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Experimental cryoconite holes as mesocosms for studying community ecology

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Abstract

Cryoconite holes are surface melt-holes in ice containing sediments and typically organisms. In Antarctica, they form an attractive system of isolated mesocosms in which to study microbial community dynamics in aquatic ecosystems. Although microbial assemblages within the cryoconite holes most closely resemble those from local streams, they develop their own distinctive composition. Here, we characterize the microbial taxa over time in cryoconite holes experimentally created from supraglacial sediments to demonstrate their utility as experimental mesocosms. We used high-throughput sequencing to characterize the assemblages of bacteria and microbial eukaryotes before melt-in, then after one and two months. Within one month of melt-in, the experimental holes, now lidded with ice, were visually indistinguishable from natural cryoconite holes, and within two months their thermal characteristics matched those of natural holes. The microbial composition of the experimental cryoconite holes declined in richness and changed significantly in the relative abundance of various taxa, consistent with possible turnover. In particular, a dominant cyanobacterium, *Nostoc* sp., further increased its dominance over the other dominant cyanobacterial phylotype, and an initially rarer *Flavobacterium* sp. became one of the dominant taxa. The eukaryotes continued to be dominated by algae and tardigrades, with the relative abundance of the dominant alga, *Macrochloris* sp., increasing notably relative to the microfauna. These changes within a single growing season in newly formed lidded cryoconite holes created from exposed supraglacial sediments are consistent with primary production and microbial turnover, and provide a promising foundation for future work using such mesocosms.

Keywords Cryoconite · Antarctic · Bacteria · Eukaryotes · Algae · Cyanobacteria

Introduction

Cryoconite holes are ice-bound water-filled holes that form on glaciers when sediments, termed cryoconite (Nordenskjöld 1875), melt into the glacial surface (Gribbon 1979; Wharton et al. 1985). They are common in snow-free areas on glaciers, covering 0.1–10% of the ablation zone of glaciers and ice sheets (Fountain et al. 2004; Anesio et al. 2009; Cook et al. 2012). Despite their small individual size, they are sufficiently numerous that the biogeochemical processes driven by microbial ecosystems within them can contribute significantly to overall carbon cycles (Anesio et al. 2009) and nutrient fluxes in oligotrophic regions such as the McMurdo Dry Valleys of Antarctica (Bagshaw et al. 2013).

Cryoconite holes develop when sediment is transported onto a glacier (Gribbon 1979) carrying with it bacteria, algae, fungi, and microfauna such as tardigrades and rotifers (Wharton et al. 1985; Cameron et al. 2012). The

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sediment, which has a lower albedo than the surrounding ice, absorbs solar radiation and melts into the glacier surface (Wharton et al. 1985; Fountain et al. 2004, 2008). Meltwater allows growth of microorganisms within the sediment, leading to communities that are often net photosynthetic (Bagshaw et al. 2016). The melting continues until the sediment layer is sufficiently deep that attenuation in radiation prevents it from melting any deeper, usually about 50 cm. At that point, melt approximately keeps pace with ablation of the ice surface (Gribbon 1979) and holes can become wider (Cook et al. 2010) in proportion to sediment volume (Cook et al. 2016).

In the McMurdo Dry Valleys, Antarctica, unlike Arctic and alpine regions, the summers remain sufficiently cold for cryoconite holes to retain a lid of ice over them, even as the sediment and a layer of water melt out within (Mueller et al. 2001). Although approximately half of the holes are connected by subsurface drainage channels (Fountain et al. 2004), many remain isolated and entombed in ice, resulting in extremely high pH values due to lack of gas exchange with the atmosphere in the presence of photosynthesis (Tranter et al. 2004; Webster-Brown et al. 2015). Distinctive and divergent microbial communities form within these isolated cryoconite holes (Stanish et al. 2013; Webster-Brown et al. 2015; Sommers et al. 2018). In Taylor Valley, one of the McMurdo Dry Valleys of Antarctica, microbial mats and sediments from the seasonal streams that flow from the glacier to the lakes have been suggested as major sources of cryoconite on the glaciers (Lancaster 2002; Stanish et al. 2013). Once the cryoconite hole forms, biological communities within them develop their own structure apart from the parent material in the streams. For instance, diatoms in cryoconite holes comprise only a subset of those found in the streams (Stanish et al. 2013), likely indicating different environmental conditions.

The purpose of our study was to determine the suitability of experimentally created cryoconite holes in Antarctica as mesocosms in which to test ecological theory. Previous work demonstrated that cryoconite holes can be artificially created by placing a thin patch of sediment on the glacial surface (MacDonell and Fitzsimons 2008). We hypothesized that the microbial communities present in sediments used to create cryoconite holes would undergo detectable changes in community structure within a single growing season as they grew in an environment distinct from that of streams or the surface of the glacier. To characterize changes, we sampled the communities in experimental holes after approximately one and two months after installation. Finally, to test whether experimental holes shared characteristics with natural holes, a replicate set of natural holes was selected for measurement of physical dimensions and community composition, and the temperature at the sediment was measured over time in a single natural and experimental cryoconite hole.

Methods

Site description and sampling

The experiment was conducted on Canada Glacier, a polar alpine glacier in the Taylor Valley, one of the McMurdo Dry Valleys in Antarctica. We selected Canada Glacier primarily for the presence of extensive information on its cryoconite holes (e.g., Christner et al. 2003; Porazinska et al. 2004; Tranter et al. 2004; Bagshaw et al. 2007; Foreman et al. 2007; Fountain et al. 2008; Tedesco et al. 2013; Telling et al. 2014) but also for its accessibility by foot from the Lake Hoare field camp.

Cryoconite holes

To construct the experimental holes, we collected sediments from the surface of the glacier ("supraglacial sediments") from the lower Canada Glacier (77.62632°S, 162.94595°E) (Fig. 1a). Before aliquoting, sediments stored at − 20 °C were allowed to thaw at 4 °C for 24 h in the field lab, then homogenized in a sterilized basin. Subsamples of 20 g (wet) (equivalent to ~ 2-mm-thick layer of sediment) were transferred to sterile plastic bags (Whirl–Pak, Nasco, WI, USA) and stored at 4 °C overnight.

On 24 November, 2016, we drilled 25 cylindrical holes less than 5 cm deep and 8 cm in diameter using a sterilized drill bit (Forstner bit). The sediments were spread into the holes in a layer about 2 mm thick (Fig. 1b). To anchor (freeze) the sediments in place, 10 ml of melted glacial water was added to each hole the following day (Fig. 1c). One subsample was reserved to characterize the community composition and structure of the initial community ("input sediment"), after treatment identical to the other samples.

Within five days' time, all sediment patches melted into the ice as expected from natural hole formation. We selected 7 out of 25 holes for sampling on 23 December (~1 month later) by gently drilling through the ice lid with an 18 v drill bit cleaned between samples by drilling into bare ice. Six out of the 7 holes contained water, and only these were sampled. We collected 3 × 1-ml sediment subsamples from each hole with a sterile 25-ml serological pipet (Fig. 1e). The three subsamples were pooled in a sterile plastic bag (Whirl–Pak: Nasco, WI, USA) and stored at − 20 °C for up to three weeks before DNA extraction.

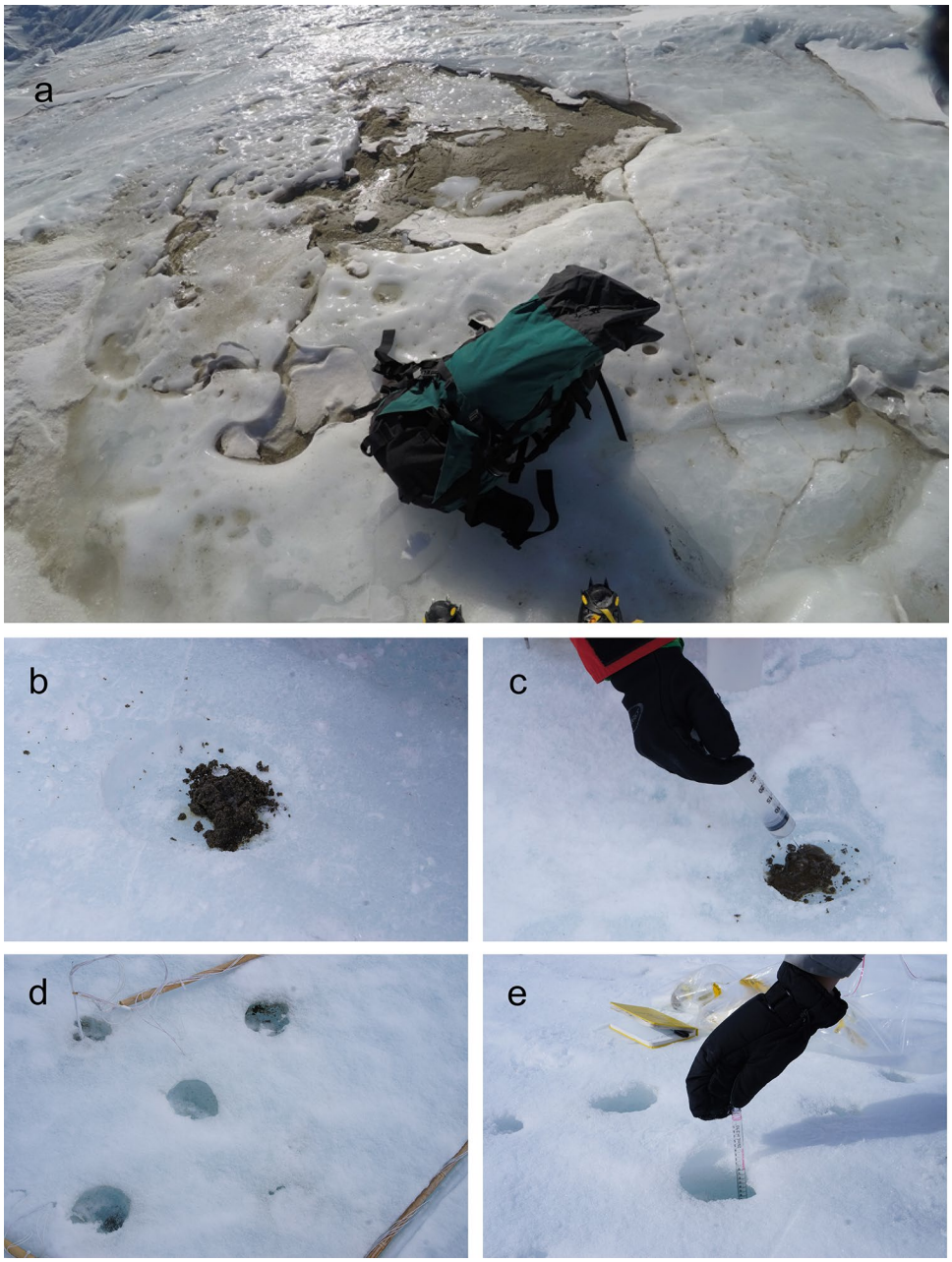

Fig. 1 The process of making experimental cryoconite holes. **a** Supraglacial sediments used as input sediments to make experimental cryoconite holes. Backpack (70 L) in the foreground for scale. **b** Input sediments placed in drilled depression on ice. **c** Melted glacial ice added to sediments. **d** Temperature probes, with wires wrapped around bamboo, mounted on masts and inserted into the ice to record temperature within experimental cryoconite holes after melt-in. **e** Melted experimental cryoconite holes being sampled with a serological pipet through a hole drilled in the ice lid

On 16 January (~ 2 months later), we sampled 8 out of initial 25 holes. Because all but one were entirely frozen, we drilled by hand into each hole with an ice auger (Kovacs, OR, USA) (cleaned in ice between samples) until the sediments evacuated to the surface, then collected sediments with an ethanol-sterilized steel scoop into sterile plastic bags (Whirl–Pak, Nasco, WI, USA). Among the 8 sampled holes, 4 had been sampled in December. All collected sediment samples were stored at − 20 °C up to three weeks before DNA extraction.

To assess thermal characteristics of the experimental holes, we installed a temperature thermistor in one hole on 27 November, 2016. The thermistor was secured to a stainless steel mast covered white Teflon tape (to minimize solar heating), and positioned 5 cm beneath the ice surface to measure the sediment temperature as it migrated downward (Fig. 1d). The instrumented hole was never sampled for microbial community structure to avoid disturbance. We also inserted a thermistor into a nearby natural cryoconite hole in the same configuration with the probe positioned at the sediment surface. The natural cryoconite hole was approximately 20 cm diameter. To measure the background ice temperature of the glacier, a thermistor was installed one meter below the surface, and for local air temperature one was mounted in a radiation shield one meter above the ice.

As a comparison to the experimental holes, we selected and sampled 8 natural cryoconite holes (14–28 cm diameter) on Canada Glacier to characterize their communities. While still frozen, these holes were sampled on 7 and 8 November, 2016 using a 10-cm diameter ice corer (SIPRE corer). Extracted cores were placed in sterile bags, and stored at − 20 °C for up to four weeks before processing. In the Crary Laboratory at McMurdo Station, the sediment puck (the disc-shared sediment portion of the core) from each core was separated from the overlaying water column and washed with Milli-Q filtered water to remove the outer layer, which was likely cross-contaminated by the corer, then allowed to melt in an acid-washed container at 4 °C for 24 h before being homogenized and subsampled for DNA extraction.

We also sampled other supraglacial sediments from different locations (8 in total) on Canada Glacier on 10 November, 2016 as reference points. An ethanol-sterilized steel scoop was used to transfer sediments into a sterile plastic bag (Whirl–Pak, Nasco, WI, USA). Sediments were stored at − 20 °C up to one month until DNA extraction.

DNA sequencing and data processing

Approximately 0.3 g (wet weight) per each collected sediment sample was used for DNA extraction using a PowerSoil DNA Isolation Kit (MoBio Inc., CA, USA) following the manufacturer's instructions. 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 Kit (Invitrogen Corp., CA, USA), and sequenced on two lanes with Illumina MiSeq V2 (Illumina Inc., CA, USA) with 2 × 250 bp chemistry at the BioFrontiers Sequencing Core Facility at the University of Colorado at Boulder.

QIIME v1.9.1 (Caporaso et al. 2010) was used to de-multiplex and quality filter the raw reads. Paired-end sequences were joined with VSEARCH (Rognes et al. 2016) and clustered into operational taxonomic units (OTUs) at 97% similarity using UCLUST (Edgar 2010). Taxonomy was assigned using QIIME's parallel_assign_taxonomy_blast.py script with the SILVA 128 Ref NR99 database (Quast et al. 2013). Based on this classification, mitochondrial and chloroplast OTUs were removed from the bacterial OTU table, and bacterial OTUs were removed from the eukaryotic OTU table. OTUs that made up at least 1% of the extraction blank sequences and were at least 1% of the samples on average were discarded as likely lab contaminants, with two bacterial exceptions (two Burkholderiales phylotypes) that were also dominant members of the cryoconite assemblage sequences and closely related to other sequences from polar environments. Singletons were removed. Bacterial OTU tables were rarefied to 8700 reads, and eukaryotic OTU tables were rarefied to 5280 reads.

Analyses

We used a linear mixed model implemented with function 'lme' in package 'nlme' (Pinheiro et al. 2018) in R v 3.5.2 (R Core Team 2018) to compare the total richness of phylotypes (OTUs) between experimental holes sampled in December ($n = 6$) and those sampled in January ($n = 8$) with a random intercept term to account for the holes that were resampled at both time points ($n = 4$) subset of December and January. We used a one-sample t test to test the null hypothesis that the mean phylotype richness in December ($n = 6$) did not differ from the phylotype richness of the input sediment ($n = 1$). Normal distributions were used for these tests because the data for the most part did not deviate significantly from assumptions of normality or show significant heteroskedasticity (Online Resource 1).

We used a redundancy analysis (RDA) on the Hellinger distances of unrarefied data (McMurdie and Holmes 2014), and a permutational ANOVA-like test with 999 permutations to ask whether days since establishment of experimental cryoconite holes were a significant correlate of community turnover. We furthermore used a permutational multivariate analysis of variance (PERMANOVA) as implemented by the function 'adonis' in package 'vegan' (Oksanen et al. 2018) with 999 permutations to compare experimental cryoconite holes sampled in different months, along with other supraglacial sediments and natural cryoconite holes. We conducted post hoc pairwise comparisons using function 'pariwise.perm.manova' from package 'RVAideMemoire' (Hervé 2018).

Finally, we compared the relative abundance of the dominant bacterial and eukaryotic phylotypes from samples collected in December to those collected in January using linear mixed-effects models and to the input sediment using a one-sample t test. Because the data generally did not deviate from assumptions of normality nor showed heteroskedacity (Online Resource 1), all tests were performed using normal distributions. However, log-transformed relative abundance was used for one eukaryotic phylotype to meet normality assumptions because negative binomial models did not converge (Online Resource 1).

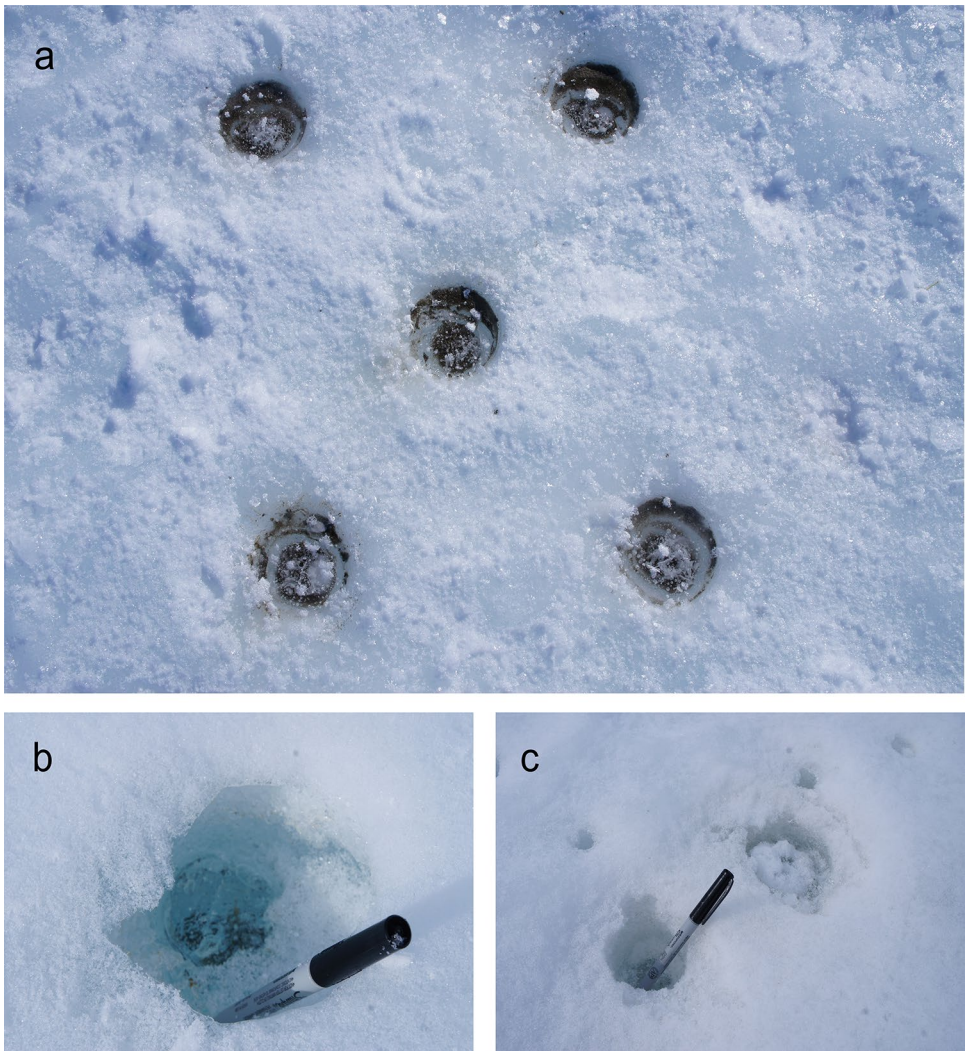

Fig. 2 Images illustrating the physical status of cryoconite holes throughout the season. **a** Initial melt two days after placement. **b** Approximately 1-month-old experimental cryoconite hole with an ice lid as observed on 23 December, 2016. **c** A nearby natural cryoconite hole of approximately same size was visually indistinguishable from the experimental hole

Results

Visual observations

The austral summer of 2016–2017 was snowy. Although the study site was initially snow-free, subsequent snow events required the site to be cleared of snow before experimental holes were installed. Snow over the ice, in this environment, reduces solar radiation into the ice and subsurface warming such that subsurface melt is absent. Fortunately, surface melt was observed at the experimental holes as early as three days after sediment placement (Fig. 2a). By the first sampling date one month later, the experimental cryoconite holes were visibly indistinguishable from natural holes (Fig. 2b, c), with a solid ice lid partially covered with a wind-crust layer of snow, and liquid water inside. By mid-January (approximately 2 months), much of the snow crust persisted, but all sampled holes were frozen.

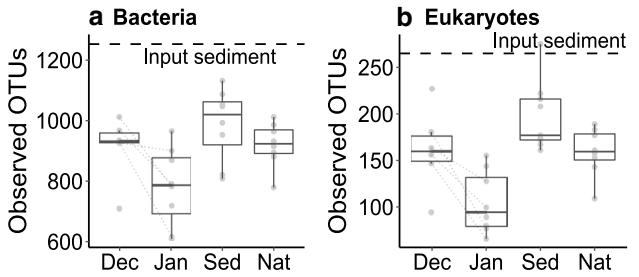

Fig. 3 Phylotype richness of **a** bacteria and **b** eukaryotes in: Input sediment ($n=1$) denoted as dashed line; approximately 1-month-old ($n=6$) and 2-month-old ($n=8$) experimental cryoconite holes denoted as "Dec" and "Jan", respectively; supraglacial sediments ($n=8$) denoted "Sed;" and natural cryoconite holes ($n=8$), denoted "Nat." Samples from the same experimental hole in December and January are indicated with dotted lines connecting points

Bacteria

In comparison to the input sediment, bacterial phylotype

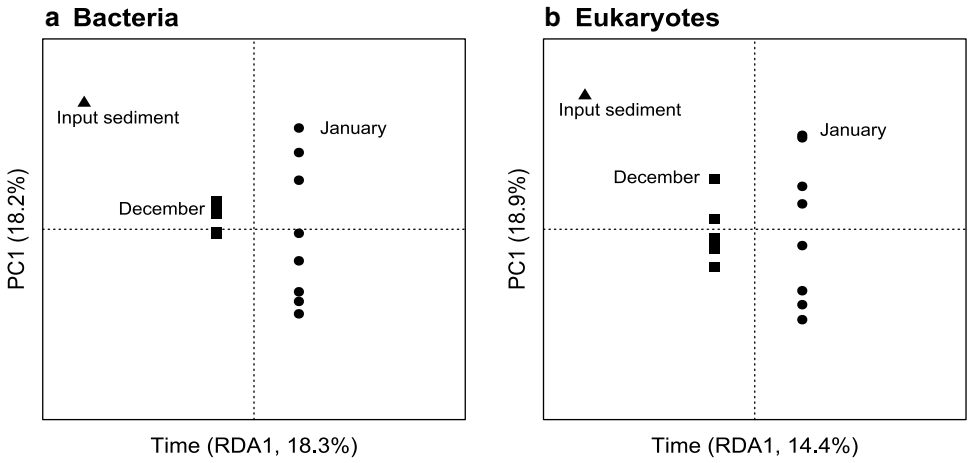

Fig. 4 Redundancy analysis of Hellinger distances of experimental cryoconite hole community structure using the days since they were established for **a** bacteria and **b** eukaryotes. Days since establishment (Time: RDA1) explained 18.3% of the variation for bacterial communities and 14.4% for eukaryotic communities

richness (Fig. 3a) declined by the December sampling (one-sample t test, $t_5 = -8$, $p < 0.001$) and remained low through the January sampling (one-sample t test, $t_7 = -10.43$, $p < 0.001$), with no difference between sampling dates (LME model Jan. to Dec., $t_3 = -2.03$, $p = 0.14$, full model results in Online Resource 3).

The community structure within the experimental cryoconite holes underwent change from the input sediment

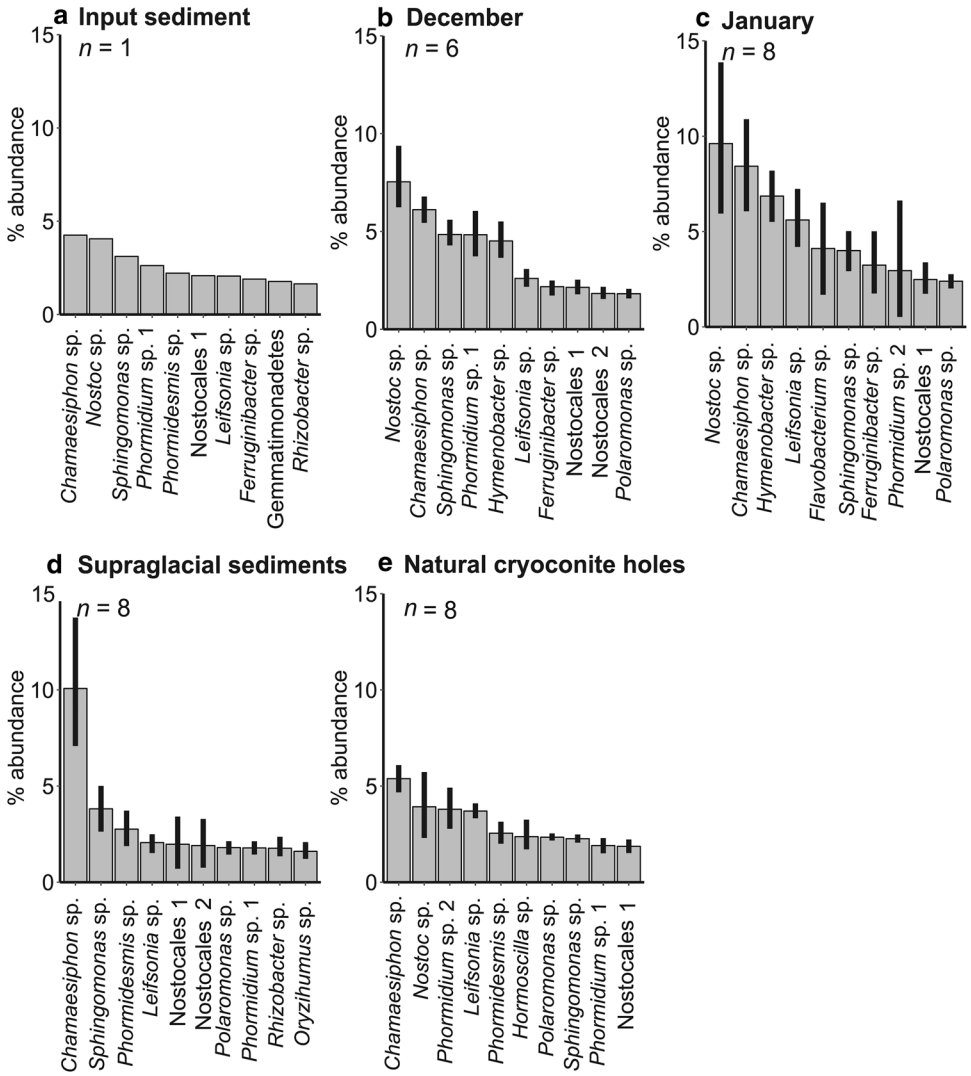

Fig. 5 Relative abundance of ten most dominant bacterial phylotypes from **a** input sediments; **b** experimental holes sampled in December; **c** experimental holes sampled in January; **d** other supraglacial sediments; and **e** naturally occurring cryoconite holes. Error bars are bootstrapped 95% confidence intervals

to December samples, and again by the January samples (RDA, $F_1 = 2.93$, $p = 0.001$, 18% of variance constrained, full model results in Online Resource 4) (Fig. 4a). Although the bacterial assemblages changed between time points, they remained distinct from natural cryoconite holes and from other supraglacial sediments (PERMANOVA, $F_3 = 5.9$, $p = 0.001$; pairwise comparisons in Online Resource 2).

The relative abundance of the dominant *Nostoc* sp. (Cyanobacteria) over the next most abundant phylotype, *Chamaesiphon* sp. (Cyanobacteria) increased (Fig. 5a–c) from the input sediment to the December sampling (one-sample *t* test, $t_5 = 3.5$, $p = 0.003$), and the *Nostoc* sp. retained its dominance into January (LME model, $t_3 = -2.03$, $p = 0.14$, full model results in Online Resource 3). A *Flavobacterium* sp. (Bacteroidetes) showed a similar pattern, increasing in relative abundance from initially rare to become one of the dominant phylotypes (one-sample *t* test of input to Dec., $t_5 = 2.4$, $p = 0.03$, LME model Dec. to Jan. $t_3 = -2.03$, $p = 0.14$, full model results in Online Resource 3) (Fig. 5a–c).

Eukaryotes

Similar to bacteria, the richness of eukaryotic phylotypes in experimental cryoconite holes declined from the input sediment to December sampling (one-sample *t* test, $t_5 = 5.9$, $df = 5$, $p = 0.002$) (Fig. 3b), and continued to decline over time (LME model Jan. to Dec., $t_3 = -4.4$, $p = 0.02$, full model results in Online Resource 3).

The assemblages of microbial eukaryotes also changed significantly over time (RDA, $F_1 = 2.20$, $p = 0.005$, 14.5% of variance constrained, full model results in Online Resource 4) (Fig. 4b). Similarly to bacteria, the eukaryotic assemblages in experimental holes were different from those in natural cryoconite holes and from supraglacial sediments (PERMANOVA, $F_3 = 5.6$, $p = 0.001$, pairwise comparisons in Online Resource 2).

Eukaryotic assemblages were dominated by a single phylotype of algae, *Macrochloris* sp., whose relative abundance increased from < 20% of the sequences in the input sediment to up to ~ 30% in December (one-sample *t* test start to Dec., $t_5 = -3.4$, $p = 0.019$) and January (LME model Dec-Jan comparison, $t_3 = 1.28$, $p = 0.29$, full model results in Online Resource 3). In contrast, one of initially dominant bdelloid rotifers, *Rotaria* sp., declined in proportion over time (one-sample *t* test, $t_5 = -5.2$, $p = 0.003$) and from December to January (LME model, $t_3 = -6.5$, $p = 0.008$, full model results in Online Resource 3). A phylotype most closely matching the tardigrade *Acutuncus antarcticus* also initially declined (one-sample *t* test, $t_5 = -4.6$, $p = 0.006$), but then rebounded from December to January (LME model: $t_3 = 1.4$, $p = 0.025$, full model in Online Resource 3) (Fig. 6a–c).

Temperature

Temperature in the experimental and natural cryoconite holes initially differed likely due to their difference in depth (Fig. 7). In December, temperatures in the natural holes were well below freezing, as expected, with diurnal fluctuations. Over this period, the ice temperature was warming and experimental holes were melting into the ice. By late December, the temperatures in the natural holes were similar to air temperatures, and warmer than air temperatures by mid-January. However, they continued to freeze daily. Temperatures in the experimental holes were warmer than in the natural holes throughout the study period until mid-January when temperatures converged, most likely due to similar depths of the probes. The probe in the natural hole was measured to be 33 cm deep at that time, and the probe in the experimental hole 32 cm deep. Sustained melting over a period of weeks did not occur, as can be seen by the diurnal fluctuations of temperature which always cooled to below freezing after initially warming to melting temperatures in mid-December.

Discussion

Our results build on past work experimentally creating cryoconite holes (MacDonell and Fitzsimons 2008) by characterizing their subsequent biological and physical dynamics over a growing season. The temperature profiles of experimental cryoconite holes fluctuated daily as they melted into the ice, forming meltwater under an ice lid. Hole appearance was already indistinguishable from natural holes by mid-season. Moreover, by the end of the season, they achieved the same temperature profile as natural holes. Changes in the microbial assemblages over that time period were indicative of microbial species turnover and growth and confirmed our hypothesis that we would be able to detect changes in community structure within a single growing season. This finding lays the foundation for the use of cryoconite holes as experimental mesocosms for studying microbial community dynamics.

Biologically, experimental cryoconite holes developed their own signatures and dynamics that differed from all sediment types collected in this study. The richness of both the bacterial and eukaryotic phylotypes in experimental holes was lower than that of the input sediment and eukaryotic richness continued to decrease over time. The reduction in richness following division of the initial sediment into smaller "islands" of sediment in individual experimental holes is consistent with patterns of island biogeography theory (MacArthur and Wilson 1967), one of the few consistent relationships in ecology, and one which cryoconite holes follow at this site and globally (Darcy et al. 2018).

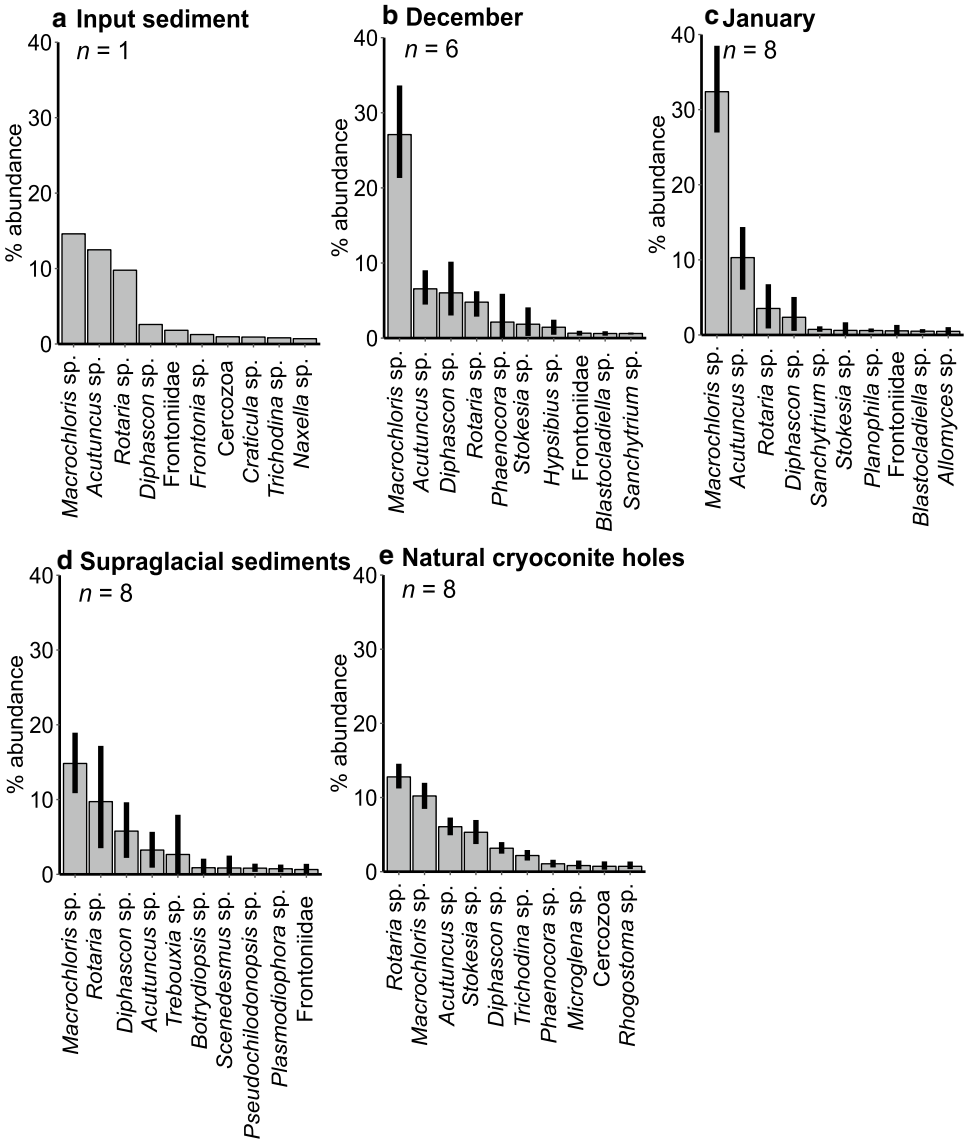

Fig. 6 Relative abundance of ten most dominant eukaryotic phylotypes from **a** input sediments; **b** experimental holes sampled in December; **c** experimental holes sampled in January; **d** other supraglacial sediments; and **e** naturally occurring cryoconite holes. Error bars are bootstrapped 95% confidence intervals

Smaller islands may reduce species richness through stochastic extinctions (MacArthur and Wilson 1967) or reduced habitat variability and thus opportunity to partition ecological niches (Hortal et al. 2009). Such niche partitioning is fundamental to stable coexistence of species (Chesson 2000). Although our current data do not allow tests of the mechanisms driving island biogeography theory, it supports the use of experimental cryoconite holes in future tests of these hypotheses.

An alternative but possible explanation for the decline in richness within experimental holes could be that some taxa in sediments are not active, but perhaps represent relic DNA atmospherically deposited by high winds that move sediment through the valley (Lancaster et al. 2002; Nylen et al. 2004; Šabacká et al. 2012; Diaz et al. 2018). Higher rates of microbial activity may be supported within cryoconite holes than on the glacial surface by the more stable temperatures we measured, buffered by the availability of liquid water for microbes, and lower light stress (Bagshaw et al. 2016). Relic DNA would therefore persist longer at the surface than in more active melted sediments, which would be consistent with previous findings of distinct phylotypes in sediments and surface ice of cryoconite holes (Sommers et al. 2019). Experimental cryoconite holes could be used in future work to test the rate at which known quantities of dead organisms' DNA is degraded in this environment.

In addition to changes in richness, both bacterial and eukaryotic assemblages underwent changes in composition throughout the growing season. For example, the relative abundance of a phylotype most closely matching *Hymenobacter antarcticus* (Bacteroidetes: accession GQ45800.1, Klassen and Foght 2011) increased to become the third most abundant phylotype by January in experimental holes. In

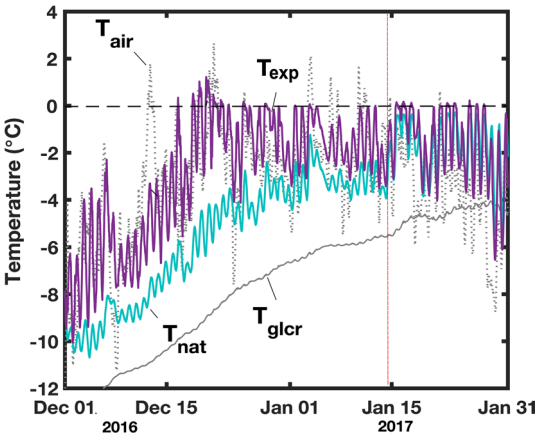

Fig. 7 Temperature profiles over the course of the season: inside the experimental cryoconite hole (T_{exp}: purple line), natural hole (T_{nat}: teal line), glacial ice at 1 m depth (T_{glcr}: black line) and air temperature (T_{air}: dotted black line). Red vertical dashed line indicates convergence of temperatures inside the experimental and natural cryoconite holes

contrast, the same phylotype was rare in natural holes and in other supraglacial sediments. Other members of this genus have been isolated in Antarctica from red snow (Kojima et al. 2016), and glacial water (Marizcurrena et al. 2017), perhaps indicating it can take advantage of shifting environmental conditions. Similarly, the relative abundance of the dominant cyanobacteria, *Chamaesiphon* sp. and *Nostoc* sp., and the dominant eukaryotic phototroph, *Macrochloris* sp., increased. Cyanobacteria from the genus *Nostoc* are commonly found in cryoconite on polar glaciers (Segawa et al. 2017). These communities did not match those of natural cryoconite holes sampled by the end of the season, and it is possible they never will due to differences in source material, as the sources and ages of the natural holes are unknown. It is also possible that these communities could be undergoing ecological selection by new environmental conditions (Vellend 2010) and that it may take more than one season for communities to mature to a stable structure, if one exists. Stable structures of bacterial communities in cryoconite holes have been observed in Greenland, despite the fact that those holes lacked ice lids and could receive continual input from the atmosphere and from supraglacial melt (Musilova et al. 2015). By contrast, bacterial communities of alpine cryoconite holes vary throughout seasons in the Alps (Franzetti et al. 2017; Pittino et al. 2018), although comparison of these results with Dry Valley cryoconite holes should be made cautiously, as alpine and Arctic cryoconite holes are typically unlidded and can have substantial hydrologic connectivity relative to cryoconite holes in the Dry Valleys (Mueller and Pollard 2004).

The dominance of primary producers could be indicative of favorable conditions for their growth, such as availability of water and photosynthetically active radiation (Bagshaw et al. 2016). It could also indicate a release from potential grazers such as rotifers and tardigrades, which decreased in their relative abundance. Amplicon sequencing data are compositional, meaning that it represents relative abundances rather than absolute abundances, so a faster increase in producers would necessarily result in an apparent reduction in microfauna, but our data cannot distinguish between rapid growth of one organism and actual decline of another. Sequencing data are furthermore influenced by differing gene copy numbers between organisms (Větrovský and Baldrian 2013), meaning that abundances of sequences should not be interpreted as the abundances of organisms. However, when the same set of samples is followed through time, as they are here, the changes in the abundance of organisms relative to one another can be used to infer changes in the relative abundance of those organisms that indicate change through time.

Despite the fact that the 2016–2017 was snowier than past years, the experimental holes exhibited shifting microbial communities indicating biological activity. Their morphology was much more stable than cryoconite holes monitored (Takeuchi et al. 2018) or manipulated (Cook et al. 2016) in Greenland, as is typical of natural cryoconite holes in the McMurdo Dry Valleys of Antarctica (Fountain et al. 2004). Also in contrast to Arctic and alpine cryoconite holes, an ice lid formed on experimental holes, consistent with natural holes in the Dry Valleys (Mueller et al. 2001). Neither experimental nor natural holes showed the same seasonal melt-development pattern that has been measured during several previous, largely snow-free summers on the same glacier (Fountain et al. 2004, 2008). Although we cleared some of the snow from the experimental study area, the remaining snow trapped in the rough ice surface greatly reduced solar heating of the ice, a critical factor in this environment because summer air temperatures are typically a few degrees below freezing (Zamora 2018; Hoffman et al. 2008). Furthermore, the ice surrounding the study site was not cleared away, creating a heat sink that further cooled the study site. This likely contributed to the temperature consistently dipping below freezing daily for both natural and experimental holes, even in January, in contrast to the previous, snow-free years (Zamora 2018).

In conclusion, within a single season, our experimental cryoconite holes mimicked the conditions in naturally formed cryoconite holes. Over that same time period, the assemblages of bacteria and microbial eukaryotes underwent compositional changes indicative of natural community dynamics, activity, and growth. Together, these data provide support for the use of experimental cryoconite holes as mesocosms to test hypotheses from community ecology theory (e.g., community assembly). Future work could, for instance, manipulate input to cryoconite holes to

test whether the order in which they arrive (priority effects) is important (Fukami 2015), or whether the relationship between taxonomic composition and ecosystem functioning differs between communities of microscopic organisms and larger organisms (Nemergut et al. 2013).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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