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Using Captain Scott's Discovery specimens to unlock the past: Has Antarctic cyanobacterial diversity changed over the last 100 years?

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Abstract

Evidence of climate-driven environmental change is increasing in Antarctica, and with it comes concern that this will propagate to impacts on biological communities. Recognition and prediction of change needs to incorporate the extent and time scales over which communities vary under extant conditions. However, few observations of Antarctic microbial communities, which dominate inland habitats, allow this. We therefore carried out the first molecular comparison of Cyanobacteria in historic herbarium microbial mat from freshwater ecosystems on Ross Island and the McMurdo Ice Shelf, collected by Captain R.F. Scott's "Discovery" Expedition (1902-03), with modern samples from those areas. Using 16S rRNA gene surveys, we found that modern and historic cyanobacteria assemblages showed some variation in community structure but were dominated by the same genotypes. Modern communities had a higher richness, including genotypes not found in historic samples, but were related to other cyanobacteria sequences from Antarctica. The results imply slow cyanobacterial 16S rRNA gene genotype turnover and a considerable community stability within Antarctic microbial mats. We suggest that this relates to Antarctic freshwater habitats requiring a capacity to withstand diverse stresses, and that this could also provide a degree of resistance and resilience to future climatic-driven environmental change in Antarctica.

Keywords: Antarctica, cyanobacteria, climatic change, 16S rRNA gene, freshwater, historic collections

Background

Climate models predict that the Polar Regions will warm faster than other parts of the globe, a scenario that frequently raises concerns over threats to the integrity of their biological communities. In the Arctic this tendency is already well developed, but while substantial warming has been seen in the Western Antarctic Peninsula since 1950's (1, 2), in Continental Antarctica warming trends are slight due to offset by changes to Southern Annular Mode of atmospheric circulation, linked to ozone depletion (3, 4). However, some evidence of change on the continent is emerging and, as the ozone hole fills, widespread warming of the continent is expected to accelerate (5). To recognize and forecast biological responses to warming in continental Antarctica, it is important to understand natural population drift and the extent and time scales over which changes in diversity, community structure and ecosystem processes occur. However, inland Antarctica is dominated by microbial ecosystems, and the absence of suitable records through time of their composition limits an assessment of their temporal stability to date.

In inland Antarctica, aquatic ecosystems such as lakes, streams and meltwater ponds are recognized as hotspots and refugia for biological productivity and diversity, and within these cyanobacterial mat communities represent the most prominent biology (6, 7, 8). Previous work on the diversity of community composition in these mats, has mostly concentrated on establishing relationships between composition, current environment and geography (9, 10, 11). No assessments along a temporal axis have been carried out in Antarctica. Archived samples of microbial communities do, however, exist that potentially allow for comparison with modern samples using molecular techniques if DNA can be extracted from them, and sufficient metadata is present to identify sampling locations. Dried herbarium specimens of

cyanobacterial mats from meltwater ponds on Ross Island and the McMurdo Ice Shelf were collected during the 1902-03 National Antarctic Expedition, also called the Discovery expedition, led by Captain Robert Falcon Scott (12). The careful collection, preservation and documentation of these samples offers a unique possibility to compare modern and century-old microbial mat communities. Such a comparison would allow the turnover of taxa to be examined and may indicate whether exotic taxa from outside of Antarctica have appeared in these ponds that could be associated with human presence over the last century.

Our goals were to determine; whether DNA can be extracted from dried mat material sufficient to determine how communities of cyanobacteria have changed over a 100-year period; whether cyanobacteria dominant in the past have been replaced with different dominate taxa in modern samples; and whether taxa that are present now but not in historic samples have an origin outside of Antarctica. To this end, we carried out the first high-throughput sequencing analysis of herbarium specimens collected from Antarctica by the "Heroic Age" expeditions. We compared these with present-day cyanobacterial mats from geographic locations as similar as possible to those sampled at the beginning of human presence, on the McMurdo Ice Shelf and Ross Island, Antarctica. The latter is a location that currently experiences one of the highest levels of human activity in East Antarctica, and perhaps most vulnerable to the introduction of human-vectored invasive taxa (13).

Material and Methods

Study sites and samples

Cyanobacterial mat samples were studied from the Ross Island and McMurdo Ice Shelf in Southern Victoria Land Antarctica (Figure 1, Supplementary Table S1 and S2). Seven cyanobacterial mat samples were collected from ponds and ice eskers during the "Discovery"

National Antarctic Expedition led by Captain Robert Falcon Scott between December 1902 and February. Six samples were from the McMurdo Ice Shelf and one from close to winter quarters on Ross Island near Hut Point, currently the location of the historic Discovery Hut and the current US Antarctic McMurdo Station (Supplementary Table S1). McMurdo Ice Shelf samples were from an area approximately half way between Brown and Black Peninsulas (12). One sample was collected by Dr Wilson and the other likely by Dr Koettlitz (12). The dried, still green-pigmented cyanobacterial mat samples (Figure 2) are held in the Natural History Museum Botanical collections, London, UK. The mm to cm thick dried cyanobacterial mats were found preserved dry on paper herbarium sheets, in the dark, at room temperature. No records are available how the specimens were dried and kept during the first half of the century. However, based on expedition records (14), herbarium specimens were likely prepared by drying and pressing of material in the field directly after collection, by Dr R. Koettlitz.

The Scott's Discovery samples were compared with present-day cyanobacteria from microbial mats collected from freshwater meltwater ponds on the McMurdo Ice Self, Cape Royds and Cape Evans on Ross Island in January 2011, and Hut Point, Ross Island near the Discovery Hut in January 2012 (Figure 1, Supplementary Table S2). Samples from the McMurdo Ice Shelf were from near Bratina Island and half way between Brown Peninsula and Black Island. Sample sites were chosen to be the same geographic regions as described from the Discovery Expedition and listed as sample locations in the Terra Nova and Nimrod expedition, as well as to cover a range of water chemistry (Supplementary Table S2). Modern samples were collected in sterile plastic containers and frozen within 24h. Frozen samples were transported to the NHM and stored at -80°C until further use.

DNA extraction, PCR, PCR-product purification, and pyrosequencing

All molecular biological work for Scott's Discovery and present-day samples were carried out in different laboratory spaces using separate pipettes and reagents to prevent cross contamination of samples. DNA from Scott's Discovery samples were extracted under an UV and ethanol sterilized laminar flow cabinet. Two to four DNA extractions were required for the Scott samples, and pooled at equal amounts prior to PCR as previously done in 16S rRNA gene surveys of cyanobacteria in benthic microbial mats (15, 16, 17). Present-day samples were also extracted in duplicate and pooled to overcome patchy distribution. All DNA extractions were carried out using the MoBio Biofilm DNA kit (Carlsbad, CA, USA) according to the manufacturer's instructions.

Cyanobacterial 16S rRNA genes were amplified using Platinum High Fidelity Taq Polymerase (Invitrogen) in triplicate using cyanobacterial specific primers 16S378F (5' GGGGAATYTTCCGCAATGGG T '3) and 16S781R (5'GAC TAC WGG GGT ATC TAA TCC C W T T '3) modified from Taton et al (18) containing the linker A (Primer A: 5' – CGT ATC GCC TCC CTC GCG CCA TCAG-MID– template specific sequence – 3') and B (Primer B: 5' – CTA TGC GCC TTG CCA GCC CGC TCAGC – template specific sequence – 3') using the recommended Roche barcodes (MID1-8). These primers provide a broad coverage across the phylum cyanobacteria (18). Barcodes were designed to be on Primer A followed by the 16S781R. PCR-products were pooled per sample and purified using Qiagen gel-purification (Qiagen, Hilden, Germany) according to the manufacturer's instructions, and quantified in duplicate by Qubit. Eight samples were multiplexed per Junior 454-pyrosequencing run (Roche) to generate approximately 450 bp long reads and was performed at the Biochemistry DNA Sequencing Facility, Department of Biochemistry, University of Cambridge, UK. We used cyanobacteria-specific protocols that allowed us to exclude any

137 other bacteria and microbial eukaryote contaminants from the century-long storage at the
138 Natural History Museum.
139
140 *Sequencing and statistical analysis*
141 454 sequencing data was analysed using QIIME v 1.8.0 (16). Split library.py was used to
142 demultiplex the samples, apply Q25 quality control and remove all sequences less than 300
143 bp long, forward and reverse primers and sequences with homopolymers of more than 6 bp.
144 Sequences from the three runs were combined and operational taxonomic units (OTUs)
145 generated using the standard operation protocol for 454 data sequencing by Qiime (19).
146 Sequences were clustered in OTUs based on 99% sequence similarity using uclust for highest
147 taxonomic resolution. OTUs were then classified to identify taxonomic annotation using the
148 Greengenes taxonomic database (20), and chimeras removed using Chimera Slayer.
149 Subsequently, sequences of unassigned, eukaryotic origin, non-cyanobacterial bacteria taxa,
150 and Cyanobacteria sequences with assignment to only phyla level ("Cyanobacteria" phyla
151 level) were removed from further analysis. The remaining OTUs were at least assigned to
152 class level (Synechococcophycideae, Oscillatoriophycideae, Nostocophycideae,
153 Gloeobacterphycideae), OTUs with abundance of less than 0.01% were filtered and removed
154 from further analysis and samples were subsequently rarefied using 1267 sequences. Chao
155 species richness using 9999 bootstraps were calculated in PAST (21). Cyanobacterial
156 communities based presence/absence and 4^{th} root transformed abundance data were analysed
157 using nonmetric multidimensional scaling (2D NMDS) based on Bray-Curtis similarities and
158 results were plotted in two-dimensions. ANOSIM was used to test if modern and Scott's
159 Discovery samples are significantly different using PAST (21). Raw sequences are available
160 at the National Center for Biotechnology Information Sequence Read Archive under the
161 accession number PRJNA323585.

Phylogenetic analysis was performed with a representative sequence for each genotype (99% clustered) that was either one of the most abundant genotypes, or only present either in the old or new samples. In addition, at least one of the closest cultured and uncultured match based using a BLASTn search (5 March 2017, 22) to GenBank was included in the analysis. For comparison, a range of cultured and environmental cyanobacterial sequences were also included from the Antarctic, Arctic and other climate zones. Sequences were aligned using Clustal X (version 2.0.9) and manual edited with Mesquite (3.04). A phylogenetic tree was constructed using maximum likelihood with RAXMLHPC2 on TG (23) as described by Jungblut et al. (15).

Results

We used cyanobacteria-specific 16S rRNA gene assessment to compare seven cyanobacterial mat specimens from Scott's Discovery expedition from the McMurdo Ice Shelf and Ross Island with 14 present-day cyanobacteria-based mat communities from the same geographic regions. In total 340 operational taxonomic units (OTUs at 99% similarity), were delineated across all sites and 267 OTUs and 312 OTUs were detected in the historic and modern samples, respectively (Supplementary Table S1 and S2). The Cyanobacterial communities in modern and historic samples were comprised of 16S rRNA gene sequences that grouped within *Leptolynbya*, *Pseudanabaena*, Pseudanabaenaceae, *Phormidium*, Nostocaceae, *Nostoc*, *Nodularia*, *Anabaena, Dolichospermum*, and Chamaesiphonaceae (Figure 3) based on Greengenes assignment in Qiime. The ten most abundant 16S rRNA genotype at 99% OTU-level made up at least 63% of the total diversity in all samples but one (Figure 4). They had highest similarity (%) to *Leptolynbya antarctica, Phormidium autumnale, Phormidium pseudopriestleyi,* and species of *Phormidesmis, Pseudophormidium Microcoleus* and

187 *Pseudanabena* based on BLAST similarity match to Genbank (Supplementary Table S3). In
188 total 29 OTUs with 1.1 % relative abundance or less were present only in Discovery
189 microbial mats, while 72 were found only in the modern samples. Modern-only OTUs were
190 present at up to 26.36% relative abundance, although the majority had less than 2.5% relative
191 abundance. The phylogenetic analysis showed that all of the OTUs found in modern but not
192 historic samples formed clades with environmental sequences or cyanobacterial isolates from
193 the Antarctic (Supplementary Figure S1).
194
195 Comparisons of community composition, based on resemblance matrices from presence-
196 absent and 4^{th} root relative abundance data, showed that for both historic and modern
197 samples, samples from Ross Island and the McMurdo Ice Shelf tended to cluster together
198 (Figure 5). There were significant differences between historic and modern samples when
199 tested by one-way ANOSIM using presence/absence (p= 0.0386, R=0.1917) and relative
200 abundance data (p= 0.0406, R=0.1917).
201
202 SIMPER analysis was performed to determine the contribution of 16S rRNA genotypes to the
203 dissimilarity of the historic and modern communities. One genotype present in the modern
204 samples only was identified to have a contribution of 1.11%, all other genotypes found only
205 in the modern samples contributed less than 0.4% of the difference (Supplemental Table S3)..
206
207 **Discussion**
208 With climatic-driven environmental change increasingly evident in continental Antarctica
209 (5), an understanding of the scale of historic community dynamics is essential to predicting
210 the extent to which future environmental changes can be expected to cascade to shifts in
211 diversity, community structure and ecosystem processes. This is especially true for keystone

organisms such as cyanobacteria which form essential habitat and drive food webs and carbon cycling in polar freshwater systems. However, our understanding of microbial diversity and ecology on a genotypic level in Polar Regions is based on a few decades of research of biogeography and the relationship between biodiversity and environmental gradients based non near-synoptic sampling. Our study represents the first comparison of cyanobacteria in Antarctic freshwater ecosystems across the 20th century by comparing the cyanobacterial 16S rRNA gene communities from microbial mats collected during Captain R.F. Scott's Discovery Expedition in 1902-03 with recent benthic microbial mat assemblages from 2011-12. The observation that robust DNA-based analyses can be made of such archived material confirms their value as a resource for understanding the dynamics of Antarctic cyanobacterial communities along temporal scales.

Our data showed that similar cyanobacteria dominated the communities with some changes from historic pond samples to modern samples. Some degree of cyanobacterial 16S rRNA genotype turnover is indicated by the presence of genotypes in only the historic (ie loss of taxa) or the modern samples (gain of taxa). However, our analysis showed that shifts in relative abundance rather than presence-absence explained most of the changes in community assembly. NMDS analyses suggest that relative-abundance changes are generally small, but the tendency of modern and historic samples to cluster in distinct groups suggest that changes may have been coherent across ponds. This coherent behaviour implies a response to an over-arching environmental variable, rather than pond-specific changes, resulting in a gradual selective shift (25). If this is a response to the relatively small environmental change that has occurred in the last 100 years, community response to changes in growth conditions may be expected to increase under accelerated climatic-driven environmental change scenarios for continental Antarctic.

237

238 The absence of a substantial drift or turnover of dominant species over 100 years is
239 surprising, since microbial communities are often described as tending to change over time
240 (25-27). Furthermore, turnover of species has been postulated as being favoured by intense
241 disturbance and variable growth conditions, and regular freezing to low, sub-zero
242 temperatures followed by a period of growth under growth conditions that vary from year to
243 year (28) might be expected to favour change. Overlain on annual stress and year-on-year
244 variability of growth conditions have been long-term trends such as increasing UV irradiance
245 in the later part of the 20th Century (29). Rather than the high stress environment promoting
246 turnover, it may be that the high stress environment has selected, over time, a metapopulation
247 of organisms that tolerate winter freezing, short cold summers as well as salinity and nutrient
248 variability (11, 30). The absolute requirement for such adaptation to persist in this habitat
249 may provide a degree of community resistance and resilience to future climate-driven
250 environmental change in Antarctic terrestrial aquatic ecosystems – so long as extreme stress
251 events continue to dictate composition. In addition, estimates of the rate of cyanobacterial
252 mat community biomass development in similar polar regimes suggest that maximum
253 biomass take many years to be attained, allowing composition to integrate many years of
254 growth conditions which may further add to limited species dynamics (31).

255

256 In addition to effects of environmental change over time, the risk of invasive species to
257 Antarctic biodiversity has been raised during the past 100 years with increased human
258 activity. Biosecurity is a recognized key issue in environmental management in Antarctica
259 (13). Cyanobacteria are likely invaders; they are inconspicuous, able to tolerate prolonged
260 desiccation and easily spread by wind once introduced. Indeed, previous work has detected
261 the cyanobacterial genus *Cylindrospermum* in this part of Antarctica, which is absent from

morphology-based inventories of species from 100 years ago, and is suggested as a recent introduction (35). However, where we found "new" genotypes in the modern samples, these had highest similarity to Antarctic environmental sequences and strains, including *Leptolynbya antarctica, Phormidium* and *Pseudanabaena* (25, 11). In addition, these are all filamentous, mat-forming oscillatorian cyanobacteria, similar to the dominant cyanobacteria in all mats described here, and would likely have similar functionalities (25, 11). Therefore, it seems likely that turnover that has occurred in cyanobacterial assemblages will have had limited affect on the functional attributes of the mat communities (26, 27). We suggest that, despite a human presence, cyanobacterial composition in our study locations has not experienced a detectable level of shift in composition due to cyanobacteria from outside of the continent.

This apparent lack of exotic taxa may be due not to an absence of propagules, but perhaps to a failure of potential invaders to establish and thrive due to more stringent environmental conditions in Antarctic ecosystems. There might also be stochastic limitations to colonization of non-native taxa due to already existing high biomass benthic cyanobacterial mat stocks and lack of niche opportunities (36), again suggesting that the microbial mats are naturally resistant to invasion. The absence of distinct alien taxa in our study is no guarantee, that they have not or cannot be introduced. It is therefore essential that current biosecurity protocols designed to limit the introduction of biological material due to human activities remain for Antarctica. There is also the risks of other, more invasive biota reaching Antarctica that allow new trophic levels to colonize these ponds that could disrupt their current biota and food webs, such as macroscopic invertebrates.

Potential problems with our analysis include the storage conditions of the herbarium samples and the limited ability to assign taxonomic units based on the short reads obtained from a single gene. It is not known if different cyanobacteria vary in their susceptibility to degradation during long term storage at room temperature, and some of the rare taxa absent from the old samples might have been lost due to degradation of the DNA. It can also not be excluded that the lower richness in the historic samples might be due to the long term storage of the samples, and more in-depth sequencing may have revealed a higher degree of community turnover. Future phylogenetic interference using multiple gene loci would provide a better taxonomic resolution, and in-depth metagenomic sequencing analysis could assist in detecting rare genotype for future comparisons of modern and historic cyanobacteria in Antarctica.

Conclusions

What are the implications for the next 100 years when more substantial climate change is expected to occur in Antarctica (37)? Our data allow us to conclude that environmental change to date has been absorbed with no substantive change in cyanobacterial composition, and that there has been no obvious impact on cyanobacterial 16S rRNA genotype composition from invasive species associated with human presence. We suggest that this ability to accommodate variability in summer growth conditions will allow the composition of cyanobacterial assemblages to retain a considerable level of stability over time, such that existing communities are likely to be resistant to a moderate degree of climate change, as long as the overwhelming stress of winter freezing and short summer growth period continues. However, the risks of other, more invasive cyanobacterial taxa and other biota reaching Antarctica remains, as does the possibility that synergy between climate change and human activity may allow new trophic levels to colonize these ponds and disrupt their current

biota and food webs. The importance of continuing biosecurity measures that not only cover animals and plant propagules but also microbial species cannot be overstressed. The work also highlights the potential of molecular analysis of historic microbial specimen collections for Antarctic microbial biodiversity and underutilized resource for temporal assessments of microbial biodiversity and ecology.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

ADJ conceived the study, ADJ and IH collected the field data, ADJ performed molecular lab and sequence analysis, and both participated in the design of the study and drafted the manuscript. All authors gave final approval for publication.

References

1. Turner J, Colwell SR, Marshall GJ, Lachlan-Cope TA, Carelton AM, Jones PD, Lagun, V, Reid, PA, Iagovikina S. 2005 Antarctic climate change during the last 50 years. *Int. J. Climatol.* **25**,279–294. (doi: 10.1002/joc.1130)
2. Nicolas JP, Bromwich DH. 2012 New Reconstruction of Antarctic Near-Surface Temperatures: Multidecadal Trends and Reliability of Global Reanalyses. *J. Climate* **27**, 8070-8093. (DOI: 10.1175/JCLI-D-13-00733.1)
3. Walsh JE. 2009 A comparison of Arctic and Antarctic climate change, present and future. *Antarc. Sci.* **3**, 179-188. (doi.org/10.1017/S0954102009001874)
4. Turner J, Barrand NE, Bracegirdle TJ, Convey P, Hodgson DA, Jarvis M, Jenkins A, Marshall GJ, Meredith MP, Roscoe HK. 2013 Antarctic climate change and the environment: an update. *Polar Rec.* **50**, 237-259. (doi.org/10.1017/S0032247413000296)
5. Fountain AG, Levy JS, Gooseff MN, Van Horn D. 2014. The McMurdo Dry Valleys: a landscape on the threshold of change. *Geomorphol.* **225**, 25-35. (doi: 10.1016/j.geomorph.2014.03.044)
6. Laybourn-Parry J, Pearce DA. 2007 The biodiversity and ecology of Antarctic lakes: models for evolution. *Phil. Trans. R. Soc.* B **362**, 2273-2289. (doi: 10.1098/rstb.2006.1945)
7. Jungblut AD, Wood SA, Hawes I, Webster-Brown J, Harris C. 2013 The Pyramid Trough Wetland: environmental and biological diversity in a newly created Antarctic protected area. *FEMS Microbiol. Ecol.* **82**, 356 - 366. (doi.org/10.1111/j.1574-6941.2012.01380.x)

8. Chown, SJ, Clarke A, Fraser CI, Cary SC, Moon KL, McGeoch MA. 2015 The changing form of Antarctic biodiversity. *Nature* **522**, 431-438. (doi:10.1038/nature14505)
9. Jungblut AD, Lovejoy C, Vincent WF. 2010 Global distribution of cyanobacterial ecotype sin the cold biosphere. *ISME J.* **4**, 191-202. (doi:10.1038/ismej.2009.113)
10. Bahl J, Lau MCY, Smith GJ, Vijaykrishna D, Craig D, Cary SC, Lacap DC, Lee CK, Papke RT, Warren-Rhodes KA, et al. (2011) Ancient origins determine global biogeography of hot and cold desert cyanobacteria. *Nat. Commun.* **2**: 163. (doi:10.1038/ncomms1167)
11. Zhang L, Jungblut AD, Hawes I, Andersen DT, Sumner DY, Mackey TY. 2015 Cyanobacterial diversity in benthic mats of the McMurdo Dry Valley lakes, Antarctica. *Polar Bio.l* **38**, 1097-1110. (10.1007/s00300-015-1669-0)
12. Fritsch FE. 1912. Freshwater algae. In: Bell, FJ (ed). National Antarctic Expedition 1901-04, Natural History Report, Zoology and Botany, (British Museum (Natural History), London, pp 1-60.
13. Chown SL, Huiskes AHL, Gremmen NJM, Lee JE, Terauds A, Crosbie K, Frenot Y, Hughes KA, Imura S, Kiefer K, *et al.* 2012, Continent-wide risk assessment for the establishment of nonindigenous species in Antarctica. *Proc. Natl. Acad. Sci. USA* **109**, 4938-4943. (doi: 10.1073/pnas.1119787109)
14. Scott RF. 1907. The South Polar Times. Smith, Elder and Co, London.
15. Jungblut AD, Lovejoy C, Vincent 2010 Global distribution of cyanobacterial cold ecotypes in the cold biosphere. *ISME J* 4, 191-202. (doi:10.1038/ismej.2009.11)
16. Jungblut AD, Wood SA, Hawes I, Webster-brown J, Harris C. 2012. The Pyramid Trough Wetland: environmental and biological diversity in a newly created Antarctic

protected area. *FEMS Microbiol Ecol* **82**, 356-366. (doi: 10.1111/j.1574-6941.2012.01380.x)

17. Kleinteich J., Hildebrand F., Wood S.A., Cirs S, Agha R, Quesada A, Pearce DA, Convey P, Kuepper FCK, Dietrich DR (2014) Diversity of toxin and non-toxin containing cyanobacterial mats of meltwater ponds on the Antarctic Peninsula: a pyrosequencing approach. *Antarct Sci* **26**, 521-532 (doi.org/10.1017/S0954102014000145)
18. Taton A, Grubisic S, Brambilla E, de Wit R, Wilmotte A. 2003 Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. *Appl. Environ. Microbiol.* **69**, 5157–5169.
19. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, *et al.* 2010 QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**, 335-336. (doi: 10.1128/AEM.69.9.5157-5169.2003)
20. McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A. 2012 An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.* **6**, 610-8. (doi:10.1038/ismej.2011.139)
21. Hammer Ø, Harper DAT, Ryan, PD. 2001 PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4: 9pp: http://palaeo-electronica.org/2001_1/past/issue1_01.htm
22. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

406 23. Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the
407 RAxML web servers. *Syst Biol* **57**, 758–771. (http://dx.doi.org
408 /10.1080/10635150802429642).
409 24. Zakhia, F., Wilmotte, A., Vincent, W.F., Taton, A., Jungblut, A.D. 2009
410 Cyanobacteria in cold environments, In: Gerday, C, Marx, JC, Schinner, F, Margesin,
411 R (eds) Psychrophiles: from Biodiversity to Biotechnology, Springer-Verlag, Berlin.
412 pp. 121-135.
413 25. Stegen JC, Lin X, Fredrickson JK, Chen X, Kennedy DW, Murray CJ, Rockhold ML,
414 Konopka A. 2013. Quantifying community assembly processes and identifying
415 features that impose them. *ISME J.* **7**, 2069-2079. (doi:10.1038/ismej.2013.93)
416 26. Shade A, Peter H, Allison SD, Baho D, Berga M, Buergmann H. 2012 Fundamentals
417 of microbial community resistance and resilience. *Front. Microbiol.* **3**, 417. (doi:
418 10.3389/fmicb.2012.00417)
419 27. Allison SD, Martiny JBH. 2008 Resistance, resilience, and redundancy in microbial
420 communities. *Proc. Natl. Acad. Sci. USA* **105**, 11512-11519.
421 28. Hawes I, Howard-Williams C, Sorrell B. 2014 Variability in ecosystem properties in
422 the ponds of the McMurdo Ice Shelf, Southern Victoria Land, Antarctica on decadal
423 timescales. *Antarc. Sci.* **26**, 219-230. (doi: 10.1073/pnas.0801925105)
424 29. McKenzie RL, Aucamp PJ, Bais AF, Bjrn LO, Ilyas M, Madronich S. 2011 Ozone
425 depletion and climate change: impacts on UV radiation. *Photochem. Photobiol. Sci.*
426 **10**, 182–198.
427 30. Sabbe K, Hodgson DA, Verleyen E, Taton A, Wilmotte A, VanHoutte K,
428 VyvermanW. 2004 Salinity, depth and the structure and composition of microbial
429 mats in continental Antarctic lakes. *Freshw. Biol.* **49**, 296–311. (doi:
430 10.1039/c0pp90034f.)

431 31. Hawes I, Howard-Williams C. 1998 Primary production processes in streams of the
432 McMurdo Dry Valleys, Antarctica. In Priscu JC (ed.) The McMurdo Dry Valleys,
433 Antarctica a cold desert ecosystem. American Geophysical Union, Washington. pp.
434 189-204.
435 32. Howard-Williams C, Pridmore R, Downes MT, Vincent WF. 1989 Microbial
436 biomass, photosynthesis and chlorophyll a related pigments in the ponds of the
437 McMurdo Ice Shelf, Antarctica. *Antarct. Sci.* **1**,125–131.
438 33. Michaud AB, Šabacká M, Priscu, JC. 2012. Cyanobacterial diversity across landscape
439 units in a polar desert: Taylor Valley, Antarctica. *FEMS Microb. Ecol.* **82**, 268-278.
440 (doi: 10.1111/j.1574-6941.2012.01297.x)
441 34. Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JBH. 2012 Beyond
442 biogeographic patterns: processes shaping the microbial landscape. *Nat. Rev.*
443 *Microbiol.* **10**, 497-506. (doi: 10.1038/nrmicro2795.)
444 35. Broady PA, Smith RA. 1994 A preliminary investigation of the diversity,
445 survivability and dispersal of algae introduced into Antarctica by human activity.
446 *Proc. NIPR Symp. Polar Biol.* **7**, 185-197.
447 36. Shea K, Chesson P. 2012 Community ecology theory as a framework for biological
448 invasions. *Trends Ecol. Evol.* **17**, 170-176. (doi: 10.1016/S0169-5347(02)02495-3)
449 37. Walsh JE. (2009) A comparison of Arctic and Antarctic climate change, present and
450 future. *Antarc Sci* **3**, 179-188.
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452

453 **Figure captions**

454

455 **Figure 1**. A) Geographic location of Ross Island (RI) and McMurdo Ice Shelf (MIS) in
456 Antarctica. B) Locations of the herbarium cyanobacteria specimens collected during Captain
457 Scott's "Discovery" National Antarctic Expedition at their winter quarters on Ross Island and
458 eskers on the McMurdo Ice Shelf halfway between Black Island (BI) and Brown Peninsula
459 (BP) in 1902-03. Modern Antarctic cyanobacterial communities were collected on Ross
460 Island (Cape Royds and Evans as well as Armitage and Hut Point adjacent to location of
461 former winter quarters) and on the McMurdo Ice Shelf including halfway between Black
462 Island and Brown Peninsula and near Brown Peninsula in 2011-2012.

463

464 **Figure 2.** Antarctic cyanobacterial communities in A) a herbarium specimen collected in
465 1902-1903 during Captain Scott's Discovery Expedition, and B) modern communities
466 growing in a meltwater ponds on the McMurdo Ice Shelf, Antarctica.

467

468 **Figure 3.** Comparison of relative composition (%) of cyanobacteria genera or lowest
469 assigned taxonomic rank in cyanobacterial mat communities collected as part of Scott's
470 Discovery expedition in 1903 (red colored names) and 14 modern samples (blue colored
471 names) collected in 1911 and 1912 on Ross Island and the McMurdo Ice Shelf, Antarctica.

472

473 **Figure 4**: Number of sequences of the 10 most abundant OTUs (99% clustering) in the
474 historic and modern Antarctic cyanobacteria mat community samples.

475

476 **Figure 5.** Non-metric dimensional scaling plots (2D) of 16S rRNA gene cyanobacteria mat
477 communities collected as part of Scott's Discovery expedition in 1903 (red circles) and 14
478 modern samples collected in 1911 and 1912 on Ross Island and the McMurdo Ice Shelf (blue
479 circles), Antarctica with convex hulls indicating smallest polygon containing all samples
480 from the Scott and modern samples respectively.

Figure 1

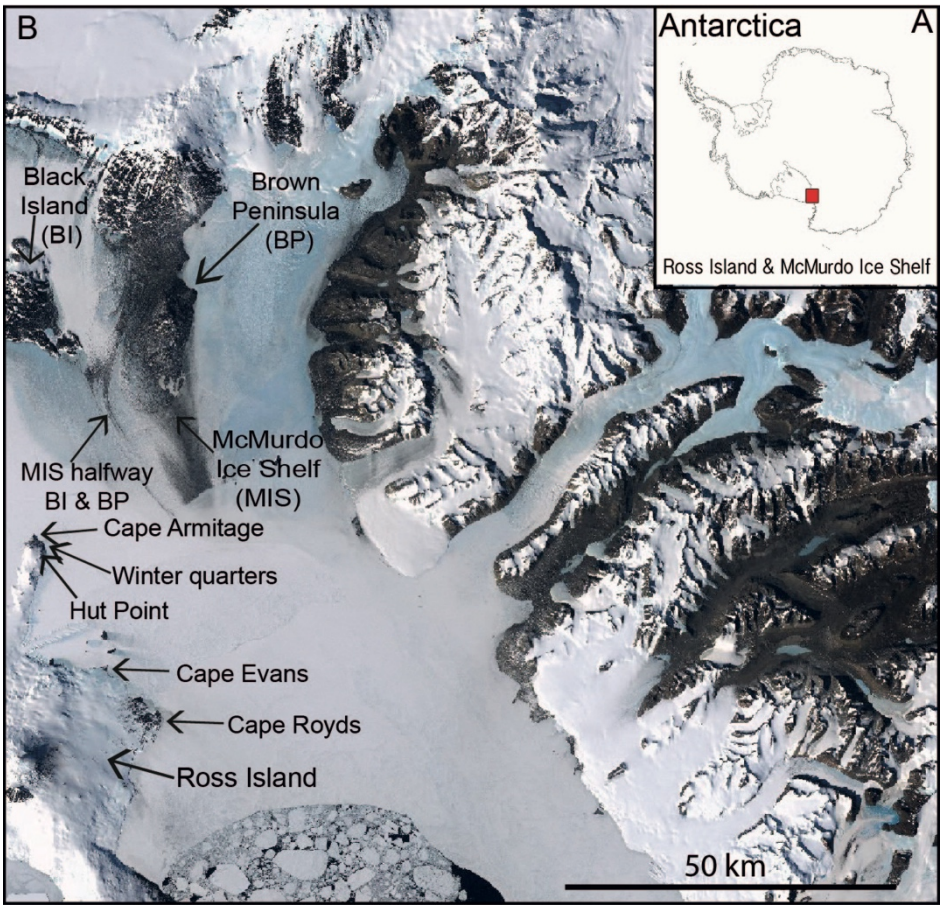
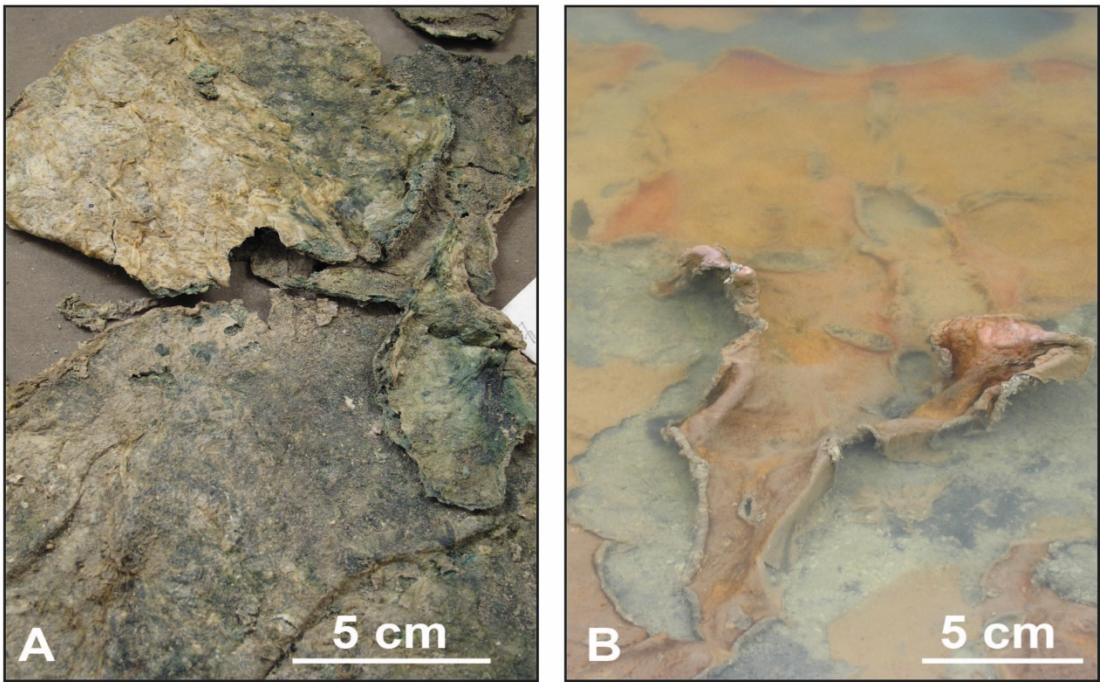

B
Antarctica
A
Ross Island & McMurdo Ice Shelf
Black Island (BI)
Brown Peninsula (BP)
McMurdo Ice Shelf (MIS)
MIS halfway BI & BP
Cape Armitage
Winter quarters
Hut Point
Cape Evans
Cape Royds
Ross Island
50 km

Figure 2



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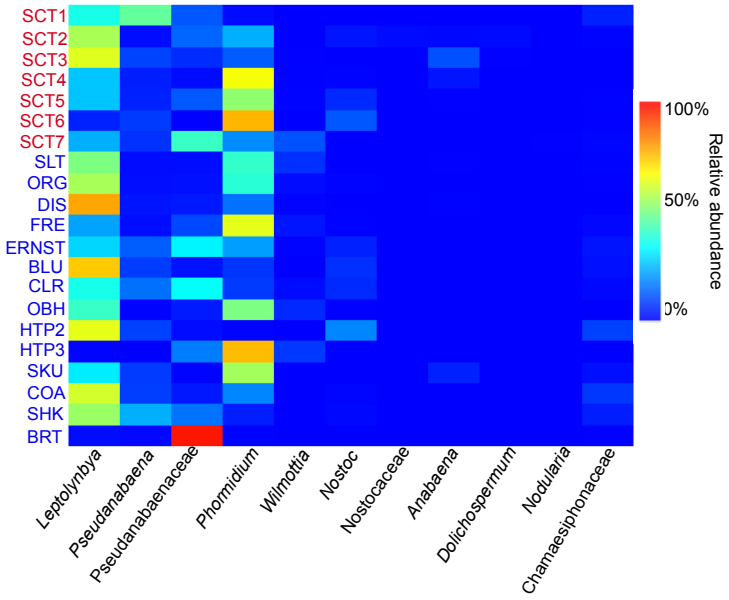
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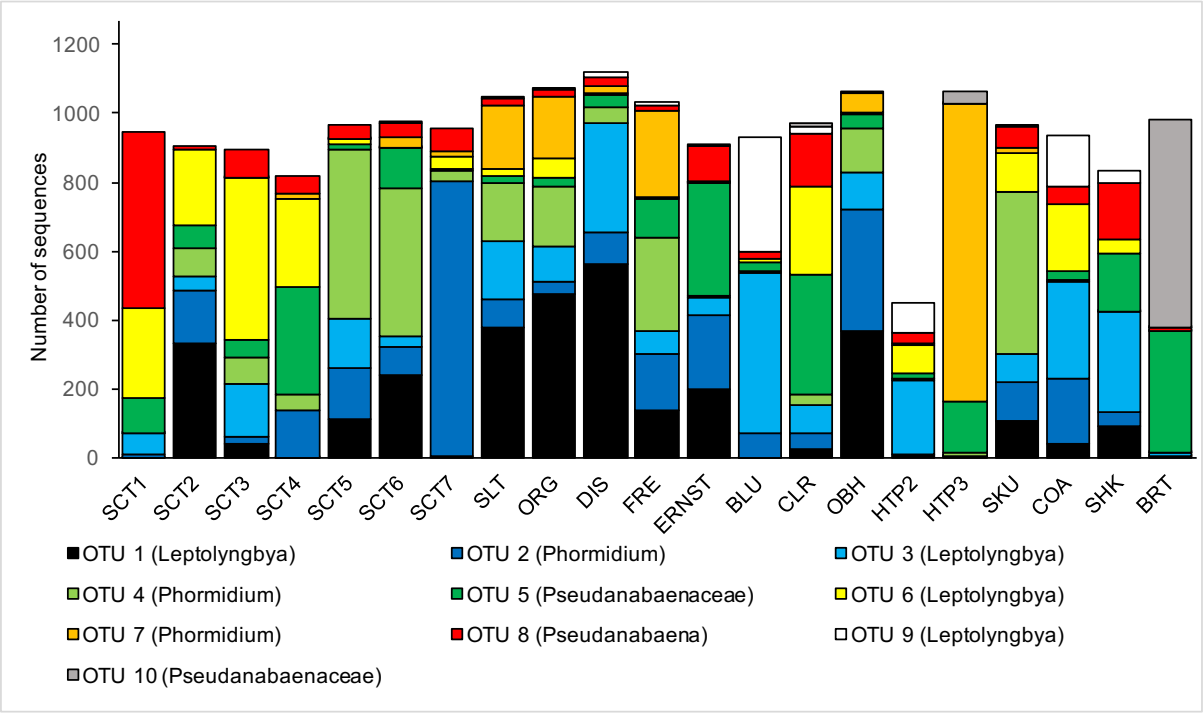
OBH
HTP2
HTP3
SKU
COA
SHK
BRT
Leptolynbya
Pseudanabaena
Pseudanabaenaceae
Phormidium
Wilmottia
Nostoc
Nostocaceae
Anabaena
Dolichospermum
Nodularia
Chamaesiphonaceae
100%
50%
0%
Relative abundance

Figure 4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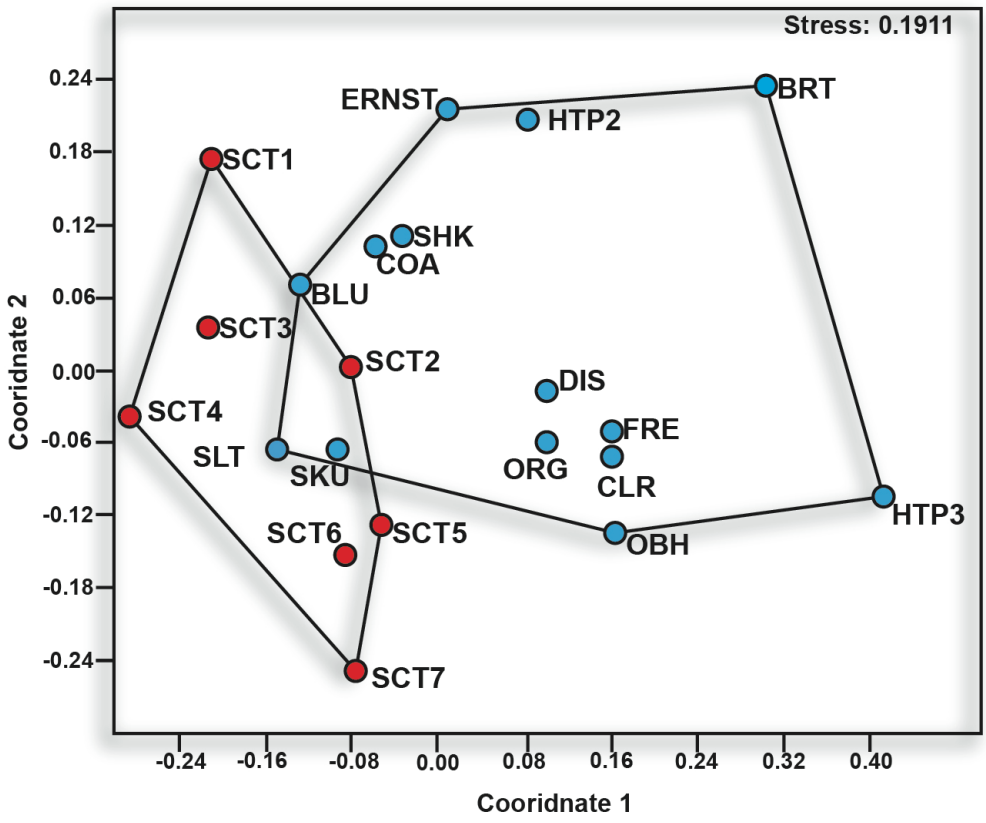
ORG
DIS
FRE
ERNST
BLU
CLR
OBH
HTP2
HTP3
SKU
COA
SHK
BRT
OTU 1 (Leptolyngbya)
OTU 2 (Phormidium)
OTU 3 (Leptolyngbya)
OTU 4 (Phormidium)
OTU 5 (Pseudanabaenaceae)
OTU 6 (Leptolyngbya)
OTU 7 (Phormidium)
OTU 8 (Pseudanabaena)
OTU 9 (Leptolyngbya)
OTU 10 (Pseudanabaenaceae)

Figure 5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Cooridnate 1