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Research Article

RESEARCH ARTICLE

Diversity patterns of microbial eukaryotes mirror those of bacteria in Antarctic cryoconite holes

Pacifica Sommers[1,*], John L. Darcy[2], Eli M.S. Gendron[1,3], Lee F. Stanish[4], Elizabeth A. Bagshaw[5], Dorota L. Porazinska[1] and Steven K. Schmidt[1]

[1]Department of Ecology and Evolutionary Biology, University of Colorado at Boulder, Boulder, CO 80309, USA, [2]Department of Botany, University of Hawai'i at Mānoa, Honolulu, HI 96822, USA, [3]Department of Molecular, Cellular, and Development Biology, University of Colorado at Boulder, Boulder, CO 80309, USA, [4]National Ecological Observatory Network, Boulder, CO 80301, USA and [5]School of Earth and Ocean Sciences, Cardiff University, CF10 3AT, UK

*Corresponding author: Department of Ecology and Evolutionary Biology, University of Colorado at Boulder, UCB 334, 1900 Pleasant St, Boulder, CO 80309, USA. Tel: +801-647-4124; Fax: +303-492-8699; E-mail: pacifica.sommers@colorado.edu

One sentence summary: Bacterial and eukaryotic alpha and beta diversity in Antarctic cryoconite holes were strongly correlated, reflecting a gradient of organic material and chemistry in the surrounding streams and soils.

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ABSTRACT

Ice-lidded cryoconite holes on glaciers in the Taylor Valley, Antarctica, provide a unique system of natural mesocosms for studying community structure and assembly. We used high-throughput DNA sequencing to characterize both microbial eukaryotic communities and bacterial communities within cryoconite holes across three glaciers to study similarities in their spatial patterns. We expected that the alpha (phylogenetic diversity) and beta (pairwise community dissimilarity) diversity patterns of eukaryotes in cryoconite holes would be related to those of bacteria, and that they would be related to the biogeochemical gradient within the Taylor Valley. We found that eukaryotic alpha and beta diversity were strongly related to those of bacteria across scales ranging from 140 m to 41 km apart. Alpha diversity of both was significantly related to position in the valley and surface area of the cryoconite hole, with pH also significantly correlated with the eukaryotic diversity. Beta diversity for both bacteria and eukaryotes was significantly related to position in the valley, with bacterial beta diversity also related to nitrate. These results are consistent with transport of sediments onto glaciers occurring primarily at local scales relative to the size of the valley, thus creating feedbacks in local chemistry and diversity.

Keywords: high-throughput sequencing; dispersal; cryoconite hole; Antarctica; bacteria; eukaryotes

INTRODUCTION

Antarctic cryoconite holes form natural mesocosms for studying the formation, assembly and functioning of microbial communities. Cryoconite holes are water-filled depressions on glacier surfaces caused by the settlement of low-albedo sediments, known as cryoconite, absorbing solar radiation and melting into the surrounding ice (Wharton *et al.* 1985; MacDonell and Fitzsimons 2008). These sediments contain microbes that become active within the holes (Foreman *et al.* 2007), undergoing succession to net phototrophic communities (Bagshaw *et al.* 2016) with a food web of heterotrophs (Porazinska *et al.* 2004), accumulating organic material and nutrients over the course of several years (Fountain *et al.* 2004; Bagshaw *et al.* 2007). Previous microscopic (Porazinska *et al.* 2004; Mieczan *et al.* 2013; Stanish *et al.* 2013) and

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geochemical studies (Foreman *et al.* 2007; Telling *et al.* 2014) confirm that cryoconite holes host an actively growing community and not simply an inactive 'seed bank' or relic DNA from dead organisms (as in Willerslev *et al.* 2004; Galotti *et al.* 2014; Carini *et al.* 2016).

Simple natural systems with a level of complexity that facilitates the study of microbial community assembly are rare. Although laboratory microcosms have been used to study community ecology (e.g. Altermatt *et al.* 2011, 2015; Carrara *et al.* 2012, 2015; Seymour and Altermatt 2014), they are often limited in their ability to generalize results to more complex communities with long-shared evolutionary history in a natural environment. By contrast, microbial communities sampled from complex natural environments, such as soils and aquatic habitats (e.g. Bates *et al.* 2013; Maestre *et al.* 2015; Ruiz-Gonzalez, Nino-Garcia and Giorgio 2015; Locey and Lennon 2016), can limit inference due to a multitude of confounding factors, such as moisture, vegetation and even the dispersal of organisms between adjacent habitat types. Cryoconite holes form a well-replicated system of limited diversity with discrete temporal and spatial boundaries. Cryoconite holes in Antarctica are uniquely tractable systems for studying microbial community assembly because unlike those in other regions, they typically form an ice lid that can isolate them from the atmosphere for years, despite melting that occurs under the lid for up to 12 weeks in the austral summer (Fountain *et al.* 2004). Antarctic cryoconite holes thus provide natural mesocosms of organisms in discrete sites and discrete growing seasons (Fountain *et al.* 2008), with long-term isolation (Fountain *et al.* 2004), and a lack of confounding variation from vertebrate and plant inputs. Their variability in age, size and connectivity to one another (Fountain *et al.* 2004) along with differing physical and chemical characteristics (Stanish *et al.* 2013; Webster-Brown *et al.* 2015) provides a natural experimental system to study patterns of community assembly.

In order to take advantage of Antarctic cryoconite holes as experimental mesocosms for studying community assembly, the composition of naturally occurring cryoconite communities must be well characterized. The bacterial communities of cryoconite holes in the McMurdo Dry Valleys generally reflect the surrounding source material of cryoconite (Webster-Brown *et al.* 2015). Furthermore, diatom diversity reflects environmental gradients of diversity in the sediments and microbial mats of nearby streams (Stanish *et al.* 2013). While the diatoms (Stanish *et al.* 2013) and meiofauna (Porazinska *et al.* 2004) of the Dry Valley cryoconite holes have been surveyed using microscopy, modern high-throughput sequencing has yet to characterize patterns of eukaryotic diversity across glaciers.

Here, we compare the large-scale spatial patterns of alpha diversity (phylogenetic diversity) and beta diversity (pairwise community dissimilarity) in cryoconite eukaryotic and bacterial communities from three glaciers in the Taylor Valley. We used high-throughput sequencing of the 16S and 18S ribosomal small subunit (SSU) genes to characterize the bacterial and eukaryotic communities, respectively. Although wind-based dispersal models suggest that the larger size of microbial eukaryotes may lead to shorter dispersal distances than bacteria (Wilkinson *et al.* 2012), that difference may not manifest at the spatial scale of this study. We therefore ask how the diversity of these communities is spatially structured, specifically (i) how closely the alpha and beta diversity patterns of eukaryotes match those of bacteria, and (ii) to what extent the alpha and beta diversity of eukaryotic and bacterial communities in cryoconite holes reflect the previously described gradients of organic material in the Taylor Valley (McKnight *et al.* 1998; Barrett *et al.* 2004) and physicochemical characteristics of the individual cryoconite holes.

METHODS

Site description and sample collection

The Taylor Valley is one of the McMurdo Dry Valleys of Antarctica, and primarily consists of poorly developed soils with glaciers spilling down the mountain passes into a series of lakes along the valley floor (Priscu 1999). The valley is ~40 km long from the eastern edge of the Antarctic ice sheet to the coast. Climate varies significantly along the length of the valley with higher temperatures and wind speeds near the ice sheet and greater precipitation near the coast (Fountain *et al.* 1999; Doran *et al.* 2002). Wind direction is governed by both onshore breezes from the Ross Sea and down-valley katabatic or föhn winds descending from the ice sheet (Nylen, Fountain and Doran 2004; Šabacká *et al.* 2012). The eastern coastal Lake Fryxell basin is relatively wide and shallow with higher soil moisture (Barrett *et al.* 2006) and greater snow accumulation (Fountain *et al.* 2010) than the western Lake Bonney basin. The eastern lake basin therefore has a greater extent of suitable stream habitats for algal mats (McKnight *et al.* 1998) that are thought to seed much of the biota within cryoconite holes. We sampled three glaciers that capture the full range of this gradient: the Commonwealth Glacier, on the east end of the valley; the Canada Glacier, to the west of the Commonwealth Glacier; and the Taylor Glacier, which descends from the ice sheet at the western end of the Taylor Valley (Fig. 1).

Nineteen cryoconite holes on these glaciers were sampled between 15 December 2007 and 4 January 2008. A core was collected from the center of each hole using a SIPRE corer. Only one cryoconite hole was completely frozen at the time of sampling, and for that hole drilling continued until clean ice below the basal debris layer was encountered. The core was then removed and stored in a Ziploc bag that had been triple-rinsed with deionized water. For the rest of the samples, in which meltwater was present at the time of sampling, the ice lid was removed with the SIPRE corer, and then a water sample was pumped out using a hand powered vacuum pump. A sample of sediment was removed and stored in a triple-rinsed Ziploc bag. On return to the field laboratory, samples were stored at –20°C until processing up to 30 days later. Samples were eventually allowed to melt out in the collection bags and water samples were drawn off using syringes, leaving the sediment behind. Electrical conductivity and pH of the water were measured in the field laboratory, and the remaining water sample was filtered and transported to the Crary Laboratory at McMurdo Station for major ion analysis within 30 days.

Homogenized cryoconite sediments collected as described above were preserved in 2-ml microcentrifuge tubes containing 1.0 ml of RNA*later* Storage Solution (Invitrogen Corp., Carlsbad, CA, USA), then filled to the top with cryoconite and stored at –70°C until further processing. Although microbial communities within the water column of cryoconite holes could differ from those in the sediment, the cryoconite itself contains an order of magnitude higher abundances of cells, (Foreman *et al.* 2007; Mieczan *et al.* 2013) and so we focused on the microbial communities of the cryoconite. Up to three samples were preserved per cryoconite hole for a total of 47 replicates from the 19 holes sampled.

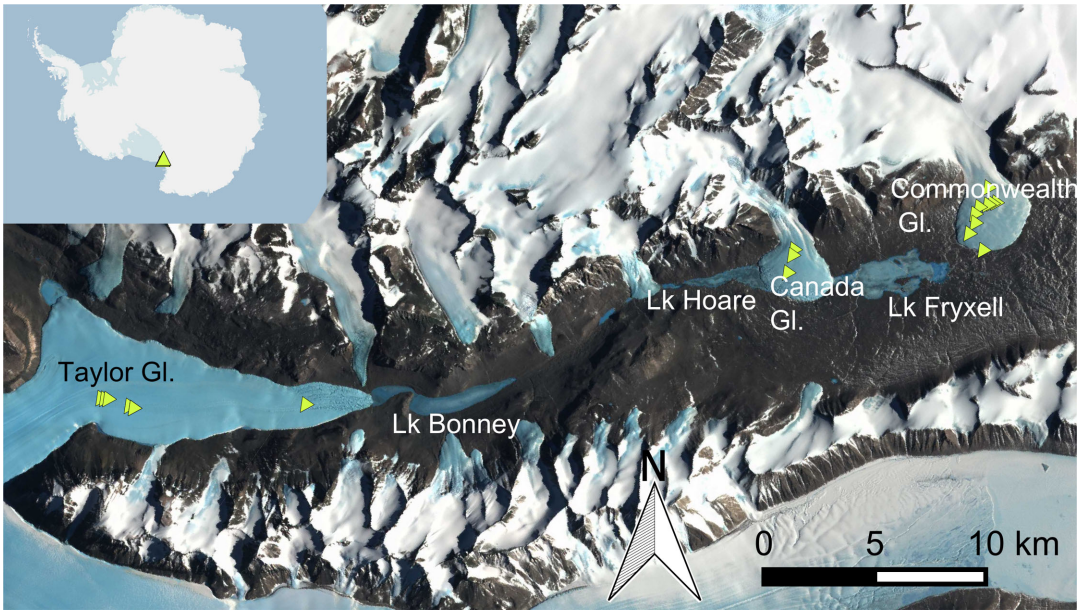

Figure 1. Map of Taylor Valley with sampling sites marked by yellow triangles made in Quantarctica (Matsuoka *et al.* 2013). Inset map of continent shows the location of the Taylor Valley.

Water chemistry

Ice samples were melted at room temperature. Approximately 150 ml of meltwater was filtered through 0.4-μm Whatman Nuclepore membranes (GE Healthcare, Pittsburg, PA, USA) and stored in three 60-ml pre-rinsed bottles, which were used for major ion, nutrient, pH and conductivity analysis. Filtrates were refrigerated at <4°C. Electrical conductivity and pH were measured on filtered samples in the field laboratory within a few hours of filtration using a YSI 30 (YSI Inc., Yellow Springs, OH, USA) and an Orion pH meter (Thermo Fisher Scientific, Inc., Lafayette, CO, USA), respectively. Major ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, SO_4^{2-} and NO_3^{-}) were measured using a Dionex DX-120 ion chromatograph (Dionex Corp. Sunnyvale, CA, USA) at the Albert P. Crary Science and Engineering Center, McMurdo Station. Full details can be found in Welch *et al.* (1996). Measurements were taken between 1 and 8 weeks after filtration. Precision was <5% for all ions. The quantification limit was taken as the concentration of the lowest standards, which are as follows: F^{-} 0.3; Cl^{-} 0.6; NO_3^{-} 0.07; SO_4^{2-} 0.4; Na^{+} 2; Mg^{2+} 5; K^{+} 0.2 and Ca^{2+} 2 μeq/l.

DNA extraction and sequencing

All cryoconite samples were stored frozen at –70°C to minimize DNA degradation (Eichmiller, Best and Sorensen 2016). Between 25 April 2016 and 2 May 2016, the frozen cryoconite sediments were thawed at room temperature and 0.4 g was processed for DNA extraction from each technical replicate separately (47 total from 19 samples) using PowerSoil DNA Isolation Kits (MoBio Inc., Carlsbad, CA, USA), according to the manufacture's protocol. Extracted genomic DNA was amplified in triplicate using 16S (515f-806r primers, Caporaso *et al.* 2012) and 18S (1391f-EukBr primers, Amaral-Zettler *et al.* 2009; Caporaso *et al.* 2012) SSU ribosomal gene markers. Amplified DNA was pooled and normalized to equimolar concentrations using SequalPrep Normalization Plate Kits (Invitrogen), and sequenced using the Illumina MiSeq V2 (2 × 250 bp chemistry) at the BioFrontiers Sequencing Core Facility at the University of Colorado at Boulder.

Data processing and analysis

Raw reads were de-multiplexed and quality filtered using the QIIME v1.9.1 bioinformatics package (Caporaso *et al.* 2010b), using paired-end sequences that were joined with VSEARCH (Rognes *et al.* 2016). Bacterial and eukaryotic sequences were separately clustered at 97% similarity using UCLUST (Edgar 2010). Taxonomy was assigned with the RDP Classifier (Wang *et al.* 2007) using QIIME's parallel_assign_taxonomy_rdp.py script for bacteria and parallel_assign_taxonomy_blast.py script with the SILVA 108 database's taxonomic information for eukaryotes (Pruesse *et al.* 2007; Quast *et al.* 2012). All mitochondrial and chloroplast OTUs based on this classification were removed from the bacterial data set, and all bacterial OTUs were removed from the eukaryotic data set. Singleton OTUs were removed, and each data set was then rarefied to the number of sequences in the least populous replicate: 14 771 bacterial sequences per replicate and 14 186 eukaryotic sequences per replicate. Bacterial sequences were aligned with pynast (Caporaso *et al.* 2010a) using the Greengenes 13.5 sequence database's reference alignment (DeSantis *et al.* 2006), and eukaryotic sequences were aligned with SINA 1.2.11 (Pruesse, Peplies and Glöckner 2012) using the SILVA 108 database as a reference (Pruesse *et al.* 2007; Quast *et al.* 2012). Phylogenetic trees for 16S and 18S sequence alignments were constructed using FastTree (Price, Dehal and Arkin 2009). Alignment failures were filtered out of each data set, and OTU tables were re-rarefied to 14 735 bacterial and 13 735 eukaryotic sequences per replicate. The taxonomic assignments of the top 22 OTUs from each data set were verified by using BLAST to search NCBI, and corrected as needed.

Alpha diversity was calculated as Faith's phylogenetic diversity (Faith 1992) and the Chao1 estimator using the rarefied 16S and 18S SSU rRNA gene datasets in QIIME. We calculated the mean phylogenetic diversity of all available technical replicates for each cryoconite hole sampled. To evaluate whether the phylogenetic diversity of microbial eukaryotic communities correlated with the phylogenetic diversity of their corresponding bacterial communities, we used a Model II regression in package

lmodel2 (Legendre 2014) with 500 permutations in R 3.3.2 (R Core Team 2016).

To test the relationship between alpha diversity (for both bacteria and eukaryotes) and abiotic characteristics of the cryoconite holes, we used multiple regression analysis. Longitude represented the underlying gradient of organic material along the longitudinal axis of the Taylor Valley observed in the streams and soils (Mcknight *et al.* 1998; Barrett *et al.* 2004). Explanatory variables were centered by subtracting their means and standardized by dividing by their means, and then tested for collinearity (Fig. B.1, Supplementary Information). Using a recommended collinearity threshold of $|r| < 0.7$ (Dormann *et al.* 2013), depth, major ions and DOC were excluded from the multiple regression.

We calculated beta diversity using the unweighted UniFrac distance metric (Lozupone and Knight 2005). As with alpha diversity, Model II regression was used to test relationships in beta diversity of eukaryotic and bacterial communities. Due to the unevenness of the surviving technical replicates (1–3) per cryoconite hole sampled and the similarity between technical replicates (Fig. A.1, Supplementary Information), all downstream analyses on beta diversity (pairwise dissimilarity), which cannot be easily averaged, were performed on a randomly selected single technical replicate from each sample. We repeated the random selection 1000 times to ensure the selection of replicates did not alter the key results (Table A.1, Supplementary Information).

Furthermore, to evaluate whether beta diversity of eukaryotic and bacterial communities were related to cryoconite hole characteristics, we performed a distance-based redundancy analysis (db-RDA, Legendre and Anderson 1999) in package vegan v.2.3–5 (Oksanen *et al.* 2013) in R v3.2.1 (R Core Team 2016) on the same subset of variables included in the multiple linear regression. We used a permutational analysis of variance to test for significance of terms in the db-RDA, in which *P*-values of vectors were corrected for multiple comparisons using the false discovery rate method (Benjamini and Hochberg 1995).

RESULTS

Considering all 47 DNA extractions across the three glaciers, including all technical replicates of the 19 cryoconite holes sampled, we obtained a total of 19 721 16S SSU ribosomal gene-based OTUs from 1101 630 high-quality reads, and 2598 18S SSU ribosomal gene-based OTUs from 1376 173 high-quality reads after removing singletons. There were 11 871 bacterial OTUs in the rarefied data set and 2294 eukaryotic OTUs. Sequence data and associated metadata from this study have been deposited in the NCBI SRA under project accession number PRJNA401941.

The dominant bacterial phyla were Bacteroidetes (33%), Proteobacteria (28%), Cyanobacteria (10%), Actinobacteria (6%), Verrucomicrobia (5%), Acidobacteria (5%), Planctomycetes (4%) and Gemmatimonadetes (4%) (Fig. 2a). Only two OTUs comprising 96 total sequences from the entire data set were assigned to Archaea. Among the 22 most abundant bacterial OTUs, all the Bacteroidetes were assigned to family Chitinophagaceae (order Sphingobacteriales), with the three main genera being *Chitinofaga*, *Ferruginibacter* and *Segetibacter* (Fig. 3a). Dominant Proteobacteria OTUs were present across all glaciers: four Betaproteobacteria (family Comamonadaceae; genera *Rhodoferax*, *Polaromonas* and *Variovorax*) and two Alphaproteobacteria (family Sphingomonadales; genus *Kaistobacter*) (Fig. 3b). Dominant Cyanobacteria OTUs from the orders Oscillatoriales, Synechococcales (likely family Pseudanabaenaceae) and Chroococcales were all present across the three glaciers in varying abundances (Fig. 3c), with greater resolution in their taxonomy difficult to determine with high confidence (Fig. 3d).

The dominant eukaryotic groups were Ciliophora (23%), Cercozoa (17%), Metazoa (16%), Discicristata (14%), Chlorophyta (10%), Ochrophyta (8%), Fungi (3%) and Euamoebida (1%) (Fig. 2b). Within the 22 most abundant OTUs, Opisthokonta dominated, among them primarily rotifers, likely *Rotifera rotatoria* (Fig. 3e). These were followed by two OTUs of tardigrades, possibly *Acutuncus antarcticus* and *Diphascon* sp. (likely *Diphascon pingue*), and a *Phaenocora*-like flatworm OTU (Fig. 3e). Many of the 22 most prevalent eukaryotic OTUs were potentially bacterivorous ciliates, flagellates and bi-flagellates (Fig. 3f and h). Dominant phototrophic eukaryotic OTUs included green algae and diatoms, except on Taylor Glacier, where diatoms were virtually absent (Fig. 3g).

Bacterial and eukaryotic alpha diversities were strongly correlated ($r = 0.99$, $P < 0.001$) (Fig. 4a). Furthermore, the beta diversities of bacterial and eukaryotic communities were also correlated ($r = 0.93$, $P < 0.001$), such that pairs of samples with similar eukaryotic communities had also similar bacterial communities, and pairs of samples with more different eukaryotic communities had more different bacterial communities (Fig. 4b).

Of all abiotic factors, longitude was the most predictive of alpha diversity for both bacteria (Table 1) and eukaryotes (Table 2). Bicarbonate had a smaller, but still significant, predictive power for both bacterial and eukaryotic alpha diversity (Tables 1 and 2), and the cryoconite hole surface area and pH were both significant predictors of eukaryotic alpha diversity. The raw values of environmental variables are provided in Table B.1 (Supplementary Information).

Permutational ANOVA of the db-RDA revealed that longitude and NO_3^- were significantly related to bacterial beta diversity (Table 3), and longitude alone (Table 4) was significantly related to eukaryotic beta diversity (Fig. 5).

DISCUSSION

We showed that the patterns of alpha and beta diversity of eukaryotic communities in Antarctic cryoconite holes in the Taylor Valley are strongly related to those of bacteria (Fig. 3). To our knowledge, no previous studies have compared bacterial and eukaryotic phylogenetic diversity of Antarctic cryoconite holes across multiple glaciers using modern, high-throughput techniques. Although the relationships between eukaryotic and bacterial diversity were expected, their strength was surprising, given that the biogeography of protists and bacteria can diverge at least at larger spatial scales (Bates *et al.* 2013), and that location-specific clustering has been more commonly found for bacterial than eukaryotic communities in Arctic cryoconite holes (Cameron, Hodson and Osborn 2012). Furthermore, models of wind dispersal suggest that the larger body size of the protists, rotifers and tardigrades found in Antarctic cryoconite holes may lead to faster settling times and lower dispersal rates than for bacteria (Wilkinson *et al.* 2012). We therefore might have expected to see the diversity patterns of larger eukaryotes diverge somewhat more from those of bacteria in a wind-dispersed system of natural mesocosms, but instead we observed that bacterial and eukaryote diversities are tightly linked across this system.

In addition to being tightly associated with each other, bacterial and eukaryotic alpha and beta diversities reflected the

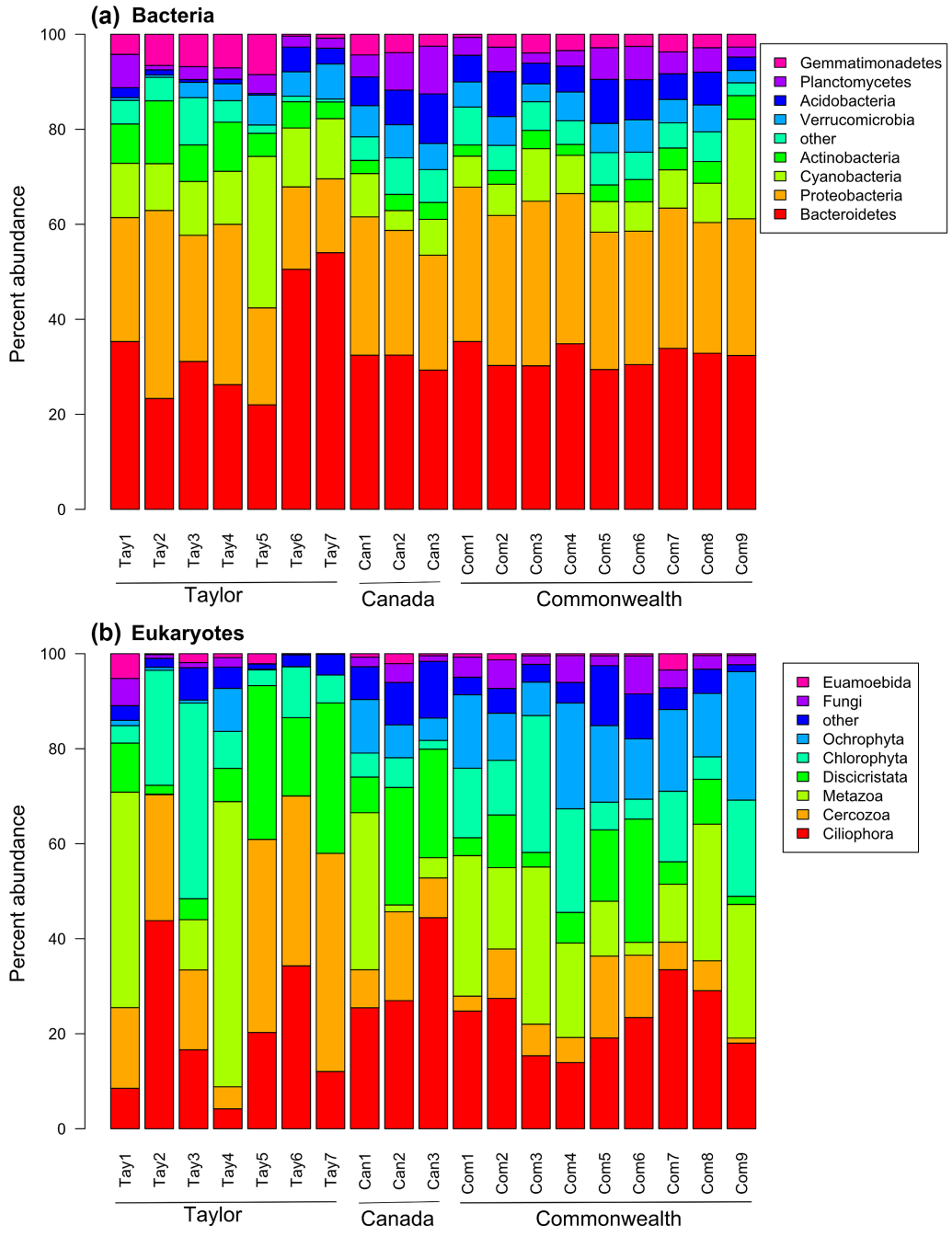

Figure 2. Relative composition of (**a**) bacterial phyla and (**b**) eukaryotic major taxonomic groupings by technical replicate along the x-axis, and then grouped by glacier.

gradient in the streams and soils of the Taylor Valley, as well as physicochemical characteristics of the cryoconite holes. The alpha diversities of both bacterial and eukaryotic communities were strongly related to the glaciers' positions in the valley, as reflected by longitude (Tables 1 and 2, Fig. 4), and significantly positively related to HCO_3^- (Tables 1 and 2). Variation in beta diversity was also strongly associated with longitude, with bacterial beta diversity also related to NO_3^- (Fig. 5, Tables 3 and 4). However, drawing inference about abiotic drivers of diversity from these observational data is impossible without further experiments. For example, the high NO_3^- values we observed can be consistent with a lack of microbial activity utilizing nitrogen, especially as the highest NO_3^- measurements were taken on the Taylor Glacier, where the lowest abundances of organisms have been previously recorded (Porazinska *et al.* 2004), and therefore better reflect a result of diversity rather than a cause.

Besides having the lowest alpha diversity of the sampled glaciers (Fig. 2), some cryoconite holes on the Taylor Glacier apparently lacked any Metazoa. Samples Tay5–Tay7 had no dominant metazoan OTUs in any of the technical replicates, whereas the dominant metazoan OTUs were found at relatively high levels in most other holes sampled (Fig. 3e). The metazoan-free samples had substantial relative abundances of flagellate OTUs (subkingdom Discoba) and ciliate OTUs not observed in other samples (Fig. 3e and f), perhaps indicating that metazoa eliminate these organisms from cryoconite holes where they

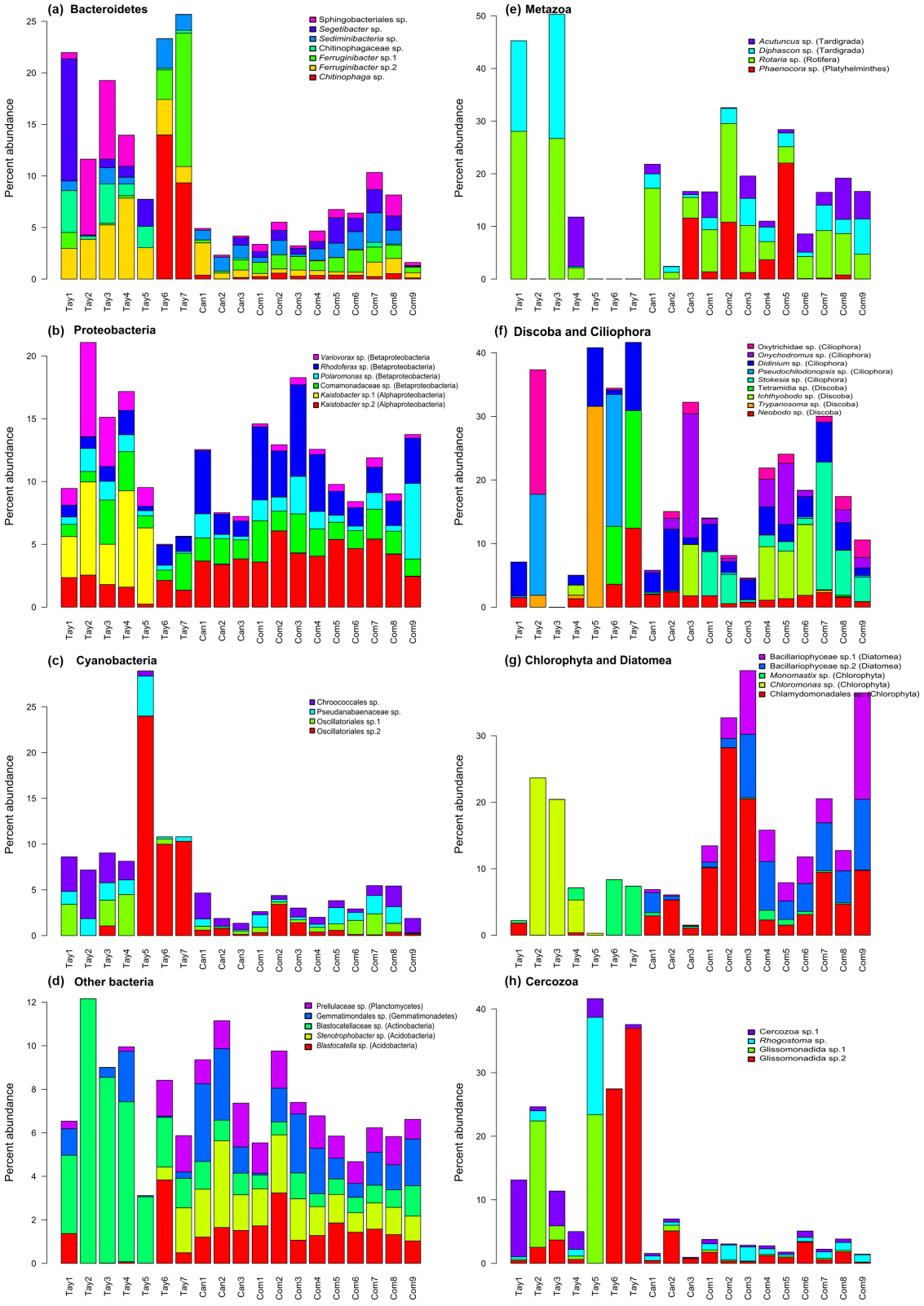

Figure 3. Relative abundance of the 22 most abundant bacterial and 22 most abundant eukaryotic OTUs within each replicate. OTUs are identified to the greatest resolution possible, and grouped for visibility into the following major groups: a) Bacteroidetes, b) Proteobacteria, c) Cyanobacteria, d) other bacteria, e) Metazoa, f) Discoba and Ciliophora, g) Chlorophyta and Diatomea, and h) Cercozoa.

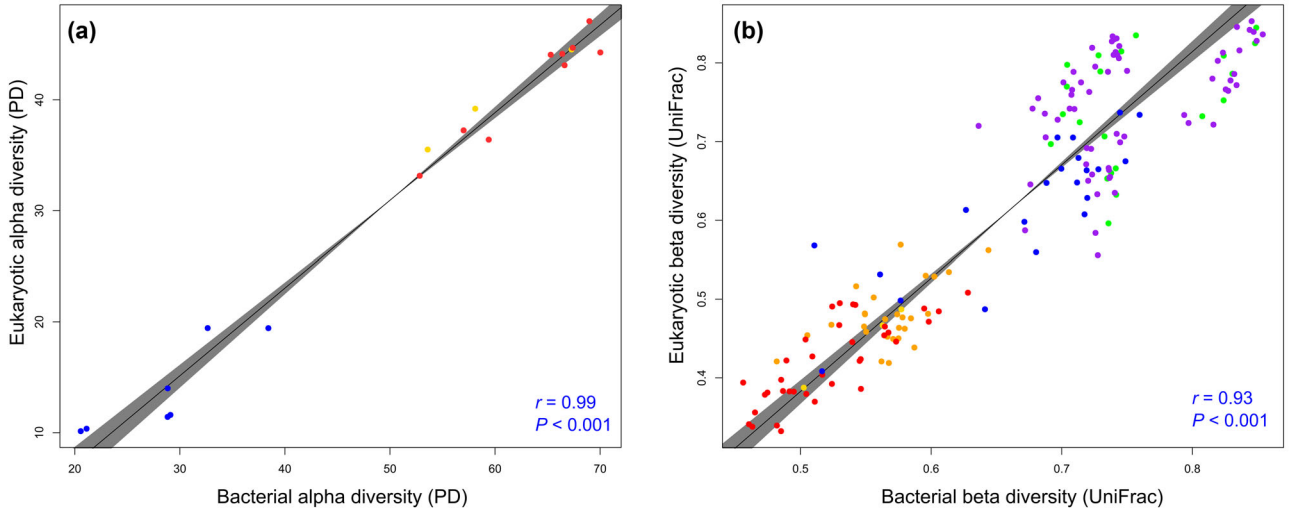

Figure 4. Model II regression of **(a)** alpha diversity measured as Faith's (1992) phylogenetic diversity (PD), and **(b)** beta diversity measured as UniFrac (Lozupone and Knight 2005) pairwise community dissimilarity of bacteria with that of eukaryotes. Colors indicate the glacier of origin for each sample: red is the Commonwealth Glacier, on the diverse east end of the valley; gold is the Canada Glacier, just west of the Commonwealth Glacier; and blue is the Taylor Glacier, which descends from the ice sheet with mixtures indicating pairwise glacial comparisons; purple is Taylor-Commonwealth; orange is Canada-Commonwealth; and green is Canada-Taylor. Gray band is 95% confidence interval.

Table 1. Results from multiple linear regression of phylogenetic diversity of bacteria against physical and chemical characteristics (centered and standardized) of the cryoconite holes.

Term	Estimate	SE	statistic	P
(Intercept)	50.13	1.06	47.35	**<0.001**
Longitude	3994.87	440.28	9.07	**<0.001**
Area	2.4	1.11	2.17	0.05
NO_3^-	−0.43	1.18	−0.37	0.72
pH	14.01	9.74	1.44	0.18
EC	1.79	3.01	0.6	0.56
HCO_3^-	−3.12	1.27	−2.46	**0.03**

Significant *P* values are bolded.

Table 2. Results from multiple linear regression of phylogenetic diversity of eukaryotes against physical and chemical characteristics (centered and standardized) of the cryoconite holes.

Term	Estimate	SE	statistic	P
(Intercept)	31.04	0.57	54.86	**<0.001**
Longitude	2969.91	235.34	12.62	**<0.001**
Area	1.84	0.59	3.11	**0.01**
NO_3^-	− 0.81	0.63	− 1.28	0.23
pH	13.56	5.21	2.6	**0.02**
EC	1.35	1.61	0.84	0.42
HCO_3^-	− 2.34	0.68	− 3.45	**0.005**

Significant *P* values are bolded.

Table 3. Results from a permutational analysis of variance on the db-RDA between environmental factors and bacterial communities, with significance adjusted for multiple comparisons using the false discovery rate method.

Term	Df	Sum of Sqs	F statistic	P
Longitude	**1**	**1.009**	**6.291**	**0.006**
pH	1	0.168	1.047	0.354
Bicarbonate	1	0.248	1.547	0.192
Nitrate	**1**	**0.321**	**2.00**	**0.048**
Area	1	0.167	1.039	0.354
EC	1	0.195	1.218	0.294
Residual	12	1.924	NA	NA

Significant terms are bolded.

Table 4. Results from a permutational analysis of variance on the db-RDA between environmental factors and eukaryotic communities, with significance adjusted for multiple comparisons using the false discovery rate method.

Term	Df	Sum of Sqs	F statistic	P
Longitude	**1**	**1.3576030**	**11.410035**	**0.006**
pH	1	0.1259959	1.058939	0.318
Bicarbonate	1	0.1700194	1.428936	0.318
Nitrate	1	0.3017957	2.536455	0.057
Area	1	0.1381269	1.160894	0.318
EC	1	0.1262669	1.061216	0.318
Residual	12	1.4277989	NA	NA

Significant terms are bolded.

initially co-occur. The dominant OTUs of cyanobacteria and algae (Chlorophyta) were also distinct in the cryoconite holes without metazoans, even from the rest of the Taylor Glacier holes sampled (Fig. 3e and g). These negative co-occurrence patterns are intriguing and may give some clue about biotic interactions occurring in cryoconite holes, but more intensive sampling will be needed in future studies to statistically test co-occurrence patterns across these glaciers.

The high-throughput sequencing of eukaryotic communities is mostly consistent with previous studies, while providing new insight through higher resolution. Perhaps the most surprising addition to this invertebrate community was a *Phaenocora*-like rhabdocoelan flatworm, sporadically present at high relative abundance across all glaciers (Fig. 3d). Rotifers, tardigrades, ciliates, fungi, flagellates, diatoms and algae, however, had all been previously found in Antarctic cryoconite holes through

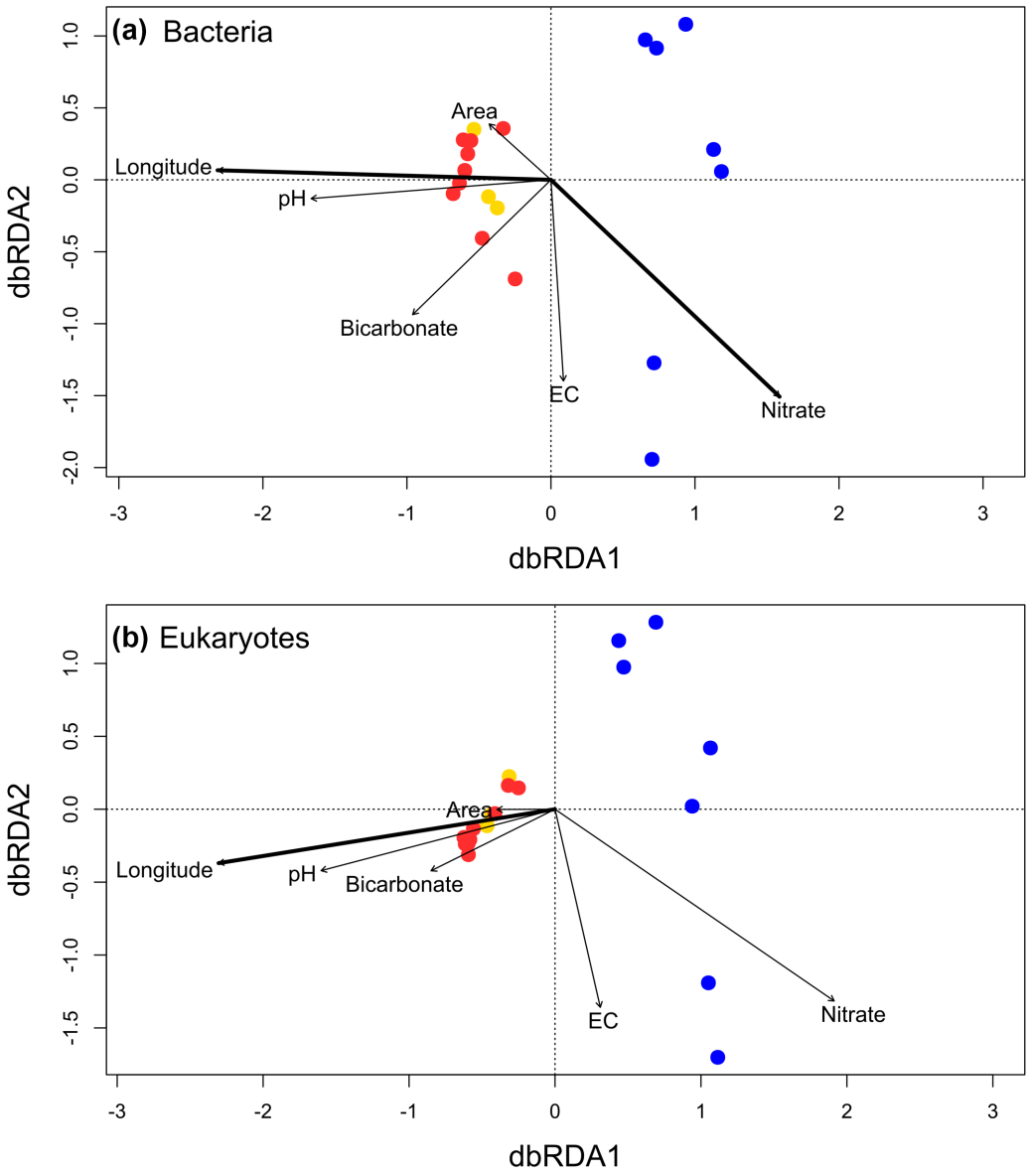

Figure 5. dbRDA biplot of UniFrac values for **(a)** bacteria and **(b)** eukaryotes, with abiotic correlations layered over community distances. Samples are colored by glacier of origin: red is the Commonwealth Glacier, on the diverse east end of the valley; gold is the Canada Glacier, just west of the Commonwealth Glacier; and blue is the Taylor Glacier, which descends from the ice sheet at the west end of the valley. Significant environmental factors are bolded.

molecular methods (Christner, Kvitko and Reeve 2003; Cameron, Hodson and Osborn 2012), microscopy (Mueller *et al.* 2001; Porazinska *et al.* 2004; Mieczan *et al.* 2013; Stanish *et al.* 2013) or both. Although Christner, Kvitko and Reeve (2003) reported nematode rRNA gene sequences in cryoconite holes, Porazinska *et al.* (2004) found no microscopic evidence of nematodes in cryoconite holes on the Taylor Valley glaciers. We detected the nematode 18S rRNA gene in our cryoconite samples, but they made up <1% of the 18S rRNA SSU reads. A more striking departure was the dominance of rotifers (8% of total reads) and tardigrades (5% of total reads) in our samples compared to Cameron, Hodson and Osborn (2012), who found no metazoan sequences in Antarctic cryoconite holes. However, the work of Porazinska *et al.* (2004) confirms that rotifers and tardigrades are common active members of cryoconite communities, and that our results do not reflect merely relic DNA.

A comparison of bacterial communities in the cryoconite holes to the streams and soils of the Taylor Valley highlights cryoconite hole communities as similar to other aquatic environments. The bacterial taxa we report are similar to those previously found in Antarctic cryoconite holes (Christner, Kvitko and Reeve 2003; Cameron, Hodson and Osborn 2012; Webster-Brown *et al.* 2015), with a few exceptions. More specifically, other surveys have found the dominant Bacteroidetes to be Cytophagales (Christner, Kvitko and Reeve 2003; Webster-Brown *et al.* 2015), whereas we detected prevalent OTUs primarily in Chitinophagales (Fig. 3a). However, many Betaproteobacteria from previous work were assigned to Burkholderiales (Cameron, Hodson and Osborn 2012; Webster-Brown *et al.* 2015), which had several dominant OTUs in our samples (Fig. 3b). Furthermore, cyanobacteria classes previously found on these glaciers (Mueller *et al.* 2001; Porazinska *et al.* 2004) and others in the Dry Valley

(Webster-Brown et al. 2015) were also represented in our dominant OTUs, including Chroococcales and Oscillatoriales (Fig. 3c). The similarity of the broad patterns of detected bacterial communities across studies provides reassurance of the persistence of these patterns despite differences in sampling protocols and sequencing platform.

Although spatial clustering of community composition by glacier within a region had not been previously found for eukaryotes in cryoconite holes (Porazinska et al. 2004; Cameron, Hodson and Osborn 2012), our higher resolution molecular methods demonstrate strong spatial structuring across glaciers (Fig. 5b). For example, at least two of the dominant ciliate OTUs on Canada and Commonwealth Glaciers are virtually absent from the Taylor Glacier (Fig. 3f), and there appears to be little overlap in the dominant algal OTUs between the Taylor Glacier and the other two glaciers (Fig. 3g). Overall alpha diversity was also lower on Taylor Glacier generally (Fig. 3a), and the communities in cryoconite holes were more variable than those on the Canada and Commonwealth Glaciers (Figs 3–5). This divide corresponds to the geography of the valley: the Taylor Glacier, in the Lake Bonney basin, is physically more distant from the other two glaciers, which are in the Lake Fryxell basin (Fig. 1). A major geologic feature, the Nussbaum Riegel, divides the Taylor Valley climatically into these basins (Fountain et al. 1999). The variable community composition of the streams in the Lake Bonney basin may lead to more variable cryoconite communities observed on the Taylor Glacier (Stanish et al. 2013). Additionally, the low alpha diversity suggests that low biomass in the streams and soils near the Taylor Glacier (Mcknight et al. 1998; Barrett et al. 2004) may result in low rates of cryoconite colonization there, contributing to the greater variability in alpha and beta diversity between holes on the Taylor Glacier (Figs 3–5).

Spatial clustering at the scale of the glaciers within the valley is consistent with previous work hypothesizing that cryoconite holes in the Dry Valleys are formed predominantly by sediments closest to the glacier (Stanish et al. 2013; Webster-Brown et al. 2015). Furthermore, cryoconite holes eventually feed back into streams especially during high melt years (Bagshaw et al. 2013), where the organisms and nutrients from these cryoconite holes then likely interact further in stream and lake habitats. Manipulative experiments on dispersal and taxonomic diversity, nutrient availability, and the physical and chemical properties of the holes would help to partition out the contribution of dispersal, abiotic factors and biotic interactions to determining microbial diversity of cryoconite holes.

CONCLUSIONS

Using modern high-throughput sequencing to characterize eukaryotes in Antarctic cryoconite holes in conjunction with bacterial communities highlights cryoconite holes as aquatic metacommunities in the McMurdo Dry Valleys. Our results show a strong correlation between eukaryotic and bacterial communities in cryoconite holes with both community types reflecting the gradient of diversity (Stanish et al. 2013) and organic material in the streams and soils along the valley (Mcknight et al. 1998; Barrett et al. 2004). These data are consistent with a cycling of biological material through the streams surrounding the glaciers (Bagshaw et al. 2013), and dispersing back onto the glaciers locally as cryoconite (Lancaster 2002; Nkem et al. 2006; Stanish et al. 2013), though future experiments will be needed to clarify the contributions of multiple processes that drive diversity in this system. Future work should therefore involve manipulations of community diversity or environmental conditions to understand why diversity patterns of bacteria and eukaryotes are so similar in these natural mesocosms.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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