RESEARCH/REVIEW ARTICLE

Contrasts between the cryoconite and ice-marginal bacterial communities of Svalbard glaciers

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Abstract

Cryoconite holes are foci of unusually high microbial diversity and activity on glacier surfaces worldwide, comprising melt-holes formed by the darkening of ice by biogenic granular debris. Despite recent studies linking cryoconite microbial community structure to the functionality of cryoconite habitats, little is known of the processes shaping the cryoconite bacterial community. In particular, the assertions that the community is strongly influenced by aeolian transfer of biota from ice-marginal habitats and the potential for cryoconite microbes to inoculate proglacial habitats are poorly quantified despite their longevity in the literature. Therefore, the bacterial community structures of cryoconite holes on three High-Arctic glaciers were compared to bacterial communities in adjacent moraines and tundra using terminal-restriction fragment length polymorphism. Distinct community structures for cryoconite and ice-marginal communities were observed. Only a minority of phylotypes are present in both habitat types, implying that cryoconite habitats comprise distinctive niches for bacterial taxa when compared to ice-marginal habitats. Curiously, phylotype abundance distributions for both cryoconite and ice-marginal sites best fit models relating to succession. Our analyses demonstrate clearly that cryoconites have their own, distinct functional microbial communities despite significant inputs of cells from other habitats.

To access the supplementary material for this article, please see Supplementary Files under Article Tools online.

On glaciers, cryoconite holes are particularly intriguing habitats (Hodson et al. 2008). They develop following the aeolian transport of dusts and debris to glacier surfaces, which become colonized by microbes. Dust-driven reduction of albedo forces localized melting and the formation of cylindrical melt-holes bearing dark, granular sediment and clear meltwater (Gribbon 1979; Cook et al. 2010). This increases the absorption of solar radiation by cryoconite granules, potentiating high rates of microbial primary production and respiration (Telling et al. 2012).

These conditions are optimal for the proliferation of a diverse range of microbial life. This microbiota is critical to the properties of cryoconite, for example, by the role of filamentous microbes and exudation of extracellular polymeric substances forming granules (Langford et al. 2010) while the deposition of dark organic matter further reduces cryoconite albedo and accentuates surface melt rates (Takeuchi, Kohshima, Goto-Azuma et al. 2001; Takeuchi, Kohshima & Seko 2001; Takeuchi 2002).

A long-standing presumption is the importance of dust-associated transfer of cells and nutrients from

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ice-marginal environments (Bayley 1891; Gajda 1958; Gerdel & Drouet 1960; Wharton et al. 1985) in the formation of cryoconite. Measurements of organic contents in great excess of the potential deposition from seasonal autochthonous activity have been used to support this notion (Stibal et al. 2008). However, in spite of the labile nature of a glacier's surface, cryoconite holes on Arctic glaciers appear remarkably stable environments, with time-lapse imagery (Irvine-Fynn et al. 2011), radionuclide composition (Tieber et al. 2009) and granule structure (Takeuchi et al. 2010) suggesting prolonged residence, up to decades, on the ice surface and the potential emergence of distinctive habitats on the glacier surface. Previously, we found at least seven bacterial phyla in Svalbard cryoconite, and associations between community structure and activity with glacier dynamics (Edwards et al. 2011). Others (Kastovska et al. 2007; Mindl et al. 2007) have implied the transfer of taxa between ice-marginal and cryoconite habitats, but the relative importance of regional and local processes in shaping the cryoconite community are poorly quantified.

As a result, the processes governing the emergence of a functional cryoconite microbiome are unclear, as are the interactions between cryoconite bacterial communities and other bacterial communities proximal to cryoconite. Therefore, we applied terminal-restriction fragment length polymorphism (T-RFLP) as a high-throughput, reproducible microbial fingerprinting method (Osborn et al. 2000; Smalla et al. 2007; Camarinha-Silva et al. 2012) to compare bacterial communities from cryoconite, moraine and proglacial tundra soils from glaciers in the Ny-Ålesund area of Svalbard.

Materials and methods

Field sites and sampling procedures

In July 2006, samples were collected from three small valley glaciers, Midtre Lovénbreen with Austre and Vestre Brøggerbreen, associated moraines and adjacent tundra on the Brøggerhalvøya peninsula, Svalbard (Fig. 1). Over the last 100 yr, the three glaciers have all retreated and thinned from their Little Ice Age maxima (ca. 1900), progressively exposing glaciogenic moraines and sediment complexes (Glasser & Hambrey 2001).

These three glaciers are adjacent to the international research settlement of Ny-Ålesund and have therefore been well characterized by intensive glaciological and microbiological studies. The reader is directed to previous investigations which have demonstrated that primary (and microbial) succession in the glacier forefields is strongly influenced by nutrient-limitation (Hodkinson et al. 2003), proto-soil development (Schutte et al. 2009) and rapid changes in stream courses and active hydrology (Moreau et al. 2008) and these glaciers' surfaces are

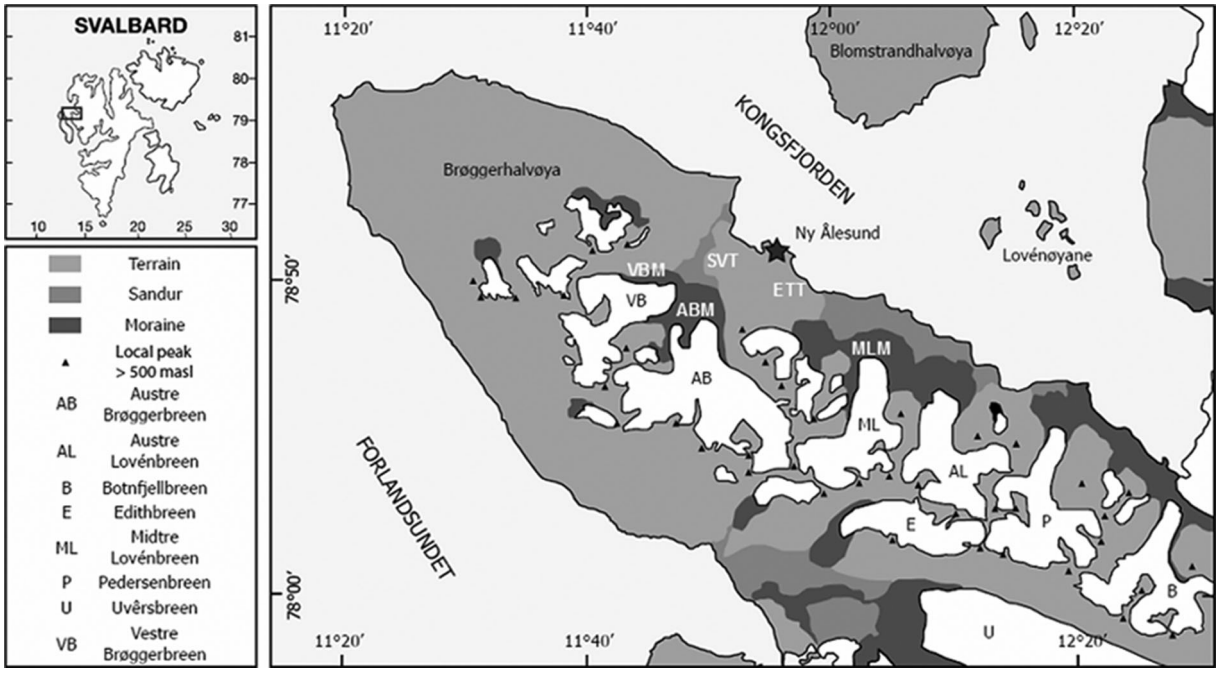

Fig. 1 Map of study area, indicating glaciers sampled from (Austre and Vestre Brøggerbreen and Midtre Lovénbreen) and the region of their moraines (ABM, MLM and VBM) as well as tundra sites (SVT and ETT).

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recognized as loci of microbial diversity and activity (Hodson et al. 2007; Edwards et al. 2011; Telling et al. 2011; Telling et al. 2012).

Cryoconite was collected aseptically from holes in midline transects of each glacier's ablation zone using sterile syringes and 15 mL centrifuge tubes, while sediments were collected from non-vegetated lateral moraines and within the little ice age terminal moraine complexes of each glacier using a flame-sterilized blade to scrape samples of near-surface sediments into centrifuge tubes; and soils were collected from neighbouring areas of vegetated tundra in the same manner. All samples were transferred to the Natural Environment Research Council Arctic Research Station on ice within 6 h and frozen at -20°C prior to air transfer frozen in insulated containers to the Aberystwyth laboratory.

Molecular analyses

Community DNA was aseptically extracted from thawed 250 mg (wet weight) subsamples of cryoconite, sediment or soil using a MoBio (Solana, CA, USA) PowerSoil DNA kit as per the manufacturer's instructions. Extracts were stored at -80°C prior to T-RFLP analysis. Two microlitres of each extract were added to 25 μL. Polymerase chain reactions (PCRs) were conducted in triplicate using primers for the bacterial 16S ribosomal RNA gene (Cy5-27F and 1389R), treated with Exonuclease I and Shrimp Alkaline Phosphatase to reduce the formation of pseudo-terminal-restriction fragments (T-RFs) and digested with *Hae*III prior to fragment analysis using a CEQ-8000 genetic analyser (Beckman Coulter, Brea, CA, USA) exactly as described in Edwards et al. (2011), with the exception that the final clean-up of T-RFs present in each sample was performed using a Montage μ96 PCR plate as per the manufacturer's instructions (Millipore, Watford, UK) prior to loading on the genetic analyser.

Statistical analyses

T-RF relative abundance profiles were extracted in MS Excel 2007. Primer-6.1.12 and PERMANOVA+1.0.2 (Primer-E, Ivybridge, UK) were used to conduct permutational analysis of variance (PERMANOVA; Anderson 2001) and canonical analysis of principal components (CAP; Anderson & Willis 2003) using Bray-Curtis similarities of fourth-root transforms of profiles. Each profile represented an independent replicate within a group (e.g., Midtre Lovénbreen cryoconites, Vestre Brøggerbreen moraines).

Finally, Bray-Curtis similarities of T-RF distributions were used to generate taxon co-occurrence networks in UCINET-6 and NetDraw (Borgatti 2002; Borgatti et al. 2002) prior to visualization of all nodes linked by edges with Bray-Curtis similarities ≥ 0.70 using Cytoscape 2.8.3 (Smoot et al. 2011). See the Supplementary File for details of phylotype abundance distribution modelling.

Results and discussion

Bacterial 16S rRNA gene T-RF profiles were generated for all 48 samples, and a significant difference was observed in peak numbers (S) between sample types (cryoconite $n=25$, median $S=18$; moraine $n=14$, median $S=23$; tundra $n=9$, median $S=6$; Kruskal-Wallis, $H=16.4$, $P<0.0001$). The number of T-RFs in each sample is provided in Supplementary Table, S1. Each T-RF was considered a distinct phylotype *sensu* Prosser (2012), and its peak height relative to total peak height per sample to represent the relative abundance of that phylotype, cf. Schutte et al. (2009). Although the detection of phylotypes comprising more than 0.1–1% of the cumulative relative abundance of a microbial community by T-RFLP is recognized (Dunbar et al. 2000; Courtney et al. 2012), it cannot be assumed that T-RFLP can accurately resolve all phylotypes present in a given environmental sample, given the long-tail distribution of taxa evident in microbial communities (Bent & Forney 2008).

Although empirical evidence supports the linkage of T-RFs detected by our T-RFLP protocol to specific 16S rRNA gene operational taxonomic units clustered at the level of 97% sequence identity frequently within cryoconite (Edwards et al. 2011), uncertainties surround the phylogenetic resolution of T-RFLP (Blackwood et al. 2007). Nevertheless, the ability of microbial community fingerprinting methods such as T-RFLP to provide ecologically-relevant insights is well recognized (Fierer 2007), as is the potential to glean information from fragmental abundance distributions (Marzorati et al. 2008). Indeed, since T-RFLP profiles can be closely correlated with physico-chemical conditions and microbial activities in the context of these glaciers (Edwards et al. 2011), we proceed with the cautious assumption that T-RFLP is no less unsuitable than any other method for the particular objectives of this study.

Profound differences in the T-RF profiles of community structures from cryoconite, tundra soil and moraines were apparent. PERMANOVA of T-RF relative abundance profiles demonstrates a clear effect of environment type (pseudo-$F=16.52$; P[perm] $=0.0001$), and furthermore between sites (pseudo-$F=6.21$; P[perm] $=0.001$) with significant differences within and between cryoconite sites and all other sites accounting for most variation (Table 1). This is confirmed by CAP analysis, which clearly

Table 1 Summary statistics of permutational analysis of variance (PERMANOVA) and canonical analysis of principal components (CAP) analyses; *P*(perm) values of less than 0.01 are in boldface to indicate significant differences between sample groups upon pairwise PERMANOVA.

	Pairwise PERMANOVA *P*(perm) values							CAP model
	ML[a] cryoconite	VB[b] cryoconite	AB[c] cryoconite	ET[d] tundra	SV[d] tundra	AB moraine	VB moraine	Misclassification error (%)
ML cryoconite	–							0
VB cryoconite	**0.001**							50
AB cryoconite	**0.001**	**0.008**						0
ET tundra	**0.001**	**0.004**	**0.001**					80
SV tundra	**0.002**	**0.006**	**0.003**	0.669				100
AB moraine	**0.003**	**0.006**	**0.001**	0.019	0.031			25
VB moraine	**0.002**	**0.002**	**0.001**	0.016	0.018	0.075		40
ML moraine	**0.001**	**0.001**	**0.001**	0.033	0.04	0.712	0.241	60

[a]Midtre Lovénbreen.
[b]Vestre Brøggerbreen.
[c]Austre Brøggerbreen.
[d]See Fig. 1 for location of the two tundra sites.

ordinates T-RFLP profiles from different environment types (Fig. 2) using a fairly robust model, correctly classifying 65.4% of samples to the correct site via leave-one-out-allocation (Table 1). Finally, PERMANOVA of T-RF profiles transformed to presence–absence (as opposed to relative abundance profiles) confirmed the above significant differences (environment type, pseudo-$F = 15.423$, $P[\text{perm}] = 0.0001$; site pseudo-$F = 5.8378$, $P[\text{perm}] = 0.0001$ with pairwise significant differences between all cryoconite sites and between cryoconite sites and all non-cryoconite sites; see Supplementary Table, S2) illustrating that the overall conclusion of differences apparent between sites and environment types appear equally robust to the application of abundance-weighted or un-weighted analyses.

As summarized in the inset in Fig. 2, the overlap in the ranges of individual T-RFs between cryoconite and other habitat types was less than would be expected if

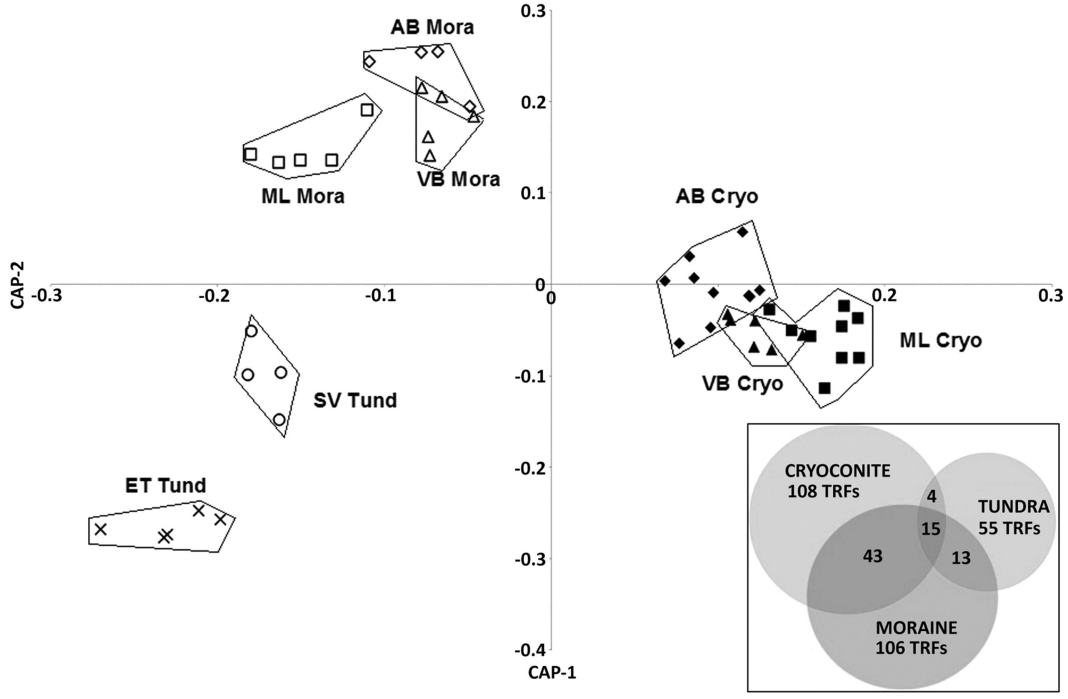

Fig. 2 Canonical analysis of principal components (CAP) ordination plot indicating the extent of differences between tundra soil (''Tund'', crosses and circles), moraine sediment (''Mora'', hollow markers) and cryoconite (''Cryo'', filled markers) bacterial community structures of the three glaciers (Austre Brøggerbreen: diamonds; Midtre Lovénbreen: squares; and Vestre Brøggerbreen: triangles) and tundra sites (SVT: circles and ETT: crosses) resolved by terminal-restriction fragment length polymorphism. The Venn diagram (inset) indicates the richness and degree of overlap between habitat types in terms of terminal-restriction fragments (T-RFs).

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T-RFs were randomly distributed, with 46 T-RFs unique to cryoconite. The greatest overlap in the ranges of individual T-RFs is found between cryoconite and moraine habitats, thus suggesting the range of some phylotypes may extend to both supraglacial and ice-marginal environments in some cases.

To assess whether the ranges of taxa detected by T-RFs extend across both cryoconite and periglacial habitats, the co-occurrence patterns of T-RFs were mapped. Networks of T-RFs exhibiting Bray-Curtis similarities ≥ 0.70 in their distribution were retained and displayed (Fig. 3). Such co-occurrence patterns have been used in terrestrial and aquatic microbial ecology to define niches for microbial taxa as represented by T-RFs, automated ribosomal intergenic spacer analysis fragments or operational taxonomic units (Fuhrman & Steele 2008; Steele et al. 2011; Barberan et al. 2012; Camarinha-Silva et al. 2012) but to our knowledge this is its first application in glacial environments. The co-occurrence networks generated are generally small, with only a few co-occurring nodes, typically linked minimally by only a few edges. Of the 17 co-occurrence networks resolved, 10 were dominated by cryoconite, moraine or soil-specific T-RFs. The two most popular networks consisted of taxa found in both cryoconite and moraine, indicating the ubiquity of these groups in both glacial environments; however, these accounted for only 19 of the 194 T-RFs represented in our data set.

Examination of these networks also suggests the presence of distinct co-occurrence patterns within habitat types. Coupled with the high ratio of total T-RFs to the median number of T-RFs per sample, this would suggest a degree of environmental heterogeneity even within the low-complexity landscape of a glacier surface. It has previously been shown that the community structure of cryoconite responds to glacier-specific factors (Mueller & Pollard 2004; Edwards et al. 2011). It is therefore possible that different cryoconite holes effectively "sample" distinct physico-chemical conditions across environmental gradients. Therefore, the potential for cryoconite ecosystems to act as sensitive indicators of spatio-temporal changes in supraglacial dynamics relating to environmental changes merits some consideration.

To help provide some insight into the phylogenetic affiliation of T-RFs resolved in this study, *in silico* predicted T-RFs for the same primer-enzyme combination applied to clone library sequences previously reported from cryoconite on the glaciers studied here (Edwards et al. 2011) and available on Genbank (accessions FN824532–FN824621) were compared to the T-RFs observed. Exact matches (± 1 bp to allow for reasonable variation from size calling) were found for the predicted T-RFs of 51 clones from a total library of 94 clones to 71 of 204 actual T-RFs detected in this study. T-RFs with matches to predicted clone library T-RFs account for 74.3, 49.6 and 56.7% of the total relative abundance for cryoconite,

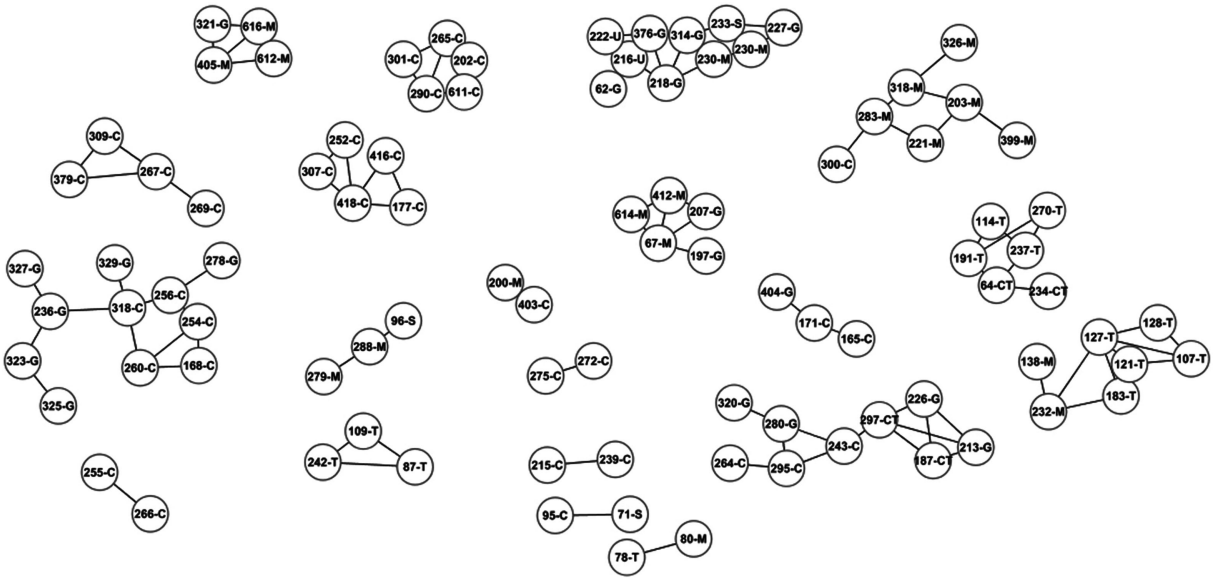

Fig. 3 Co-occurrence pattern networks for bacterial 16S ribosomal RNA gene terminal-restriction fragments (T-RFs) resolved in this study; each node is labelled with the size of the 1-bp bin of the T-RFs (in bp) and a code letter designating the origin of the T-RF (C: cryoconite, M: moraine, T: tundra, G: moraine and cryoconite, S: moraine and tundra, CT: cryoconite and tundra). Only nodes linked by edges with a strength of association exceeding a Bray-Curtis similarity of ≥ 0.70 are shown.

moraine and tundra habitats, respectively, consistent with the contention that T-RFLP has provided effective resolution of phylotypes making significant contributions to the communities sampled. Although the putative nature of these matches must be emphasised, this lends support for the validity of T-RFLP as a tool for the comparison of bacterial communities in this study. The potential bias from only comparing to cryoconite libraries is recognized, and therefore the discrepancy between habitats in terms of T-RF-clone match representation is of the expected distribution considering the confirmation of hypotheses of differences between habitats. Yet further support is gained from the proportions of matches to different taxa observed which are comparable to other analyses of cryoconite bacterial diversity, for example the dominance of *Alphaproteobacteria* and relative abundance of *Cyanobacteria* broadly consistent with both clone libraries (Edwards et al. 2011), the clone library of Midtre Lovénbreen cryoconites recently reported (Cameron et al. 2012) and 454-pyrosequencing (Edwards et al. unpubl. ms.) of Arctic and alpine cryoconites. Statistically significant shifts between habitat types are apparent, both at the level of individual T-RFs (PERMANOVA pseudo-F = 10.82; P[perm] = 0.0001) and for the cumulative relative abundances of T-RFs aggregated by phylum or proteobacterial class, which are displayed in Fig. 4. Despite the support rendered by these several lines of evidence, further work is required to test the related, but distinct, hypothesis of differential representation of higher ranked bacterial taxa between cryoconite and ice-marginal habitats, since our understanding of the ecological coherence of higher ranked bacterial taxa is less than complete, even within intensively studied habitats such as soil (Fierer et al. 2007; Philippot et al. 2010; Prosser 2012).

Finally, considering that (i) the presence of cosmopolitan taxa in cryoconite (Edwards et al. 2011); (ii) the strong dispersal vectors seen in polar environments (Alsos et al. 2007); (iii) the continual deposition and

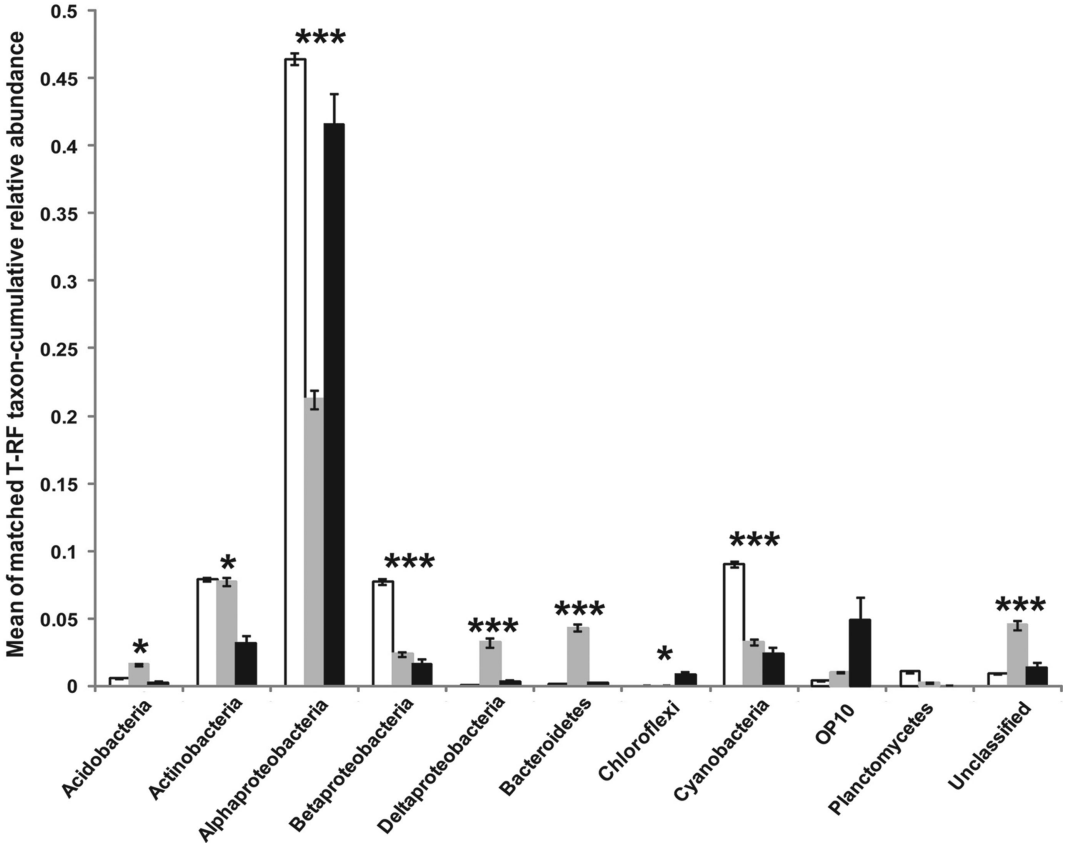

Fig. 4 Phylum and proteobacterial class distribution of putative matches (exact or ±1 bp) of terminal-restriction fragments (T-RFs) to 16S rRNA gene clone library sequences amplified from cryoconite on Austre and Vestre Brøggerbreen and Midtre Lovénbreen (Genbank FN824532–FN824621; published by Edwards et al. [2011]). Columns are mean values for cryoconite (white), moraine (grey) and tundra (black) habitats with error bars representing ±1 standard error of the mean. The values represent the proportion of total relative abundance accounted for by matches to each phylum or class. Significant (P <0.05) and highly significant differences (P <0.001) returned by Kruskal–Wallis tests comparing the total relative abundance of hits putatively affiliated with each taxon in each habitat type are indicated by one or three asterisks, respectively.

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"archiving" of cells in glacial environments (Xiang et al. 2009); and (iv) the mass transfer of debris from adjacent habitats (Gerdel & Drouet 1960; Stibal et al. 2008), it is unlikely that Arctic glacier surfaces act to limit potential dispersal of taxa. Indeed, measurements of aeolian deposition on High-Arctic glaciers suggest significant cell fluxes on to the ice surface, with rates of deposition in the order of 10^7 cells m^{-2} h^{-1} to the bare ice of Midtre Lovénbreen apparent (Irvine-Fynn et al. 2012). Therefore, our analysis of T-RF profiles of bacterial community structure would be consistent with the occupation of distinct niches by bacterial phylotypes specific to either cryoconite or ice-marginal habitats, with the exception of a specific subset of phylotypes with a broadly defined niche spanning both habitat types. It is likely that dispersal of this minority of generalist phylotypes between these habitats will permit successful colonization of substrates, and the potential of ice-marginal habitats to inoculate cryoconite and vice versa cannot be excluded for some phylotypes. Nevertheless, in summary it appears clear that despite the substantial potential for transfer of cells from ice-marginal habitats, cryoconite holes are home to a distinctive yet functional bacterial community.

To explore the properties of the cryoconite bacterial community further, we conducted phylotype abundance distribution modelling of T-RF profiles in relation to null, pre-emption, log-normal, Zipf, Zipf-Mandelbrot and Zero-Sum models (see the Supplementary File). Zipf and Zipf-Mandelbrot distributions fit the T-RF profiles of cryoconite best in all cases according to Akaike Information Criterion scores, fitting 46 of the 48 samples profiled in this study; log-normal distributions were marginally the best-fit for two soil samples (Supplementary Table S1). These distributions are consistent with the predominance of deterministic (niche-based) processes over neutral processes in structuring a community. However, the coarser phylogenetic resolution of T-RFLP may reduce its sensitivity to neutral processes impacting at fine scales. Nevertheless, T-RFLP profiles have supported a role for neutral processes in other microbial communities (Dumbrell et al. 2009; Ofiteru et al. 2010; Caruso et al. 2011), indicating that further study into the relative roles of deterministic and neutral processes in structuring cryoconite communities is merited. Zipf and Zipf-Mandelbrot distributions, which differ only slightly in relation to the predicted length of the tail in the species abundance distribution (Wilson 1991), relate that as phylotype rank increases, phylotype abundance decreases exponentially. These distributions have been associated with communities in later stages of ecological succession (Wilson 1991; Aoki, 1995). Although the paradigm of primary succession in glacier forefields and hence subsequent to deglaciation is well documented, even for bacteria (Schutte et al. 2009), the fit of Zipf and Zipf-Mandelbrot distributions to cryoconite bacterial communities (i.e., habitats formed prior to deglaciation) is especially intriguing. On these grounds, it is tempting to hypothesize that primary succession may be initiated prior to deglaciation. Since cryoconite influences the mass balance of glaciers (Takeuchi, Kohshima & Seko 2001; Takeuchi 2002; Fountain et al. 2004), a supraglacial succession could result in a significant microbial influence on the fate of glaciers responding to a warming climate.

In summary, our data suggest that the cryoconite microbiome is essentially distinct from neighbouring habitats, with most, but not all, phylotypes occupying distinct niches in ice-surface and ice-marginal environments. Future work should concentrate on (i) defining the cryoconite microbiome at high resolution, perhaps by deep pyrosequencing; (ii) relating the dynamics of the microbiome to its functionality and the supraglacial habitat to resolve the niches of cryoconite taxa in detail; and (iii) evaluation of the role of neutral and non-neutral processes in governing cryoconite bacterial community assembly. Such efforts could prove useful in understanding the interactions between glacier wastage and glacial ecosystems.

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