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ORIGINAL PAPER

Cyanobacterial diversity in benthic mats of the McMurdo Dry Valley lakes, Antarctica

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Abstract Perennially ice-covered, meromictic lakes in the McMurdo Dry Valleys, Antarctica, are useful models to study the relationship between cyanobacterial and environmental variables. They have rich benthic cyanobacterial mat accumulations and stable stratification of physical and chemical conditions. Here, we evaluated cyanobacteria from benthic mats from multiple depths in three geographically separated ice-covered lakes, Lakes Vanda, Hoare and Joyce, using 16S rRNA gene clone libraries. We identified 19 ribotypes, mostly Oscillatoriales and several Chroococcales, as well as potentially novel cyanobacterial ribotypes. The majority of ribotype diversity was shared between lakes, and only a weak relationship between ribotype community structure and environmental variables was evident. Multivariate analysis of all lake–depth combinations implied that photosynthetically active radiation, dissolved reactive phosphorus and conductivity were potentially important for shaping benthic communities in McMurdo Dry Valley lakes. Cyanobacterial-specific pigment signature analysis by high-performance liquid chromatography showed that the cyanobacterial communities responded to light conditions similarly, irrespective of community composition. The results imply a capability within a suite of cyanobacteria to colonise, adapt and grow across broad environmental ranges and geographic space, and such adaptability may provide a high degree of community resistance and resilience to future climate-driven environmental change in Antarctic terrestrial aquatic ecosystems.

Keywords Cyanobacteria · 16S rRNA gene · Microbial mat · Pigment · Diversity · Lake · Antarctica

Introduction

Habitats in the Antarctic continent are characterised by the extreme environmental conditions that challenge phototrophic organisms, including prolonged darkness and subzero temperatures. Within this region, benthic cyanobacteria are of particular interest because they are often the predominant phototrophs in aquatic ecosystems, frequently accumulating to very high biomass (Vincent 2000; Zakhia et al. 2008). These mat communities form highly pigmented layers that coat the benthic substrata and may accumulate to several tens of centimetres in thickness, sometimes forming complex structures and microbialites (Vincent 2000, Andersen et al. 2011, Hawes et al. 2011, 2013a, b). In the last decade, increased effort has been made to better describe the cyanobacterial taxonomy (morphological and molecular), community structure, biogeographical distribution and physiology in the Antarctic

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benthic mats (Taton et al. 2003, 2006; Jungblut et al. 2005; Novis and Smissen 2006; Strunecky et al. 2013); however, despite the ubiquitous presence and ecological importance of cyanobacteria in the benthic habitats in Antarctica, the factors regulating their diversity and community composition are still not well understood.

The McMurdo Dry Valleys (MDV) region, Southern Victoria Land, Antarctica (Fig. 1), holds several closed-basin, meromictic lakes that are perennially ice covered. The persistent ice cover imparts specific characteristics, including a reduction in the penetration of light to the waters beneath the ice (McKay et al. 1994; Howard-Williams et al. 1998) and a lack of wind induced mixing (Wharton et al. 1993). Together with the absence of mixing, low but variable water influx has allowed formation of intense stratification of solutes, including major ions and nutrients (Quesada et al. 2008; Hawes et al. 2011). Thus, micro-organisms may be confronted by markedly different growth conditions in the same lake at different depths (Vincent et al. 2008). However, there have been few attempts to explore how the cyanobacterial mat community composition at different depths varies in response. The two extreme hypotheses governing species assembly within the microbial mats in these lakes are that (a) taxa with performance optima that best suit a given environment dominate at specific locations and that environmental stratification is mimicked by community distribution, or that (b) in this extreme environment, opportunistic species with broad habitat tolerances dominate, leading to similarities in species assemblage across wide environmental ranges. Understanding how important environmental conditions are in determining the communities' composition will assist in predicting the response of cyanobacterial diversity to climatic-driven environmental change in Antarctic terrestrial aquatic ecosystems.

This study tested hypothesis (a) by (1) describing the benthic cyanobacterial genetic diversity and community composition in MDV lakes with depth and (2) determining whether environmental variables correlate with diversity and community composition in samples from a range of lakes and depths. The cyanobacterial communities were compared using cyanobacterial 16S rRNA gene clone library analyses, and the patterns of community composition and diversity were analysed to assess the effect of environmental factors on the communities in MDV lakes.

Materials and methods

Study sites and sampling

The McMurdo Dry Valleys (MDV) are located in Southern Victoria Land, west of McMurdo Sound. The region is noted for its low humidity, with mean annual precipitation below 50 mm/year water equivalent (Fountain et al. 2009). Perennially ice-covered lakes are major landscape features in the MDV, and most of the lakes are covered by 4–7 m of ice. Below the perennial ice cover, these lakes are meromictic, with an upper layer of dilute freshwater overlying saline deeper waters (Green and Lyons 2009;

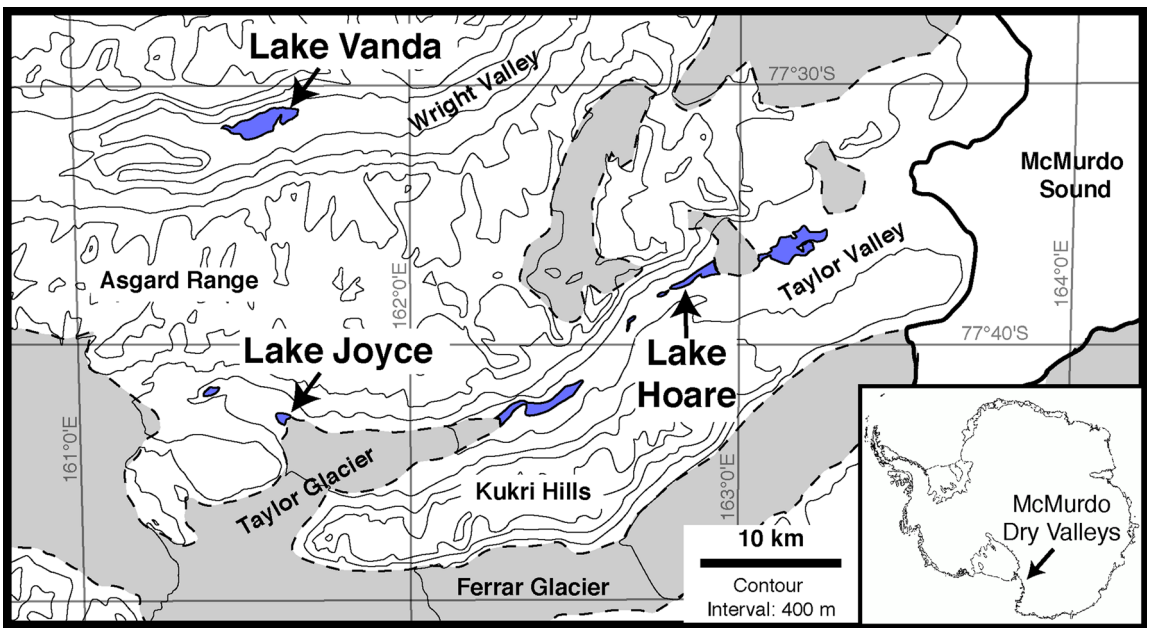

Fig. 1 Map showing location of Lakes Vanda, Joyce and Hoare in the central McMurdo Dry Valley area. *Inset* shows approximate position of detailed map relative to Antarctica

Suppl. Table S1 and Fig. S1). This study focused on Lakes Joyce, Hoare and Vanda. Lake Joyce lies along the northern side of Taylor Glacier in Pearse Valley, Lake Hoare is located in Taylor Valley, and Lake Vanda is in Wright Valley.

Sampling took place in November and December 2010. Mat material was sampled from four depths at Lake Joyce (LJ 6, 11, 14 and 21 m) and Lake Hoare (LH 8, 12, 16 and 21 m), and two samples were obtained from Lake Vanda (LV 18 and 27 m), for a total of ten samples. Sample depths in the lakes were selected to provide, as much as possible, a matrix of environmental variables that allowed comparisons to be made with some or all of these variables within similar ranges, across lakes. Samples are hereafter referenced by a lake–depth combination, as LJ11, LH16 etc. Mat samples were collected from each depth by SCUBA divers, using a 38-mm-diameter coring device, and in all cases, only the cohesive upper component of the mat was taken, avoiding underlying, organic-rich sediment. Mat samples were frozen at −20 °C after collection and stored at −80 °C after return to the laboratory until further analysis.

Environmental variables

Photosynthetically active radiation (PAR) was measured using a LI-Cor LI-192 underwater quantum sensor connected to a LiCor LI-1400m (Li-Cor, Lincoln, NB, USA), located in a waterproof housing. Readings were taken at 1-m vertical depth intervals by a diver swimming along the lake bottom. A Li-Cor Li-190 terrestrial quantum sensor was used to obtain simultaneous measures of incident irradiance to allow underwater readings to be recalculated as percentage of surface irradiance. Conductivity, pH and temperature of Lake Vanda and Joyce water were measured *in situ* using a freshly calibrated YSI 6600V2 multiparameter data sonde (YSI, Inc., Yellow Springs, OH, USA) lowered through the water column. Similar data for Lake Hoare were obtained from the McMurdo Dry Valley Long-Term Ecological Research Programme (http://www.mcmlter.org/data_home.htm). Water column samples were taken and analysed for dissolved inorganic carbon (DIC), dissolved reactive phosphorus (DRP), NH_4–N and NO_3–N. For DIC, 2-mL samples were injected into gas-tight serum tubes containing nitrogen at 1-atm pressure and 0.2 mL concentrated phosphoric acid. Acidification converted the DIC dissolved in the water to CO_2, which then partitioned between the water and the headspace. After equilibration, CO_2 in the headspace was measured by injecting a known volume into a stream of nitrogen passing through the measuring cell of a LiCor LI-820 CO_2 Analyzer based upon a single-path, dual-wavelength infrared detection system. Calibration was made with freshly prepared solutions of $NaHCO_3$. DRP, NH_4–N and NO_3–N were measured using an Astoria autoanalyzer.

Biomass

For the analysis of biomass components from Lake Vanda and Lake Hoare, known area core samples were transported frozen (−20 °C) to New Zealand and freeze-dried. Data for Lake Joyce were obtained from Hawes et al. (2011). Weighed aliquots of four mat samples from each lake–depth combination were analysed for the loss of mass on acidification (LoAc—inferred to approximate calcite content), loss of mass on ignition (LoI) and chlorophyll-*a*. A single composite subsample from each location, made from equal parts of each of the four replicates, was also analysed for organic carbon and nitrogen content and determination of other acetone-soluble pigments by high-performance liquid chromatography (HPLC). Methods are fully described in Hawes et al. (2011), but briefly, LoAc was determined as % weight change before and after addition of 10 % HCl and subsequent rinsing to oven-dried samples (60 °C), and % ash was determined after subsequent combustion at 450 °C for 3 h and LoI calculated as oven-dry mass less LoAc and ash. Organic C and N contents were measured on weighed aliquots (after acidification) using a Carlo Erba Automated CHN analyser and again expressed as % oven-dry weight.

For chlorophyll-*a* and HPLC analysis, aliquots were extracted by ultrasonication (15–20 W for 30 s) in ice-cold 95 % acetone and left in the dark for 24 h to complete extraction. After clarification by centrifugation, chlorophyll-*a* concentration was determined by spectrophotometry, without acidification, and normalised to core area. HPLC analysis used a Dionex system, with PDA-100 diode array detector (300–800 nm), and separated pigments according to the chromatographic method of Zapata et al. (2000). Pigments were quantified by absorption at 436 nm, with calibration by reference to commercially available standards. Statistical treatment of the HPLC data used multidimensional scaling (MDS) based on Bray–Curtis similarities of pigment concentrations after normalisation to HPLC-derived chlorophyll-*a* and used the PRIMER 6 software package (PRIMER-E, Ltd., UK). Non-metric MDS was undertaken with 100 random restarts, and results were plotted in two dimensions.

DNA extraction and cyanobacterial-specific polymerase chain reaction (PCR)

DNA was extracted from about 200 mg of mat from ten sites (Table 1) with three replicates each, using a MoBio PowerBiofilm DNA Isolation kit (Carlsbad, CA) according to the manufacture's protocol. Triplicate DNA extracts

Table 1 Physical and chemical properties of the sampling sites at different depths of Lake Joyce (LJ), Lake Hoare (LH) and Lake Vanda (LV) in the McMurdo Dry Valley (MDV) regions, Antarctica

Sample	Depth (m)	PAR (%)	pH	Conductivity (μS/cm)	Temperature (°C)	DIC (mg/L)	DRP (mg/L)	NH_4–N (mg/L)	NO_3–N (mg/L)
LJ6	6	0.38	10.05	337	0.42	4.7	1.0	1.0	1090
LJ11	11	0.21	10.05	392	0.24	5.1	1.0	1.0	1090
LJ14	14	0.11	9.71	590	0.26	6.1	1.0	1.0	1130
LJ21	21	0.09	8.22	4221	0.25	31.4	1.0	1.0	1070
LH8	8	0.41	8.70	524	0.49	33.9	2.5	2.4	1.1
LH12	12	0.32	8.40	570	0.40	48.3	3.4	2.4	1.3
LH16	16	0.15	7.60	723	0.14	68.2	3.7	5.6	39.3
LH21	21	0.08	7.40	724	0.16	74.5	4.3	14.7	62.4
LV18	20	7.30	8.52	603	4.25	8.6	1.8	4.2	29.3
LV27	30	4.40	8.62	970	6.36	9.9	2.2	1.3	34.6

PAR photosynthetically active radiation, *DIC* dissolved inorganic carbon, *DRP* dissolved reactive phosphorus

were pooled after quantification of DNA using NanoDrop ND8000 (Labtech International, UK). Three different DNA concentrations of the pooled DNA were used for PCR amplification. PCR amplification of cyanobacterial 16S rRNA gene was performed using 1 unit of DNA Taq polymerase (Bioline, Taunton, MA) in a 20-μL reaction mix containing 1 μL Bioline PCR buffer, 2.5 mM $MgCl_2$, 0.4 μg BSA, 0.2 mM dNTPs and 0.5 μM of each cyanobacteria-specific primer 27F1 (5′-AGAGTTTGATC CTGGCTCAG-3′) and 809R (5′-GCTTCGGCACGGCTC GGGTCGATA-3′). As described by Jungblut et al. (2005), these primers provide broad coverage of cyanobacterial taxa. Thermal cycling conditions were 94 °C for 2 min followed by 30 cycles of 94 °C for 20 s, 55 °C for 30 s, 72 °C for 1 min, repeated for 30 cycles with a final extension of 72 °C for 7 min.

Cloning, restriction fragment length polymorphism (RFLP) analysis and sequencing

The amplified PCR products were verified by gel electrophoresis. Prior to cloning, PCR products amplified from different concentrations of DNA were pooled for each sample and purified with the QIAquick PCR Purification kit (Qiagen, Hilden, Germany). PCR products were cloned using the Stratagene cloning kit (Agilent Technologies, Cedar Creek, TX). Ligation and transformation were performed according to the manufacture's instructions. Positive (insert containing) clones were transferred to 96-well plates containing Luria-Bertani medium with 7.5 % glycerol. The inserted 16S rRNA sequences were amplified using vector-specific primers M13F and M13R, and the correct-sized amplicons were subjected to RFLP screening as described in Jungblut et al. (2012). Two clones for each unique RFLP pattern were sequenced (or one clone sequenced with a unique RFLP pattern) using the vector-specific T7 universal primer (single read) at the Natural History Museum sequencing facility using Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster city, CA, USA). The 16S rRNA gene sequences are available under GenBank accession numbers KM112100–KM112167.

Sequence processing and diversity calculations

Sequences were checked for chimeras using Bellerophon (Huber et al. 2004), and chimeras were excluded from further analysis. Sequences were edited using 4Peaks (version 1.7.2) and aligned using ClustalX (version 2.0.9). Manual editing was performed with MacClade (version 4.08). For each sequence, a closest cultured and uncultured match based on a BLASTn search (Altschul et al. 1990) to GenBank was determined. Individual ribotypes or operational taxonomic units (OTUs) were defined as groups of sequences with at least 97 % similarity using MOTHUR (version 1.25.1, Schloss et al. 2009). Library coverage, Chao1 and ACE nonparametric richness estimates, Shannon–Wiener diversity index and rarefaction curves were calculated using MOTHUR (Schloss et al. 2009).

Phylogenetic analysis

Phylogenetic trees were constructed using maximum-likelihood method implemented in RaxML-HPC2 (version 7.3.2; Stamatakis et al. 2008) through the CIPRES portal (version 3.0). The GTRCAT substitution model was used for the bootstrapping phase and the GTRGAMMA for the final tree inference. A best-scoring ML tree was obtained

based on 1000 bootstraps. One representative for each ribotype from each sample was included in the phylogenetic analysis. For each sequence included, the closest cultured and uncultured match based on a BLASTn search (Altschul et al. 1990) to GenBank was selected as reference sequences. 16S rRNA gene sequences from isolates of the identified morphotypes and environmental 16S rRNA gene sequences from Antarctic and Arctic sites obtained in some published studies were also included in the phylogenetic tree for comparison. The 16S rRNA gene sequence of *Gloeobacter violaceus* PCC 7421 (BA000045) was used as an outgroup. The 11-bp insertion (5′ AGTTGTGAAAG-3′, Nadeau et al. 2001) was removed form the alignment for the phylogenetic analysis.

Statistical analysis

Correlation analyses were carried out in R using nonparametric Spearman's correlations. UniFrac (Lozupone et al. 2006) was used to compare the microbial communities based on the established cyanobacterial-specific 16S rRNA clone libraries using the UniFrac distance metric (Lozupone and Knight 2005). Samples were considered significantly different if the UniFrac distance for the real tree was greater than would be expected if the sequences were randomly distributed between the compared samples. Based on 1000 permutations, the significance of difference between each pair of samples was estimated. A principal coordinate analysis (PCoA) was performed on the established clone libraries using the UniFrac distance matrix.

Canonical correspondence analyses (CCAs) were carried out in Canoco 4.5 for windows (after Braak and Smilauer 2002) to test the influences of environmental variables (Table 2) on the cyanobacterial community structure. The OTU relative abundance matrix was treated as "species" data. The default log transformation (i.e. log $(y + 1)$) was applied to the relative abundance data (on 0–100 scale), PAR, conductivity, temperature, DIC, DRP, NH_4–N and NO_3–N (Table 2). Environmental variables to be included in the model were chosen by forward selection at a 0.10 baseline. Using only the chosen variables, the significance of the whole canonical model was estimated with 999 permutations.

Results

Environmental properties

The ten sampling sites showed considerable variations in physical and chemical properties (Table 1). Lake Vanda had the most transparent lake ice cover that enabled roughly 15 % of PAR to pass through (Vincent et al. 1998) and thus led to an underwater light regime much brighter than the other two lakes (Table 1). PAR was 7.3 % of the incident irradiance at LV18 and 4.4 % at LV27, whereas Lake Joyce and Lake Hoare samples were exposed to PAR ranging from 0.09 % (LJ21) to 0.38 % (LJ6), and 0.08 % (LH21) to 0.41 % (LH8), respectively. The two Lake Vanda samples with ≥4.25 °C water temperatures were warmer than Lakes Joyce and Hoare, which had temperatures of ≤0.5 °C. Lake Joyce samples had the highest pH values ranging from 8.22 (LJ21) to 10.05 (LJ6 and LJ11) (Table 1). DIC at LJ21, though lower than Lake Hoare samples, was higher than those at the other depths of Lake Joyce. LJ21 was also high in conductivity (4221 µS/cm), contrasting all the other samples ranging from 337 µS/cm (LJ6) to 970 µS/cm (LV27). The water in Lake Joyce was rich in inorganic nitrogen (NO_3–N ≥ 1070 mg/L), whereas Lake Hoare samples had very low inorganic nitrogen but

Table 2 Chlorophyll-*a* concentration, loss on acidification, carbon and nitrogen content of the microbial mats from the ten lake–depth combinations

Sample	Chlorophyll-*a* (µg/cm^2)		Loss on acid (%)		%C	%N	C:N
	Mean	SD	Mean	SD			
Hoare 8 m	11.3	3.2	45.8	5.2	12.0	1.18	10.2
Hoare 12 m	8.5	2.0	40.7	2.8	12.0	1.24	9.7
Hoare 16 m	7.8	2.6	26.2	15.9	16.8	2.25	7.5
Hoare 21 m	6.7	3.9	24.1	9.9	9.5	1.34	7.1
Joyce 6 m	4.7*	2.3	2.3	1.1	0.93	0.09	10.3
Joyce 11 m	3.1*	1.5	30.9	13.1	1.63	0.14	11.6
Joyce 14 m	1.7*	0.5	14.8	3.9	1.49	0.15	9.9
Joyce 21 m	1.8*	0.7	17.8	9.3	1.13	0.13	8.7
Vanda 18 m	10.7	6.8	21.9	6.1	6.1	0.55	11.1
Vanda 27 m	5.5	1.8	60.0	20.0	14.0	1.33	10.5

Means are calculated for four samples, and where no mean is calculated, these are blended samples

* Data for Lake Joyce were obtained from Hawes et al. (2011)

higher dissolved inorganic carbon (≥33.9 mg/L) and phosphorus concentrations (≥2.5 mg/L, Table 1) than Lake Joyce. Largely driven by nitrate concentration, the ratios of inorganic N:P in the lakes were very different, with Lake Joyce consistently exceeding 1000:1, Lake Hoare (shallow) approaching 1:1, whereas Lake Vanda and the deeper Hoare samples were between 10:1 and 20:1.

Biomass and cyanobacterial-specific pigments

Using chlorophyll-*a* as an indicator of biomass, within each lake the biomass declined with depth, and Lakes Vanda and Hoare tended to have higher biomass than Joyce (Table 2). However, ANOVA showed that within-depth variance was such that there were no significant depth-specific differences in biomass in any of the three lakes, but that the two deepest Lake Joyce samples were significantly lower than any biomass values in Lakes Vanda or Hoare. Loss on acidification is taken as a metric of calcite content, and this was high, at 15–45 % dry weight, in most samples. ANOVA identified Joyce at 6 m as unusually low, and Vanda at 27 m as unusually high. Organic carbon and nitrogen contents of the Lake Joyce mats were lower than in Lakes Vanda and Hoare, reflecting the large amount of inorganic material that was incorporated into Lake Joyce mats. Organic C:N ratios were rather constant across the samples, at close to 10 (by mass).

As shown in Suppl. Fig. S2, multidimensional scaling plot of the cyanobacterial-specific pigments data clustered all of the samples but LH21 within a 60 % similarity envelope. Within this, samples tended to cluster at a high degree of similarity (75 %), with one outlier, the deepest sample from Lake Joyce. The pigments that most drove these outliers were different: a high concentration of a myxoxanthophyll-like pigment and low concentration of caloxanthin in the Lake Hoare 21-m sample, and a high concentration of caloxanthin in the Lake Joyce 21-m sample. Separation of the samples along the two axes in Suppl. Fig. S2 was mostly attributable to these two pigments, and Lake Hoare samples separated in depth order along axis 1 and Lake Vanda samples were near identical, and Lake Joyce samples separated in depth order along both axes.

16S rRNA gene cyanobacterial diversity and phylogenetic analysis

A total of 559 clones with the correct insert size were obtained from the ten microbial mat samples (Table 3). Nineteen OTUs were identified, defined at a 0.03 distance cut-off (97 % 16S rRNA gene similarity). Rarefaction curves of LJ11, LJ14 and LV18 appeared to reach an asymptote (Suppl. Fig. S3) with an estimated library coverage of 100 % (Table 3). In contrast, rarefaction curves of LJ6, LH8, LH12, LH16, LH21 and LV27 did not reach asymptote, which suggested that the cyanobacterial diversity may be underestimated for these samples to different degrees. Correspondingly, coverage ranged from 89.3 % (LV27) to 97.4 % (LH8) in these six samples and non-parametric richness estimations were higher than the observed diversity (Table 3). Only seven clones with the correct insert size were obtained for LJ21, which accounted for 71.4 % of the total cyanobacterial diversity (Table 3) based on Good's coverage index.

Seven cyanobacteria 16S rRNA gene ribotypes were present in all three MDV lakes, one (MDVcy09) in two of the lakes, and the remainder were identified from only one lake. Five OTUs were restricted to Lake Vanda, four to Lake Hoare and two to Lake Joyce. The largest number of OTUs (12) occurred in LV27, which also had the highest Shannon–Wiener diversity index ($H' = 2.04$; Table 3). The lowest diversity index ($H' = 0.11$) was found in LJ11, which contained only two OTUs. MDVcy14 was the most widespread, detected in nine of ten mat samples across the three lakes, whereas MDVcy02, MDVcy03 and MDVcy18 were identified in eight mat samples, and the other ribotypes were found in six or fewer mat samples (Fig. 2).

Fifteen of the nineteen OTUs, comprising 95.9 % of all of the clones, had highest similarity to other Antarctic and Arctic sequences within the genera *Leptolyngbya*, *Phormidium*, *Oscillatoria*, *Limnothrix*, *Pseudanabaena* and *Tychonema* of the order Oscillatoriales (Suppl. Table S2). Four OTUs were related to the genera *Chamaesiphon*, *Synechococcus* and an uncharacterised genus (isolate cyanobacterium cCLB-9, HQ230230) confirmed to belong to the order Chroococcales via the phylogenetic analysis (Fig. 3a, b).

Two ribotypes MDVcy04 (92 % *Leptolyngbya* sp. FYG (FJ933259)) and MDVcy13 (92/93 % *Phormidium uncinatum* SAG 81.79 (EF654086)) from Lake Vanda and ribotype MDVcy16 (93 % *Phormidium mucicola* IAM M-221 (AB003165) and 92 % *Pseudanabaena* sp. 1a-03 (FR798944)) that were present in all lakes had low 16S rRNA gene similarity to sequences of cultured cyanobacteria in the public database, suggesting the presence of potentially uncultured, novel cyanobacterial taxa (Suppl. Table S2). Of these, both MDVcy04 and MDVcy16 formed clusters with other uncultured cyanobacterial sequences in the phylogenetic tree (Fig. 3a, b). MDVcy16 clustered within Oscillatoriales, while MDVcy04, which grouped between *Leptolyngbya* and *Chamaesiphon* clade, could not be assigned with confidence at the order level.

Oscillatorian diversity was likely under-represented by the OTU definition of ≥97 % similarity as sequences

Table 3 Measures of α-diversity for the ten cyanobacterial-specific 16S rRNA gene clone libraries from Lake Joyce (LJ), Lake Hoare (LH) and Lake Vanda (LV), MDV, Antarctica

Sample	Number of clones	Number of OTUs	Coverage (%)	Chao1 richness	ACE richness	Shannon–Wiener diversity (H')
LJ6	51	9	94.1	10.5	12.1	1.75
LJ11	88	2	100	2.0	2	0.11
LJ14	46	5	100	5.0	5	1.21
LJ21	7	3	71.4	4.0	10.7	0.8
LH8	76	8	97.4	8.3	9.5	1.41
LH12	81	8	96.3	11.0	12.5	1.53
LH16	74	8	97.3	8.3	9.7	1.4
LH21	31	6	90.3	9.0	13.7	1.34
LV18	49	7	100	7	7	1.59
LV27	56	12	89.3	19.5	44.2	2.04

An OTU was defined at 97 % 16S rRNA gene similarity using average neighbour algorithm. Coverage is the Good's coverage index calculated as $C = [1–(n/N)] \times 100$, where n is the number of OTUs with a single clone and N is the total number of clones in the library

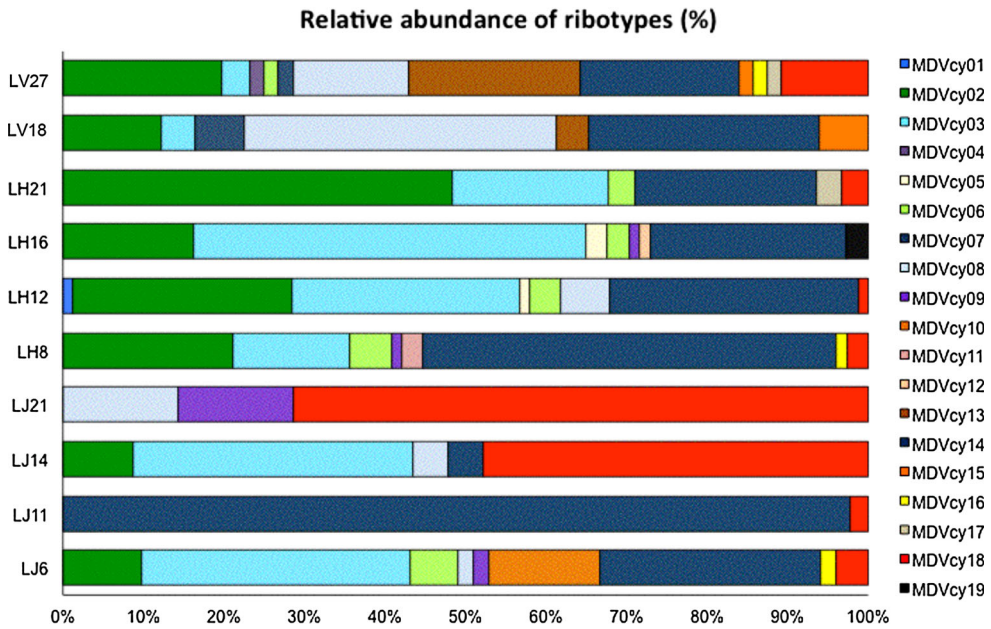

Fig. 2 Relative distribution of OTUs based on 16S rRNA gene clone libraries from benthic cyanobacterial communities at different depths of Lake Joyce (LJ), Lake Hoare (LH) and Lake Vanda (LV), MDV, Antarctica

clustered within the OTU MDVcy14 grouped with the *Phormidium* and *Tychonema* clusters separately in the phylogenetic tree. Similarly, MDVcy17 formed clades with *Limnothrix* and *Pseudanabaena* (Fig. 3a, b).

Cyanobacterial community comparisons and the influence of environmental factors

Based on the relative abundance of OTUs in samples from the three lakes (Fig. 2), Lake Hoare mat samples showed similar cyanobacterial community composition, with the relative abundances of MDVcy02 (97–99 % *Phormidium* sp. PMC301.07 (GQ859651)) and MDVcy03 (98/99 % *Leptolyngbya antarctica* ANT.L18.1 (AY493607)) generally increasing, and MDVcy14 decreasing, with increasing depth, although correlations between abundance and depth were not statistically significant ($r = 0.4$, $P = 0.75$; $r = 0.4$, $P = 0.75$; $r = -1$, $P = 0.08$, respectively, $N = 4$ for all). Also, a similar number of OTUs were found in Lake Hoare samples, with LH8, LH12, LH16 each having eight OTUs and LH21 having six OTUs. In Lake Joyce, the OTU richness was highest in LJ6 (nine OTUs) with samples from the other depths having two (LJ11) to five (LJ14) OTUs. LJ6 had a similar OTU composition to the Lake Hoare samples (Fig. 2). In Lake Vanda samples, up to 12 OTUs (LV27) were determined, and LV18 and LV27

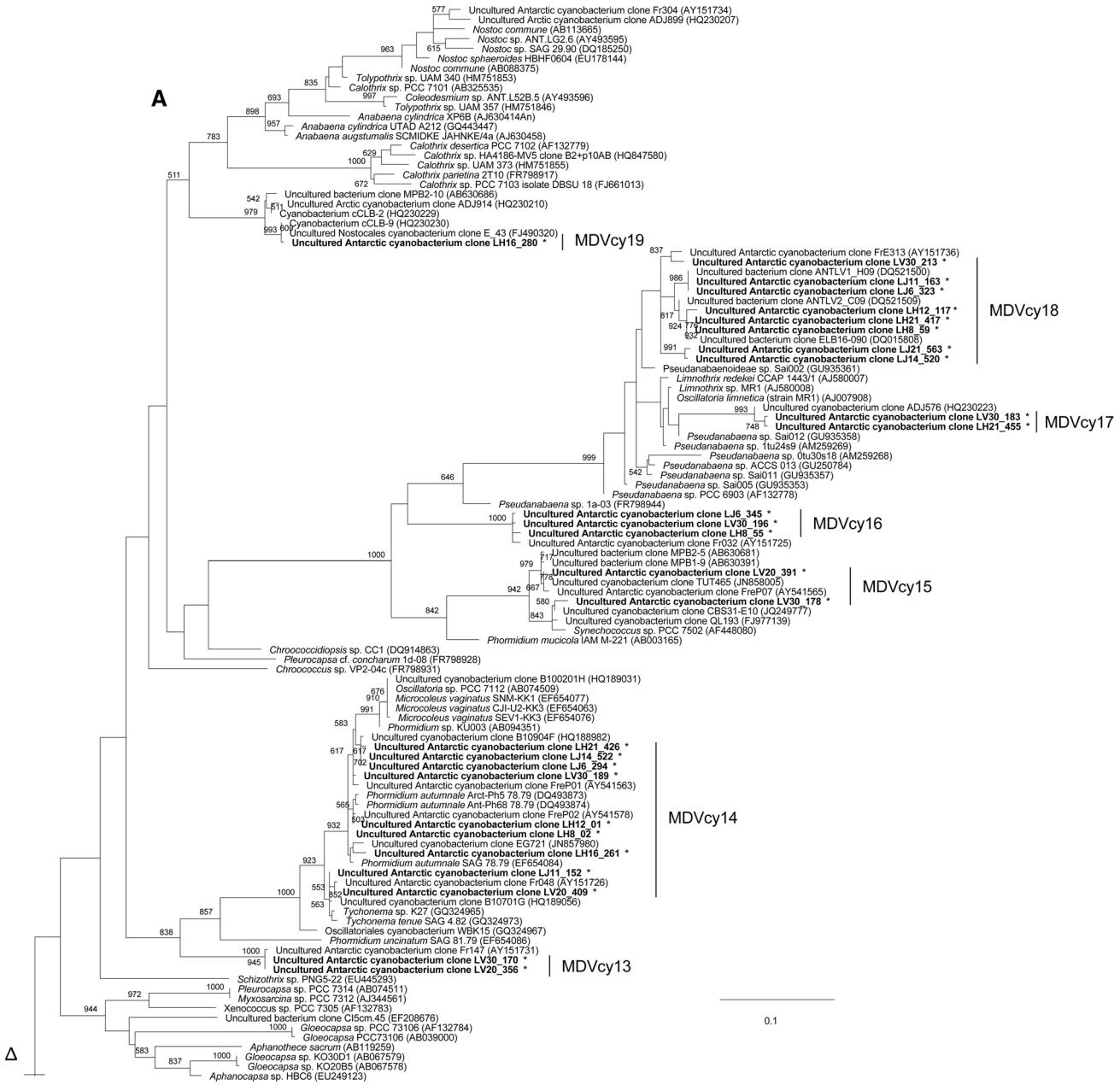

Fig. 3 Phylogenetic analysis of the identified cyanobacterial 16S rRNA gene ribotypes from benthic microbial mats in Lake Joyce (LJ), Lake Hoare (LH) and Lake Vanda (LV), MDV, Antarctica. The 16S rRNA gene sequence of *Gloeobacter violaceus* PCC 7421 (BA000045) was used as an outgroup. Bootstrap support (based on 1000 bootstrap replicates) for nodes is shown for values greater than 500. Sequences obtained in the present study are highlighted in *bold* and with a *star* at the end. The *triangle* indicates where **a** joins **b**

shared the majority of OTUs including MDVcy07 (98 % *Leptolyngbya frigida* ANT.L53B.2 (AY493576)), MDVcy13 (92/93 % *Phormidium uncinatum* SAG 81.79 (EF654086)) and MDVcy15 (97–99 % *Synechococcus* sp. PCC 7502 (AF448080)), which were exclusive to the two Lake Vanda samples (Fig. 2).

The UniFrac significance test, based on 1000 permutations, compared each pair of samples (Suppl. Table S3), and a principal coordinate analysis (PCoA) was performed using a distance matrix derived from the UniFrac metric (Fig. 4). LJ21 was excluded from the UniFrac analyses due to the very small number of clones obtained for this sample. The pairwise comparisons between samples incorporating phylogenetic information and the ordination diagram largely agreed with the community composition patterns observed at the OTU level. LJ11 had a distinct phylogenetic community structure and was separated from the remaining samples along the first and second PCoA, which

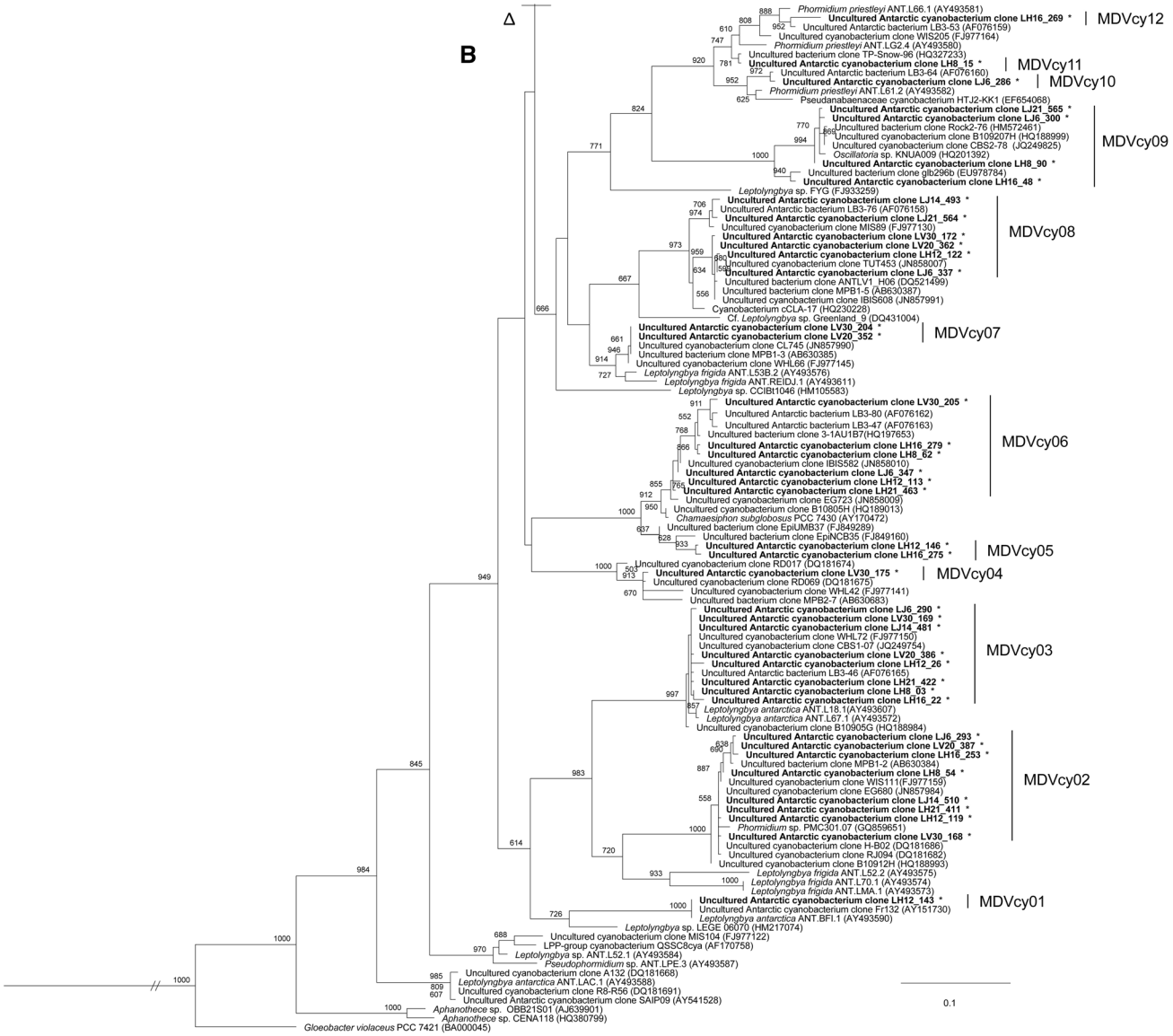

Fig. 3 continued

together explain 60.96 % of the total cyanobacterial community variability among all the samples (Fig. 4). LH16 also appeared to be separated from the other samples (Fig. 4) which could be ascribed to the presence of MDVcy19 (99 % cyanobacterium cCLB-2 (HQ230229); 99 % cyanobacterium cCLB-9 (HQ230230)). LJ6 again appeared to have an affinity with the Lake Hoare samples (Fig. 4). No significant difference was detected between the two Lake Vanda samples, and they were separated from the other samples along the second PCoA (Fig. 4).

Canonical correspondence analyses (CCAs), based on relative abundance of OTUs as taxa data and all environmental data, were used to examine which environmental variables most closely corresponded with cyanobacterial community composition (Fig. 4).Of the variables measured, PAR (photosynthetically active radiation; $P = 0.0060$), DRP (dissolved reactive phosphorus; $P = 0.0300$) and conductivity ($P = 0.0890$) were found to be most strongly corresponding, using a forward stepwise approach (Fig. 5; test of significance of all canonical axes: $P = 0.0010$).

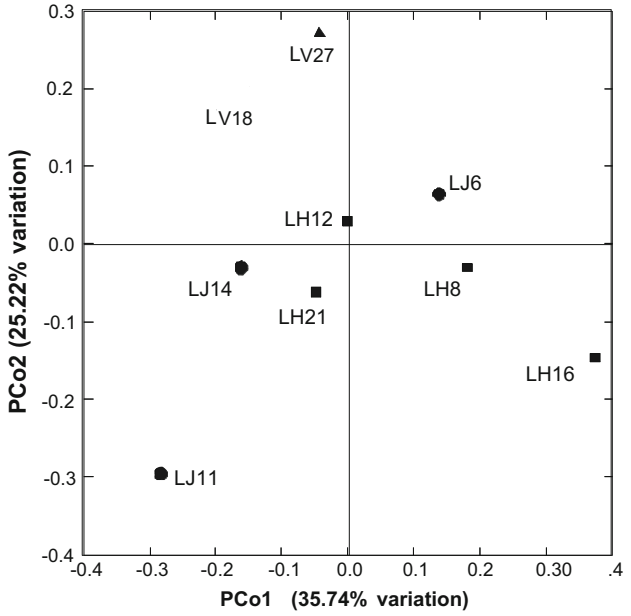

Fig. 4 Principal coordinate analysis (PCoA) of the nine clone libraries from benthic microbial mats of Lake Joyce (LJ), Lake Hoare (LH) and Lake Vanda (LV), MDV, Antarctica, as revealed by cyanobacteria-specific 16S rRNA gene sequences, based on UniFrac metric. *Circles* symbolise samples from Lake Joyce, squares for Lake Hoare and *triangles* for Lake Vanda

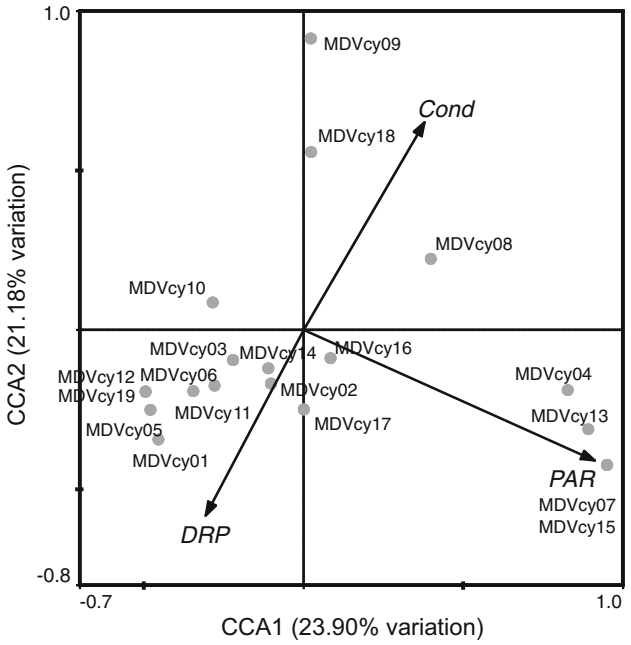

Fig. 5 Canonical correspondence analysis (CCA) ordination plots of the relationships between the cyanobacterial community structure and the environmental variables at different depth of Lake Joyce, Lake Hoare and Lake Vanda, MDV, Antarctica, by assessing the cyanobacterial-specific 16S rRNA gene OTUs. Data points for samples were omitted from the graph for clarity. *PAR* photosynthetically active radiation, *Cond* conductivity, *DRP* dissolved reactive phosphorus

Discussion

Cyanobacterial diversity in benthic mats of McMurdo Dry Valley lakes

A total of 19 cyanobacterial OTUs (97 %) were identified in Lakes Joyce, Hoare and Vanda from microbial mats at depths ranging from 6 to 27 m. All of the cyanobacterial mat communities were dominated by members of the filamentous order Oscillatoriales, well known as fundamental components of polar mat communities (Quesada and Vincent 2012). The most common taxa were *Phormidium* and *Leptolyngbya*, in agreement with previous morphological studies of cyanobacteria in these three lakes (Wharton et al. 1983; Hawes and Schwarz 1999; Hawes et al. 2011), and other terrestrial inland freshwater ecosystems across the Antarctic continent (Broady and Kibblewhite 1991; Taton et al. 2003, 2006; Jungblut et al. 2005, 2012). *Pseudanabaena* was present in all lakes, though less abundant than *Phormidium* and *Leptolynbgbya,* and was also previously identified in mats from lakes in the MDV lakes (Hawes et al. 2013a, b) and East Antarctica (Taton et al. 2006). The genus *Oscillatoria,* detected in Lake Joyce and Lake Hoare in this study, was also described previously in benthic mats in these two lakes (Hawes and Schwarz 1999; Hawes et al. 2011). Four ribotypes belonging to the unicellular order Chroococcales were also detected. Wharton et al. (1983) reported the presence of Chroococcales, specifically *Cyanothece* in Lake Hoare. However, in the current study, the sequences are most phylogenetically related to the genus *Synechococcus*, which is a picocyanobacterium. It is typically present as phytoplankton in the water column of Antarctic lakes (Vincent 2000) and may have settled out of the water column via sedimentation into benthic communities rather than have grown *in situ*.

In general, the communities in the three lakes were not diverse, with an average of seven OTUs in each sample and a maximum of ten in LV27. We note that LJ11 was unexpectedly OTU poor and this may have been due to incomplete sampling. Only seven of the recognised OTUs were present in all three lakes, while Lake Hoare had five OTUs not found in the other lakes, Vanda four and Joyce one. Rarefaction curves (Fig S3) suggested that cyanobacterial OTU diversity was slightly underestimated in several samples, but overall was low compared to other Antarctic mats. Taton et al. (2003) described 15 OTUs (97.5 %) from mat samples from a single depth in the shallow margin of Lake Fryxell, also a perennially ice-covered MDV lake. The overall number of OTUs recovered from the three MDV lakes was also lower than that described previously from the Pyramid Trough in the MDV

where 21 ribotypes were detected (Jungblut et al. 2012), albeit across a much larger conductivity range (65–32,700 μS/cm) and in shallow freshwater ecosystem types with less stringent light conditions.

A significant reason for the lack of diversity in the under-ice lake mats may be the absence of Nostocales, unlike in shallow meltwater ponds or shallow moat zones of larger lakes (Taton et al. 2003; Jungblut et al. 2005; Taton et al. 2006; Sutherland and Hawes 2009), that no Nostocales were observed in the MDV benthic mats. This discrepancy between above and below ice mats in terms of Nostocales is consistent with microscopic observations from Lake Vanda (Hawes et al. 2013a, b), Lake Hoare (Wharton et al. 1983) and Lake Fryxell (Wharton et al. 1983; Taton et al. 2006). Whether the absence of nitrogen-fixing Nostocales beneath the thick perennial ice cover of these lakes is due to their dependence on high light, particularly to provide sufficient energy for the nitrogenase system (Allnutt et al. 1981; Wharton et al. 1983), or due to a competitive exclusion under dim light, cannot be determined from our data. Allnutt et al. (1981) also suggested that relatively high concentration of NH_4–N and NO_3–N could preclude heterocystous forms, which was the case for all but the two shallowest samples from Lake Hoare. The C:N ratios for the mats were all close to ten and would not appear to indicate any nitrogen limitation that could make the ability to fix nitrogen advantageous. An alternative possibility could be that the heterocystous taxa of Nostocales were present at very low abundance in the benthic mat communities we investigated, but clone library methods failed to detect them. The molecular approach used here can introduce bias into the observed diversity patterns (Jungblut et al. 2005), which may be overcome by better coverage through high-throughput sequencing of cyanobacterial diversity and PCR-free metagenomic analysis (Sogin et al. 2006; Varin et al. 2010).

Our findings suggest that the diversity of cyanobacterial mats under perennial ice cover tends to be lower than in well-lit, seasonally frozen ponds and lake margins. Nostocales are particularly rare under ice. In addition, we find that while many OTUs are shared among lakes, some lake-to-lake differences did occur. In particular, samples from Lake Vanda tended to be clearly distinguishable from those from Lakes Hoare and Joyce, whereas the Lake Joyce communities tended to show some degree of convergence with Lake Hoare, particularly in shallow samples. In terms of our hypothesis under test, the sharing of 50 % of the OTUs among all three lakes, including all of the abundant ones, does imply the existence of a wide dispersal of rather broadly tolerant, dominant cyanobacteria within the region, that are particularly effective in dominating under-ice communities. However, the between-lake differences in quantitative community composition do imply a degree of local selection, and differences between rare taxa suggest some degree of population divergence between lakes. The low OTU richness and dominance by a few common taxa may also reflect the methods used, and it is likely that future studies using high-throughput techniques will allow a more robust analysis of the importance of rare taxa in defining inter-lake differences in community composition.

Cyanobacterial diversity and environmental factors

Canonical correspondence analyses implied that irradiance, DRP and conductivity correlated with the benthic cyanobacterial community structure in the three investigated perennially ice-covered Antarctic lakes. These inferences are to some extent confounded by the fact that relatively few samples are available and that systematic differences exist between lakes (such as Lake Vanda having a much higher irradiance than the other two at all depths and only Lake Joyce having very low DRP), such that correlations with environmental variables may in part reflect between lake differences (and vice versa). Light regime is, however, likely to play a significant role in selecting microbiota. PAR has been previously suggested to influence benthic cyanobacterial communities, and benthic mats under the perennial ice cover have a conspicuous pink appearance because of their high content of phycoerythrin, a light-harvesting pigment facilitating photosynthesis under the dim, blue-green light regime beneath ice (Hawes and Schwarz 1999, 2001). However, the four ribotypes that were associated with high irradiance in PCoA (MDVcy04, 07, 13 and 15) were all found only in Lake Vanda and it is uncertain whether their correlation with PAR is genuinely a function of irradiance or one of biogeography. Only analysis of samples from deeper in Lake Vanda, with reduced irradiance, could discriminate between these two interpretations.

Conductivity has been shown to play a role in the cyanobacterial community composition in benthic mats in Antarctica (Broady and Kibblewhite 1991; Simmons et al. 1993; Jungblut et al. 2005; Taton et al. 2006; Verleyen et al. 2010; Jungblut et al. 2012), and evidence was provided that some cyanobacterial taxa were restricted to a specific range of conductivity (Broady and Kibblewhite 1991; Jungblut et al. 2005; Taton et al. 2006). Mechanisms involved in response to salinity in cyanobacteria include the usage of some organic osmolytes such as glycine betaine to balance extracellular ions (Jungblut and Neilan 2010). This also agrees with the finding that sequences with highest match to Leptolyngbya frigida were not identified in LJ21 (4221 μS/cm), which would agree with Wharton et al. (1983) who suggested salinity, in combination with other environmental variables, could preclude the presence of this cyanobacterial taxa.

Cyanobacterial diversity also showed an apparent relationship with DRP in the water column, though the range of DRP in our data set was narrow. Nutrient limitation is a feature of most Antarctic lakes (Lyons and Finlay 2008), and it was demonstrated previously that Lake Vanda is phosphorus deficient (Vincent and Vincent 1982), whereas Lake Hoare is nitrogen deficient (Priscu 1995). This is consistent with the differences in inorganic N:P ratio in lake water reported here, which in turn implies that Lake Joyce is also more likely to be P than N limited. However, other authors argue that the growth of cyanobacteria in benthic mats may not be nutrient limited, as benthic communities are regarded as accumulators of carbon, nitrogen and phosphorus over time and nutrient concentrations within mat interstitial waters are typically much higher than those in the overlaying water Quesada et al. (2008). At present, our data suggest that DRP may play a role in cyanobacterial selection, but a more comprehensive data set is required to properly address this question.

Implications for cyanobacterial diversity in terrestrial aquatic ecosystems and future climatic-driven environmental change

Environmental change has been documented for terrestrial aquatic ecosystems in the MDV (Bomblies et al. 2001). This has included increased water levels for the MDV lakes over many decades (Hawes et al. 2011, 2013a, b). Increased water levels may lead to the upward movement of photic zones in the MDV lakes as documented for Lake Joyce, where microbialite structures that grow over decade and are fixed to specific location in the lakes have already reduce their rates of phototrophic biomass accumulation and photosynthetic activity (Hawes et al. 2011). However, our data suggest that such chemical and physical characteristics of MDV lakes may have limited impact on the overall cyanobacterial diversity in the MDV. Although relationships between environmental variables and the cyanobacterial assemblages were identified statistically, the cyanobacterial 16S rRNA gene diversity based on relative abundance and phylogenetic distance showed that nearly 50 % of the taxa were shared across MDV lakes, despite differences in environmental conditions. Two factors therefore appear to underpin resilience in MDV lake mat communities.

Firstly, the similarity in the lake cyanobacterial ribotype diversity across broad geographic distances implies that most of the OTUs in the lakes are drawn from the same metapopulation. This may in part be a legacy of previous low lake levels, when dried cyanobacterial mats from littoral zones could have been eroded by strong winds, distributed across the landscape and entered lakes coupled with sedimentation through the thick ice cover (Squyres et al. 1991; Priscu et al. 1998; Jepsen et al. 2010). This mechanism also implies some degree of genetic exchange among lakes. Cyanobacterial assemblages in MDV lakes may also overlap with mats from terrestrial shallow inland meltwater ponds and ice shelf meltwater ponds (Jungblut et al. 2005, 2012) through aerial transport of dried microbial mats (Parker et al. 1982; Michaud et al. 2012). Dispersal of the cyanobacterial communities across the MDV may therefore assist in maintaining cyanobacterial diversity in the McMurdo Dry Valley lakes even as habitats change (Howard-Williams et al. 1989; Michaud et al. 2012). While there is growing evidence of endemism and population structure in Antarctic microbial communities (De Wever et al. 2009; Vyverman et al 2010; Peeters et al. 2011), and the identification of possibly unique sequences in our data supports this growing evidence, dispersal appears to be an effective blending mechanism for cyanobacterial ribotype diversity at least at the scale of the MDV region.

Secondly, our 16S rRNA gene data provide further support that many cyanobacteria have broad environmental tolerance, which agrees with previous reports from Pyramid Trough region, Southern Victoria Land, where cyanobacterial ribotype diversity varied little from conductivities from 65 to 5900 μS (Jungblut et al. 2012). Various physiological traits have been identified in polar cyanobacteria to overcome environmental stress, which potentially might even act as a selection pressure in favour of multitolerant taxa in Antarctic cyanobacteria (Vincent 2000). This, coupled to dispersal, further supports the conclusion that Antarctic cyanobacteria have a high degree of resistance to climate change (Allison and Martiny 2008). Only extreme conditions as seen for very high conductivities (>30,000 μS/cm) have been shown to reduce richness and induce change in taxonomic diversity towards more rare and niche-specific cyanobacterial groups (Jungblut et al. 2005, 2012).

Convergent cyanobacterial community composition in these three lakes thus supports a mix of the two hypotheses that common species are widely distributed within the MDV area and have broad habitat tolerance, in particular, the dominance of most mat communities in geographically separated lakes by a small number of *Leptolyngbya* and *Phormidium* ribotypes. However, it also appears that some rare cyanobacterial taxa show a degree of habitat specificity, including the absence of Nostocales from under-ice habitats and the apparent relationship of community composition with conductivity and DRP.

In summary, the current study evaluated the cyanobacterial diversity in benthic habitats of three McMurdo Dry Valley lakes in Antarctica. We showed that geographically isolated lakes share the majority of their most abundant cyanobacteria, suggesting that dominant lake cyanobacteria are well dispersed within the MDV, and have broad habitat

tolerance. However, rare species can show a degree of localisation and these may have more specific habitat requirements, and we identified underwater PAR, dissolved reactive phosphorus and salinity as environmental factors explaining a large amount of the variations in community composition across lakes. Overall, we conclude that the hypothesis that "taxa with performance optima that best suit a given environment dominate at specific locations and that environmental stratification is mimicked by community distribution" is not supported. Rather, we suggest that our data support the alternate that "taxa with broad habitat tolerances dominate, leading to similarities in taxa assemblage across wide environmental ranges". The present findings help understand how further climates could influence the cyanobacteria in Antarctica. They provide preliminary basis for a testable hypothesis that the broad tolerance and wide distribution of cyanobacteria may confer a degree of resistance and resilience to future climate-driven environmental changes in Antarctic terrestrial aquatic ecosystems.

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Conflict of interest The authors do not have any conflict of interest.

References

Allison SD, Martiny JBH (2008) Resistance, resilience, and redundancy in microbial communities. Proc Natl Am Soc 105:11512–11519

Allnutt FTC, Parker BC, Seaburg KG, Simmons GM (1981) In situ nitrogen (C2H2) fixation in lakes of southern Victoria Land, Antarctica. Hydrobiol Bull 51:99–109

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Andersen DT, Sumner DY, Hawes I, Webster-Brown J, MCKay CP (2011) Discovery of large conical stromatolites in Lake Untersee, Antarctica. Geobiology 9:280–293

Bomblies A, McKnight DM, Andrews ED (2001) Retrospective simulation of lake-level rise in Lake Bonney based on recent 21-yr record: indication of recent climate change in the McMurdo Dry Valleys, Antarctica. J Paleolimnol 25:477–492

Broady P, Kibblewhite A (1991) Morphological characterisation of *Oscillatoria* (Cyanobacteria) from Ross Island and southern Victoria Land, Antarctica. Antarct Sci 3:35–45

De Wever A, Leliaert F, Verleyen E, Vanormelingen P, Van der Gucht K, Hodgson DA, Sabbe K, Vyverman W (2009) Hidden levels of phylodiversity in Antarctic green algae: further evidence for the existence of glacial refugia. Proc R Soc B 276:3591–3599

Fountain AG, Nylen TH, Monaghan A, Basagic HJ, Bromwich D (2009) Snow in the McMurdo Dry Valleys, Antarctica. Int J Climatol 30:633–642

Green WJ, Lyons WB (2009) The saline lakes of the McMurdo Dry Valleys, Antarctica. Aquat Geochem 15:321–348

Hawes I, Schwarz A-M (1999) Photosynthesis in an extreme shade environment: benthic microbial mats from Lake Hoare, a permanently ice-covered Antarctic lake. J Phycol 35:448–459

Hawes I, Schwarz A-M (2001) Absorption and utilization of low irradiance by cyanobacterial mats in two ice-covered Antarctic lakes. J Phycol 37:5–15

Hawes I, Sumner DY, Andersen DT, Mackey TJ (2011) Legacies of recent environmental change in the benthic communities of Lake Joyce, a perennially ice-covered Antarctic lake. Geobiology 9:394–410

Hawes I, Sumner DY, Andersen DT, Jungblut AD, Mackey TJ (2013a) Timescales of growth response of microbial mats to environmental change in an ice-covered Antarctic lake. Biology 2:151–176

Hawes I, Howard-Williams C, Sorrell B (2013b) Decadal variability in ecosystem properties in the ponds of the McMurdo Ice Shelf, Southern Victoria Land, Antarctica on decadal timescales. Antarct Sci 26:219–230

Howard-Williams C, Pridmore R, Downes M, Vincent VF (1989) Microbial biomass, photosynthesis and chlorophyll a related pigments in the ponds of the McMurdo Ice Shelf, Antarctica. Antarct Sci 1:125–131

Howard-Williams C, Schwarz A-M, Hawes I, Priscu JC (1998) Optical properties of the McMurdo Dry Valley lakes. In: Priscu JC (ed) Ecosystem dynamics in a Polar Desert: the McMurdo Dry Valleys, Antarctica, Antarctic research series, vol 72. American Geophysical Union, Washington DC, pp 189–203

Huber JA, Faulkner G, Hugenholtz P (2004) Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 20:2317–2319

Jepsen SM, Adams EE, Priscu JC (2010) Sediment melt-migration dynamics in perennial Antarctic lake ice. Arct Antarct Alp Res 42:57–66

Jungblut AD, Neilan BA (2010) Cyanobacteria mats of the meltwater ponds on the McMurdo Ice Shelf (Antarctica). In: Seckbach J, Oren A (eds) Microbial mats: modern and ancient microorganisms in stratified systems. Cellular origin, life in extreme habitats and astrobiology. Springer, Berlin, pp 499–514

Jungblut AD, Hawes I, Mountfort D, Hitzfeld B, Dietrich DR, Burns BP, Neilan BA (2005) Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environ Microbiol 7:519–529

Jungblut AD, Lovejoy C, Vincent WF (2010) Global distribution of cyanobacterial ecotypes in the cold biosphere. ISME J 4:191–202

Jungblut AD, Wood AD, Webster-Brown J, Harris C (2012) The pyramid trough wetland: environmental and biological diversity in a newly created Antarctic protected area. FEM Microb Ecol 82:356–366

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71:8225–8235

Lozupone C, Hamady M, Knight R (2006) UniFrac—An online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinform 7:371

Lyons WB, Finlay JC (2008) Biogeochemical processes in high-latitude lakes and rivers. In: Vincent WF, Laybourn-Parry J (eds) Polar lakes and rivers: limnology of Arctic and Antarctic aquatic ecosystems. Oxford University Press Inc., New York, pp 137–156

McKay C, Clow G, Andersen D, Wharton R (1994) Light transmission and reflection in perennially ice-covered Lake Hoare, Antarctica. J Geophys Res Oceans 99:20427–20444

Michaud AB, Sabacká M, Priscu JC (2012) Cyanobacterial diversity across landscape units in a polar desert: Taylor Valley, Antarctica. FEMS Microbiol Ecol 82:268–278

Nadeau TL, Milbrandt EC, Castenholz RW (2001) Evolutionary relationships of cultivated Antarctic oscillatorians (cyanobacteria). J Phycol 37:650–654

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 G, Love FG (1982) Removal of organic and inorganic matter from Antarctic lakes by aerial escape of bluegreen algal mats. J Phycol 18:72–78

Peeters K, Hodgson DA, Convey P, Willems A (2011) Culturable diversity of heterotrophic bacteria in Forlidas Pond (Pensacola Mountains) and Lundstrom Lake (Shackleton Range), Antarctica. Microb Ecol 62:399–413

Priscu JC (1995) Phytoplankton nutrient deficiency in lakes of the McMurdo Dry Valleys, Antarctica. Freshw Biol 34:215–227

Priscu JC, Fritsen CH, Adams EE, Giovannoni SJ, Paerl HW, McKay CW, Doran PT, Gordon DA, Lanoil BD, Pinckney JL (1998) Perennial Antarctic lake ice: an oasis for life in a polar desert. Science 280:2095–2098

Quesada A, Vincent WF (2012) Cyanobacteria in the cryosphere: snow, ice and extreme cold. In: Whitton BA (ed) Ecology of Cyanobacteria II: their diversity in space and time. Springer Science & Business Media, Dordrecht, pp 387–399

Quesada A, Fernandez-Valiente E, Hawes I, Howard-Williams C (2008) Benthic primary production in polar lakes and rivers. In: Vincent WF, Laybourn-Parry J (eds) Polar lakes and rivers: limnology of Arctic and Antarctic aquatic ecosystems. Oxford University Press Inc., New York, pp 179–196

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541

Simmons GM, Wharton RA, Vestal JA (1993) Environmental regulators of microbial activity in continental Antarctic lakes. In: Green WJ, Friedmann EI (eds) Physical and Biogeochemical processes in Antarctic lakes. American Geophysical Union, Washington, D.C., pp 165–195

Sogin M, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc Natl Am Soc 103:12115–12120

Squyres SW, Andersen DW, Nedell SS, Wharton RA (1991) Lake Hoare, Antarctica: sedimentation through a thick perennial ice cover. Sedimentology 38:363–379

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web server. Syst Biol 57:758–771

Strunecky O, Komárek J, Johansen J, Lukešová A, Elster J (2013) Molecular and morphological criteria for revision of the genus *Microcoleus* (Oscillatoriales, cyanobacteria). J Phycol 49:1167–1180

Sutherland D, Hawes I (2009) Annual growth layers as proxies of past growth conditions for benthic microbial mats in a perennially ice-covered Antarctic lake. FEMS Microb Ecol 67:279–292

Taton AS, Grubisic E, Brambilla R, Wit De, 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 AS, Grubisic E, Balthasart P, Hodgson DA, Laybourn-Parry J, Wilmotte A (2006) Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. FEMS Microbiol Ecol 57:272–289

ter Braak CJF, Smilauer P (2002) CANOCO reference manual and CanoDraw for Windows User's Guide: software for Canonical Community Ordination (Version 4.5). Microcomputer Power, Ithaca, NY

Varin T, Lovejoy C, Jungblut AD, Vincent WF, Corbeil J (2010) Metagenomic profiling of Arctic microbial mat communities as nutrient scavenging and recycling systems. Limnol Oceanogr 55:1901–1911

Vincent WF (2000) Cyanobacterial dominance in polar regions. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Kluwer Academic Publishers, The Netherlands, pp 321–340

Vincent WF, Vincent C (1982) Factors controlling phytoplankton production in Lake Vanda (77°S). Can J Fish Aquat Sci 39:1602–1609

Vincent WF, Rae R, Laurion I, Howard-Williams C, Priscu JC (1998) Transparency of Antarctic lakes to solar ultraviolet radiation. Limnol Oceanogr 43:618–624

Vincent WF, Maclntyre S, Spigel RH, Laurio I (2008) The physical limnology of high-latitude lakes. In: Vincent WF, Laybourn-Parry J (eds) Polar lakes and rivers: limnology of arctic and antarctic aquatic ecosystems. Oxford University Press Inc., New York, pp 65–81

Vyverman W, Verleyen E, Wilmotte A, Hodgson DA, WillemA Peeters K, Van de Vijver B, De Wever A, Leliaert F, Sabbe K (2010) Evidence for widespread endemism among Antarctic microorganisms. Polar Sci 4:103–113

Wharton RA, Parker BC, Simmons GM (1983) Distribution, species composition and morphology of algal mats in Antarctic Dry Valley lakes. Phycologia 22:355–365

Wharton RA, McKay C, Clow G, Andersen D (1993) Perennial ice covers and their influence on Antarctic lake ecosystems. Antarc Res Ser ANTSA 4:53–70

Zakhia F, Jungblut AD, Taton A, Vincent WF, Wilmotte A (2008) Cyanobacteria in cold ecosystems. In: Margesin R, Marx JC, Gerday C (eds) Psychrophiles from biodiversity to biotechnology. Springer, Berlin, pp 121–135

Zapata M, Rodriguez F, Garrido JL (2000) Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using reversed phase C8 column and pyridine-containing mobile phases. Mar Ecol Prog Ser 195:29–45