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ORIGINAL PAPER

Endolithic microbial diversity in sandstone and granite from the McMurdo Dry Valleys, Antarctica

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Abstract Cryptic microbial communities develop within rocky substrates in Antarctica's McMurdo Dry Valleys as a stress avoidance strategy. They may be cryptoendolithic within pore spaces of weathered rocks, or develop in cracks and fissures as chasmoendolithic communities and are characterised by coloured bands of colonisation. Here we used a precision drill to recover fractions from black, white, green and red layers within colonised granite and sandstone. We combined backscattered scanning electron microscopy and high-throughput sequencing to identify major taxa in each band. We confirmed the presence of algal and fungal lichen symbionts, cyanobacteria and free-living algae, plus a diverse heterotrophic bacterial and archaeal component. A clear delineation at the community level was observed. The relatively biodiverse and heterogenous lichen communities occurred in weathered sandstone cliffs, whilst in granite and sandstone boulders, cyanobacterial communities were dominant. Differences between coloured bands of colonisation within each community were less clear. The study demonstrates that endolithic microbial communities can be recovered using a drill technology similar to that planned for the search for endolithic biosignatures on Mars.

Keywords Antarctica · Astrobiology · Chasmoendolith · Cryptoendolith · Cyanobacteria · Lichen

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Introduction

The McMurdo Dry Valleys are the largest ice-free terrestrial environment in Antarctica and comprise the coldest, driest and windiest desert on Earth (Wynn-Williams 1990; Moorhead et al. 1999; Pointing et al. 2015). Life in these valleys is dominated by microbial communities that colonise oligotrophic mineral soils (Cary et al. 2010) and exposed rocks (de los Ríos et al. 2014a). The harsh moisture, thermal and UV stress limit colonisation of exposed surfaces and instead communities develop in cryptic habitats within soil and rocks as a stress avoidance strategy (Pointing and Belnap 2012; Pointing et al. 2014). These communities have emerged as highly distinct from each other depending upon whether they occur in exposed soil, beneath translucent rocks in soil contact as hypoliths, in cracks and fissures within rocks as chasmoendoliths, or in pore spaces of weathered rocks as cryptoendoliths (Pointing et al. 2009). Soil communities are dominated by actinobacteria and other cosmopolitan soil bacteria (Aislabie et al. 2006; Smith et al. 2006; Niederberger et al. 2008; Lee

et al. 2012; Niederberger et al. 2012; Stomeo et al. 2012), whilst soil-associated hypolithic communities (Chan et al. 2012; Pointing 2016) are dominated by relatively unstructured cyanobacteria-dominated biofilms (de los Ríos et al. 2014b). In contrast, the chasmoendolithic and cryptoendolithic colonisation of rock involves development of more complex biofilms structured by the porosity of the rock, and here, communities are dominated by cyanobacteria or by chlorophyte algae and ascomycete fungi in putative lichen symbioses (Friedmann 1982; de la Torre et al. 2003; de los Ríos et al. 2014a; Yung et al. 2014).

Since the earliest reports of endolithic colonisation (Friedmann and Ocampo 1976; Friedmann 1982), it has been recognised that a clearly visible layered community develops with different coloured bands of colonisation occurring at different depths within the rock (Wierzchos et al. 2012). In cyanobacteria-dominated chamsoendoliths and cryptoendoliths, this is usually a simple transition from a pink/red layer near the surface, assumed to be due to production of UV-protective carotenoid pigments, to a green layer of unprotected biomass deeper within the fissure (Friedmann 1982; Yung et al. 2014). Chasmoendolithic lichens are visualised as a green layer near the surface, but the white mycobiont colonisation extends deeper in the fissure (de los Ríos et al. 2005). In cryptoendolithic lichen-dominated communities, the near-surface layer appears black due to the presence of melanised fungi, a deeper white layer is attributed to lichen growth, and the deepest green layer has been identified as supporting free-living chlorophyte algae and cyanobacteria (Friedmann 1982; de los Ríos et al. 2014). This light-driven community assembly has been termed the 'microbial cabana' strategy (Pointing and Belnap 2012).

Major advances in understanding the spatial organisation of cryptoendolithic communities have been made using electron and confocal microscopy (Friedmann 1982; de los Ríos et al. 2004; Wierzchos et al. 2005), including insight into the physiological state and viability of cells (de los Ríos et al. 2004; Wierzchos et al. 2004). Communities were differentiated into black-white-green lichen dominated and pink-green cyanobacteria dominated based upon microscopic observation (Friedmann et al. 1988). Subsequent biodiversity assessment via sequencing of rRNA genes from environmental DNA samples confirmed the dominance of chlorophyte algae and cyanobacteria in lichen and cyanobacterial cryptoendoliths, respectively (de la Torre et al. 2003). Furthermore, microscopy as well as rRNA gene-based studies have also confirmed the dominance of cyanobacteria in pink-green cryptoendoliths (de los Ríos et al. 2004; Pointing et al. 2009) and chasmoendoliths (de los Ríos et al. 2007; Pointing et al. 2009; Yung et al. 2014). These studies did not attempt to discriminate which taxa occupy the different coloured bands within these communities, and so confirmation of the dominant taxa responsible for bands of colonisation remains unconfirmed.

Here we report a study to identify the taxonomic diversity within coloured bands of cryptoendolithic and chasmoendolithic communities (collectively referred to herein as endoliths) for the McMurdo Dry Valleys, Antarctica. The use of high-throughput sequencing for bacterial, archaeal and eukaryal rRNA genes allowed greater resolution than previous studies that have used chromatographic or clone library approaches to identify taxa.

Materials and methods

Sample retrieval

Colonised rock samples were collected from rock substrates at three locations in the McMurdo Dry Valleys Antarctic Special Managed Area (SCAR 2004). These comprised a cryptoendolithic community inhabiting a heavily weathered sandstone cliff in University Valley (December 2009) and chasmoendolithic communities occurring in weathered sandstone boulders on the valley floor of McKelvey Valley (December 2008) and weathered granite boulders on the valley floor of Miers Valley (January 2010). Rock samples were excised using a geological hammer and rock chisel that had been surface-sterilised using 70 % ethanol. Samples were immediately placed in sterile whirl–pak (Nasco) bags with heat-sterilised silica bead desiccant in the field and then stored frozen (−80 °C) and in darkness until processed. Each colonised rock sample was divided into two in the laboratory using sterile tools in a sterile laminar flow cabinet, with one half used for microscopy and the other half for molecular analysis.

Scanning electron microscopy with backscattered electron imaging (SEM-BSE)

Colonised rock samples were processed following (Wierzchos and Ascaso 1994).

Briefly, rock-colonised fragments were fixed in glutaraldehyde (3 % v/v) and osmiumtetroxide solutions (1 %w/v), dehydrated in a graded ethanol series (from 30 to 100 %v/v) and embedded in LR White resin. Blocks of resin-embedded rock-colonised samples were finely polished, carbon-coated and observed using a ZeissDSM-960 microscope.

Environmental rRNA gene-defined diversity assessment

Samples from distinct coloured bands of microbial colonisation from each rock were obtained using a

Dremel® 4000 drill with a Dremel Diamond point drill bit (3/32 in.; Part#: 7134) under aseptic conditions. Drilling was undertaken in a laminar flow cabinet with a 70 % ethanol-sterilised drill bit. The rock was held within a sterile petri dish (Fisher Scientific) and fine particles and debris collected within the petri dish. The cover of the petri dish was held over the top of the rock sample to avert airflow and the deflection of rock debris. The collected debris within the petri dish was assimilated with diethyl dicarbonate (DEPC) inactitreated sterile water (MOBIO) and DNA extracted from the slurry using the Powersoil® DNA Isolation Kit as per manufacturer's instructions (MOBIO).

All three domains (i.e. archaea, bacteria and eukaryota) were detected in validation amplifications for all extracted DNA's using commonly utilised polymerase chain reaction (PCR) methods. All PCR reagents were purchased from Sigma-Aldrich (USA). Bacterial amplifications consisted of: 3 µl of 25 mM MgCl2; 2.5 µl of 10× buffer; 2.5 µl of 2 mM dNTPs; 0.75 µl of 10 mM primers (27F: AGA GTT TGA TCC TGG CTC AG and 1492R: GGT TAC CTT GTT ACG ACT T); 2.5 µl of 10 mg/ml BSA; 0.5 µl of 2.5 U/µl JumpStartTM Taq DNA polymerase; 5 µl of template DNA; and the volume adjusted to 25 µl with DEPC-treated water (MOBIO). Eukaryotic amplifications consisted of: 1.5 µl of 25 mM MgCl2; 2.5 µl of 10× buffer; 2.5 µl of 2 mM dNTPs; 0.75 µl of 10 mM primers (Euk1A: CTG GTT GAT CCT GCC AG and Euk516r: ACC AGA CTT GCC CTC C); 2.5 µl of 10 mg/ml BSA; 0.5 µl of 2.5 U/µl JumpStartTM Taq DNA polymerase; 5 µl of template DNA; and the volume adjusted to 25 µl with DEPC-treated water (MOBIO). Archaeal amplifications consisted of: 1.5 µl of 25 mM MgCl2; 2.5 µl of 10X buffer; 2.5 µl of 2 mM dNTPs; 0.5 µl of 10 mM primers (A751F: CCG ACG GTG AGR GRY GAA and UA1406R: ACG GGC GGT GWG TRC AA); 2.5 µl of 10 mg/ml BSA; 0.5 µl of 2.5 U/µl JumpStartTM Taq DNA polymerase; 5 µl of template DNA; and the volume adjusted to 25 µl with DEPC-treated water (MOBIO). Templates for positive and negative PCR control amplifications consisted of 5 µl of DNA from Antarctic Dry Valley hyporheic soil microbial mat and DEPC-treated water, respectively. PCR were visualised via electrophoresis (240 V for 7 min) in 1× SB agarose gel containing ethidium bromide. Partial lengths 16S and 18S rRNA genes amplicons (Table 1) from the bacteria/archaea and eukarya were sent to the Research and Testing Laboratory in Lubbock, TX, USA, for barcode-tagged amplicon pyrosequencing. PCR primer pairs used for these amplifications were: 28F/519R (bacteria), 517F/909R (archaea) and euk7F/euk570R (eukarya).

Bioinformatic and statistical analysis

Tags and primers were removed from the pyrosequencing data, and any untargeted sequences were removed. Bacterial, archaeal, and fungal amplicons were processed separately. Remaining sequences were processed using AmpliconNoise version 1.0 for quality filtering, denoising and chimera removal (Quince et al. 2009). Briefly, raw flowgrams (sff files) with perfectly matching primer and barcode sequences were filtered for a minimum flowgram length of 360 cycles (including primer and barcode sequences) before the first noisy signal (i.e. 0.5–0.7 or no signal in all four nucleotides). All flowgrams were then truncated at 360 bases and clustered to remove sequencing noise using PyroNoise (Quince et al. 2009; Quince et al. 2011). Noise introduced by PCR was removed using SeqNoise (Quince et al. 2009), and PCR chimeras were removed using Perseus (Quince et al. 2009). Resulting dereplicated sequences from Perseus were processed using Mothur 1.17.0 (Schloss et al. 2009) to create a unique sequence and names file. Pairwise alignments and distance

Table 1 Diversity metrics for bacterial 16S rRNA gene pyrosequencing

Samples	Reads	Number of OTUs	Estimated richness[a]		Diversity indices[a]		Coverage[a]
			Chao1	ACE	Shannon	Simpson	Good
A.1	4963	151	133	164.4746	4.269154	0.887787	0.970229
A.2	6122	80	58.16667	60.12277	3.093815	0.799859	0.989313
B.1	2219	28	22	22.45111	2.780655	0.785687	0.999237
B.2	16,149	23	6	6.6	1.267273	0.529547	0.999237
C.1	3234	126	113.0714	117.709	4.814297	0.934556	0.979389
D.1	7485	107	121.6667	125.6122	1.757146	0.384589	0.974809
E.1	14,042	106	57	56.80225	1.096015	0.237471	0.98855
F.1	3520	122	96.06667	108.4781	2.356483	0.523766	0.977863
F.2	1310	54	100.4286	105.1453	2.609808	0.649519	0.980153
G.1	3805	58	63.14286	76.02285	1.608188	0.568092	0.984733

[a] Samples were rarefied to an even depth of 1310 reads for richness, diversity and coverage estimations

were calculated using Espirit (Sun et al. 2009). Mothur was then used to cluster sequences into operational taxonomic units (OTUs) defined at the average neighbour Jukes–Cantor distance of 0.03 ($OTU_{0.03}$). Rank-abundance data were generated for each sample. The representative bacterial and archaeal OTU sequences were classified, with RDP classifier (Wang et al. 2007) and the taxonomy from the SILVA database (Release 102). For eukaryal sequencing, relatively few OTUs were generated and these sequences were manually classified based on BLASTn search of the NCBI non-redundant database.

The bacterial communities were analysed and visualised with the QIIME pipeline 1.9.1 (Caporaso et al. 2010). To prevent uneven sampling depth causing bias in the assessment of the communities, the samples were evenly rarefied to the depth of 1310 reads before diversity/richness statistics (Table 1) were generated. Binary Sørensen–Dice index was used to assess the difference between the communities. The distances generated were then used for principal coordinate analysis (PCoA) using QIIME. Further analysis and interpretation of diversity data focus mainly on bacterial assemblages in the rock substrates because of the very low richness observed for archaeal and eukaryal assemblages in this study.

Results

A visual assessment of colonised rock was made for weathered sandstone cliffs (University Valley), sandstone boulders (McKelvey Valley) and granite boulders (Miers Valley) (Fig. 1). Microscopic examination using SEM-BSE revealed all coloured bands in sandstone- and granite-supported microbial colonisation, and putative cyanobacterial cells were associated with an extracellular polymeric substance (Fig. 2). For University Valley samples, the different colour bands were dominated by distinct morphotypes, although co-occurrence of these in different coloured bands made differentiation of diversity between bands somewhat diffuse (Fig. 2a). Lichenised chlorophyte algal cells were visualised (white asterisks in Fig. 2b) in the colonisation green-yellow layer. Free-living algal cells resembling *Hemichloris antarctica* were also observed beneath lichen symbionts (black asterisks in Fig. 2b). Coccoid cyanobacteria (black arrow in Fig. 2b) were also detected in green-yellow layer, situated under free-living algae. Lichen symbionts, free-living algae and cyanobacteria occupied the same sandstone pores (Fig. 2a) in close proximity to each other, as well as black fungi (white arrows in Fig. 2c in the green-yellow layer.

Cyanobacteria-dominated the green-yellow bands of chasmoendolithic communities in granite (Figs. 1d, 2c). Different morphotypes of cyanobacteria were detected in the fissures (Fig. 2c, d). Diversity was highly variable at the micro-habitat scale: Some fissures were occupied by single colonies of coccoid cyanobacteria (Fig. 2c), whilst other fissures showed a great diversity of forms including thick filamentous cyanobacteria cells (black arrow in Fig. 2d) and heterotrophic bacteria (Fig. 2d), especially when pink-red layers were present. Free-living algae were visualised in granite fissures corresponding to green-yellow layer without cyanobacteria colonisation.

In the molecular survey, a total of 62,849 bacterial 16S rRNA gene reads (ranging from a minimum of 1310 to a maximum of 16,149 reads) passed filtering and were used for community analysis (Table 1). Overall bacterial DNA sequencing coverage was >97 % in all samples (Table 1). A total of 501 bacterial OTUs resulted from clustering. ACE richness estimates indicated that the total community was represented from all samples except E.1, F.2 and G.1 and that >80 % of the community was represented according to Chao1. Thus, sandstone cryptoendoliths were fundamentally more biodiverse than granite chasmoendoliths.

Samples yielded a broad range of Shannon diversity index figures with no apparent trends between valleys, substrates or band colours (Table 1). A 2D principal coordinate analysis (PCoA) (Fig. 3) based on total pyrosequencing data allowed the visualisation of community variation between samples. The strongest variation occurred between University Valley and the other valleys across axis PC1. The next strongest variance in community structure occurred across axis PC2, separating University Valley endolith samples. Miers and McKelvey valleys were moderately separated from one another across axis PC3.

As expected, those samples with the highest Shannon diversity, comprising green and black bands from sandstone (A.1, A.2 and C.1), displayed the most complex phyla structure (Fig. 4). Among the bacteria, the dominant phylum were the Cyanobacteria. The Scytonemataceae (Nostocales) were the dominant taxa in McKelvey Valley sandstone cryptoendoliths (samples D, E) and Miers Valley granite chasmoendoliths (samples F, G), but absent in the University Valley cryptoendoliths (samples A, B). A different polyphyletic suite of cyanobacteria was dominant in green bands of University Valley endoliths, although they did also occur with low abundance in McKelvey and Miers samples. Cyanobacteria comprised less than 0.01 % of OTUs in the red bands of both sandstone and granite endoliths. These differences in dominant Cyanobacteria mirror the PCoA observations (Fig. 3) where there is greater variance between University Valley and the other two valleys across axis PC1 as well as the similarity of Miers and McKelvey valleys and the difference between University Valley endolith samples A and B on axis 2.

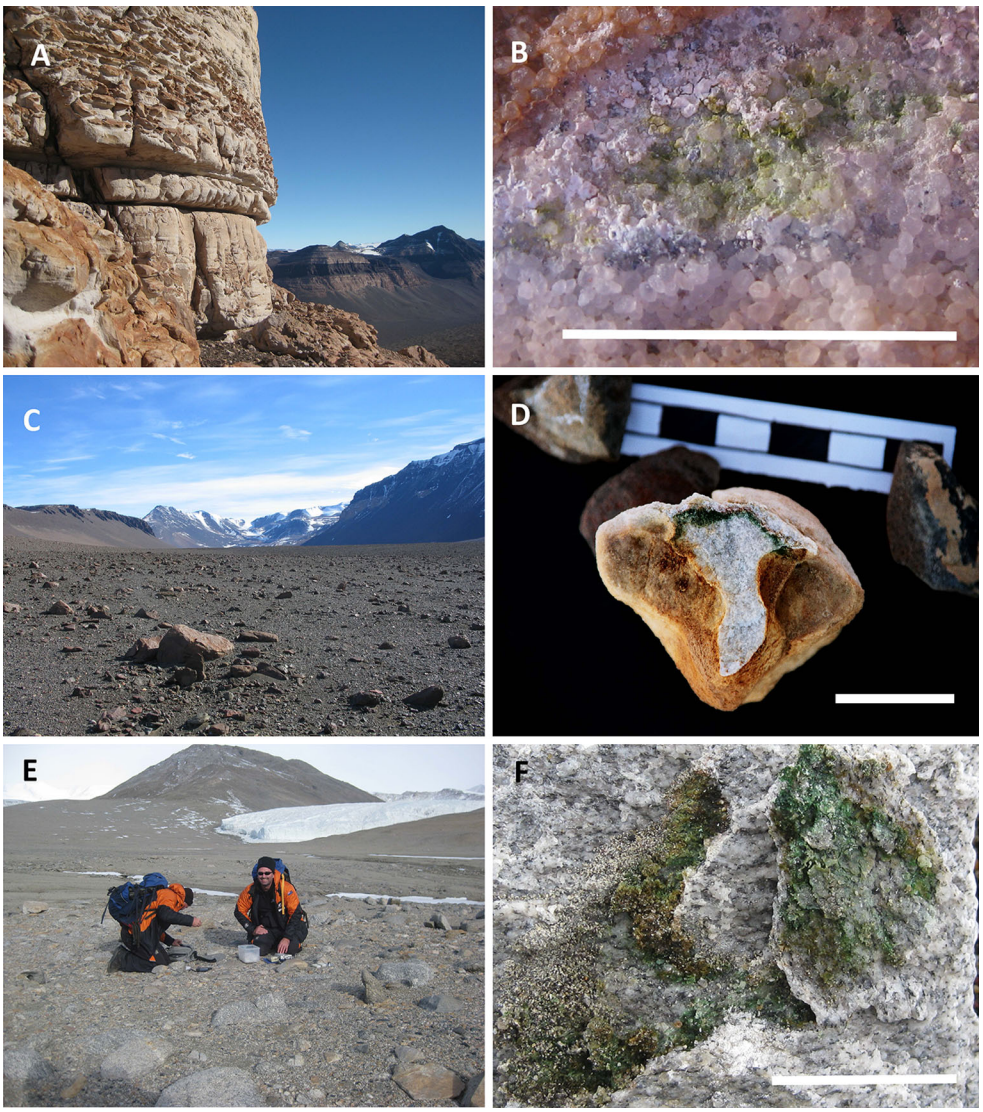

Fig. 1 Substrates for endolithic microbial colonisation in the McMurdo Dry Valleys, Antarctica. **a** Weathered sandstone cliff in University Valley. **b** Colonised sandstone in University Valley. **c** Sandstone boulders on valley floor of McKelvey Valley. **d** Colonised sandstone in McKelvey Valley. **e** Granite boulders on floor of Miers Valley. **f** Colonised granite in Miers Valley. *All scale bars* represent 20 mm

Other cyanobacterial taxa displayed patchy occurrence. For example, one green band from University Valley was dominated by two unidentified cyanobacterial taxa, and these were absent in all other samples, and a *Leptolyngbya*-like taxon was detected only in one sample (sample A) from University Valley (Fig. 4).

A total of nineteen other bacterial phyla were encountered across all samples. The *Actinobacteria*, *Bacteriodetes* and *Chloroflexi* were present in all samples with *Proteobacteria* only absent in one Miers Valley granite green band (sample G). The most abundant heterotrophic bacteria in University Valley samples were alpha proteobacteria, whereas actinobacteria were the most abundant heterotrophs in all samples from McKelvey and Miers valleys. Other notable differences were the relative abundance of photosynthetic chloroflexi in McKelvey and Miers valleys and the abundance of Lentisphaerae in a single University Valley sample. Other phyla were relatively minor components of the community and displayed no clear pattern in their distribution between coloured bands in different substrates.

Eukaryotic signals were detected in all bands (except for A1 which failed to sequence) and all substrates, although with a rather low diversity comprising just 22 OTUs (Online Resource 1). Two OTUs representing 97.8 % of sequences recovered were identified as belonging to the green algae Chlorophyta. The most abundant OTUs, representing 75.2 % of sequences (99 % sequence identity to

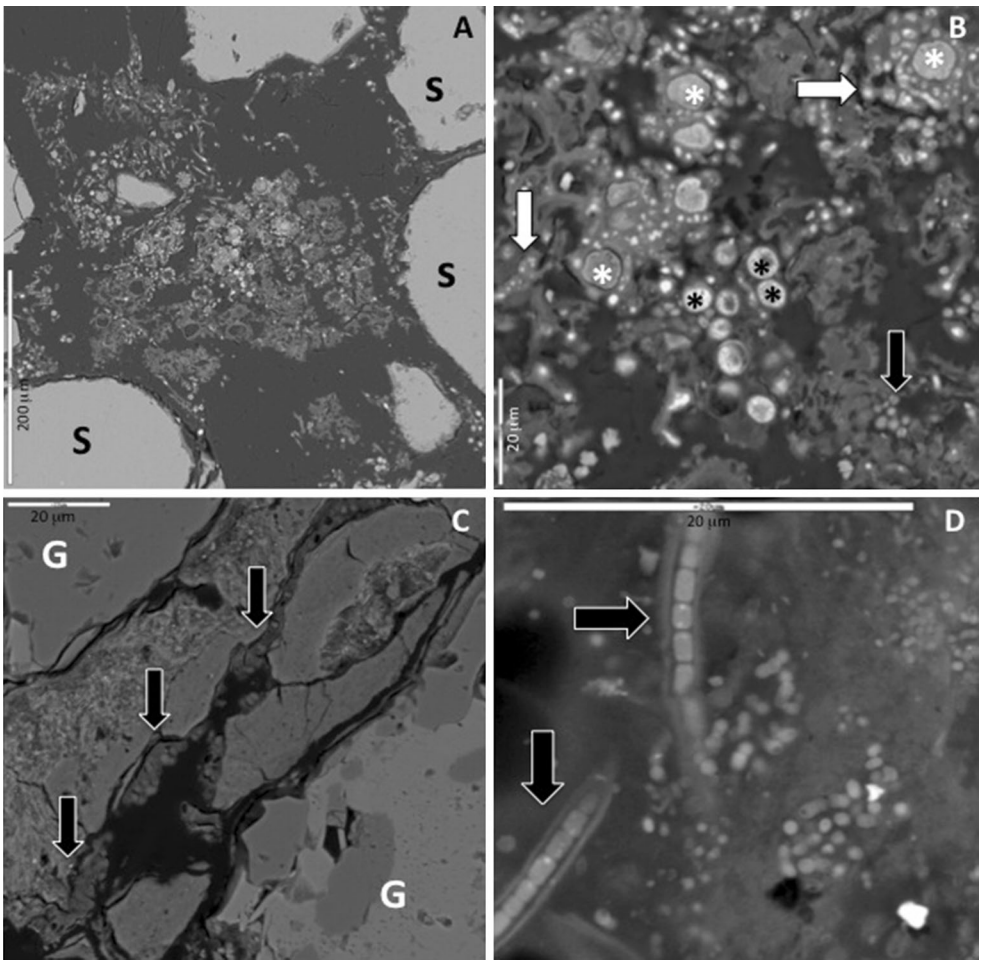

Fig. 2 SEM-BSE images of lithobiontic Antarctic microbial communities. **a** Cryptoendolithic microbial community in between sandstone grains (*S*) from University Valley. **b** Magnified view of the community shown in *A*, composed by lichenised algae (*white asterisks*) and fungi, free-living algae (*black asterisks*), coccoid cyanobacteria (*black arrow*) and darkly pigmented fungi (*white arrows*). **c** Chasmoendolithic microbial community from Miers Valley showing coccoid cyanobacteria cells (*black arrows*) within a granite (*G*) fissure. **d** Magnified view of a cyanobacteria-dominated chasmoendolithic community harbouring a great diversity of morphological cellular types. *Arrows* show thick cyanobacterial filaments. Cyanobacterial cells are surrounded by an extracellular polymeric substance. *Black areas* are regions of shadow due to the three-dimensional nature of this substrate

Diplosphaera sp.), were present in McKelvey and Miers valleys but was absent in University Valley, whilst the most abundant algal taxon in University Valley corresponded to a free-living alga (100 % identity to *Hemichloris antarctica),* which was absent in McKelvey and Miers Valleys. Whilst *Trebouxia*-like algal cells were observed with our microscopy, other algal OTUs could not be resolved confidently to lichen-forming taxa. Fungal taxa were detected in all substrates with low abundance and diversity. Lichen-forming ascomycetes (*Lecidea* genus) were detected only in University Valley, whilst non-lichen-forming ascomycetes were detected in University and McKelvey valleys. Basidiomycete OTUs were detected in University and Miers valleys. Very few archaeal OTUs were confidently identified with 98.1 % of sequences (95 % identity to *Nitrososphaera gargensis*) representing an ammonia-oxidising Thaumarchaeota present in McKelvey and Miers valleys but absent in University Valley (Online Resource 2).

Discussion

Endolithic communities form a significant part of standing biomass in the McMurdo Dry Valleys, since they occupy approximately 4 % of sandstone boulders (Pointing et al. 2009), up to 30 % of granite boulders (Yung et al. 2014), and may occupy 100 % of available substrate at some sandstone cliff locations (Friedmann 1982). We have identified the bacterial, algal, fungal and archaeal taxa that comprise the different coloured bands in multiple substrates, thus providing new insight into endolithic

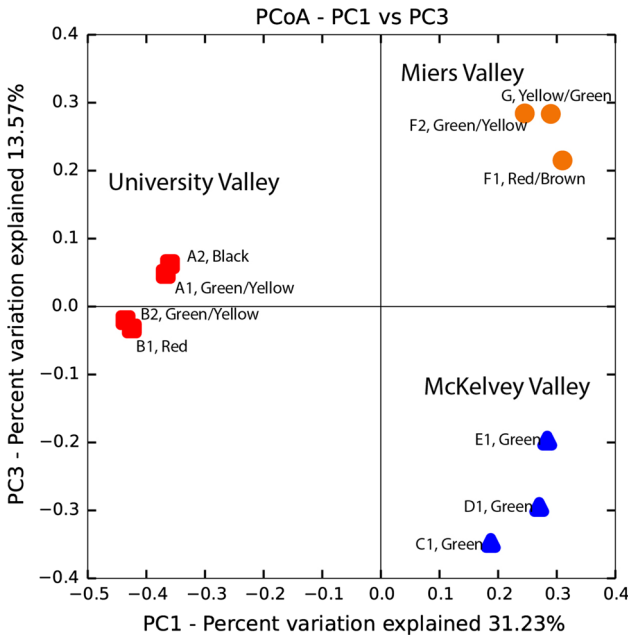

Fig. 3 Dissimilarity between communities as measured by binary Sørensen–Dice index and visualised via principal coordinate analysis (PCoA). The analysis explained 67.75 % of total variance among the communities, and the 2D projection shown here illustrates 54 % of that variation

community assembly in the McMurdo Dry Valleys. The approach used different PCR primers for each domain, and so we were unable to acquire data on the relative abundance between domains; nonetheless, our diversity data provide important evidence that cyanobacteria are likely the major biotic driver of community assembly for endolithic communities. This is supported by two studies that used quantitative PCR to demonstrate that cyanobacteria are numerically dominant across all domains in cryptoendoliths in the Antarctic dry valleys (Pointing et al. 2009) and in the Tibetan plateau (Wong et al. 2010). It also mirrors the finding that hypolithic biofilms in polar and non-polar deserts are also shaped largely by cyanobacterial diversity (Caruso et al. 2011; Valverde et al. 2015). The relatively smaller cyanobacteria have the ability to grow at lower light and oxygen levels than green algae, making it possible to colonise deeper areas in rocky substrates (Broady 1981).

Overall diversity patterns indicate the unique and relatively biodiverse nature of University Valley sandstone cryptoendoliths compared to McKelvey Valley sandstone cryptoendoliths and Miers Valley cyanobacterial granite chasmoendoliths. This was visibly demonstrated by the black-white-green banding in University Valley versus the pink-green banding in the other locations, and this indicates lichen-dominated versus cyanobacteria-dominated communities, respectively (de los Ríos et al. 2005, 2014a). We identified lichen symbionts, free-living algae and cyanobacteria coexisting in endolithic microbial communities from University Valley sandstones. This finding supports the observations made in earlier studies of cryptoendoliths from Battleship Promontory in the Convoy Range, Antarctica (Friedmann et al. 1988; de la Torre et al. 2003). Shannon estimates were consistent with previous clone library studies of different endolith types from the McKelvey Valley (Pointing et al. 2009) and Miers Valley (Yung et al. 2014). They were also consistent with previous investigations of endoliths in Swedish Lapland (Marnocha and Dixon 2014) and non-polar locations in Hawaii and Nanjiang, China (Gomez-Alvarez et al. 2007; Tang et al. 2012).

A study of cryptoendoliths in Tibetan limestone may provide insights into the abiotic drivers of the differences in diversity between University Valley and other sites (Wong et al. 2010). Quantitative PCR data demonstrate that whilst algal and fungal lichen symbionts dominated limestone of the cliff face, in rocks on the valley floor, the same substrate was dominated by cyanobacteria. Furthermore, their study showed that the main driver of colonisation was porosity of the colonised substrate (Wong et al. 2010). Cámara et al. (2014) have also recently shown how differences in textural rock properties, including the porosity, can condition the microbial composition. In our study, we envisage the more diverse University Valley community assembles at least in part because the sandstone substrate is highly weathered and more porous than in the McKelvey or Miers valleys. However, we cannot discard biogeographic and other environmental influences.

Another important finding was that the University Valley community supported distinct cyanobacterial taxa compared to the Scytonemataceae-dominated communities in Miers and McKelvey valleys. This likely reflects that whilst in the lichen community of University Valley, the black layer offers UV protection for biomass below, in cyanobacterial endoliths, the absence of this layer selects for cyanobacteria capable of producing their own UV-protecting compounds such as scytonemin and carotenoids (Vincent et al. 1993; Gao and Garcia-Pichel 2011). The cyanobacterial diversity in our Antarctic samples also differed markedly from that of non-polar cryptoendoliths where the genus *Chroococcidiopsis* is assumed to dominate (Friedmann 1980). Earlier descriptions of Antarctic cryptoendoliths visibly recorded this genus (Friedmann et al. 1988), but subsequent molecular surveys have failed to detect this organism (de la Torre et al. 2003; Pointing et al. 2009). Whilst this genus does appear to be ubiquitous in non-polar lithic biofilms (Bahl et al. 2011), it is becoming evident that this may not be true for Antarctic communities. An important observation was that all cyanobacteria cells were associated with extracellular polymeric substances. Since these polymeric substances are highly

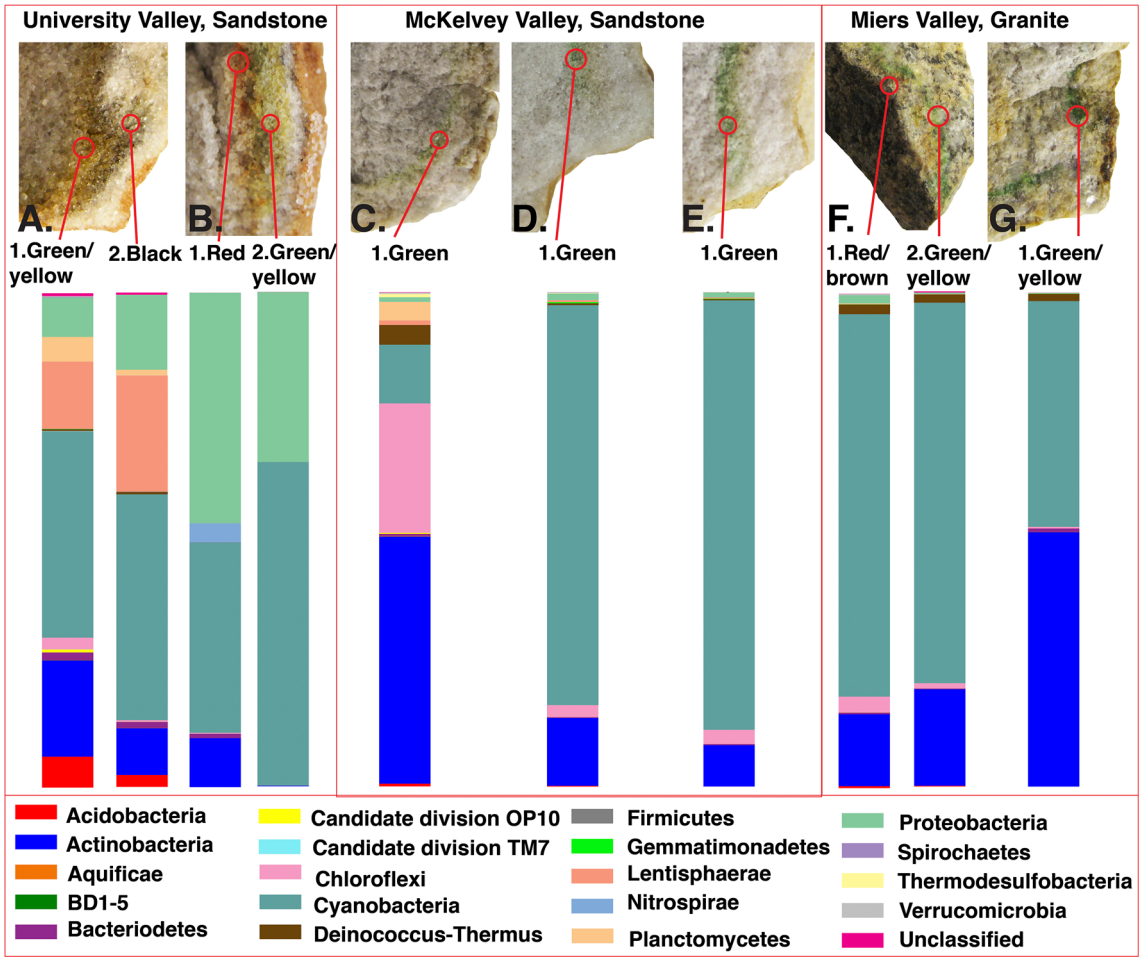

Fig. 4 Phylum-level distribution of bacteria in University Valley weathered sandstone cryptoendolith (**a**, **b**), McKelvey Valley sandstone chasmoendolith (**c**, **d**, **e**) and Miers Valley granite chasmoendolith (**f**, **g**). The 16S rRNA $OTUs_{0.03}$ were classified with RDP classifier and the taxonomy from the SILVA database (Release 102). Colonised rock sample images showing *coloured bands* are all orientated with the surface of the rock to the *right*

hygroscopic (Nicolaus et al. 1999), it reasonable to conclude that in addition to being the source of primary productivity to the system (Chan et al. 2013), they also likely play a key role in freeze–thaw and desiccation tolerance for the community in this xeric environment (de los Ríos et al. 2004).

Among the eukaryotes, the free-living alga *Hemichloris antarctica* (Darienko et al. 2015) occurred only in University Valley and was absent from cyanobacterial endoliths, whilst the genus *Diplosphaera* was found only in 'cyanobacterial' endoliths from Miers and McKelvey valleys. The genus *Diplosphaera* genus has been reported as a lichen photobiont (Thus et al. 2011), but the lack of lichen-forming ascomycete OTUs in these localities suggest that they may not correspond to lichenised forms. The microhabitat occupied by free-living algae in University Valley sandstones and Miers Valley granite is physically different, which may indicate different light and nutrient requirements for these two genera. *Hemichloris* cells were situated in the proximity of lichen symbionts and cyanobacteria cells, whilst free-living algae in Miers Valley granites were isolated in different fissures of the rock. The identification of lichen mycobiont taxa only in University Valley in our molecular survey further highlights the difference between this substrate and location versus the cyanobacterial endoliths in granite and sandstone at other sites.

The clear difference in the most abundant heterotrophic bacterial phyla may reflect in part the physical orientation of the substrate and local recruitment. In lichen cryptoendoliths from a cliff location, the alpha proteobacteria were dominant and have been previously associated with cryptoendolithic substrates (Pointing et al. 2009), whilst in cyanobacterial endoliths from sandstone and granite boulders on the valley floor, the ubiquitous Antarctic soil bacterial phylum Actinobacteria was most commonly encountered (Lee et al. 2012). Since the alphaproteobacterial OTUs included diazotrophic taxa, this difference may in part reflect that the cyanobacteria encountered in

University Valley were not diazotrophs, whereas in McKelvey and Miers valleys, putatively diazotrophic cyanobacteria from the Nostocales were dominant.

Some unexpected findings suggest further work is necessary in resolving the spatial arrangement of eukaryotes in endolithic communities. Lichen associations were demonstrated in University Valley samples by microscopy, but our molecular analysis revealed the presence of lichen mycobionts but failed to detect lichen photobionts, and this may reflect PCR bias. Another interesting point was that the superficial black layer we observed has previously been attributed to free-living melanised fungi (Friedmann 1982; Ruisi et al. 2007), but we also recorded a diverse bacterial assemblage for this layer. These findings suggest that the traditional delineation of these communities and coloured bands into 'fungal', 'lichen' and 'cyanobacterial' may require some revision as clearly they all support a diverse multi-domain assemblage. Our approach using a precision drill to recover discreet bands of colonised substrate was validated in this study and has interesting application towards future efforts to search for life on the surface of Mars where sandstone and other substrates occur that are believed to have been capable of supporting cryptoendolithic life (Wierzchos et al. 2012).

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