Running Title: Antarctic microbial mats and climate change

The structuring role of climate-related environmental factors on Antarctic microbial mat communities

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ABSTRACT

Both ground based and satellite data show that parts of Antarctica have entered a period of rapid climate change, which already impacted on the functioning and productivity of limnetic ecosystems. In order to predict the consequences of future climate anomalies for lacustrine microbial communities, we not only need better baseline information on their biodiversity but also on the climate-related environmental factors structuring these communities. Here we applied Denaturing Gradient Gel Electrophoresis (DGGE) of the SSU rDNA to asses the genetic composition and distribution of cyanobacteria and eukaryotes in 37 benthic microbial mat samples from East Antarctic lakes. The lakes were selected to span a wide range of environmental gradients governed by differences in lake morphology and chemical limnology across five ice-free oases. Sequence analysis of selected DGGE bands revealed a high degree of potential endemism among the cyanobacteria (mainly represented by Oscillatoriales and Nostocales), and the presence of a variety of protists (alveolates, stramenopiles, and green algae), fungi, tardigrades and nematodes, which corroborates previous microscopy-based observations. Variation partitioning analyses revealed that the microbial mat community structure is largely regulated by both geographical and local environmental factors of which salinity (and related variables), lake water depth, and nutrient concentrations are of major importance. These three groups of environmental variables have previously been shown to change drastically in Antarctica in response to climate change. Together, these results have obvious consequences for predicting the trajectory of biodiversity under changing climate conditions and call for the continued assessment of the biodiversity of these unique ecosystems.

Key words: Antarctica, climate change, lake, microbial mats, DGGE

INTRODUCTION

Both ground based and satellite data show that parts of Antarctica have entered a period of rapid climate change (Steig et al. 2009). In some regions such as the Antarctic Peninsula, temperatures are rising at 0.55°C per decade, which is six times the global mean. This warming trend has already had a detectable impact on the cryosphere; eighty seven percent of Antarctic Peninsula glaciers have retreated in the last 60 years (Cook et al. 2005) and >14 000 km^2 of ice shelves have collapsed (Hodgson et al., 2006a), with some of the disintegration events being unprecedented during the past 11,000 years (Domack et al. 2005). Other regions in Antarctica are, in contrast, showing a rapid net cooling trend, such as the McMurdo Dry Valleys, where temperature dropped by 0.7°C per decade between 1986 and 2000 (Doran et al. 2002). In East Antarctica many regions are similarly experiencing marked changes in their weather, including increased wind speeds (Gillett & Thompson 2002) and changing patterns of snow and ice accumulation (Hodgson et al. 2006b).

The recent temperature and climate anomalies have also had impacts on both terrestrial and marine ecosystems in the Antarctic (Walther et al. 2002). Experiments measuring the ecological changes occurring at inland nunataks, dry valleys, and coastal ice-free areas, have likened these ecosystems to ‘canaries in a coalmine’ and ‘natural experiments’ with which to identify biological responses to changing climate variables that are applicable on a wider (global) scale (see Convey 2001, Robinson et al. 2003, Lyons et al. 2006 for reviews). Already lacustrine ecosystems in some ice free regions have been shown to respond quickly to air temperature variability. For example, long term monitoring of maritime Antarctic lakes between 1980 and 1995 has revealed extremely fast ecosystem changes associated with increased nutrient concentrations and primary production in response to climate warming (Quayle et al. 2002). In East Antarctica, paleolimnological analyses of three lakes in the Windmill Islands have revealed a rapid salinity rise during the past few

74 decades, which has been linked to regional increases in wind speed and enhanced evaporation
75 and sublimation of water and ice from the lakes and their catchments (Hodgson et al. 2006b).
76 Conversely, the long-term cooling trend in the McMurdo Dry Valleys resulted in lake level
77 fall, increased lake-ice thickness, and decreased primary production (Doran et al. 2002). A
78 short episodic warming event during the Austral summer of 2001-2002 reversed these
79 environmental changes and altered the biogeochemistry of the lakes (Foreman et al. 2004).
80 The most obvious feature of almost all lakes in polar oases is the extensive benthic
81 microbial mats, which develop in the absence or rarity of grazers, and often dominate primary
82 production (Ellis-Evans et al. 1998, Fig.S1). In order to be able to predict the effects of future
83 climate and concomitant environmental changes on these benthic microbial mats we not only
84 need better baseline information on their biodiversity, but also on the environmental factors
85 structuring their communities. This information is becoming available for soil and lake
86 bacterial communities (e.g., Pearce 2005, Yergeau et al. 2007), but is still largely lacking for
87 autotrophic biota inhabiting limnetic ecosystems. What is known comes from regional diatom
88 inventories (Verleyen et al. 2003, Gibson et al. 2006a), local biodiversity assessments (e.g.,
89 Jungblut et al. 2005) and surveys of the surface pigment composition, for example in east
90 Antarctic lakes (Hodgson et al. 2004), which revealed that lake water depth (and lake ice
91 dynamics and light climate related variables such as turbidity), salinity and nutrient
92 concentration are the most important environmental variables structuring the microbial
93 communities. However, it is still unclear which factors influence the taxonomic composition
94 of those microorganisms which are difficult to identify to species level by microscopy, such
95 as the cyanobacteria and green algae (Vincent 2000, Taton et al. 2003, Unrein et al. 2005).
96 These data are however urgently needed, because these organisms (particularly cyanobacteria)
97 not only constitute the bulk of the biomass in most Antarctic lakes (Broady 1996), but also
98 include a large number of endemics (e.g., Gibson et al. 2006b, Taton et al. 2006a, b).

Cyanobacteria further efficiently recycle nutrients, and form the fabric of the microbial mats in which fungi, protists and other bacteria are embedded (Vincent et al. 1993).

Here we used Denaturing Gradient Gel Electrophoresis (DGGE), a culture independent molecular fingerprinting technique to analyse the genetic diversity of 37 microbial mat samples inhabiting 26 lakes in different ice-free regions of East Antarctica and the Ross Sea region, including the McMurdo Dry Valleys and four ice-free oases in the Prydz Bay region, namely the Vestfold Hills, the Larsemann Hills, the Bølingen Islands and the Rauer Islands (see Fig.1 for a map). The lakes were selected to span a wide range of environmental gradients (see Table 1 for the data measured), which are governed by lake morphometry and chemical limnological factors. We aimed to assess the importance of these different environmental factors in structuring the genetic composition of cyanobacteria and eukaryotes inhabiting the microbial mat communities in these climate sensitive water bodies.

MATERIALS AND METHODS

Study sites

The McMurdo Dry Valleys (DV, 77°00'S-162°52'E) consist of three main valleys (Taylor, Wright and Victoria Valley) located on the West coast of McMurdo Sound and are the largest relatively ice-free area (approximately 4800 km²) in Southern Victoria Land (Fig.1). The perennially ice-covered lakes, ephemeral streams and extensive areas of exposed soil within the DV are subject to limited precipitation and limited salt accumulation.

The Vestfold Hills (VH, 68°30' S-78°00' E) form a 400 km^2 ice-free area on the Prydz Bay (PB) coast and consist of three main peninsulas (Mule, Broad and Long Peninsula) and a number of offshore islands (Fig.1). Over 300 lakes with varying limnological properties are found in the region, many of which have been intensively studied (Laybourn-Parry 2003). The Larsemann Hills (LH, 69^{o}23’ S-76^{o}53’ E) in PB is a 50 km^2 large ice-free area located

approximately midway between the eastern extremity of the Amery Ice Shelf and the southern boundary of the VH. The region consists of two main peninsulas (Stornes and Broknes), together with a number of scattered offshore islands. More than 150 lakes are found in the LH. The lakes are mainly fresh water and range from small ephemeral ponds to large water bodies (Gillieson et al. 1990). The Bølingen Islands (BI, 69°30'S – 75°50'E) is a smaller ice-free archipelago in PB, which is situated approximately 15 km to west-south-west of the LH and north of the Publications Ice Shelf. The BI includes two medium-sized islands (> 1km^2), and numerous minor islands. Seven shallow lakes and ponds are found in the region of which four have been analysed for pigment and diatom community structure (Sabbe et al. 2004, Hodgson et al. 2004). The Rauer Islands (RI, 68°50' S - 77°45'E) are an ice-free coastal archipelago in PB, situated approximately 30 km away from the VH, and includes 10 major islands and promontories together with numerous minor islands covering a total area of some 300 km^2. A detailed description of the RI and of the microbial communities inhabiting 10 out of more than 50 shallow lakes and ponds are given in Hodgson et al. (2001).

Sampling

Microbial mats from the littoral and/or deep spot within the oxygenated euphotic zone in the stratified lakes in the VH and the DV were sampled during the Austral summer of 1999 using a custom-made scoop. Samples in the LH, BI and RI were taken manually from the littoral zone in shallow lakes (< 2m), and using a Glew gravity corer from the deepest spot in the deep lakes during the Austral spring and summer of 1997-1998. Replicates were taken in the littoral and deeper (yet still oxygenated) parts of some lakes from the VH and LH in order to account for microhabitat heterogeneity (Table 1). All the samples were frozen in the field and kept frozen at -20°C prior to analysis.

DNA extraction, PCR, DGGE and DGGE band sequencing protocols

Nucleic acid extraction

Nucleic acids were extracted using a combined mechanical-chemical method. One gram of mat material, 0.5 g of zirconium beads (0.1 mm diameter), 0.5 ml 1X TE buffer, pH 8 (10 mM Tris, pH 7.6, 1 mM EDTA) and 0.5 ml buffered phenol (pH 7 to 8) were added to a 2 ml eppendorf tube which was shaken 4 times at high frequency (30 times/s) during 1.25 min with intermittent cooling on ice. After 5 min centrifugation at 10,000 rpm, the aqueous supernatant was extracted twice with phenol-chlorophorm-isoamylalcohol (25:24:1 v/v). The DNA in the aqueous phase was precipitated (commercial solution of 1/10 v of 3 M sodium acetate pH 5, 2 v/v of 96 % ethanol and 3 µl glycogen; Boehringer Mannheim), concentrated (30 min centrifugation after overnight storage at -20ºC) and washed (1 ml of 70 % ethanol was added to the pellet and centrifuged for 5 min at 13000 rpm). The ethanol was removed and the pellet was air-dried for 20 min. The DNA was purified after resuspension in 50 µl of 1X TE at 55°C and incubation for 20 min at 55°C according to the protocol of the wizard DNA clean-up Kit (Promega). Template DNA was stored at -20°C.

Polymerase Chain Reaction (PCR)

16S rRNA gene fragments that were 422 bp long were generated by seminested PCR, as described by Boutte et al (2006). The primers used for the first PCR were 16S378F and 23S30R (Table 2). PCR amplification was performed in a 50 µl (total volume) reaction mixture containing 0.5µl of mat DNA, 1X Super Taq Plus PCR buffer, the deoxynucleoside triphosphate at a concentration of 0.2 mM, 0.5µM primer 16S27F (Table 2), 0.5µM primer 23S30R (Table 2), and 1 mg of bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) ml-1, and 1 U of Super Taq Plus polymerase with proofreading activity (HT Biotechnology, Cambridge, United Kingdom). Amplification was carried out with a Gene Cycler (Bio-Rad,

Hercules, Calif.) as follows: incubation for 5 min at 94°C, followed by 30 cycles of 45 s at 94°C, 45 s at 54°C, and 2 min at 68°C and then a final elongation step of 7 min at 68°C. The resulting PCR products (0.5µl) served as templates for the second PCR, which was performed with forward primer 16S378F and reverse primers 16S781R(a) and 16S781R(b) (Table 2), which, respectively, target filamentous cyanobacteria and unicellular taxa (Boutte et al. 2006). A 38-nucleotide GC-rich sequence was attached to the 5' end of each of the reverse primers. The reaction conditions were the same as those described above except that amplification was carried out as follows: incubation for 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 68°C and then a final elongation step of 7 min at 68°C. Two distinct reactions were performed for each reverse primer. The negative control for the first PCR was used in the second PCR to check for contamination.

A eukaryotic 18S rDNA fragment of approximately 260 bp was amplified using the universal eukaryote specific primers GC1 and GC2 designed by Van Hannen et al. (1998; Table 2). The 50 µl reaction mixture contained 100 ng of template DNA, 10X PCR-buffer (Perkin Elmer), 20 mM $MgCl_2$, 0.5 µM of each primer, 4 mM of each deoxynucleotides, 10 µg/µl of bovine serum albumin, and 2.5 U DNA Polymerase (AmpliTaq; Perkin Elmer) and sterile water (Sigma) to adjust the final volume. A touchdown PCR amplification was performed using a Tgradient cycler (Biometra) with the following conditions: 94°C for 5 min followed by 20 cycles of 94°C for 1 min, 65°C for 1 min (this temperature was decreased every cycle by 0.5°C until the touchdown temperature of 55.5°C was reached), 72°C for 1 min, 5 additional cycles were carried out at an annealing temperature of 55°C, and a final extension step of 72°C for 10 min. The size of the amplified DNA was estimated by analysing 5 µl of PCR product on 1.5 % agarose gel, staining with ethidium bromide and comparing it to a molecular weight marker (Smart-Ladder; Eurogentec).

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE of the cyanobacterial SSU rDNA fragments was carried out following the protocol of Nübel et al. (1997) with a Dcode System (Bio-Rad). The PCR products obtained with two different primers 16S781R(a) and 16S781R(b) were applied separately onto a 1 mm thick 6% polyacrylamide gel. The gel contained a linear 45 to 60% denaturant gradient (100 % denaturant corresponded to 7 M urea and 40 % (v/v) formamide). The pH of the TAE buffer was adjusted to 7.4, and electrophoresis was performed for 16 h at 45 V and 60°C.

DGGE of the eukaryotic SSU rDNA fragments was performed as described by Muylaert et al. (2002). Full PCR products were loaded onto a 1 mm thick 8 % (wt/v) polyacrylamide gels in 1X TAE (20 mM Tris-acetate pH 7.4, 10 mM acetate, 0.5 mM disodium EDTA). The denaturing gradient contained 30 to 55 % denaturant. The pH of the TAE buffer was adjusted to 7.4, and electrophoresis was performed for 16 h at 75 V and 60°C.

On each gel, we ran three standard lanes (samples from temperate lakes) in parallel with the study samples in order to aid the alignment of the bands. The DGGE gels were stained with ethidium bromide and photographed on a UV transillumination table with a charge-coupled device camera. Automatic band matching using standard settings and manual inspection of the band-classes was performed using the Bionumerics 5.1 software package (Applied Maths BVBA, Belgium).

DGGE bands sequence determination and analysis

The cyanobacterial DGGE bands that could be properly cut out were excised with a surgical scalpel rinsed with ethanol on a UV transillumination table. Each small gel block was placed in 100 µl of sterile water for 2 h at room temperature. This solution was used as a template for PCR amplification as described above (second PCR). Sequencing was carried out using the primer 16S784R (derived from Nübel et al. 1997; Table 2) by GenomeExpress (Paris, France)

with an ABI PRISM system 377 (PE Applied Biosystems, Foster City, CA, United States). Chimera detection was performed by using Check Chimera in the Ribosomal Database Project (Maidak et al. 2001).

Eukaryotic DGGE bands with more than 40% relative band intensity in at least two samples were selected for sequencing. These bands were excised and sequenced after re-extraction and amplification. Sequencing was performed with the ABI-Prism sequencing kit (PE Biosystems) using the primer GC3 (5'-TCTGTGATGCCCTTAGATGTTCTGGG-3') and an automated sequencer (ABI-Prism 377).

A nucleotide BLAST search (Altschul et al. 1998) available at the NCBI website was performed in order to obtain sequences that were most similar. New sequence data were deposited in the GenBank database. 43 Partial 16S rDNA gene sequences of cyanobacteria were deposited under the accession numbers EU009658, EU009659, EU009664 to EU009666, EU009668, EU009674 to EU009679, EU009681 to EU009685, EU009689 to EU009695, EU009698, EU009699, EU009701, EU009703, EU009705, EU009706, EU009709 to EU009717, EU009719, EU009721 to EU009723 and the 22 partial 18S rDNA eukaryotic sequences under the accession numbers EU004828 to EU004849 (Table S1).

Multivariate analysis

Two biotic matrices were developed and consist of the presence absence data of the DGGE data obtained using universal eukaryotic and cyanobacteria specific primers (Table 2). The datasets of the cyanobacteria identified using the two different primers were combined into one single matrix as both primers were shown to target different cyanobacterial groups (i.e. unicellular versus filamentous taxa) and allow a more complete assessment of the diversity of the cyanobacterial flora (Boutte et al. 2006). The correlation coefficient between the number of bands obtained using each primer was calculated in Statistica 6.0 in order to assess the

amount of overlap between both primers. If the correlation coefficient is low or insignificant, both primers likely target different members of the cyanobacterial community.

In order to assess the amount of within-lake variability in the genetic composition of the lakes in relation to the entire variability in these biotic matrices we applied cluster analysis (Bray-Curtis, group average) using the computer programs PC-ORD 4.32 (McCune & Mefford 1999). In order to identify those factors that structure the genetic composition of cyanobacteria and eukaryotes in our studied Antarctic water bodies we applied direct ordination analyses using the program CANOCO 4.5 for Windows (ter Braak & Smilauer 2002). Five different matrices were used: the two biotic incidence matrices, a matrix with the environmental data, one with geographical factors and one representing the date of sampling. The matrix with the geographical variables was created because dispersal and migration have recently been shown to be important in structuring microbial communities on a regional Antarctic (Verleyen et al. 2003) and a global scale (Vyverman et al. 2007; Verleyen et al. 2009). The matrix with the date of sampling was included as Lake Fryxell was sampled during the late austral summer, whereas the other lakes were sampled during the late Austral spring or early summer which might potentially influence their taxonomic composition. Below we detail how these matrices where developed.

The environmental matrix contains 12 limnological variables (Table 1). Samples for the analysis of nutrients and major ion concentrations were taken during the field campaigns described above for the majority of the lakes (LH, BI and RI) and are extracted from Sabbe et al. (2004) and Hodgson et al. (2001, 2004). For the lakes in the VH and Lake Fryxell the environmental variables were extracted from Roberts & McMinn (1996) and Green et al. (1989) and in these cases were not measured at the same time as the sampling of the microbial mats. The seasonal matrix contained the ordinal date of sampling, with negatives values denoting dates before January. The matrix with the geographical factors consists of the

eigenvectors corresponding to the positive eigenvalues (V1-V3) after principal coordinate analysis of a truncated matrix of the geographic distances among the sampling sites (Borcard and Legendre 2002), which approximates the connectivity between sites. This approach was recently shown to be the proper method to test the importance of geographical variables in explaining turnover patterns in communities (Jones et al. 2008).

First a principal component analysis (PCA) of the standardized and centred environmental variables was applied in order to assess correlations between environmental variables and to reveal whether environmental properties varied between the lakes in different ice-free regions. We subsequently applied indirect and direct ordinations on the biotic data. Detrended correspondence analyses (DCA), with detrending by segments, were used to determine the length of the gradient in the biotic data sets. The length of gradient of the first axes equalled 4.352 and 3.957 for the cyanobacteria and 3.540 and 6.185 for the eukaryotes respectively, implying that unimodal ordination methods are most appropriate (ter Braak & Smilauer 2002). Canonical correspondence analysis (CCA) with forward selection of log-transformed environmental factors and unrestricted Monte Carlo permutation tests (999 permutations, $P \leq 0.05$) was used to select the minimal number of variables explaining the largest amount of variation in the biotic data. The relative contribution of the environmental variables to the ordination axes was evaluated by the canonical coefficients (significance of approximate t-tests) and intraset correlations (ter Braak & Smilauer 2002). Variance inflation factors were used to construct the most parsimonious model. In CCAs the ordination axes are dependent on the selected environmental variables; different samples derived from the same lake (i.e. with the same environmental variables) are therefore forced to cluster together. In order to assess differences in the occurrence of the DGGE bands between (and within) the lakes independently from environmental variability between the water bodies, CAs were run

with the significant environmental variables, selected by the CCAs, as supplementary (passive) variables.

Variation partitioning analysis (cf. Borcard et al. 1992) was subsequently used to assess the unique contribution of the environmental, geographical and seasonal variables in structuring the microbial communities (Laliberté 2008). The forward selection procedure using Monte Carlo Permutation tests (999 permutations) in CANOCO 4.5 was used to select only those variables (geographical, seasonal and environmental variables selected separately) that significantly explain variation in DGGE band occurrence between the lakes. The variation partitioning analyses results in 8 fractions if at least one variable is significant in each of the different factor classes, namely (1) the unique effect of geographical variables, (2) the unique effect of environmental variables, (3) the unique effect of seasonal variables, and the combined variation (4-7) due to joint effects of (1) and (2), (2) and (3), (1) and (3), and the three groups of variables combined, and (8) the unexplained variation in DGGE band patterns. Monte Carlo permutation tests (999 permutations) were used to assess the significance of the ordination axes in each model.

RESULTS

Environmental properties

Our dataset contains water bodies ranging from small shallow ponds to deep and large lakes (z-max between 0.7 and 39m; lake area between 0.27 and 708 ha) and spans a wide salinity gradient from freshwater to hypersaline (between 0.1 and 140 ppt; Table 1). PCA of the standardized and centred environmental variables revealed that the environmental diversity is mainly structured by conductivity-related variables (major ions and salinity), morphological variables (lake depth and area) and nutrient concentrations (NO_3-N and PO_4-P; Fig.2); PO_4-P is important on the third axis (figure not shown) and discriminates the relatively nutrient rich

Firelight Lake in the BI from the other sites. The four axes explain 93% of the total variance; the first, second and third axes explain 63%, 17% and 8% respectively. The salinity gradient is important along the first axis and negatively correlated with altitude. Geographic differences in environmental properties are present; saline lakes are mainly restricted to the RI and the nearby VH, whereas freshwater lakes dominate in the LH and the BI. Lake depth is important along the second axis, with the lakes in the VH and Lake Fryxell in the DV being larger and deeper than the shallow ponds in the RI and the generally smaller and shallower lakes and ponds in the LH and BI.

Molecular richness and community composition

An average of 13 DGGE bands per sample was found using both cyanobacteria specific primers, with a maximum of 24 (Sunset Lake in the BI) and a minimum of 6 (Lake Sibthorpe in the LH and Highway Lake in the VH). The use of both primers allowed a more complete assessment of the cyanobacterial diversity. The relationship between the molecular richness obtained using both primers is not significantly correlated (R^2_{Adj}=-0.03, *P=0.984*) implying that both primers are complimentary, which is in agreement with Boutte et al. (2006). Most bands were relatively rare; over 50% of the bands occurred only in 1 or 2 samples. Only 2 bands occurred in over 50% of the samples, which were generally derived from saline lakes. Another 5 bands occurred in over 25% of the samples.

The average yield of DGGE bands per sample using the universal eukaryotic primer was 15. The maximum number of bands was 29 (Highway Lake in the VH) whilst only 1 band was observed in a hypersaline lake in the RI. Over 30% of the bands occurred in one or two samples. Only 4 bands occurred in 25% or more of the samples.

Sequence analysis of the DGGE bands and a subsequent BLAST search revealed the presence of a variety of protists (alveolates, stramenopiles, unicellular green algae), fungi,

tardigrades, and nematodes among the eukaryotes (Table S1). For the cyanobacteria many representatives of *Leptolynbya* and *Nostoc* were found. Interestingly, a large number of the closest relatives of the cyanobacteria sequences in BLAST (in % similarity) were sequences which are currently only reported from Antarctica and can thus be considered as potential endemics. The sequences related to Nostocales did not follow this general trend, and have closest relatives with sequences reported from outside Antarctica and can thus be considered to have a cosmopolitan distribution. A picocyanobacterial sequence (*Synechococcus* sp.) was found in the cyanobacterial mat of Firelight Lake. This taxon might however be derived from the pelagic zone as a relatively well-developed planktonic community was observed in this lake, likely due to the high phosphorous concentrations, present as a result of nutrient input from the excreta of snow petrels nesting in the catchment (Sabbe et al. 2004).

No potential endemism was found for the eukaryotic sequences, as most of the sequences or operational taxonomical units (OTU) had a high sequence similarity to genotypes found in various regions. Yet, one of the OTUs (E70.3) had the highest sequence similarity to *Chlamydomonas raudensis* isolated from Lake Bonney in the McMurdo Dry Valleys.

Patterns in microbial community structure

The variability in taxonomic composition between lakes was assessed using CA and cluster analysis (Fig. S2 and S3). The results of both methods are comparable. In the CA biplot of the cyanobacteria the saline lakes from the RI and VH are situated on the right side of the diagram, whereas the generally shallower and freshwater lakes from the LH and BI are plotted on the left side (Fig.3). The relatively low amount of sequences prevents us from identifying those bands underlying the differences in cyanobacterial community composition. One of the bands generally found in saline lakes appeared to be related to *Leptolynbya*. The differences

between samples from the same lake are small relative to the variability between lakes; the multiple samples from Highway Lake, Lake Pendant and the majority from Ace Lake are highly similar and grouped in well-defined clusters (Fig. S2). However, ordination and cluster analyses revealed that 2 samples from Ace Lake (one of which is a littoral sample; Table 1) and the two samples from Lake Reid and Ekho Lake are clearly separated.

CA of the eukaryote DGGE band patterns revealed that the saline lakes from the RI are situated on the positive side of the second axis (except R02; Fig.4). The freshwater lakes from the LH and the BI are generally situated on the right side of the first axis in the CA biplot, whereas the lakes from the VH are clustered along the left side of this axis, which is negatively correlated with the concentration of the major ions and NO_3-N. Although a relatively small amount of DGGE bands were sequenced, some general observations can be made regarding the taxonomic composition of the eukaryotic communities. Fungi belonging to the Basidiomycota and Ascomycota occur in almost every lake (except L. Fryxell). The lakes in the VH are characterised by the presence of ciliates belonging to the Spirotrichea and Colpodea and a pennate diatom, which is virtually absent in the other lakes (Table S1; Fig. 4). The lakes in the LH are, in contrast, characterised by the presence of Tardigrades belonging to the Macrobiotidae, which are virtually absent in the studied water bodies from the other regions (except Ekho Lake in the VH). Green algae are widespread in every region and largely dominated by taxa belonging to the Chlamydomonadales, yet a difference in species composition is present between the saline (RI and VH) and freshwater (BI and LH) lakes. Members of the Ulvophyceae are generally more abundant in the VH lakes and rare in the lakes from the RI and LH. The within-lake variability is similarly low in the eukaryotic dataset, except for the samples from Lake Reid, one littoral sample from Pendant Lake and two samples from Ace Lake, which belong to different groups than the other samples from these particular lakes in the cluster analysis (Fig.S3).

CCA with forward selection and unrestricted Monte Carlo permutation tests of the cyanobacteria dataset revealed that sulphate (positively correlated to salinity and the other major ions; Fig. 2)), NO_3-N, and lake water depth significantly explain 10.9% of the variation in DGGE bands in the different lakes. CCA of the eukaryote data revealed that variation in the DGGE band patterns is best explained by SO_4, NO_3-N, chloride and calcium concentration and altitude. The latter is negatively correlated with salinity related variables (Fig. 2) as the PB lakes, which are situated below c. 10 m, have mostly been isolated from the sea due to isostatic uplift (Verleyen et al. 2005) and therefore in general are more saline. Combined, the environmental variables explain 19.9% of the eukaryote DGGE band patterns. The variance inflation factors were low (< 11 for all variables) in the final models, implying that parsimonious models were selected. The species-environment correlation for all the axes is relatively high in both datasets despite the small amount of variation explained (> 90% in both datasets).

Variation partitioning analysis

Variation partitioning analyses allowed us to statistically assess the unique contribution of environmental versus geographical and seasonal variables in explaining differences in the occurrence of the DGGE bands in the lakes (Fig. 5). The seasonal variable was only selected in the eukaryote dataset in the forward selection procedure. However, it failed to explain a significant unique part of the variation in community structure after accounting for the environmental and geographical variables. The environmental variables explained 16.9% and 9.1% of the total variance, independent of the geographical and seasonal variables in the eukaryote and cyanobacteria datasets respectively (all ordination axes were significant at $P \leq 0.01$ in both models). The geographical variables were less important and explained 10% and 5.8% of the total variance independent of the environmental and seasonal variables in the

eukaryote and cyanobacteria dataset respectively [all ordination axes were significant at $P \leq 0.05$ in the eukaryote dataset, but marginally insignificant in the cyanobacteria dataset ($P = 0.078$ for all four ordination axes together)]. These results imply that although environmental variables are more important than geographical factors, the latter partly underlie differences between the microbial communities of the different ice-free oases, independent of environmental and seasonal factors. In addition, geographical factors are apparently more important in structuring eukaryote communities compared with cyanobacterial communities at the SSU rDNA level.

DISCUSSION

Although our dataset contains only 26 lakes, and not all environmental (e.g. pH) and biological (e.g. biotic interactions) variables were measured, we are confident that it covers the most important ecological gradients known to structure east Antarctic lacustrine communities, namely salinity (Gibson et al. 2006a) and lake water depth and related variables, such as light regime and the amount of physical disturbance by lake ice (Fig. S1; Verleyen et al. 2003, Sabbe et al. 2004). Furthermore our dataset contains the most abundant lake types known to occur in these Antarctic ice-free oases, when water bodies are classified according to their geomorphological origin (i.e., glacial lakes formed in hollows during ice recession versus isolation basins formed as a result of postglacial isostatic rebound). Although not exactly known for each water body, lake age is similarly highly variable and ranges from >120,000 years (Hodgson et al. 2005) to c. 2000 years (Verleyen et al. 2004a, b). Apart from epishelf lakes (Smith et al. 2006) and sub- and supraglacial water bodies (e.g., Hawes et al. 1999, Siegert et al. 2005), our dataset thus likely spans much of the environmental gradient in this region, implying that our results can be cautiously extrapolated to the East Antarctic biogeographical province.

448 Sequence analyses and BLAST searches revealed that the cyanobacteria genera
449 *Leptolyngbya* and *Nostoc*, and eukaryotes belonging to different taxonomic groups, such as
450 alveolates, stramenopiles (e.g. diatoms), green algae, fungi, tardigrades, and nematodes
451 dominate the microbial mat communities. Our taxonomic inventory corroborates previous
452 phenotype-based (e.g., Vinocur & Pizaro 2000, Sabbe et al. 2004) and genetic assessments
453 (e.g. Taton et al. 2006b; Jungblut et al. 2005), and autotrophic community composition
454 fingerprinting studies based upon HPLC analysis of photosynthetic pigments (e.g. Hodgson et
455 al. 2004). However, our molecular methods enabled, for the first time, a more accurate and
456 relatively complete assessment of the biodiversity at a lower taxonomic level for some groups
457 than is usually achieved using traditional microscopy (e.g., Vincent 2000, Unrein et al. 2005).
458 This is particularly the case for the green algae and cyanobacteria, which dominate these
459 ecosystems (Fig. S1) and constitute much of the structural fabric of the microbial mats and
460 thus provide the habitat for the other inhabiting biota (Broady 1996). The improved
461 performance of these methods becomes clear when our results are compared with
462 microscopy-based taxonomic inventories. For example in the lakes from the Larsemann Hills,
463 a total of 89 bands were found using our cyanobacteria specific primers. Although some
464 different bands might represent the same OTU as a result of the presence of ambiguities in the
465 sequences, this number clearly exceeds the number of phenotypes (27) present in a taxonomic
466 inventory of the same lakes based upon light microscopic observations (Sabbe et al. 2004). In
467 addition, the superiority of molecular methods in analysing cyanobacterial biodiversity
468 corroborates a polyphasic study of 59 strains isolated from a set of Antarctic lakes, where a
469 total of 21 OTU belonged to 12 cyanobacterial phenotypes (Taton et al. 2006b).
470 Interestingly, 23% of the new cyanobacterial sequences have no relatives in GenBank
471 from non-Antarctic environments that share more than 97.5% of similarity in sequence data.
472 In particular sequences from *Leptolynbya* were generally most closely related to sequences

473 which are restricted to Antarctica. The Nostocales were in contrast largely related to
474 sequences derived from other regions. The observed provinciality here is in agreement with
475 various studies that reported a relatively high number of potential Antarctic endemics (e.g.,
476 Taton et al. 2003, 2006a, b, Jungblut et al. 2005). Restricted distribution patterns are however
477 absent in the eukaryotic dataset. This is however likely due to the fact that the SSU rDNA is
478 insufficient to discriminate to the species level because of its low taxonomic resolution. In
479 fact, previous studies reported a relatively high number of endemics belonging to a variety of
480 eukaryotic taxonomic groups (Barnes et al., 2006, Gibson et al. 2006b), such as diatoms
481 (Sabbe et al. 2003, Esposito et al. 2006), nematodes (Bamforth et al. 2005), ciliates (Petz et al.
482 2007), mites and springtails (Convey & Stevens 2007), flagellates (Boenigk et al. 2006) and
483 recently also green algae (De Wever et al. 2009).
484 The high number of rare bands in our dataset (particularly among the cyanobacteria)
485 corroborates recent findings based upon the molecular analysis of 4 contrasting Antarctic
486 lakes where 20 out of the 28 cyanobacterial OTU occurred in only one site (Taton et al.
487 2006a). The abundance of singletons and doubletons might be related to various factors, yet
488 does not necessarily mean that organisms are restricted to particular lakes as DGGE is known
489 to potentially suffer from methodological artefacts (e.g. Boutte et al. 2006) and is unlikely to
490 detect sequences present in low abundances (e.g., Muyzer et al. 1993, Fromin et al. 2002).
491 The restricted distribution patterns thus need to be confirmed using state-of-the art molecular
492 techniques such as QRT-PCR (Ahlgren et al. 2006) and dot-blot hybridization (Gordon et al.
493 2000), which allow the detection of sequences present in low quantities. Despite these
494 methodological problems, the rarity of a large number of bands suggests that at least the
495 dominance of the various taxa is different between the lakes. Fungi and green algae belonging
496 to the Chlamydomonadales are present in the majority of the lakes, yet different sequences
497 were obtained in saline versus freshwater lakes. In addition, tardigrades seem to be largely

498 restricted to the freshwater lakes from the Larsemann Hills, whereas they are absent or too
499 rare to be detected in the saline water bodies. Salinity appears thus to be the main
500 environmental variable in structuring these communities. Importantly, together with the other
501 variables significantly explaining differences in taxonomic composition, such as lake water
502 depth (Doran et al. 2002, Foreman et al. 2004) and nutrient concentrations (Quayle et al.
503 2002), salinity (and related variables; Hodgson et al., 2006b) have previously been shown to
504 change drastically in response to climate changes. Although within-lake dissimilarities are
505 present, and likely related to the origin of the samples (i.e. littoral samples are clustered apart
506 from their deep water counterparts), we cannot assess the importance of sample depth as it
507 was not systematically recorded during sampling. Despite the observed within-lake
508 variability, the environmental factors significantly explain part of the variation in DGGE band
509 patterns. This corroborates previous findings in particular taxonomic groups, such as diatoms
510 studied at the morphospecies level in east and maritime Antarctic lakes (e.g., Jones et al.
511 1993, Verleyen et al. 2003, Sabbe et al. 2004, Gibson et al. 2006a) and cyanobacteria
512 genotypes in supraglacial meltwater ponds on the McMurdo Ice Shelf (Jungblut et al. 2005)
513 whose community structure exhibited a close relationship with environmental factors. HPLC
514 analysis of the photosynthetic pigment composition in east Antarctic microbial mats similarly
515 revealed that the major groups of autotrophic organisms are constrained by these groups of
516 climate-related environmental factors (Hodgson et al. 2004). Interestingly, a microscopy
517 based taxonomic inventory of the cyanobacterial community composition in 56 lakes in the
518 Larsemann Hills revealed that lake depth and pH (not available for all studied lakes here)
519 were the most important variables (Sabbe et al. 2004), and that salinity (or conductivity) was
520 of minor importance in explaining the distribution of cyanobacterial morphotypes. In contrast,
521 our data revealed that salinity is important, as observed in other taxonomic groups, which
522 underscores the need to apply molecular techniques rather than classical microscopy, as

morphological characteristics are insufficient to discriminate between cyanobacterial OTUs (e.g., Taton et al. 2006b; Jungblut et al. 2005).

Although the environmental factors explain more of the community structure than the geographical variables, the structuring role of dispersal limitation in microbial communities is confirmed by the variation partitioning analysis; 10% of the variance in the eukaryotic DGGE bands, and 5.8% of the cyanobacterial DGGE bands were explained by geographical variables. This is in agreement with similar studies of diatoms at an Antarctic regional scale (Verleyen et al. 2003) and on a global scale (Vyverman et al. 2007; Verleyen et al. 2009), and with other organisms in which environmental factors generally dominate over geographical factors (Cotenie 2005). Although we acknowledge that our dataset represents only a cross-section of the biodiversity of east Antarctic lakes, both eukaryotic and cyanobacteria communities are structured by geographical factors, after environmental variables are factored out. This, together with the relatively high amount of cyanobacterial sequences that have no relatives from non-Antarctic environments in GenBank, and the presence of Antarctic endemics in at least three other taxonomic groups, namely diatoms (Sabbe et al. 2003), flagellates (Boenigk et al. 2006) and green algae (De Wever et al. 2009) appears to contradict previous claims that for microorganisms everything is everywhere (Baas Becking 1934). Our results thus suggest that Antarctic microbial communities are probably structured by the same processes as those occurring in macroorganisms, as has been observed in studies of global diatom communities (Vyverman et al. 2007; Verleyen et al. 2009).

Together, our results thus have important implications for the distribution of taxa and for predicting the biodiversity trajectory under changing climate conditions. In some regions experiencing increased wind speeds, and in regions experiencing increasing temperatures, the precipitation-evaporation balance will remain negative, which is expected to influence the salinity and thus the future structure and composition of the microbial mat communities. It

548 remains uncertain how these climate changes will affect the dispersal and establishment
549 capacities of the microbial organisms, and whether this will lead to more introductions of
550 exotic species into these often unique ecosystems.
551

ACKNOWLEDGEMENTS

This research was funded by the EU project MICROMAT and the Belgian Federal Science Policy (BelSPO) project AMBIO 'Antarctic Microbial Biodiversity: the importance of geographical and ecological factors'. EV is a postdoctoral research fellow of the Fund for Scientific Research Flanders, Belgium (FWO). AT was funded by the Fund for Research Formation in Industry and Agriculture (FRIA, Belgium). AW was Research Associate of the National Fund for Scientific Research FNRS. We thank K. Welch, P. Noon, W. Quayle, J. Laybourn-Parry, G. Murtagh, P. Dyer, T. Henshaw, and I. Janse who collected the samples. The material was collected with the support of the Long Term Ecosystem Research Program (LTER) and the Australian Antarctic Division (ASAC project 2112). Three anonymous reviewers and Dr. R. De Wit are thanked for their constructive comments on an earlier version of the manuscript.

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Table 1: Chemical and morphological characteristics of the studied lakes. Biological samples were taken at different areas in the lakes indicated with an asterisk. Sampling locations are littoral zone (lit) and the deepest spot in the oxygenated zone (ds). REI1 and REI2 correspond to ReidJ and ReidD in Taton et al. (2006a) respectively. Multiple samples from the same lake have identical environmental variables, although lake depth can vary slightly (but was not consistently measured during the time of sampling).

Lake Name	Sample Code	Sampling location	region	Lake area (ha)	altitude (m a.s.l.)	Lake depth; z-max (m)	NO3-N (µg/L)	PO4-P (µg/L)	Salinity (ppt)	Na (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Cl (mg/L)	SO4 (mg/L)
Firelight L.	FIR	lit	BI	0.88	30.0	1.5	0.0	6.3	2.1	850	25	50	96	1500	50
Sunset L.	SUN	lit	BI	1.12	10.0	1.8	0.1	0.1	0.5	161	4	26	20	275	27
L. Fryxell	FRY	lit	DV	708.00	19.0	20.0	1.0	0.1	1.3	172	23	42	108	640	40
L. Burgess	BUR	ds	LH	4.00	40.0	16.0	0.3	0.0	0.1	28	2	2	3	44	5
Fold L.	FOL	lit	LH	0.27	30.0	1.0	0.6	0.1	0.4	180	8	8	17	303	34
-	GE2	lit	LH	0.25	65.0	1.0	0.6	0.0	0.1	31	1	2	3	55	9
-	GRO	ds	LH	3.50	50.0	16.0	0.2	0.0	1.4	530	18	47	63	860	195
L. Jack	JAC	lit	LH	4.20	85.0	2.0	0.5	0.0	0.1	19	0	1	2	25	4
L. Sibthorpe	SIB	lit	LH	12.50	60.0	0.7	1.0	0.0	0.1	25	6	1	3	38	5
-	L52b	lit	LH	0.45	80.0	1.0	0.5	0.0	0.1	40	1	3	5	67	9
-	L67	ds	LH	4.50	45.0	5.0	0.5	0.0	0.9	310	10	21	32	481	60
Long L.	LON	ds	LH	5.00	80.0	11.0	0.2	0.2	0.1	25	1	1	3	41	5
-	MAN	lit	LH	0.42	30.0	1.0	0.1	0.0	0.2	51	3	4	8	108	20
Pup Lagoon	PUP	ds	LH	1.00	5.0	4.6	1.1	0.1	0.5	190	10	15	18	277	55
L. Reid*	REI1	unknown	LH	5.50	30.0	3.8	0.7	0.2	4.1	1900	58	50	176	2660	105
	REI2	ds													
Sarah Tarn	SAR	ds	LH	1.00	75.0	2.5	0.5	0.1	14.0	6200	160	193	824	10400	480
-	R02	lit	RI	2.53	10.0	3.0	0.0	0.0	140.0	63000	1234	350	5600	113270	2790
-	R05	lit	RI	4.30	2.0	4.0	0.0	0.0	100.0	42000	1149	450	3768	62230	6650
-	R07	lit	RI	1.09	15.0	1.5	0.0	0.1	24.9	10000	213	93	351	10350	8420
-	R08	lit	RI	1.09	18.0	1.0	0.0	0.0	4.6	1200	49	29	137	2380	1040
-	R09	lit	RI	1.02	8.0	1.5	0.0	0.0	12.4	4000	136	90	272	6010	1780
Ace L.*	ACE1	unknown	VH	13.10	8.9	23.0	1.3	0.2	19.5	4420	404	58	1170	9100	312

	ACE2	ds												
	ACE3	ds												
	ACE4	ds												
	ACE5	lit												
Ekho L.*	EKH1	ds	VH	44.40	0.0	39.0	0.1	0.4	52.0	13210	1940	430	3360	26100	1975
	EKH2	ds												
Highway L.*	HIW1	ds	VH	20.00	8.3	17.4	1.6	0.2	2.5	510	43	26	97	940	105
	HIW2	ds												
	HIW3	ds												
	HIW4	ds												
-	PEN1	ds	VH	16.00	3.0	18.4	2.9	0.6	13.6	4250	296	178	870	7400	1320
	PEN2	lit												
	PEN3	lit												
Watts L.	WAT	ds	VH	38.00	0.0	29.5	0.1	0.2	2.3	610	105	25	215	1200	187

Table 2: Primers used in this study. R (reverse) and F (forward) designations refer to the primer orientation in relation to the rRNA. W indicates an A/T nucleotide degeneracy.

Primer	**Sequence (5' – 3')**
universal eukaryote forward (Van Hannen et al. 1998)	CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCTCTTGTGATGCCCTTAGATGTTCTGGG
universal eukaryote reverse (Van Hannen et al. 1998)	GCGGTGTGTACAAAGGGCAGGG
16S378F (Nübel et al. 1997)	GGGGAATTTTCCGCAATGGG
16S781R(a) (Nübel et al. 1997)	CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCGACTACTGGGGTATCTAATCCCATT
16S781R(b) (Nübel et al. 1997)	CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCGACTACAGGGGTATCTAATCCCTTT
16S784R (derived from Nübel et al. 1997)	GGACTACWGGGGTATCTAATCCC
23S30R (Taton et al. 2003)	CTTCGCCTCTGTGTGCCTAGGT

Figure legends

Fig. 1: The studied lakes in the Larsemann Hills, Vestfold Hills, Rauer Islands, Bølingen Islands, and the McMurdo Dry Valleys. Inset shows a map of Antarctica with the study regions in the Prydz Bay area and the McMurdo Dry Valleys.

Fig. 2: Principal component analysis (PCA) of the studied lakes showing the inter-regional differences in limnology and the structuring role of conductivity and morphological related variables, which account for a large part of the environmental variation in the dataset. White squares: the Bølingen Islands, black triangles: the Larsemann Hills, white triangles: the Dry Valleys, white circles: the Rauer Islands, and black diamonds: the Vestfold Hills. For a key to the lake names and environmental variables the reader is referred to Table 1.

Fig. 3: Correspondence analysis biplot showing the variation in the presence-absence of DGGE bands obtained using the cyanobacteria specific primers, with the significant geographical (V2 and V3) and environmental variables plotted as supplementary variables. Symbols are as in Fig. 2. For a key to the lake names and environmental variables the reader is referred to Table 1.

Fig. 4: Correspondence analysis biplot showing the variation in the presence-absence of DGGE bands obtained using the universal eukaryote primer, with the significant environmental, geographical and seasonal variables selected in the variation partitioning analysis plotted as supplementary variables. Symbols are as in Fig. 2. For a key to the lake names and environmental variables the reader is referred to Table 1.

Fig. 5: The amount of variation in the taxonomic structure of the eukaryotic (A) and cyanobacterial (B) communities uniquely explained by the geographical, local environmental and seasonal variables and the overlap between the different fractions as assessed using variation partitioning analysis.

Fig. 1:

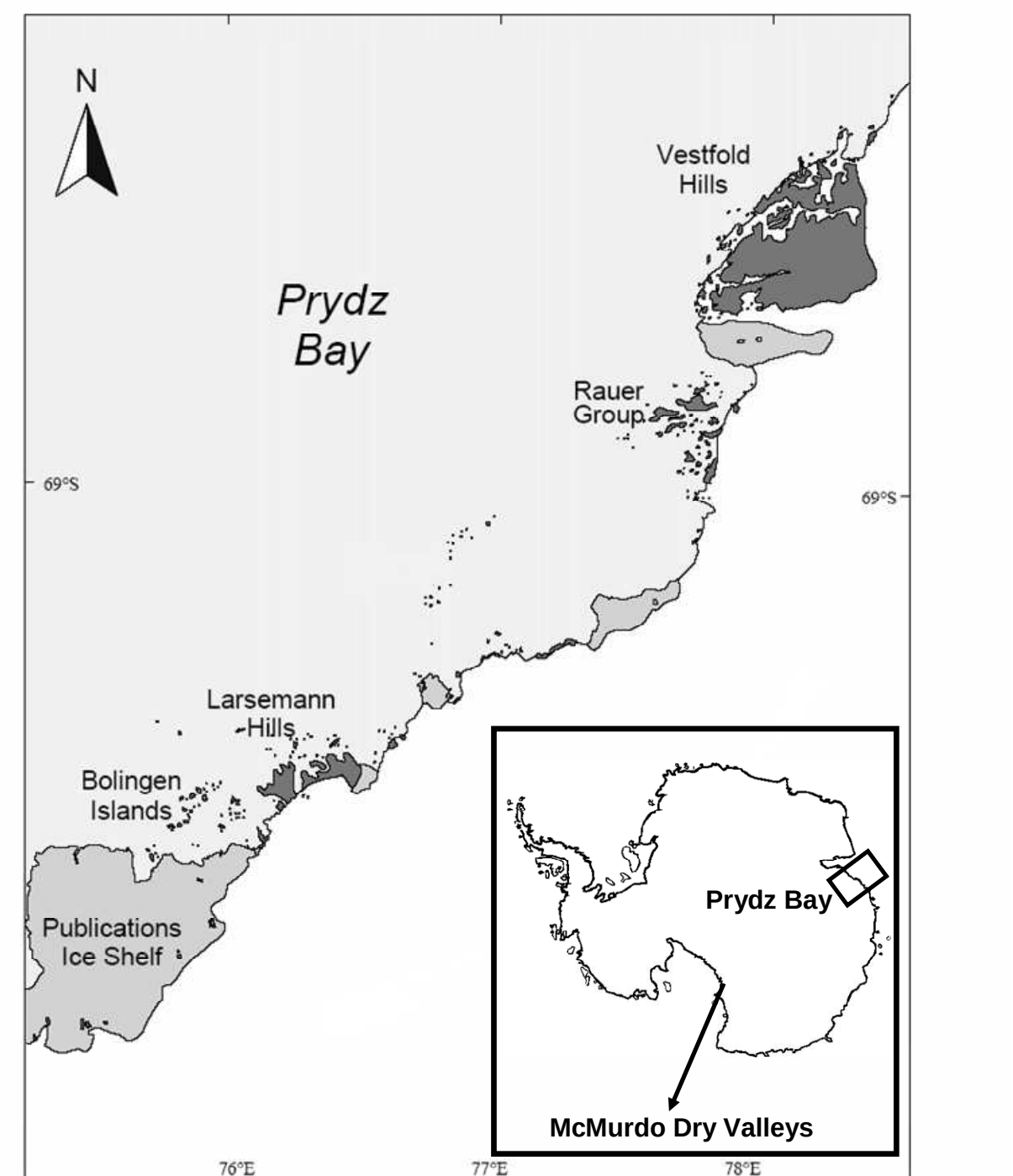
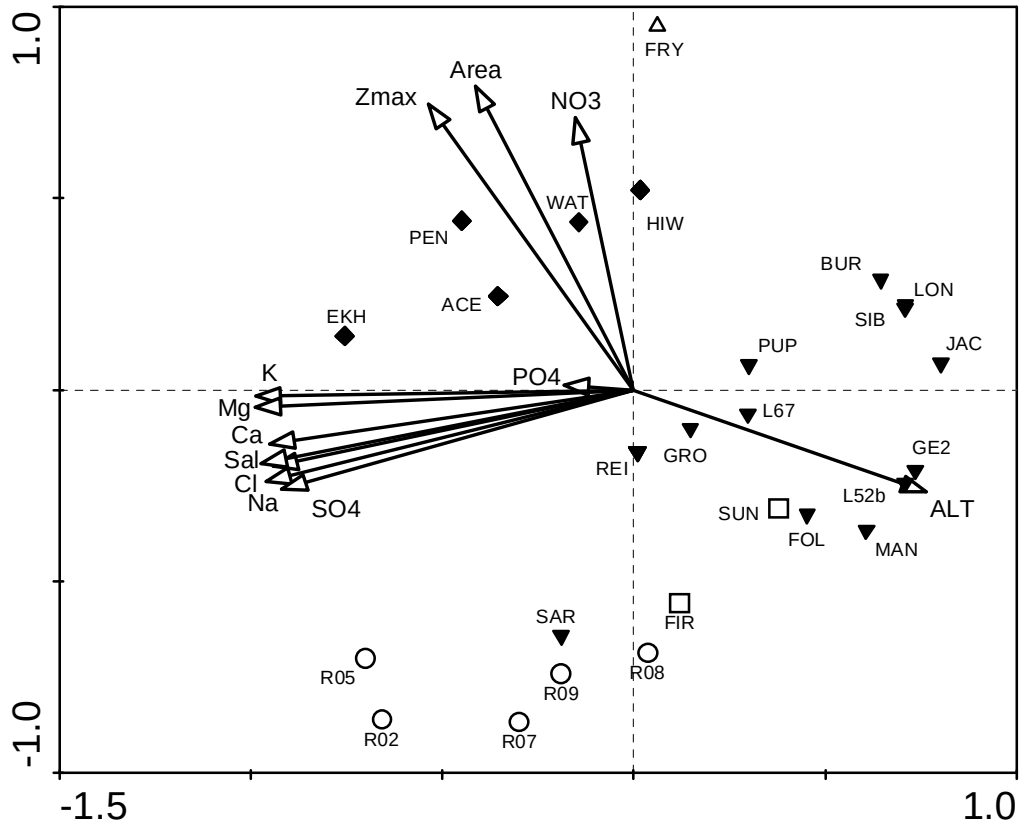

N
Vestfold
Hills
Prydz
Bay
Rauer
Group
69°S
69°S
Larsemann
Hills
Bolingen
Islands
Publications
Ice Shelf
Prydz Bay
McMurdo Dry Valleys
76°E
77°E
78°E

Fig. 2: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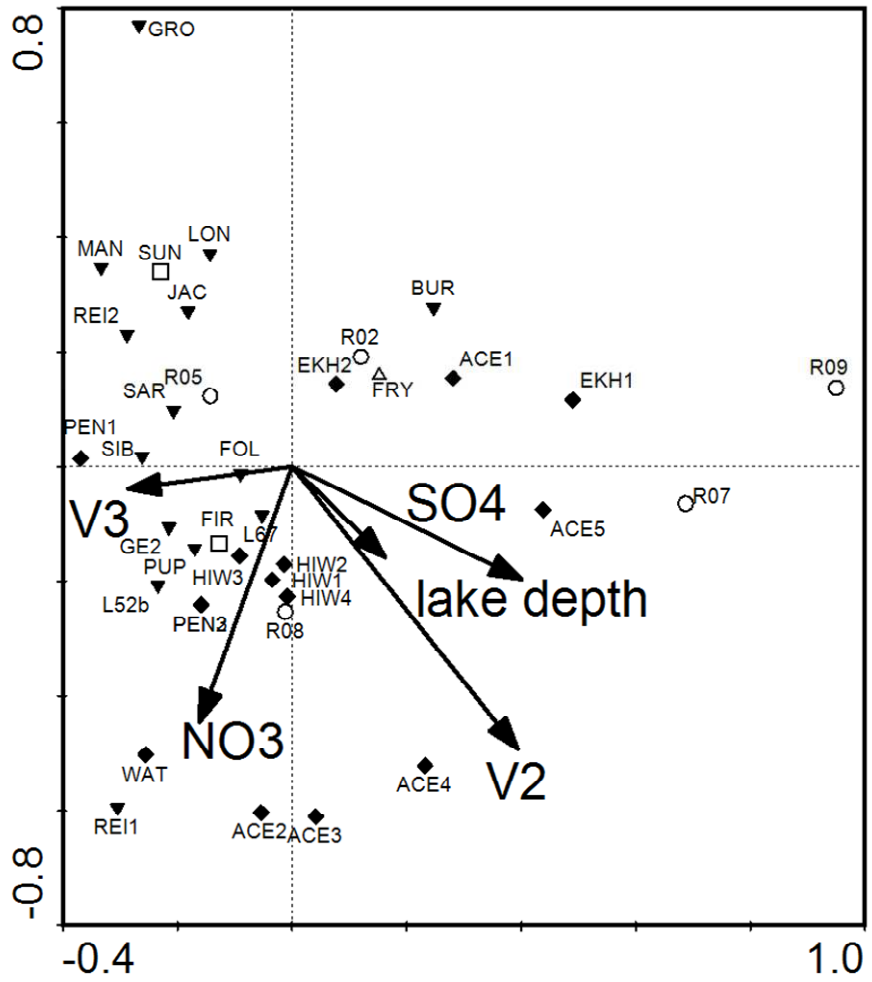
WAT
HIW
PEN
ACE
EKH
BUR
LON
SIB
JAC
PUP
K
PO4
Mg
Ca
Sal
Cl
Na
SO4
L67
GRO
REI
GE2
ALT
L52b
SUN
FOL
MAN
SAR
FIR
R05
R08
R09
R02
R07

Fig. 3: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SUN
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JAC
REI2
BUR
R02
EKH2
FRY
ACE1
EKH1
R09
SAR
R05
PEN1
SIB
FOL
V3
SO4
R07
ACE5
FIR
L67
GE2
PUP
HIW3
HIW2
HIW1
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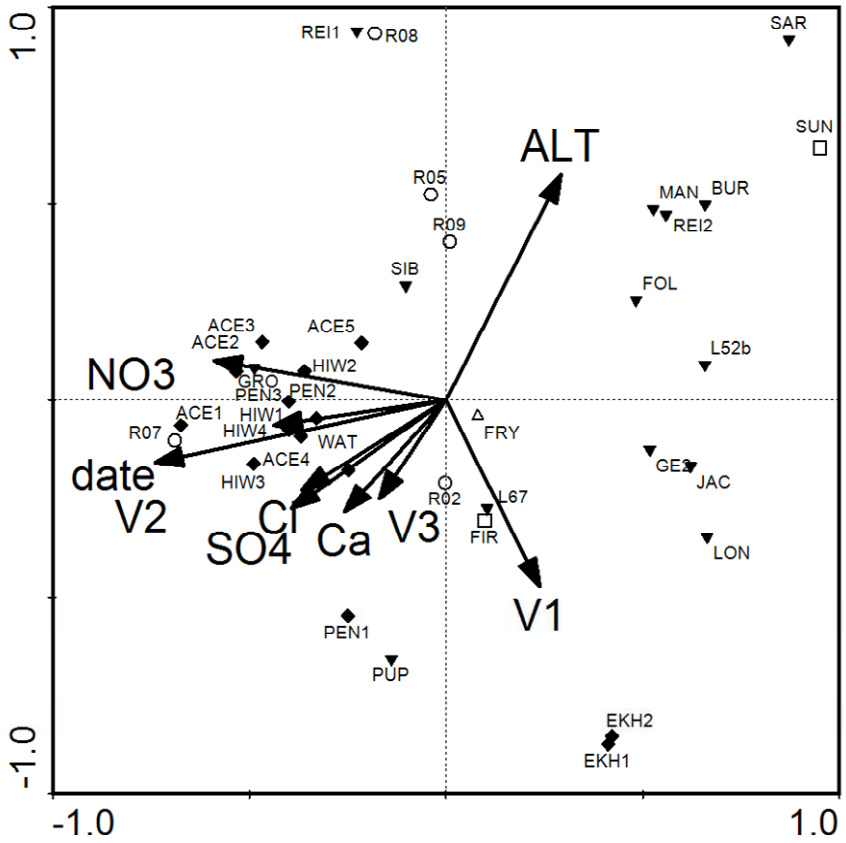
WAT
ACE4
V2
REI1
ACE2
ACE3

Fig. 4: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1.0
REI1
R08
SAR
SUN
ALT
R05
MAN
BUR
REI2
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SIB
FOL
ACE3
ACE5
ACE2
L52b
NO3
HIW2
GRO
PEN3
PEN2
ACE1
HIW1
FRY
R07
HIW4
WAT
GE2
date
ACE4
JAC
HIW3
R02
L67
V2
Cl
Ca
V3
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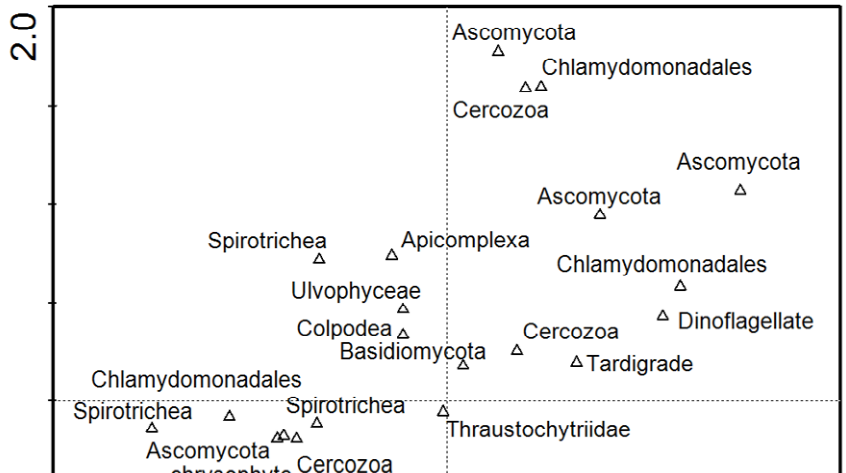
SO4
LON
V1
PEN1
PUP
EKH2
EKH1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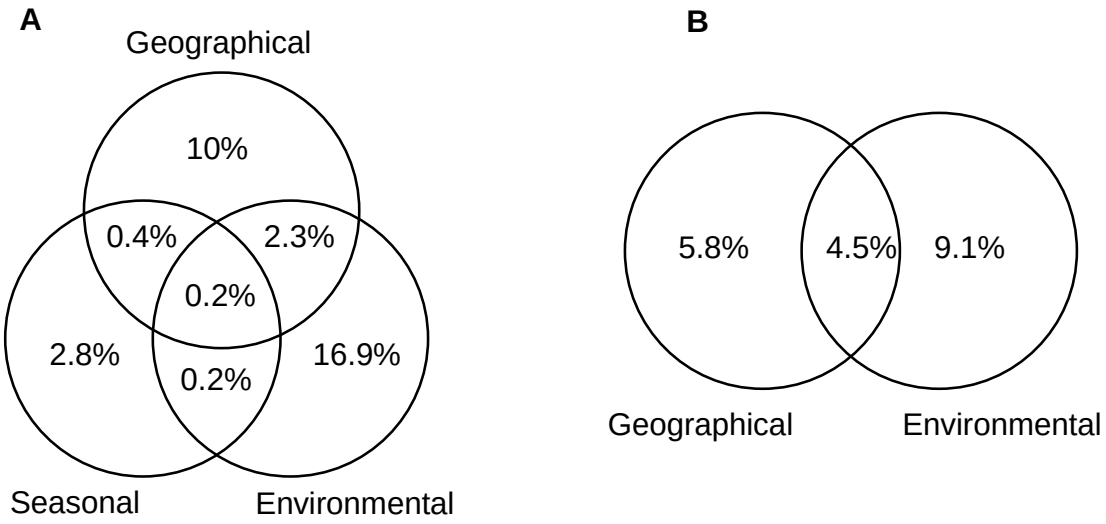
Chlamydomonadales
Cercozoa
Ascomycota
Ascomycota
Spirotrichea
Apicomplexa
Chlamydomonadales
Ulvophyceae
Dinoflagellate
Colpodea
Cercozoa
Basidiomycota
Tardigrade
Chlamydomonadales
Spirotrichea
Spirotrichea
Thraustochytriidae
Ascomycota
Cercozoa

Fig.5:


A
Geographical
10%
0.4%
2.3%
0.2%
2.8%
16.9%
0.2%
Seasonal
Environmental
B
5.8%
4.5%
9.1%
Geographical
Environmental

SUPPLEMENTARY INFORMATION

Table S1: BLAST hits of sequences obtained from DGGE bands. For each DGGE band sequence, the cyanobacterial hits included the first sequence indicated by BLAST; if this sequence was from an uncultivated cyanobacterium, the first strain sequence was added. When the first hit was isolated from an Antarctic environment, the first hit that share more than 97.5% similarity with the query and isolated from a non-Antarctic environment was added. For eukaryotes the closest match is given.

DGGE band sequence[a]	Possible taxonomic affiliation	Hit indicated by BLAST[b]	Similarity (%)[c]
E82.8	Eukaryota; Metazoa; Tardigrada; Eutardigrada; Apochela; Macrobiotidae	*Ramazzottius oberhauseri* (AY582122)	98
E93.54	Eukaryota; Viridiplantae; Chlorophyta; Chlorophyceae; Chlamydomonadales	*Carteria* sp. UTEX2 (AF182817)	96
E70.3		*Chlamydomonas raudensis*, isolate CCAP 11/131 from Lake Bonney, Antarctica (AJ781313)	100
E67.1 [20b]	Eukaryota; Viridiplantae; Chlorophyta; Ulvophyceae	*Pseudendoclonium submarinum* (EF591129)	100
E64.72	Eukaryota; Viridiplantae; Chlorophyta; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas.	*Chlamydomonas pulsatilla* from northwest Spitzbergen (AF514404)	100
E59=58.77=57.73	Eukaryota; Fungi	Uncultured fungus clone F5f2 (AY937464)	95
E80.71	Eukaryota; Fungi; Basidiomycota; Hymenomycetes	*Mrakia frigida* AFTOL-ID 1818 (DQ831017)	100
E71.2	Eukaryota; Fungi; Basidiomycota; Hymenomycetes; Heterobasidiomycetes; Tremellomycetidae; Filobasidiales	*Filobasidium globisporum* (AB075546)	100
E62.51	Eukaryota; Fungi; Ascomycota	Uncultured *Pezizomycotina* clone Sey062 (AY605205)	99
E90.2		Uncultured Sarcosomataceae clone Amb_18S_1472 (EF023999)	100
E51.5	Eukaryota; Alveolata; Apicomplexa; Colpodellidae	*Colpodella edax* (AY234843)	99

E42.88	Eukaryota; Alveolata; Dinophyceae; Gymnodiniales	Uncultured eukaryote isolate E230 permanently anoxic Cariaco Basin (Caribbean Sea) (AY256288)	100
E53.29	Eukaryota; Cercozoa	Uncultured eukaryote clone Amb_18S_1283 (EF023834)	99
E24.95		*Spongomonas minima* strain ATCC 50404 (AF411280)	97
E36.02		Uncultured cercozoan clone LEMD111 (AF372739)	99
E48.32	Eukaryota; stramenopiles; Labyrinthulida; Thraustochytriidae	*Thraustochytriidae* sp. MBIC11075 (AB183658)	91
E21.6	Eukaryota; stramenopiles; Bacillariophyta; Bacillariophyceae; Bacillariophycidae	*Eolimna minima* isolate SNA15 (AJ243063)	95
E52.7	Eukaryota; stramenopiles; Oomycetes; Peronosporales	*Peronospora corydalis* from *Corydalis speciosa* Max. (AF528564)	100
E37.94	Eukaryota; stramenopiles; Chrysophyceae	Uncultured marine eukaryote clone M4_18F06 (DQ103808)	98
E37.33	Eukaryota; Alveolata; Ciliophora; Intramacronucleata; Colpodea	*Bursaria truncatella* (U82204)	99
E49.35	Eukaryota; Alveolata; Ciliophora; Intramacronucleata; Spirotrichea; Stichotrichia; Stichotrichida	Oxytrichidae environmental sample clone Amb_18S_1444 (EF023975)	99
E42.25		Oxytrichidae environmental sample clone Amb_18S_1444 (EF023975)	99
E49.93=50.5	Eukaryota; Alveolata; Ciliophora; Intramacronucleata; Spirotrichea; Stichotrichia; Stichotrichida; Oxytrichidae	*Onychodromopsis flexilis* (AY498652)	99
Rauer7-124b* & LH-Pup23-126b*	Cyanobacteria; Uncultured cyanobacterium	Uncultured cyanobacterium clone H-B02*	100
Grovness-11b		Uncultured cyanobacterium isolate DGGE gel band C1	94.5
Rauer7-96a_1*, Pup23-100a*, Pup23-102a*, Pup23-103a* & L70-0-2cm-64a*		Uncultured cyanobacterium clone H-A07*	98.9-100.0
Gentner2-25a		Uncultured soil crust cyanobacterium clone lichen13	98.4
Ace-106a	Cyanobacteria; Oscillatoriales; *Phormidium* spp.	*Phormidium murrayi* ANT.ACEV5.2*	100
		Uncultured bacterium clone CD29	97.6
Ace-129b, Rauer7-123b, Rauer8-48b, Firelight-45b, Sarah-Tarn-	Cyanobacteria; Oscillatoriales; *Leptolyngbya* spp.	*Leptolyngbya antarctica* ANT.ACEV6.1*	100
		Leptolyngbya sp. CCMEE6037	98.5

121b & Rauer7-96a		*Plectonema* sp. HPC-49	98.8
L11(Reid/Big)-13b*		*Leptolyngbya antarctica* ANT.LH18.1*	99.7
Rauer9-110b		Uncultured cyanobacterium clone R8-B31*	100
		LPP-group MBIC10597	99
Fold-5b, Fold-6b & Fold-7b		*Leptolyngbya frigida* ANT.JACK.1*	99.7-100.0
		Leptolyngbya sp. CCMEE6119	99.4-99.7
Jack-2b*, L67-44b*, Manning-4b		Uncultured cyanobacterium clone H-D28*	99.7-100
		Uncultured cyanobacterium clone H-C16*	99.7-100
		Uncultured bacterium Tui1-3	96.5-97.6
		Leptolyngbya frigida ANT.LH52.3*	96.0-97.5
Firelight-57a & Manning-28a	Cyanobacteria; Oscillatoriales; *Geitlerinema* spp.	Uncultured Antarctic cyanobacterium clone BGC-Fr005*	99.6-100
		Geitlerinema splendidum 0ES34S4	99.3-99.7
Rauer9-84a	Cyanobacteria; Nostocales; *Nodularia harveyana*	*Nodularia harveyana* strain CCAP 1452/1	99.6
Burgess-90a, Burgess-91a & Burgess-92a	Cyanobacteria; Nostocales; *Nostoc* spp.	*Nostoc* sp. 152 partial	98.2
L67-49a, L67-50a, L67-51a, L67-54a, L67-52a, Long-146a & L67-53a		*Nostoc* sp. PC2 partial	99.2-99.4
		Nostoc sp. *'Pseudocyphellaria crocata* cyanobiont' strain Pcro436	98.8-99.4
		Uncultured Antarctic cyanobacterium DGGE gel band FrF1*	99.2-100
Sarah-Tarn-94a & Fold-29a		*Nostoc* sp. *'Peltigera canina* 2 cyanobiont'	99.7-100
		Nostoc commune EV1-KK1	99.4-100.0
Jack-26a & Jack-27a	Cyanobacteria; Nostocales; *Coleodesmium* spp. / *Cylindrospermum* spp.	*Coleodesmium* sp. ANT.LH52B.5*	99.7
		Cylindrospermum sp. A1345	97.9
		Uncultured cyanobacterium clone LV60-CY1-1	99.1
Firelight-39b	Cyanobacteria; Chroococcales; *Synechococcus* sp.	*Synechococcus* sp. PS845	100
L70-0-2cm-46b	Cyanobacteria; Chroococcales; *Chamaesiphon* spp.	Uncultured cyanobacterium clone CSC9*	99.7
		Chamaesiphon subglobosus PCC 7430	98.2

[a]The DGGE bands sequences were grouped using the average neighbor clustering algorithm of the software Dotur (http://www.plantpath.wisc.edu/fac/joh/dotur.html) with a threshold of 97.5% binary similarity according to a similarity matrix made with the software package ARB (http://www.arb-home.de) and based on an alignment that includes the positions 380 to 730 relative to *E. coli.* Insertion-

deletions and ambiguities were not taken into account. An asterisk denotes sequences that do not have relatives with at least 97.5% binary similarity from a non-Antarctic environment and therefore could potentially be considered as endemic to Antarctica.

[b]An asterisk denotes sequences isolated from an Antarctic environment.

[c]A range of similarities is given when multiple DGGE band sequences were included in the same group. Levels of similarity were determined by the computation of similarity matrixes as described above.

Figure legends

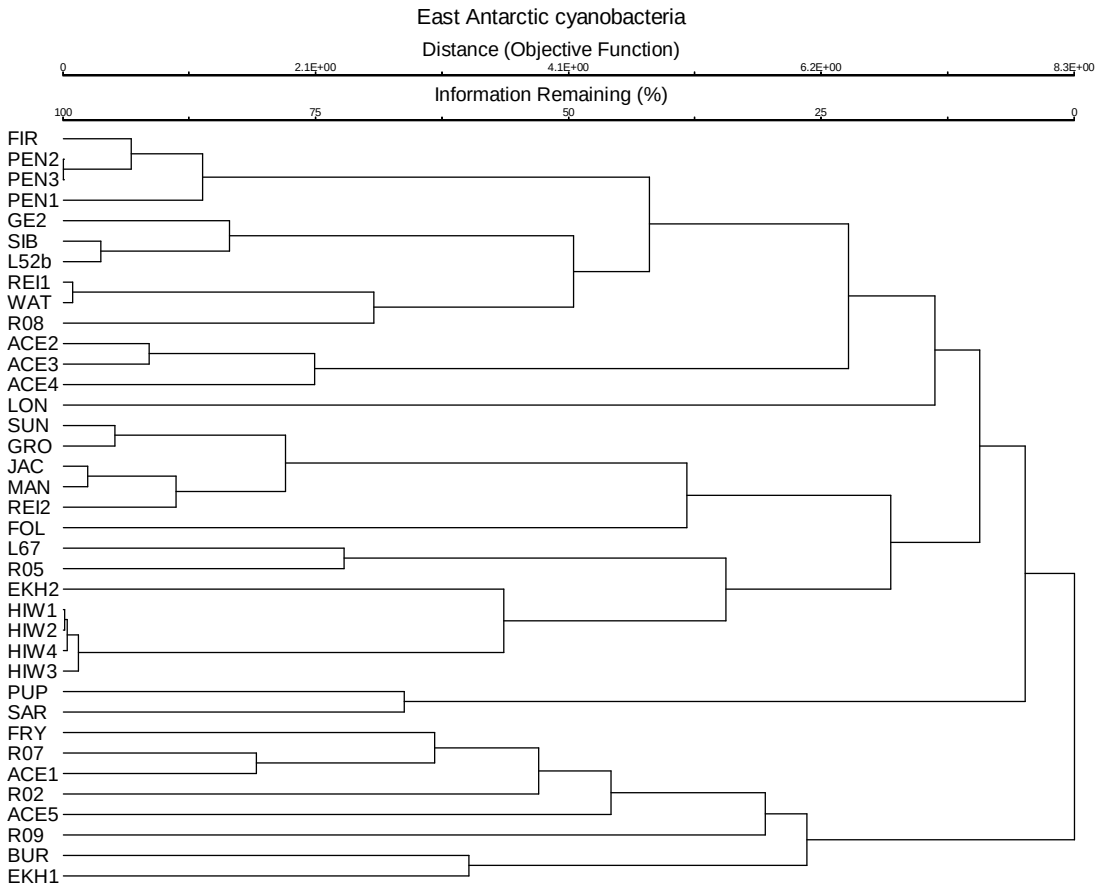
Fig. S1: Microbial mats dominated by cyanobacteria in Kobachi Ike (Lützow-Holm Bay, east Antarctica). The mat is detached as a result of the wind-induced redistribution of melting lake ice, which bulldozes on the shoreline. This physical disturbance is a major factor in shaping the community structure and physiognomy of microbial mats in Antarctic lakes (e.g., Sabbe et al. 2004) and partly underlies the importance of lake water depth in structuring these communities. Inset: Microphotograph of a *Nostoc* sp. colony surrounded by thin oscillatorians of the genus *Leptolyngbya*. The picture was taken using a light microscope from a microbial mat sample collected in Lake Reid (Larsemann Hills, east Antarctica).

Fig. S2: Cluster analysis of DGGE bands derived using our cyanobacteria specific primers showing that in-lake variability is generally low, except for Ekho Lake, Lake Reid and 2 samples from Ace Lake (one of which is a littoral sample)

Fig. S3: Cluster analysis of DGGE bands derived using our universal eukaryote primers showing that in-lake variability is generally low, except for the two samples from Lake Reid and Ace Lake, which were similarly different in DGGE bands of cyanobacterial sequences, and one littoral sample from Pendant Lake. The samples from Ekho Lake are highly similar in contrast to the cyanobacterial sequence composition.

Fig. S1:

Fig. S2:


East Antarctic cyanobacteria
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6.2E+00
8.3E+00
Information Remaining (%)
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75
50
25
0
FIR
PEN2
PEN3
PEN1
GE2
SIB
L52b
REI1
WAT
R08
ACE2
ACE3
ACE4
LON
SUN
GRO
JAC
MAN
REI2
FOL
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HIW1
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FRY
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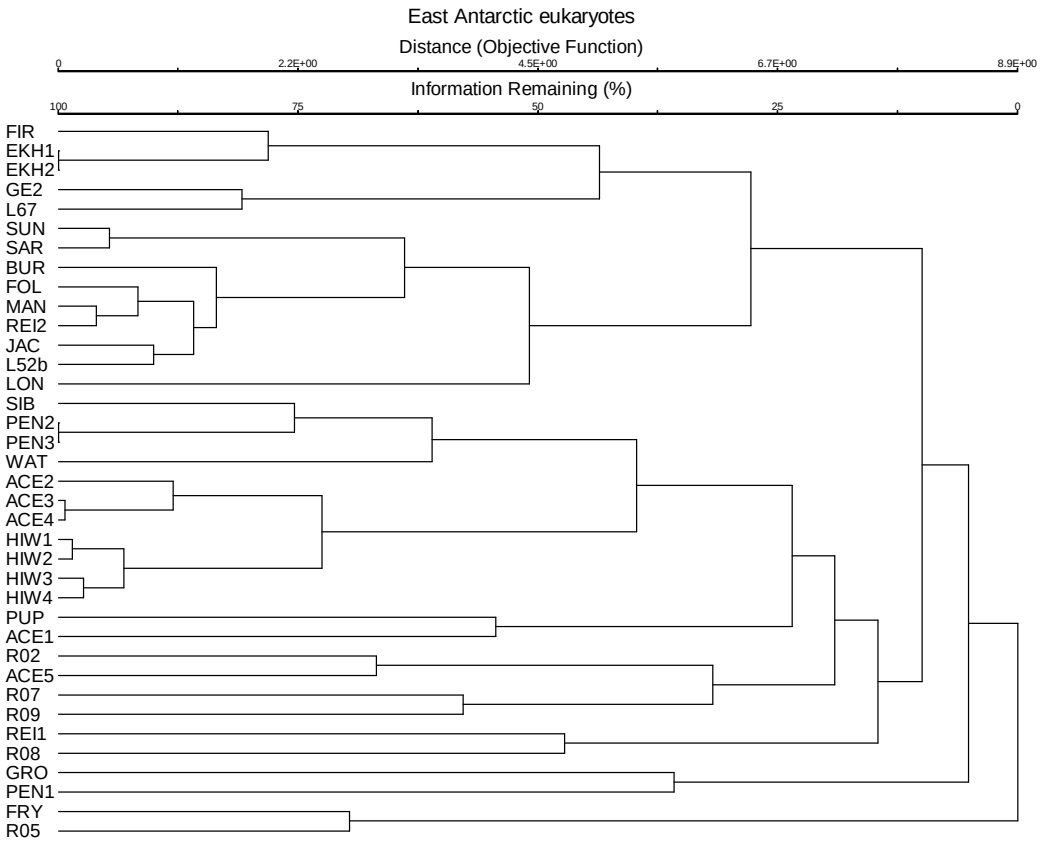
Fig. S3: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GE2
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SAR
BUR
FOL
MAN
REI2
JAC
L52b
LON
SIB
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