Structure and diversity of bacterial, eukaryotic and archaeal communities in glacial cryoconite holes from the Arctic and the Antarctic

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Abstract

The cryosphere presents some of the most challenging conditions for life on earth. Nevertheless, (micro)biota survive in a range of niches in glacial systems, including water-filled depressions on glacial surfaces termed cryoconite holes (centimetre to metre in diameter and up to 0.5 m deep) that contain dark granular material (cryoconite). In this study, the structure of bacterial and eukaryotic cryoconite communities from ten different locations in the Arctic and Antarctica was compared using T-RFLP analysis of rRNA genes. Community structure varied with geography, with greatest differences seen between communities from the Arctic and the Antarctic. DNA sequencing of rRNA genes revealed considerable diversity, with individual cryoconite hole communities containing between six and eight bacterial phyla and five and eight eukaryotic ‘first-rank’ taxa and including both bacterial and eukaryotic photoautotrophs. Bacterial Firmicutes and Deltaproteobacteria and Epsilonproteobacteria, eukaryotic Rhizaria, Haptophyta, Choanomonada and Centroheliozoa, and archaea were identified for the first time in cryoconite ecosystems. Archaea were only found within Antarctic locations, with the majority of sequences (77%) related to members of the Thaumarchaeota. In conclusion, this research has revealed that Antarctic and Arctic cryoconite holes harbour geographically distinct highly diverse communities and has identified hitherto unknown bacterial, eukaryotic and archaeal taxa, therein.

Introduction

The cryosphere comprises ~10% of the earth’s terrestrial surface (Paterson, 1994). Within polar regions, despite sub-zero temperatures, months of darkness and low nutrient conditions, a variety of cold habitats have been found to harbour microbial communities including permanently ice-covered lakes (Priscu et al., 1998), sea ice (Thomas & Dieckmann, 2002), snow (Jones et al., 2001; Amato et al., 2007), permafrost soils (Rivkina et al., 2000) and rock environments (de la Torre et al., 2003), as well as glacial moraine (Stibal et al., 2006) and subglacial and supraglacial environments (Hodson et al., 2008). In supraglacial systems, cryoconite holes are emerging as an important microbial ecosystem, comprising water-filled depressions on the glacial surface containing small spheres (0.1–3 mm in diameter) of dark granular material (cryoconite) at the hole base (Gerdel & Drouet, 1960; Takeuchi et al., 2001a). Cryoconite holes can cover 0.1–10% of the surface of a glacier (Anesio et al., 2009), can range in size from a few centimetres to more than a metre in diameter and can be up to 50 cm in depth (Gerdel & Drouet, 1960; Fountain et al., 2004). Cryoconite holes form when dark material (e.g. soil or dust) is deposited onto the surface of the glacier. The low albedo of this material causes increased absorption of solar energy, relative to the surrounding ice, resulting in melting of the ice below. As the ice melts, a water-filled hole...
is created into which the dark material sinks (Gajda, 1958; McIntyre, 1984).

Microorganisms present in cryoconite holes are predominantly found within the cryoconite granular material, although smaller numbers of microorganisms are found within cryoconite hole water; for example, Säwström et al. (2002) found $4.5 \times 10^3$ cells mL$^{-1}$ in a cryoconite hole from Svalbard. Original observations of microorganisms within cryoconite holes identified 'brown polycellular' algae (Leslie, 1879). Subsequently, observational, microscopy- and culture-based techniques have been used to study cryoconite hole microbial assemblages and have shown considerable diversity, identifying several bacterial and eukaryotic phyla (Gerdel & Drouet, 1960; Mueller & Pollard, 2004; Porazinska et al., 2004) together with viruses (Säwström et al., 2002; Anesio et al., 2007).

There have been two prior studies that have employed molecular approaches to investigate cryoconite community composition (Christner et al., 2003; Edwards et al., 2011). In a study of a cryoconite community within a single hole on Canada glacier, Taylor Valley, Antarctica (Christner et al., 2003), bacterial and eukaryotic rRNA gene clone libraries were generated from DNA isolated from cryoconite, revealing a diverse microbial community therein. More recently, Edwards et al. (2011) demonstrated, via terminal restriction fragment length polymorphism (T-RFLP) analysis, the occurrence of distinct bacterial communities within cryoconite holes on either cold (Austre Brøggerbreen) or polythermal (Midre Lovénbreen and Vestre Brøggerbreen) valley glaciers that are located < 10 km apart in Svalbard, in the High Arctic. However, it is not known the extent to which the composition of these communities is representative of other cryoconite holes, either locally or over great distances. Three additional studies have directly compared the microbial communities of cryoconite holes from different polar locations; whilst these studies were limited to microscopy-based approaches and focused on a limited range of primarily eukaryotic taxa (together with cyanobacteria), the composition of cryoconite communities was found to be similar between holes in close proximity (Taylor Valley; Porazinska et al., 2004) and to vary between holes from an Arctic and an Antarctic glacier (Mueller et al., 2001; Mueller & Pollard, 2004). Similarly, studies of snow algae and bacteria have highlighted both environmental variability, for example, altitude (Yoshimura et al., 1997) and also the distribution of local biological material (Liu et al., 2009), as factors that can influence the structure and composition of snow microbial communities.

In this study, we have explored the structure and diversity of cryoconite bacterial, eukaryotic and archaeal communities from multiple Arctic and Antarctic locations using a molecular (rRNA gene)-based approach. The specific aims of this study were first to investigate whether cryoconite hole microbial communities exhibited spatial variability within and between Arctic and Antarctic glacial regions. Second, this research sought to identify the dominant bacterial, eukaryotic and, for the first time, archaeal taxa that are present within cryoconite holes from different polar locations. Together, these studies aimed to improve our understanding of the diversity and possible origins, functions and trophic interactions of microbial communities in cryoconite holes.

**Materials and methods**

**Sampling sites and cryoconite hole sampling**

Cryoconite was sampled from a total of 39 individual holes across 10 glacial locations, with holes separated by distances ranging from a few metres up to $\sim 1.5$ km apart on individual glaciers, in northern and southern hemisphere polar regions (Table 1, Fig. 1).

It is important to note that at several locations (A-S, S-R, S-F, S-V and N-J; see Table 1), only one or two communities were sampled because of the opportunistic nature of securing samples from remote locations. All cryoconite samples were taken from holes ranging from 5 to 30 cm in diameter. All Arctic communities were sampled between late July and early September, within a year, and all Antarctic communities were sampled in January. The age of individual hole was unknown, and glacial locations could not be revisited to enable investigation of temporal variability. Note that only two Antarctic communities were available for analysis: Signy Island (A-S), a maritime island glacier; and Vestfold (A-V), a mainland coastal glacier.

Total C and N (%) were determined by freeze-drying samples overnight, followed by sample grinding and subsequent isotope ratio mass spectrometry analysis using a Sercon PDZ Europa 20-20 ANCA-GSL IRMS (Cheshire, UK). Solid cryoconite for molecular analysis was harvested using a sterile plastic Pasteur pipette. Material was placed into sterile Eppendorf tubes, and any excess liquid was removed. Samples were frozen at $-20$ °C on return to the UK, and on arrival, the samples were stored at $-80$ °C.

**Nucleic acid extractions from cryoconite granules**

Genomic DNA was extracted from cryoconite sampled from individual holes using the PowerSoil™ DNA isolation kit (Mo Bio laboratories, Cambridge, UK) in
Table 1. Location, situation and carbon and nitrogen content of cryoconite hole sampling sites

<table>
<thead>
<tr>
<th>Location</th>
<th>Glacier identification</th>
<th>Site code</th>
<th>No. of holes sampled</th>
<th>Latitude and Longitude</th>
<th>Mean total carbon (%)*</th>
<th>Total carbon (%)†</th>
<th>Total nitrogen (%)‡</th>
<th>C/N ratio‡</th>
<th>Characteristics of location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antarctica – Signy Island</td>
<td>Tuva Glacier (unofficial name)</td>
<td>A-S</td>
<td>1</td>
<td>60° 41' S 45° 38' W</td>
<td>0.46 (n = 1)</td>
<td>0.46 (A-S1)</td>
<td>0.06 (A-S1)</td>
<td>7.67</td>
<td>Small, west-facing outlet glacier from Signy Island Ice Cap.</td>
</tr>
<tr>
<td>Antarctica – Vestfold Hills</td>
<td>Sæsdal Glacier</td>
<td>A-V</td>
<td>9</td>
<td>68° 39' S 78° 21' E</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>North-west-facing flanks of outlet glacier from East Antarctic Ice Sheet</td>
</tr>
<tr>
<td>Greenland – South West Ice Sheet</td>
<td>Kangerlussuaq</td>
<td>G-K</td>
<td>6</td>
<td>67° 09' N 50° 01' W</td>
<td>0.69 ± 0.17 (n = 6)</td>
<td>0.62 (G-K5)</td>
<td>0.10 (G-K5)</td>
<td>6.2</td>
<td>West-facing flanks, central-west Greenland Ice Sheet</td>
</tr>
<tr>
<td>Greenland – Kronprins Christian Land</td>
<td>Greenland Ice Sheet</td>
<td>G-Kp</td>
<td>3</td>
<td>79° 55' N 24° 06' W</td>
<td>2.15 ± 0.91 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>North-east-facing flanks, north-east Greenland Ice Sheet</td>
</tr>
<tr>
<td>Svalbard – Central Spitsbergen</td>
<td>Longyearbreen</td>
<td>S-L</td>
<td>6</td>
<td>78° 10' N 15° 30' E</td>
<td>2.69 ± 0.56 (n = 3)</td>
<td>3.26 (S-L2)</td>
<td>0.21 (S-L2)</td>
<td>15.5</td>
<td>Small valley glacier, central Svalbard</td>
</tr>
<tr>
<td>Svalbard – central Spitsbergen</td>
<td>Reiperbreen</td>
<td>S-R</td>
<td>1</td>
<td>78° 7' N 16° 4' E</td>
<td>1.54 (n = 1)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Small valley glacier, central Svalbard</td>
</tr>
<tr>
<td>Svalbard – central Spitsbergen</td>
<td>Foxonna</td>
<td>S-F</td>
<td>2</td>
<td>78° 08' N 16° 07' E</td>
<td>1.69 ± 0.59 (n = 2)</td>
<td>2.11 (S-F2)</td>
<td>0.02 (SF-2)</td>
<td>105.5</td>
<td>East-facing flank of small mountain ice cap, central Svalbard</td>
</tr>
<tr>
<td>Svalbard – north-west Spitsbergen</td>
<td>Midre Lovønbreen</td>
<td>S-M</td>
<td>8</td>
<td>78° 53' N 12° 03' E</td>
<td>2.51 ± 0.57 (n = 8)</td>
<td>2.78 (S-M2)</td>
<td>0.25 (S-M2)</td>
<td>11.42</td>
<td>Small valley glacier, north-west Svalbard</td>
</tr>
<tr>
<td>Svalbard – west Nordaustlandet</td>
<td>Vestfonna</td>
<td>S-V</td>
<td>2</td>
<td>79° 48' N 18° 24' E</td>
<td>6.42 ± 0.58 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>West-facing flank of ice cap, north Svalbard</td>
</tr>
<tr>
<td>Norway – Jostedalsbreen Ice Cap</td>
<td>Austerdalsbreen</td>
<td>N-J</td>
<td>1</td>
<td>61° 35' N 06° 58' E</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>South-east-facing outlet glacier from Jostedalsbreen Ice Cap, central-east Norway</td>
</tr>
</tbody>
</table>

ND, not determined.

*Mean total carbon ± SE was determined from multiple holes (as indicated) that were sampled on each glacier.

†(%) Total carbon and total nitrogen.

‡C/N ratios are presented within individual cryoconite holes from which rRNA gene libraries were generated (hole locations as indicated).
accordance with the manufacturer’s instructions and using approximately 0.8 g (dry weight) of solid cryoconite material. DNA was eluted into a final volume of 30 μL of nuclease-free water (Ambion, Warrington, UK).

**PCR amplification**

16S and 18S ribosomal RNA (rRNA) genes were amplified using PCR. Reactions (50 μL) comprised 0.4 μM of each forward and reverse oligonucleotide primer, 200 μM of each deoxyribonucleotide triphosphate (dNTPs), 1 mM MgCl2, 1x reaction buffer (Bioline, London, UK), 2.5 U of Taq polymerase (Bioline) and 1 μL of DNA (ranging from 14.7 to 45.6 ng DNA μL⁻¹). For 16S rRNA gene amplification, primers 27F (5′-AGA GTT TGA TCM TGG CT C AG-3′) (Lane, 1991) and 1389R (5′-ACG GGC GGT GT G TAG AAG-3′) (Osborn et al., 2000) were used. For 18S rRNA gene amplification, primers Euk1a (5′-CTG GTT GAT CCT GCC AG-3′) (Amann et al., 1990) and 516R (5′-ACC AGA CTT GCC CTC C-3′) (Sogin & Gundersen, 2006) were used. For archaeal 16S rRNA gene amplification, primers Arch21F (5′-TTC CGG TTG ATC CYG CCG GA-3′) and Arch958R (5′-YCC GCC GGT GTG GAM TCC AAT T-3′) (DeLong et al., 1994) were used. For T-RFLP analysis, forward primers were labelled at the 5′ end with 6′-carboxyfluorescein (6-FAM). For 16S rRNA gene T-RFLP analysis, FAM-63F (5′-CAG GCC TAA CAC ATG CAA GTC-3′) (Marchesi et al., 1998) was used in combination with the reverse primer 1389R. For 18S rRNA T-RFLP analysis, the 6-FAM-labelled Euk1a primer was used in combination with the reverse primer 516R.

The PCR cycling conditions for 16S and 18S rRNA gene amplifications consisted of an initial denaturation of 94 °C for 2 min, followed by 25 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min 30 s, with a final extension at 72 °C for 10 min. Archaeal rRNA genes were amplified using an initial denaturation at 98 °C for 1 min followed by 30 cycles of 92 °C for 45 s, 45 °C for 45 s and 72 °C for 45 s and a final extension at 72 °C for 5 min. PCR products were visualized by gel electrophoresis with ethidium bromide staining to ensure that the correct size fragment was amplified.

**T-RFLP analysis**

Microbial communities were analysed by T-RFLP analysis (Liu et al., 1997; Osborn et al., 2000). Fluorescently labelled PCR products were purified using a QIAquick PCR purification kit (Qiagen, Crawley, UK) prior to restriction digestion at 37 °C for 3 h in reactions containing 5 μL of PCR product, 10 U of CfoI (Roche, Hertfordshire, UK), 1x reaction buffer and made up to a total volume of 20 μL with sterile deionized water; 5 μL of the digested product was desalted by addition of 0.25 μL of glycogen (20 mg mL⁻¹) and 75 μL of 0.2 mM MgSO4.7H2O in 70% ethanol wash solution. The mixture was vortexed thoroughly and incubated at room temperature for 15 min before centrifugation at 11 300 g for 15 min. The pellet was air dried and resuspended in 5 μL of nuclease-free water (Ambion); 0.25 μL of digested desalted DNA was added to 9.75 μL of a formamide mix containing a GeneScan™ 500 ROX™ size standard.
run time was 20 min. GenEMAPPER
and 15-sec injection. Then, 10 voltage ramps were per-
(Applied Biosystems) in POP7 polymer using a 2-volt
electrophoresed on an ABI 3730 Genetic Analyzer
Digestion products were denatured at 95 °C for 3 min
and electrophoresed on an ABI 3730 Genetic Analyzer
et al. identified using Mallard version 1.02 (Ashelford
sion 1.34 (www.technelysium.com.au). Chimeras were
vector primers on an ABI 3730 Genetic Analyzer.
Sequencing Kit (Applied Biosystems) using T3 and T7
nál restriction fragments (T-RFs) that were larger than 50
bases long and with peak heights of 50 fluorescence units
or greater were included in the analysis. The relative
abundance of the peak area of each individual T-RF was
calculated as a proportion of the total peak area of all
peaks from within each individual T-RFLP profile.
T-Align (Smith et al., 2005) was then used to determine
the relative abundances of each peak size between all the
community samples. Primer 6, version 6.1.10 software
(Applied Biosystems, Warrington, UK) was used to calculate a
Bray–Curtis resemblance matrix from square root-transformed
data sets and then displayed as cluster dendro-

Clone library construction, sequencing and analysis
PCR products, amplified from DNA isolated from cryo-
conite in individual holes, were purified using the QIA-
quick PCR purification kit (Qiagen), ligated into the
pCR2.1-TOPO® TA cloning vector (Invitrogen, Paisley,
UK) and transformed into One Shot® Chemically Com-
potent Escherichia coli TOP10 F’ cells (Invitrogen) in accordance with the manufacturer’s instructions. Trans-
formed cells were plated on Luria–Bertani (LB) agar con-
taining ampicillin (50 μg mL⁻¹) and X-gal (20 μg mL⁻¹)
and incubated overnight at 37 °C. White colonies were
picked at random and used to inoculate 100 μL of LB
broth containing ampicillin. After 2-hour incubation at
37 °C, 1 μL of each culture was used in PCR to amplify
dNA using vector-specific primers T3 (5’-ATT
AAC CCT CAC TAA AGG GA-3’) and T7 (5’-TAA TAC
GAC TCA CTA TAG GG-3’) (Invitrogen). The amplified
vector inserts were purified using SureClean (Bioline) and
and were sequenced using a BigDye® Terminator v3.1 Cycle
Sequencing Kit (Applied Biosystems) using T3 and T7
vector primers on an ABI 3730 Genetic Analyzer.
Sequences were edited manually using CHROMAS PRO
version 1.34 (www.techneylsium.com.au). Chimeras were
identified using Mallard version 1.02 (Ashelford et al.,
2006). FASTA-formatted 16S and 18S rRNA gene
sequences were compared to the GenBank database using
BLASTN (Altschul et al., 1990). Note that initial use of
RDP-Classifier (rdp.cme.msu.edu/classifier/) resulted in
unclassified matches for > 12% of bacterial sequences
(data not shown), whilst this software cannot currently be
used for classification of eukaryotic RNA gene sequences.
BioEDIT version 7.0.5.3 (Hall, 1999) was used to align the
library of sequences using CLUSTALW (Thompson et al.,
1994). MEGA version 4.1 (Tamura et al., 2007) was used to
calculate evolutionary distances and create phylogenetic
trees from the cryoconite hole sequences generated during this
study, using the neighbour-joining method (Saitou &
Nei, 1987) with bootstrap analysis (1000 replicates).
Operational taxonomic units (OTUs) were defined using a
cut-off of 97% identity. Nonparametric taxon richness
estimates (Chao1 and the Abundance-based Coverage
Estimator: ACE) were determined using DOTUR (Schloss &
Handelsman, 2005).

Nucleotide accession numbers
rRNA gene sequences were deposited in the GenBank
database with accession numbers GU298304–GU298966
(bacteria), GU297612–GU298216 (eukaryotes) and
GU298217–GU298303 (archaea).

Results
Geographical variation in cryoconite bacterial and eukaryotic community structure
T-RFLP analysis of 16S rRNA genes was conducted using
cryoconite DNA isolated from individual cryoconite holes
across 10 glacial locations in the Arctic and the Antarctic
(Table 1 and Fig. 1). T-RFLP analysis yielded a total of
209 distinct T-RFs from the 34 cryoconite holes. Cluster
analysis revealed two main clusters of bacterial cryoconite
communities (Fig. 2a). The first cluster comprised solely
of communities taken from the Antarctic Vestfold Hills
(cluster I), whilst the second cluster contained all of the
communities from the Arctic as well as the cryoconite
community from Signy Island, Antarctica (cluster II).
Within cluster II, communities from northern Svalbard
and northern Greenland (Kronprins Christian Land)
shared the highest similarity (> 44%).

T-RFLP analysis of eukaryotic 18S rRNA genes yielded
a total of 200 distinct T-RFs from 36 cryoconite holes.
Four clusters of eukaryotic cryoconite communities were
identified on the basis of T-RFLP analysis from the 10
Arctic and Antarctic glacial locations (Fig. 2b). Two of
the clusters consisted of cryoconite communities from the
southern hemisphere (clusters I and II), and two clusters
comprised of the communities from the northern

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hemisphere (clusters III and IV). Communities from the two Antarctic sampling locations were distinct from each other with communities from the Vestfold Hills forming a single cluster sharing at least 44% similarity (cluster II), whilst the cryoconite community from Signy Island clustered separately (cluster I). Twenty-four of the 26 cryoconite communities sampled from the northern hemisphere comprised a single cluster (cluster III). Within this cluster, only the communities from northern Svalbard at Vestfonna showed location-specific clustering (cluster IIIb); otherwise, there was no location-based clustering of the communities from or within Greenland, Svalbard or mainland Norway. Two communities from the northern hemisphere clustered as outliers (cluster IV).

Composition and relative abundance of bacterial taxa within cryoconite communities

Six cryoconite holes were chosen for further analysis on the basis that they represented distinct geographical locations in the Arctic and Antarctic and/or that they were representative of particular clusters following T-RFLP analysis. The composition of the communities within these holes was studied further through sequencing of 16S rRNA gene clone libraries. All of these bacterial cryoconite communities contained sequences that were related to those from members of between six and eight phyla (Fig. 3a). In each community, sequences related to those from both autotrophic and heterotrophic bacteria were identified, and all six communities included sequences related to Alphaproteobacteria, Betaproteobacteria and Deltaproteobacteria, as well as to members of the Bacteroidetes, Cyanobacteria and Actinobacteria. When compared to the other communities, the Signy Island community included a large proportion of betaproteobacteria-related sequences (45%), whilst the Kangerlussuaq community was dominated by Cyanobacteria-related sequences (52%). Most of the communities contained sequences that were related to either two or three different phyla comprised of autotrophic bacteria. However, within the Antarctic Vestfold Hills community, only cyanobacteria-related sequences were identified as likely bacterial primary producers. Additionally, the Vestfold cryoconite sample was the only community in which Gemmatimonadetes-related sequences were found.

Of the 663 16S rRNA gene clones that were sequenced across the six cryoconite communities, a total of 209 bacterial OTUs were identified using a cut-off of 97% similarity (Supporting Information, Fig. S1). The majority (79%) of the OTUs were present within multiple locations. Of these OTUs, 29 were identified within both an Arctic and an Antarctic community, and 14 were identified within two or more Arctic communities. The diversity of
species found within each particular phylum ranged from two OTUs (Planctomycetes) to 92 OTUs within the Proteobacteria, which included 42 and 24 OTUs within the Beta-proteobacteria and Alphaproteobacteria, respectively. A further 40 OTUs were identified within the phylum Bacteroidetes (Fig. S1). Despite considerable OTU diversity within many phyla, the majority of the OTUs within a particular phylum or class were most closely related to members of the same order, for example, Burkholderiales within the Betaproteobacteria, Sphingobacteriales within the Bacteroidetes and Actinomycetales within the Actinobacteria. An exception to this trend was seen in the Alphaproteobacteria, in which OTUs were identified that were related to bacteria within five different orders (Sphingomonadales, Rhizobiales, Rhodobacterales, Rhodospirillales and Caulobacterales).

To provide an estimate of ‘global’ bacterial diversity within cryoconite holes, richness estimates were determined across the meta-data set comprising sequences from the six cryoconite hole communities, with a total of 451 and 449 OTUs predicted using Chao1 and the abundance-based coverage estimator (ACE), respectively (Table S1). Hence, the 209 bacterial OTUs identified within this study represent up to ~46% of the total number of bacterial OTUs present within these cryoconite communities.

Based on the sampling efforts, estimates of taxon richness within individual communities were highest in cryoconite holes from the two Antarctic locations (Table S1).

**Composition and relative abundance of eukaryotic taxa within cryoconite communities**

Clone libraries were constructed using 18S rRNA genes amplified from five cryoconite communities that were sampled from different geographical locations and/or from holes that were representative of clusters identified by T-RFLP analysis. Taxonomic assignment was initially made at ‘[Super-group]’ and ‘first rank’ (Fig. 3b), and subsequently at lower ranks, in accordance with the classification proposed by Adl et al. (2005). Sequences related to Cercozoa constituted around a third of the communities at each location (Fig. 3b). Alveolata-related sequences were also present within all five communities, although their relative abundance varied from 4.5% to 23.5%. All three Arctic communities contained sequences related to Metazoa (Tardigrada phylum), and these comprised a significant proportion (45%) of the sequences within the Longyearbyen community. In contrast, Metazoa were not
identified within the Antarctic communities. The greatest diversity at the ‘first-rank’ taxonomic level was found in the Antarctic Signy Island community, where sequences related to representatives of eight different first-rank taxa were identified. Sequences related to members of the taxa Haptophyta, Choanomonada and Centrohelidae [Incerta sedis] were found only at this site. Sequences related to those from photosynthetic organisms (members of the Chloroplastida and Stramenopiles) constituted a large proportion of the communities on the Antarctic Signy Island and at Kangerlussuaq (48% and 39%, respectively). In contrast, the Midre Lovénbreen community contained no Chloroplastida-related sequences and <1% of sequences related to Stramenopiles. Generally, Tubulinea were absent or made up <1% of cryoconite community members, with the exception of the Antarctic Vestfold community, in which 30% of the sequences were related to members of this taxon.

Of the 605 18S rRNA gene clones that were screened across the five cryoconite communities investigated, a total of 96 eukaryotic OTUs were identified, using a cut-off of 97% similarity (Fig. S2). Of these, <15% (14 OTUs) were common to two or more communities; seven of these were found in both an Arctic and an Antarctic community, and a further seven were found only amongst two or more Arctic communities. Within the cryoconite community libraries, there were several first-/second-rank taxa that contained OTUs that were related to members of multiple classes. For example, of the 14 OTUs identified within the second-rank taxon Ciliophora [Alveolata], there were representatives of the fourth rank (classes): Spirorichaea, Nassophorea, Litostomatea, Phyllopharyngea, Prostomatea and Colpodea. Greatest diversity was seen within the Cercozoa [Rhizaria] in which 33 different OTUs were identified amongst the five communities investigated. These Cercozoa OTUs were related to members of four main lineages, within the second-rank taxa: Cercomonadida, Silicofilosea and Phaeodarea, and within the family Vampyrellidae, with Cercomonadida-related sequences being the most common, represented by 24 different OTUs.

To provide an estimate of ‘global’ eukaryotic diversity within cryoconite holes, richness estimates were determined across the meta-data set comprising sequences from five cryoconite hole communities with a total of 147 and 140 OTUs predicted using Chao 1 and ACE, respectively (Table S2). Therefore, the 96 eukaryotic OTUs identified within this study represent up to 65% of the total number of eukaryotic OTUs present amongst these cryoconite communities. Estimates of taxon richness within individual communities were highest in cryoconite holes from Longyearbyen, Svalbard and Signy Island, Antarctica (Table S2).

**Presence and diversity of archaea within cryoconite holes**

PCR was used to screen for the presence of archaea within the cryoconite hole communities. Archaeal 16S rRNA gene sequences were only amplified from the two Antarctic communities, from which both *Thaumarchaeota* (formerly mesophilic *Crenarchaeota*, see Brochier-Armanet et al., 2008) and *Euryarchaeota*-sequences were identified (Fig. 4). The majority (77%) of the sequences were related to *Thaumarchaeota*. However, there was limited diversity amongst this group of organisms with 63 of the 68 clones sharing >97% similarity. Of these *Thaumarchaeota*, the closest related sequences in the databases originated from diverse environments, including the pores of Alpine dolomite rock (AB255764), a cold sulphur-rich water spring (FJ968078) and the rhizosphere of freshwater macrophytes associated with elevated rates of nitrification (EU309862). Within the *Euryarchaeota*, sequences were related to members of two methanogenic classes: *Methanobacteriaeae* and *Methanomicrobia*.

**Determination of total carbon and nitrogen content within cryoconite holes**

Total carbon contents were highest in cryoconite holes from Svalbard and lowest in the single cryoconite hole sampled at Signy Island, Antarctica (Table 1). No obvious patterns in total nitrogen content could be identified between different geographical regions (Table 1), although C/N ratios were higher in Svalbard than in Greenland or Antarctica.

**Discussion**

**Arctic and Antarctic cryoconite holes harbour distinct bacterial and eukaryotic communities**

The structure of bacterial and eukaryotic cryoconite communities was found to vary according to their geographical location, as shown by 16S and 18S rRNA gene T-RFLP analysis (Fig. 2). Whilst prior research had demonstrated local (glacier to glacier) variation within Svalbard cryoconite microbial communities (Edwards et al., 2011), our research has demonstrated variability across a global scale. The communities from cryoconite holes in individual glacier locations often clustered together, suggesting localized sources of organisms and/or similar environmental selection pressures within a number of glacial locations. The most pronounced differences were found upon comparison between Arctic and Antarctic cryoconite communities, which may be a consequence of bipolar variation in mean total carbon content (Table 1).
Within the Arctic, location-specific clustering was more commonly found with respect to bacterial than eukaryotic communities. These findings reflect earlier microscopy-based studies that showed cyanobacterial, algal and invertebrate composition to be similar between cryoconite holes from five different glaciers within Taylor Valley, Antarctica (Porazinska et al., 2004), whilst communities present within an Arctic glacier and an Antarctic glacier were found to be different (Mueller & Pollard, 2004).

Aeolian transport has previously been proposed to account for the similarities across cryoconite communities over short distances (Porazinska et al., 2004). However, more recent molecular characterization of bacterial cryoconite communities present in three Svalbard glaciers (< 10 km apart) suggests that more subtle abiotic variability (e.g. in temperature, pCO₂ and cryoconite inorganic content) leads to local variation in bacterial community structure (Edwards et al., 2011). If the microbial communities within cryoconite holes are seeded in

Fig. 4. Neighbour-joining phylogeny of archaeal 16S rRNA gene sequences cloned from Antarctic Signy Island (A-S Arch) and Antarctic Vestfold Hills (A-V Arch) locations. Sequences are identified with a unique clone number, or where branches of the phylogeny have been collapsed, the number of clones within a cluster is shown in bold type. Closest related database sequences (with accession number) are indicated by a circle. Bootstrap values of > 70% are shown (from 1000 replicates). Scale bar represents 5% sequence divergence.
ways similar to their original formation, as described by Gajda (1958), then aeolian transportation of local terrestrial or aquatic microorganisms onto the glacial surface will be an important contributor to the communities that establish.

In this study, Cercozoa-related sequences were the most abundant and diverse eukaryotic lineage within the cryoconite holes. Note we recognize that identification of the most abundant sequences from eukaryotic communities that comprise both unicellular and multicellular communities can be biased by multiple copies of rRNA genes being amplified from DNA from different cells from the same organism, that is, the relative abundance of multicellular organisms may be overestimated in such communities. Typically, Cercozoa are found in high abundances and are important within soil, freshwater and marine ecosystems (Bass & Cavalier-Smith, 2004). The high abundance of diverse Cercozoa within cryoconite communities would support their aeolian transport from other ecosystems onto these glacial surfaces. Similarly, Chlorophyta have been found to be transported from soil onto snow surfaces (Stibal & Elster, 2005), and terrestrial bacteria, including cyanobacteria, have been proposed to colonize permanent lake ice (Gordon et al., 2000). Cryoconite communities have also previously been noted for their similarities to local permanent lake ice communities and microbial mat communities (Christner et al., 2003).

As the geographical location of a cryoconite hole varies, so will the composition of microbial communities within the local environment that are available to seed them (Mueller et al., 2001). As a consequence, unique communities that are characteristic to each region may develop. It is, however, possible that biological inputs are not solely restricted to the neighbouring environment but that material can be transported over far greater distances, on trade winds (Rousseau et al., 2005; Price, 2009). Whilst local winds (Lyons et al., 2003) and polar easterlies and westerlies might cause community mixing within either Arctic or Antarctic ecosystems, foreign biological material could also travel from temperate regions to be deposited onto polar ice surfaces (Bovallius et al., 1978). However, the extent of this biological input and the ability of these foreign organisms to tolerate and survive the cryosphere’s extreme conditions are limited, although studies of bacteria isolated from Antarctic ice cores (e.g. Christner et al., 2000) suggest that survival is possible.

Taxonomic composition of cryoconite bacterial and eukaryotic communities

Sequencing of rRNA genes was used to study the composition and diversity of cryoconite microbial communities that were representative of different geographical locations and of T-RFLP-derived clusters (Fig. 2). With the proviso that all PCR-based assessments of microbial community diversity are subject to potential biases (von Wintzingerode et al., 1997; Sipos et al., 2007), these cryoconite communities were found to contain members of between six and eight bacterial phyla, five and eight eukaryotic first-rank taxa and, at the two Antarctic locations only, representatives of two archaean phyla. Interestingly, sequences related to both bacterial (Cyanobacteria and/or Chloroflexi) and eukaryotic (Archaeplastida and/or Stramenopiles) photoautotrophs were identified within every location studied, which together will provide a source of organic carbon to these environments.

All of the communities also contained organisms related to both bacterial heterotrophs and eukaryotic grazers, suggesting a self-contained multi-level trophic web. Metazoa (tardigrades) as top grazers were found in Arctic cryoconite holes (Fig. 3 and Fig. S2), but were not identified at the two Antarctic locations, in contrast to Christner et al. (2003), who found tardigrade-related sequences together with those from nematodes and rotifers within a cryoconite community in Taylor Valley, Antarctica. Additionally, the previous observation and identification of viruses within cryoconite holes (Säwström et al., 2002; Anesio et al., 2007) will contribute further to carbon cycling within cryoconite trophic webs (Säwström et al., 2006).

We identified representatives of all of the bacterial phyla and classes that were previously identified, using molecular analysis, within Antarctic (Christner et al., 2003) and Arctic (Svalbard) cryoconite holes (Edwards et al., 2011) were also found in this study. In addition, Delta- and Epsilonproteobacteria and Firmicutes were found in the majority of holes from both the Arctic and Antarctic that were sampled, whilst Epsilonproteobacteria were also present in cryoconite communities in Svalbard. Perhaps, the most striking discovery within this study is the ubiquity, high relative abundance (26–35% of clones sequenced) and diversity of Rhzidaria-related sequences in all of the Arctic and Antarctic locations studied. In contrast, no Rhizaria-related sequences were identified by Christner et al. (2003), perhaps due to differences in specificity of the primers used in their study. Moreover, members of the first-rank lineages Haptophyta, Choaomonada and Centroheliozoa have also been found for the first time within cryoconite communities in our study.

Despite the extreme conditions of the cryosphere, taxon diversity within these cryoconite communities was surprisingly high. Taxon richness estimates (at an OTU threshold of 97% similarity) indicate that up to approximately 46% of the total estimated number of bacterial species and 67% of the total estimated number of eukaryotic species within these cryoconite communities were...
identified within this study. The overall estimated taxon richness (i.e. 449 bacterial and 139 eukaryotic OTUs) across these communities was comparable to that in bacterial communities found in more hospitable climates within arable and grassland soils (Ovřeás, 2000; Hughes et al., 2001), although substantially lower than global estimates of bacterial species in soil (4 x 10^6) and in the oceans (8000) (Curtis et al., 2002). Bacterial taxon richness was not estimated in the two prior studies of cryoconite holes (Christner et al., 2003; Edwards et al., 2011), in which clone libraries were smaller in size (18 and 36 per hole, respectively), in contrast to the larger libraries generated within our current study (mean of 112 clones per hole; Table S1).

The reasons for the relatively high taxon diversity observed within cryoconite holes are unclear. Cryoconite holes are recognized as being important hydrological and biological systems, providing a harbour from extreme polar conditions (MacDonell & Fitzsimons, 2008), and provide a refugia for biota from physical factors, such as wind, desiccation, freezing, glacial flush-out (Fountain et al., 2004, 2008) and high UV intensities (Vincent et al., 2004). Moreover, the development of cryoconite holes helps to enhance localized water and nutrient availability (Takeuchi et al., 2001b; Säwström et al., 2002; Mueller & Pollard, 2004; Hodson et al., 2005; Stibal et al., 2006). Such resulting physicochemical conditions are biologically favourable and thus may help to support elevated taxon richness.

Taxon diversity was particularly high within the Comamonadaceae (Betaproteobacteria) and the Sphingobacteriales (Bacteroidetes), although the majority of such sequences were only found at a single cryoconite location (data not shown). Within the Cyanobacteria, members of two orders, Oscillatoriales and Nostocales, were identified (Fig. S1). However, sequences related to members of the order Chroococcales were not identified within any of the six cryoconite communities, despite descriptions of these organisms within other cryoconite studies (Mueller et al., 2001; Christner et al., 2003; Porazinska et al., 2004 and Mueller & Pollard, 2004). Many of the closest relatives of the bacteria present within the cryoconite communities were related at genus level to sequences within the microbial mat communities of Lake Fryxell, Antarctica (Brambilla et al., 2001).

**Identification of archaea within Antarctic cryoconite holes**

Archaea were identified within the Antarctic (but not the Arctic) cryoconite communities. To our knowledge, there have been no other reports of archaea within cryoconite holes; archaea were not detected by PCR-based approaches in a recent molecular investigation of cryoconite holes on three Svalbard glaciers (Edwards et al., 2011). However, archaea have previously been identified on Alpine glaciers (Battin et al., 2001) and have been found frequently within many cold environments (DeLong, 1998; Cavicchioli, 2006), including Antarctic rock structures (Smith et al., 2000; de la Torre et al., 2003), within polar oceans (DeLong et al., 1994) and in Arctic rivers and the Arctic Ocean (Galand et al., 2006). Representatives of two archaeal phyla were found (Fig. 4), with the majority identified as *Thaumarchaeota*, related to sequences from a range of environments, including 49 of the 63 sequences that were most closely related to an archaeon found in the cold sulphur-rich springs of Lake Erie (FJ968078; Chaudhary et al., 2009). Other *Thaumarchaeota* sequences were related to an endolithic archaeon from the pores of European Alpine dolomite rock (Horath & Bachofen, 2009). A further 11 clones from Signy Island, Antarctica, were most closely related to archaea within the rhizosphere of macrophytes within freshwater sediments that were thought to contribute towards elevated nitrification via ammonia oxidation (Herrmann et al., 2008). It is therefore possible that some of the *Thaumarchaeota*, identified within this cryoconite system, are similarly contributing towards ammonia oxidation and nitrification, facilitating nitrogen cycling within these environments, as is further evidenced via PCR amplification of *Thaumarchaeota*-related amoA genes from this site (Cameron, Hodson and Osborn; in preparation).

Members of the methanogenic *Euryarchaeota* classes *Methanobacteriaceae* and *Methanomicrobia* (Woese, 1987; Boone et al., 1993) were also identified within the Antarctic cryoconite holes. *Methanomicrobiaeae* have been previously found within the microbial mats of Antarctica’s Lake Fryxell (Brambilla et al., 2001).

**Conclusions**

In conclusion, we have demonstrated that cryoconite holes harbour highly diverse bacterial and eukaryotic and, in the case of Antarctic holes, archaeal communities, potentially comprising a multi-level trophic web. Taxa within individual cryoconite communities were often found to be specific to individual regions, suggesting that they may be seeded primarily via localized aeolian transportation and/or during glacial melt from nearby surrounding environments, in addition to subsequent variation owing to localized selection pressures.

Additionally, long-distance aeolian transport may provide a mechanism for immigration into cryoconite holes, although it should be recognized that some of the taxa detected via the DNA-based methods used herein may not be active in the cryosphere. Nevertheless, as pioneer
communities on the glacial surface, cryoconite hole communities have recently been shown to have levels of primary production during summer months comparable to those in soils (Anesio et al., 2009) and are suggested to contribute to seeding of glacial forelands (Schütte et al., 2009). Hence, this study provides important new insight into our understanding of the structure and diversity of the microbial communities inhabiting cryoconite holes and their potential contribution to wider glacial ecosystems.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Neighbor-joining phylogeny showing bacterial diversity within six cryoconite communities.

Fig. S2. Neighbor-joining phylogeny showing eukaryotic diversity within five cryoconite communities.

Table S1. Bacterial taxon richness estimates (represented by number of OTU at 97% identity) for bias-corrected Chao1 (Chao), abundance-based coverage estimator (ACE), interpolated jackknife (Jack), and bootstrap (Boot) calculations, determined using DOTUR (Schloss & Handelsman, 2005).

Table S2. Eukaryotic taxon richness estimates (represented by number of OTU at 97% identity) for bias-corrected Chao1 (Chao), abundance-based coverage estimator (ACE), interpolated jackknife (Jack), and bootstrap (Boot) calculations, determined using DOTUR (Schloss & Handelsman, 2005).

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