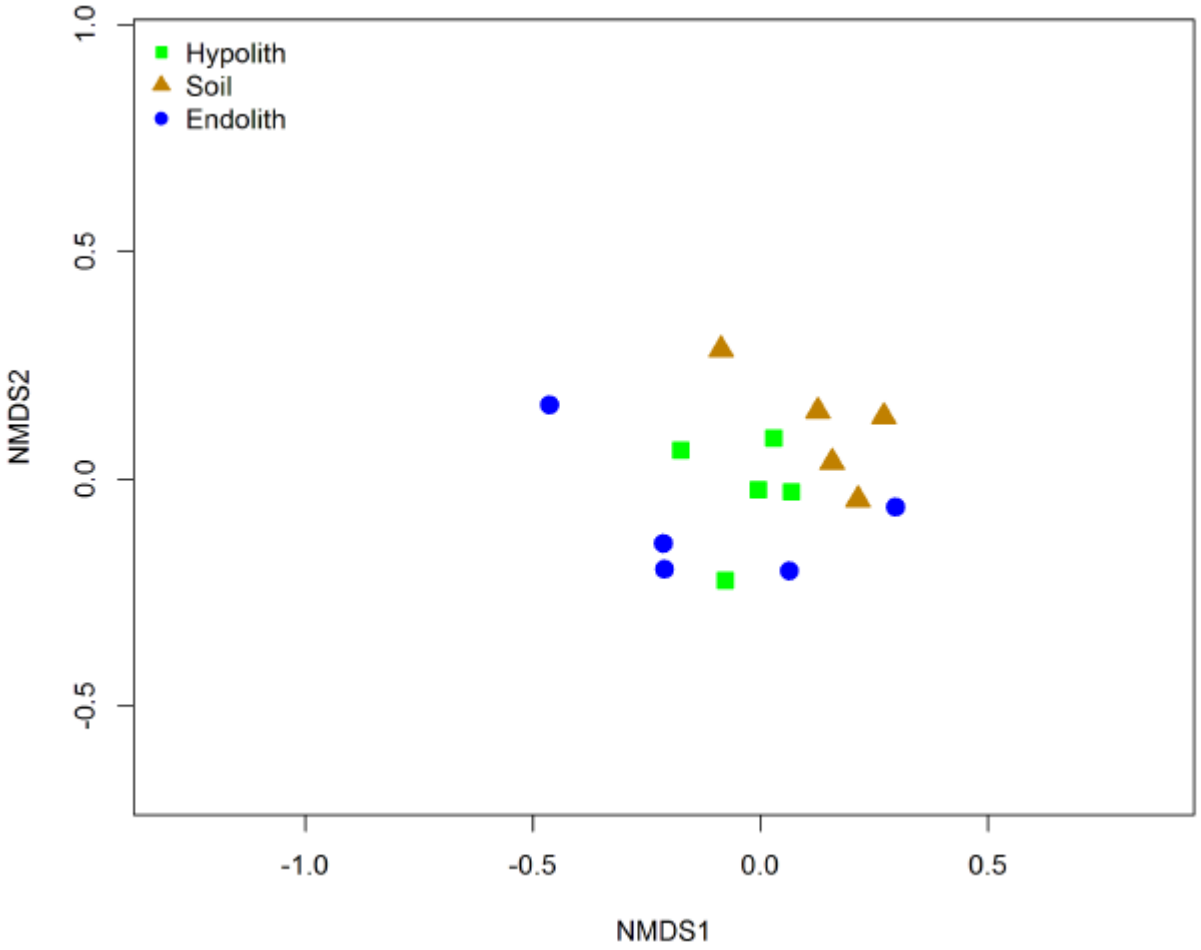

Figure 3. The bacterial community structures of the unique edaphic habitats (hypolith, endolith and soil) are visualised in an nMDS ordination plot. Single points represent entire community fingerprints on the basis of Bray-Curtis dissimilarities of 16S rRNA variation. The distances between samples reflect dissimilarity thus proximally clustered samples are less dissimilar in community composition.

Table 1. Univariate models of phylotype richness and diversity metrics on the basis of T-RFLP analysis.

	Hypolith	**Endolith**	**Soil**
$\bar{\alpha}$	31.6±5.9	31.2±11.4	24.6±4.7
γ	48	41	56
β ($\gamma/\bar{\alpha}$)	1.56	1.98	1.71
Shannon (H')	3.77±0.6	3.50±1.0	3.42±0.4
Gini-Simpson (1-λ')	1.048±0.02	1.063±0.01	1.064±0.03
betadisper	0.1815	0.2505	0.2024

terrestrial communities in Miers Valley, Antarctica (Wood et al., 2008). We used pyrosequencing to identify the dominant bacterial phyla from each habitat and to explore whether there is evidence of cyanobacterial phylotypes which may have originated from aquatic sources.

Pyrosequencing analysis of lithonbionts and soils

After read curation and chimera removal, a total of 724 bacterial OTUs (97% identity) were obtained from 10,665 sequences, the majority of which belonged to *Actinobacteria* (41% of total OTUs). *Cyanobacteria* (18%) represented the primary photoautotrophs in each community, while ubiquitous heterotrophic members included *Gemmatimonadetes* (15%), *Verrucomicrobia* (13%) and *Acidobacteria* (4%) (Table 2). These bacterial groups are common colonists of terrestrial Dry Valley habitats (Lee et al., 2012, Bottos et al., 2014, Yung et al., 2014) and are prevalent in other desert soils (Makhalanyane et al., 2013b, Robinson et al., 2013), albeit at different relative abundances. Overall, bacterial diversity spanned 11 phyla (Fig. 4), all of which, barring *Chlorobi*, were present in the soil community. The lithobionts harboured fewer bacterial phyla than the soil community, and were colonised by between six to eight phyla. Surprisingly, heterotrophic members, rather than *Cyanobacteria*, comprised the majority of recoverable phylotypes in lithobionts. Rarefaction curves and Chao1 estimates of richness suggest that sequencing had not reached saturation, which may have led to underestimates of diversity. Univariate tests were used to estimate sample richness and showed that soils were the most diverse communities at the phylum level (Table 3).

Approximately 12% of the classified sequences were present fewer than 5 times in the global dataset, underlining the importance of rare members to community structure. Rare groups included the candidate phyla WS3 and TM7 which were not present in all samples. We could not detect archaeal signatures in any samples, which may be the result of extreme xeric stress in Victoria Valley. Critically low water bioavailability has been suggested to prevent Archaea from persisting in hyperarid deserts (de la Torre et al., 2003, Pointing et al., 2009). Consistent with expectations, we found copiotrophic taxa (including members of the *Bacteroidetes* and *Proteobacteria* phyla) to be under-represented in these highly oligotrophic habitats (Fierer et al., 2012a). Members of these phyla are reliant on high organic soil carbon content, while the majority of Antarctic habitats, particularly in the Dry Valleys, are extremely nutrient poor (Cowan et al., 2014). We observed the opposite distribution for oligotrophic *Acidobacteria* which were found in all communities.

The phylum-assigned sequences indicated that there were diverse microbial taxa in all habitat types, with both similarities and significant differences between communities. In all three communities, the most dominant sequences were *Actinobacteria*, although we found significant over-representations of the phylum in sample E5.1 (endolith) in comparison to the other samples (Fisher's exact test; $P < 0.0001$). Furthermore, the proportions of *Cyanobacteria* ($P < 0.05$) and *Verrucomicrobia* ($P < 0.05$) were significantly under-represented in E5.1 relative to all other communities. We also found unclassified bacteria to be significantly more abundant in lithic-associated consortia relative to the soil community (S1) ($P < 0.05$), while the

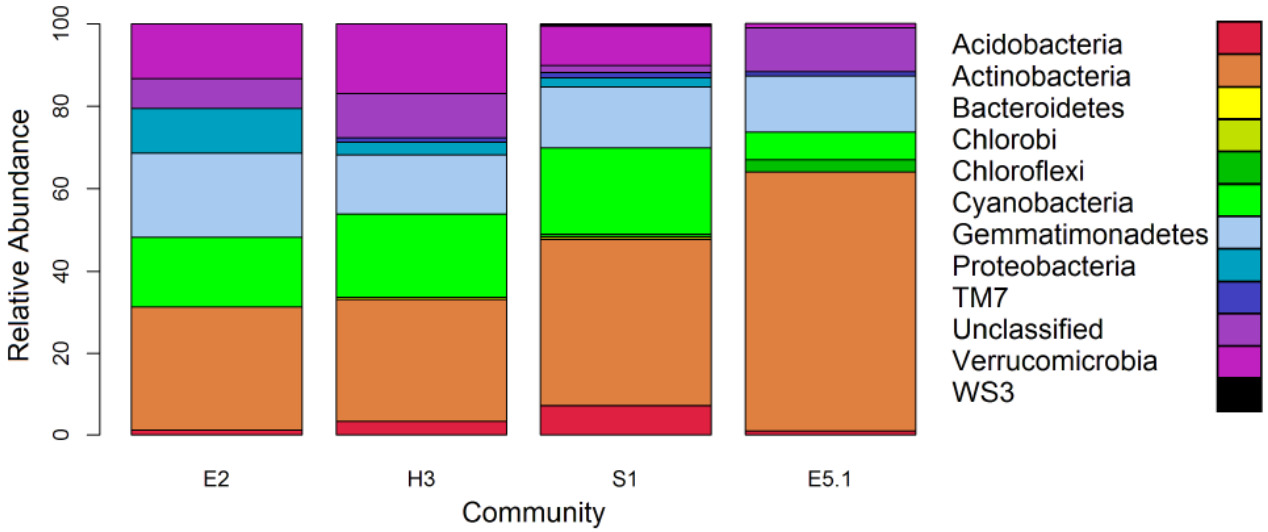

Figure 4. The taxonomic distribution of bacterial OTUs on the basis of 16S rRNA gene assignments obtained from single samples selected from each habitat (hypolith, soil, fungal-dominated endolith and *Cyanobacteria*-dominated endolith). Bars show the relative abundances of bacteria assigned at the phylum level. Taxonomies were provided after comparisons to the latest Ribosomal Database Project (RDP) Release at a confidence of 80%.

Table 2. The most abundant OTU taxa are presented, sequences were detected at least 5 times in the dataset. Numbers in parenthesis indicate the relative abundance of each genus in the sample. No detection is marked with a 0.

	H3 (%)	S1 (%)	E2 (%)	E5.1 (%)	Overall (%)
Actinobacteria					
Actinobacteria					
Acidimicrobiales					
Acidimicrobineae					
Aciditerrimonas	17 (4.68)	5 (2.86)	2 (2.33)	9 (9.00)	33 (4.56)
Iamiaceae					
Iamia	40 (11.02)	12 (6.86)	3 (3.49)	10 (10.00)	65 (8.98)
Acidomicrobiaceae					
Ilumatobacter	15 (4.13)	12 (6.86)	5 (5.81)	3 (3.00)	35 (4.83)
Actinomycetales					
Nocardioidaceae					
Aeromicrobium	1 (0.28)	6 (3.43)	0	0	7 (0.97)
Marmoricola	3 (0.83)	7 (4.00)	0	6 (6.00)	16 (2.21)
Nocardioides	9 (2.48)	16 (9.14)	0	3 (3.00)	28 (3.87)
Kineosporiaceae					
Angustibacter	2 (0.55)	0	1 (1.16)	2 (2.00)	5 (0.69)
Unclassified Kineosporiaceae	3 (0.83)	0	2 (2.33)	0	5 (0.69)
Micrococcaceae					
Arthrobacter	1 (0.28)	1 (0.57)	2 (2.33)	1 (1.00)	5 (0.69)
Sporichthyaceae					
Sporichthya	2 (0.55)	1 (0.57)	1 (1.16)	1 (1.00)	5 (0.69)
Mycobacteriaceae					
Unclassified Mycobacteriaceae	1 (0.28)	2 (1.14)	0	2 (2.00)	5 (0.69)
Euzebyales					
Euzebyaceae					
Euzebya	7 (1.93)	0	3 (3.49)	18 (18.00)	28 (3.87)
Gemmatimonadetes					
Gemmatimonadetes					
Gemmatimonadales					
Gemmatimonadaceae					
Gemmatimonas	52 (14.33)	25 (14.29)	18 (20.93)	14 (14.00)	109 (15.06)

Table 3. Estimates of community richness including the total number of sequences obtained and OTU distributions on the basis of pyrotag data.

Sample	Habitat	Reads	OTUs	% of Unique OTUs	Chao1	1-λ'	H'
H3	Hypolith	5089	362	18	305.5 [±94.6 (SD)]	0.28	2.0
E5.1	Endolith	3273	103	21	107.1 [±43.0 (SD)]	0.20	2.3
E2	Endolith	1340	83	22	67.1 [±20.2 (SD)]	0.06	3.8
S1	Soil	963	176	14	165.1 [±53.3 (SD)]	0.02	4.3

abundance of members belonging to *Proteobacteria* differed significantly between the two endolithic communities ($P < 0.001$).

Gemmatimonas phylotypes (phylum *Gemmatimonadetes*) were highly represented in all samples and contributed 109 OTUs across the four samples (15% of the total assigned sequences). Previous surveys have found this genus to be abundant and widespread in Antarctic mineral soils, including in the McMurdo Dry Valleys (Babalola et al., 2009), in Northern Victoria Land (Niederberger et al., 2008) and Schirmacher Oasis (Shivaji et al., 2004). Members of the *Gemmatimonas* may contribute to local biogeochemical cycling through phosphate metabolism (Zhang et al., 2003), while predicted cold tolerance proteins have been linked with the group (Grzymski et al., 2006).

The order Acidimicrobiales (phylum *Actinobacteria*) was also abundant in all samples (133 OTUs). Three genera within the Acidimicrobiales, *Iamia*, *Aciditerrimonas* and *Ilumatobacter*, were abundant in all samples, suggesting that these taxa may have important functional roles. Members of the *Aciditerrimonas* genus, such as *A. ferrireducens*, are thought to be capable of reducing iron in environmental soils (Itoh et al., 2011). *Actinobacteria* are major colonists of inland Dry Valley soils (Cary et al., 2010) and are minor components of high-altitude hypolith communities (Wong et al., 2010). Largely consistent with other studies (Smith et al., 2006, Niederberger et al., 2008) we found *Proteobacteria* to be poorly represented (4% of total assigned sequences) in all communities.

Cyanobacteria are the dominant bacterial members of lithic and microbial mat communities in hyperarid deserts (Warren-Rhodes et al., 2006, Cary et al., 2010, Cowan et al., 2014). Studies have shown that Cyanobacteria are important colonisers of soil communities in high-altitude Dry Valleys (Smith et al., 2006) and in hot hyperarid soils of the Namib desert (Makhalanyane et al., 2015a). *Cyanobacteria* were abundant in all samples analysed here, comprising 7 – 21% of the sequences. For example, the genus *Leptolyngbya* accounted for 14 – 54% of cyanobacterial populations (Fig. 5). *Leptolyngbya* are frequently identified in lake communities (Taton et al., 2006, Biondi et al., 2008) and in maritime Antarctic assemblages (Komárek et al., 2008). Another oscillatorian genus, *Phormidium*, was abundant (27 – 38%) in most communities, but was completely absent from one endolithic consortium. Members of *Phormidium* are some of the most common colonists of water-saturated soils in low elevation valleys, similar to mat communities in ponds (Broady, 2015). Altogether, these results appear to indicate that the diversity of terrestrial communities in Victoria Valley may be influenced by proximal aquatic systems, as has been suggested in Miers Valley (Wood et al., 2008).

While Oscillatoriales phylotypes dominated all samples, *Chroococcidiopsis* were found exclusively in soils and hypoliths. This is in contrast to a survey showing *Chroococcidiopsis* phylotypes to dominate endolithic consortia (Pointing et al., 2009). The process of primary production in both hot and cold deserts is thought to be strongly mediated by members of *Chroococcidiopsis* (Tracy et al., 2010, Bahl et al., 2011). Although members of the phyla *Chlorobi* (found in hypoliths) and *Chloroflexi* (found in soils and endoliths) may also mediate anoxygenic photosynthesis in those communities (Bryant et al., 2012).

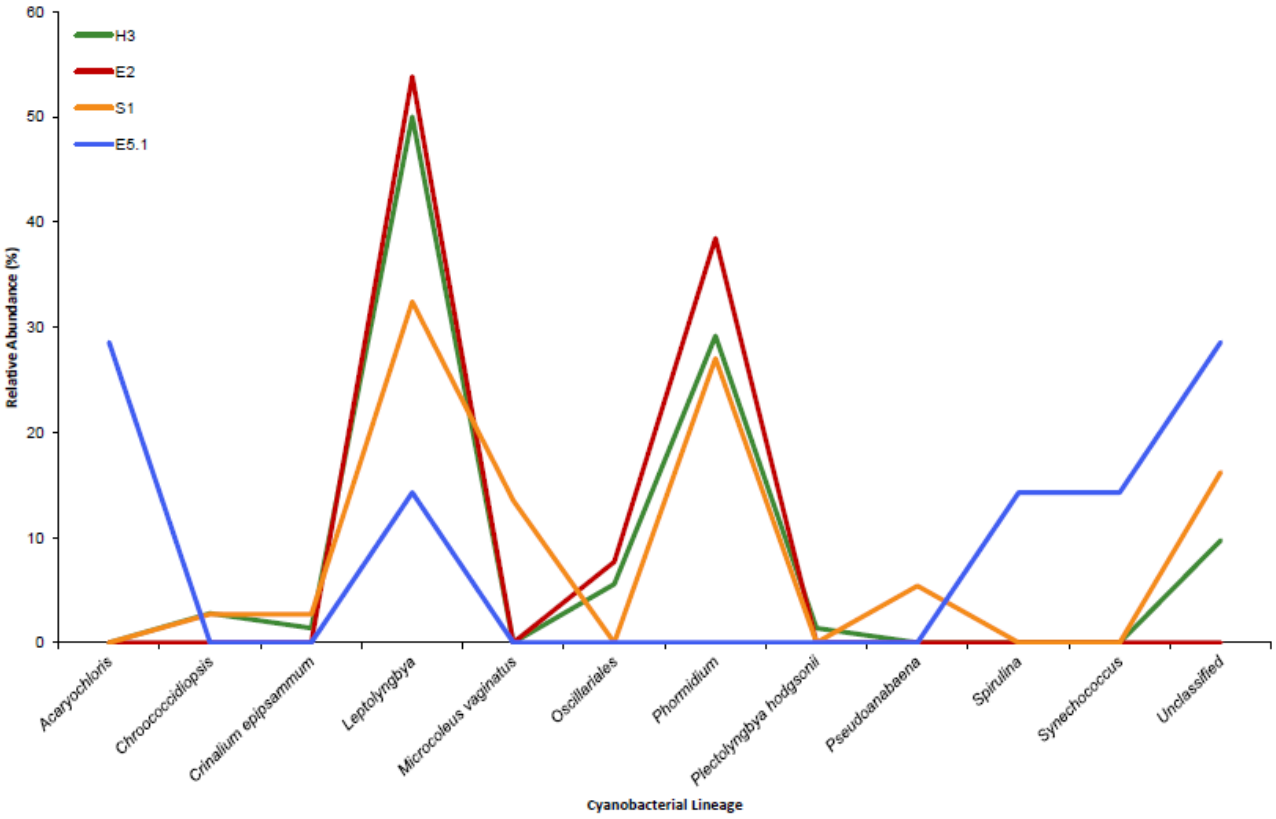

Figure 5. Line graph of the cyanobacterial lineages present in the four samples (97% cut-off). Taxonomic affiliations were provided by the Ribosome Database Project Classifier (RDP) at 80% confidence.

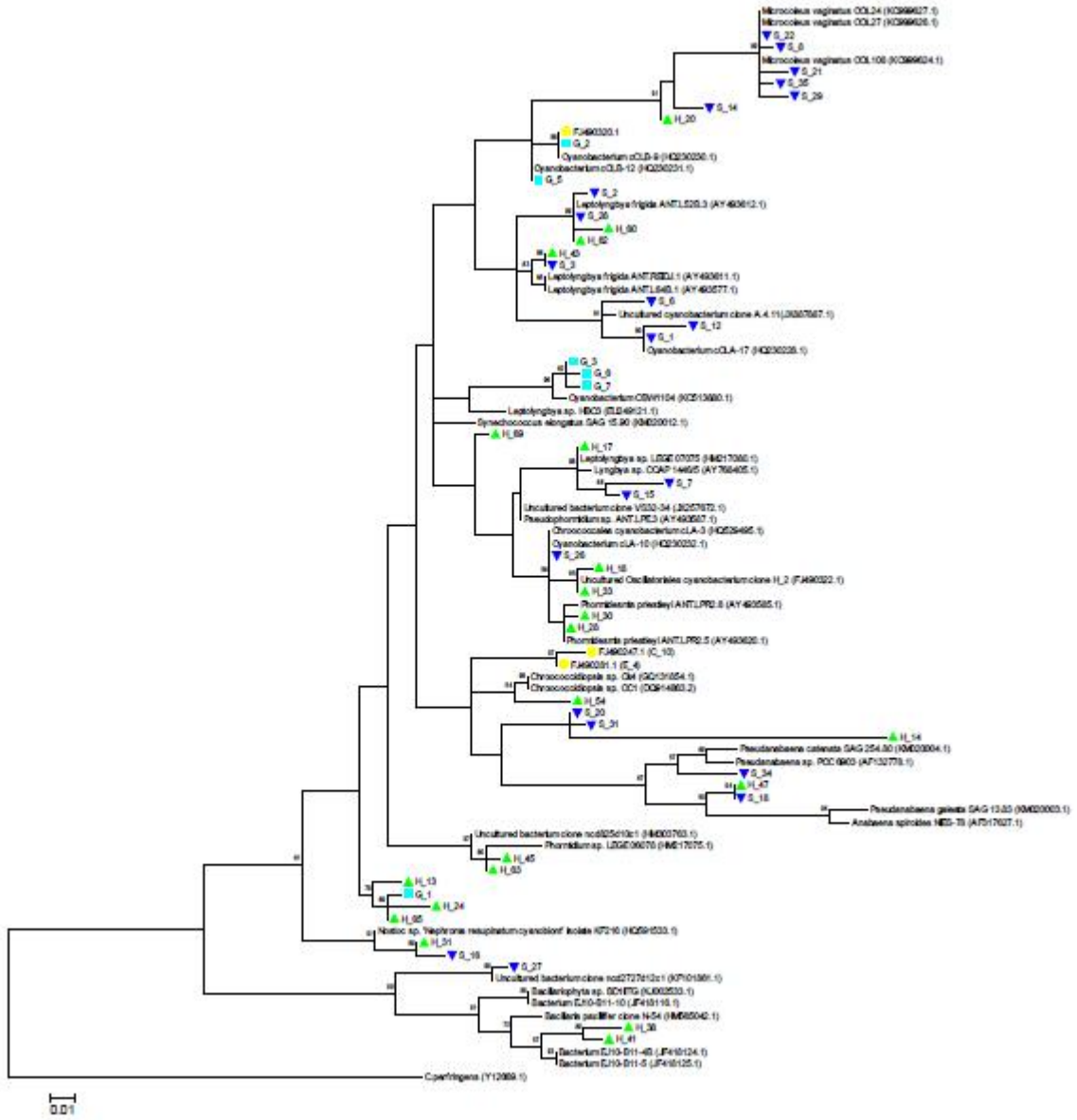

Figure 6. Maximum likelihood tree showing the phylogenetic relationships between cyanobacterial phylotypes recovered from Victoria Valley samples.

Interestingly, soils shared 88% and 85% of the total bacterial OTUs with hypolithic and endolithic communities, respectively. The high number of shared OTUs between soil and lithic communities may be due to a number of possible reasons, including positive synergistic ecological interactions between species or due to proposed mechanisms in which lithobionts serve as reservoirs of terrestrial biota (Pointing et al., 2009). An alternative explanation may be the aeolian transport of viable cells in the Dry Valley aerosphere. Many of the lineages found here, such as Oscillatoriales and Chroococcales, are common colonists of aquatic habitats in the Dry Valleys (Wood et al., 2008, Kleinteich et al., 2014), but are typically absent from hyperarid soils (Pointing et al., 2009, Makhalanyane et al., 2013a). This concept is further supported by the detection of *Microcoleus vaginatus* in the soil community. *M. vaginatus* phylotypes have been found in maritime Antarctic locations such as Schirmacher Oasis and Windmill Hills (Pankow et al., 1991, Ling and Seppelt, 1998), and are important components of Wright Valley ponds and streams (Novis et al., 2015). The *M. vaginatus* phylotypes from this study clustered strongly with Mojave and Chihuahuan desert sequences (Fig. 6). We propose that aeolian transport may reduce the strong deterministic effects of soil physicochemistry and habitat type in structuring the edaphic microbial communities of Victoria Valley. By implication, random introduction and colonisation events are likely to disproportionately affect the food web structure in this environment (Bottos et al., 2014, Pointing and Belnap, 2014).

Hypolithic and endolithic communities were shown to cluster separately from soils despite sharing a majority of the bacterial OTUs found here. Even though generalist lineages colonised all of the edaphic habitats analysed in this desert, we observed higher variation in lithic community composition compared to exposed soils which are likely driven primarily by stringent abiotic factors such as very low soil pH (Fierer et al., 2012b, Van Horn et al., 2013). Many phylotypes were unclassified (~8%) although this is not uncommon for desert microbial communities which are hindered by rRNA gene variability (Niederberger et al., 2008, Lee et al., 2012, Makhalanyane et al., 2013a). We suggest that lakes and ponds in the Dry Valleys may serve as sources of cyanobacterial diversity for terrestrial habitats which is largely consistent with the concept proposed by Wood et al. (2008). This work provides insights into the bacterial community structures of Victoria Valley habitats for the first time. Future studies are required to expand the understanding of these systems by assessing the biological functions of these important consortia.

Conflict of Interest

The authors declare no conflict of interest.

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