Microbial composition, diversity, and activity varies across different types of basal ice

Shawn M. Doyle^a and Brent C. Christner^b

^aDepartment of Oceanography, Texas A&M University, College Station, TX, USA.

^bDepartment of Microbiology and Cell Science, University of Florida, Gainesville, FL, USA.

Running title: **Microbial ecology of different basal ice types**

Abstract word count: 214

Main text word count: 6579

*Corresponding author:

Shawn M. Doyle

Department of Oceanography

Texas A&M University

Eller O&M Bldg, Rm 716D

College Station, TX, 77843-3146

Tel: 979-845-5105

E-mail: shawndoyle@tamu.edu

Competing Interests: The authors declare no competing financial interests.

ABSTRACT

Basal ice often contains entrained subglacial debris and sediment which can serve as a source of nutrients and organic matter and provide habitat for microorganisms adapted to frozen conditions. However, basal ice comes in many different forms and comparatively little is known about how microbial composition, diversity, and activity vary across different types of basal ice. Here, we investigated these parameters in four different types of basal ice from two different glaciers and then used a meta-analysis to compare our findings with microbiome studies of other permanently frozen environments. We found microbiome composition varies substantially between basal ice types, even within the same glacier. Further, the microbiomes of sediment-rich basal ices were distinct from those found in glacial ice and instead were most like those found in permafrost. Consistent with this, microbial diversity was also comparable to that found in permafrost and was much higher relative to glacial ice. Patterns of 16S rRNA read abundance from RNA relative to DNA implicated certain taxa as potentially active in basal ice with ice temperature appearing to be an important predictor for the diversity of taxa inferred to be active. Our results improve our understanding of the microbial ecology of different basal ice types and provide insight into which types are likely habitats for metabolizing microbial communities.

1. INTRODUCTION

Basal ice has a chemistry and physical structure that is directly affected by its proximity to and interaction with material beneath the glacier (Knight, 1997). Basal ice layers are typically entrained with debris from the underlying substrate that ranges in size from fine-grained clays and silts to coarse sands, gravels, and boulders. Basal ice formation can produce layering and stratification patterns that become deformed by the folding and shearing forces associated with glacier flow. These processes of entraining and restructuring of subglacial debris combine to produce various types of basal ice. This ranges from relatively debris-poor types containing <1% (w/v) sediment to debris-rich types composed almost entirely of sediment with only interstitial ice (Knight, 1997). When compared to overlying englacial ice (i.e., derived from snowfall and deposited aerosols), the sediment-rich basal ice contains higher concentrations of microbial substrates such as formate, acetate, ferrous iron, and ammonia (Skidmore *et al.*, 2000; Wadham *et al.*, 2004; Tung *et al.*, 2006; Yde *et al.*, 2010). These compounds are hypothesized to be an important source of electron donors for heterotrophic and lithotrophic microorganisms entrapped in subglacial environments, including the basal ice itself (Montross *et al.*, 2013, 2014).

Before there was much interest in the microbiology of the cryosphere, basal ice microbial communities were assumed to exist in a persistent state of metabolic dormancy while frozen. This view began to change around the turn of the century after mounting evidence from several pioneering studies revealed microorganisms are not only abundant in subglacial environments but actively participate in weathering processes (Sharp *et al.*, 1999; Skidmore *et al.*, 2000; Tranter *et al.*, 2002, 2005; Sheridan *et al.*, 2003; Miteva *et al.*, 2004; Yung *et al.*, 2007). The metabolic activity of basal ice microorganisms is now thought

to play an important role not only in the biogeochemistry of basal ice but also downstream terrestrial, freshwater, and marine environments which receive seasonal discharges of basal meltwater (Wadham *et al.*, 2010; Barker *et al.*, 2018; Hopwood *et al.*, 2019; Vick-Majors *et al.*, 2020). Indeed, basal meltwater is one of the largest sources of labile dissolved organic carbon and iron to coastal oceans adjacent to glaciated watersheds (Hood *et al.*, 2009; Bhatia *et al.*, 2013).

Due to accelerated rates of melt in many regions, basal ice microorganisms may also play an important role in global carbon cycling. The Greenland and Antarctic Ice sheets alone are estimated to contain ~10.2 Pg of organic carbon (Priscu *et al.*, 2008), ~0.5% of the estimated world's total soil organic carbon pool (Batjes, 2014). The subglacial production of biogenic methane and its subsequent release during periods of ice sheet retreat could represent a positive feedback mechanism that accelerates further climate warming (Wadham *et al.*, 2012). Indeed, basal ice sediments from John Evans Glacier (Skidmore *et al.*, 2000) and Robertson Glacier (Boyd *et al.*, 2010) have been found to contain viable methanogens and methanotrophy has been documented in subglacial water from the Greenland (Dieser *et al.*, 2014) and Antarctic ice sheets (Michaud *et al.*, 2017). Microbial activities in the subglacial and basal ice zones of the world's glaciers and ice sheets may thus be an overlooked component of Earth's climate oscillations over geologic timeframes. Despite this potential importance, accessing the basal zone of glaciers is logistically difficult and our understanding of basal ice microorganisms and how their composition and activity varies between different types of basal ice is still limited.

In this study, we used 16S rRNA gene amplicon sequencing to profile the composition and structure of microbial assemblages within several different basal ices and

RNA-based approaches to identify potentially active taxa. To explore how these microbial assemblages compare to those in other permanently frozen environments, we performed a meta-analysis that combined our data with that from other studies. Many of these studies sequenced different variable regions of the 16S rRNA gene (e.g. V4, V2, V3V4) that do not fully overlap, hindering the use of traditional OTU-based approaches. To address this, we used the SATé-enabled phylogenetic placement (SEPP) technique to insert exact amplicon sequence variants (ASVs) from these heterogeneous variable regions into the same reference phylogeny (Janssen *et al.*, 2018). This enabled a phylogenetically stable comparison of the microbial assemblages inhabiting these different frozen environments. In contrast to the aeolian-source microbes in englacial ice, the microorganisms in basal ice appear to represent “communities” in an ecological sense. We discuss the implications of microbial processes in an important transient environment of the cryosphere.

2. METHODS AND MATERIALS

2.1 Ice sampling

Taylor Glacier

Taylor Glacier is an outlet glacier of the East Antarctic Ice Sheet that terminates at the western end of Taylor Valley (McMurdo Dry Valleys, Victoria Land, Antarctica). During the austral summers of 2007 and 2009, two subglacial tunnels were excavated into the basal zone of the northern lateral margin of the glacier (Figure S1). The tunnels were initiated on fresh ice aprons and extended ~8 m in from the glacier face. At the end of each tunnel, a vertical shaft (2007 expedition) or large chamber (2009 expedition) was excavated to expose a stratigraphic sequence of multiple basal ice types (Montross *et al.*

2014). Electric chainsaws equipped with unlubricated carbide tipped chains were used to collect samples of the ice sequence from each basal ice type. Ice samples were wrapped in plastic, packed into insulated containers, and shipped frozen to Louisiana State University where they were subsequently stored at -20 °C.

Matanuska Glacier

Matanuska Glacier is a 43km valley glacier located in south-central Alaska, approximately 140 km northeast of Anchorage. In July 2013, a horizon of debris-rich basal ice exposed near the glacier terminus was sampled using an electric chainsaw (Figure S2). After collection, the ice samples were packaged in polypropylene flat wrap and shipped frozen to Louisiana State University for storage at -20 °C.

2.2 Ice decontamination

Within a temperature-controlled laboratory (-5°C), ~2 kg subsamples were cut from large samples of the basal ice using either a band saw (for the sediment-poor basal ice) or a diamond-bladed masonry saw (sediment-rich basal ice). The surface of the freshly cut samples was then cleaned following previous methods (Christner *et al.*, 2005). Briefly, the outermost surface was physically removed by scraping with an autoclaved microtome blade and the newly exposed ice surface was then washed with chilled (-5°C), 0.22 µm filtered 95% ethanol. Microtome scraping was omitted when processing sediment-rich samples due to the presence of coarse granules and stones embedded in the sediment-layers. Following the ethanol wash, samples were washed extensively with ice-cold, 0.22 µm, twice-autoclaved deionized water until a minimum of ~5 mm of the outer surface had been removed based on the ice weight. Large, sterilized surgical forceps were used to handle all ice samples during decontamination and were exchanged frequently during the

procedure to prevent carryover contamination. Sample weights before and after decontamination were used to estimate the amount of material removed from each sample during processing, which was typically between 15% and 25% of the initial mass.

The effectiveness of the decontamination procedure was monitored by coating ice samples beforehand with a tracer solution that consisted of three components: (1) $\sim 10^8$ cells mL^{-1} of *E. coli* JM109 cells transformed with a pETBlue-2 plasmid (Novagen) containing the gene for alcohol dehydrogenase (ADH) from *Drosophila melanogaster*, (2) fluorescein (1000 ppm), and (3) 33% (v/v) glycerol to prevent freezing on the ice at -5°C . The removal of the tracer was monitored after each step of the decontamination procedure (i.e. scraping, ethanol wash, H_2O wash). The fluorescein fluorophore component was monitored by quantifying blue-green fluorescence in 200 μL of rinse-water or meltwater using a BioTurner 20/20n Luminometer (P/N 2030-002) equipped with the blue fluorescence module (BioTurner 2030-041). The pETBlue-2 plasmid component was monitored using a PCR with the following conditions: 1 U of Taq DNA polymerase (5PRIME), $1\times$ MasterTaq buffer, $1\times$ TaqMaster PCR enhancer, 1.5 mM Mg^{2+} , 0.2 μM of each primer (TF7: 5'-TAATACGACTCACTATAGGG -3'; pETBlue-DOWN: 5'-GTAAATTGCTAACGCAGTCA-3'), 200 μM dNTPs, and ~ 100 pg of template DNA. Thirty cycles of PCR were done with a 30 s denaturation step at 94°C , 60 s annealing step at 55°C and extension at 72°C for 60 s, followed by a final extension at 72°C for 10 min. The presence of viable *E. coli* was monitored by spread plating rinse-water and meltwater on agar-solidified LB media containing ampicillin ($100 \mu\text{g mL}^{-1}$) followed by incubation at 37°C . Samples in which any component of the tracer solution was detected in the final meltwater were discarded and reprocessed from a new subsample.

2.3 ATP analysis

Immediately after melting, samples were filtered through 0.22 μm pore size polyethersulfone (Supor; Pall) filters using a bench-top vacuum manifold (≤ 20 kPa). Clean ice samples were filtered through a 25 mm diameter filter while banded, solid, and dispersed ice samples were processed through either a 47 mm or 90 mm diameter filter, depending on total sediment content. The filters were cut into small pieces using a sterile scalpel and extracted on a vortex mixer for 10 seconds with 5 mL of boiling Tris-acetate buffer (TAB; 50mM Tris base adjusted to pH 7.75 with acetic acid). The filters were then incubated in a boiling water bath for 5 minutes and immediately placed on ice for 30 minutes. Extracts were centrifuged for 5 minutes at 4500 $\times$ g to remove debris and the supernatant was collected.

The concentration of ATP was measured in the extracts using a modification of the firefly luciferase-luciferin assay described by (Amato and Christner, 2009). Briefly, 10 μL of extract was added to an equal volume of triethanolamine buffer (TEOA buffer; 200 mM triethanolamine pH 7.6, 2 mM MgCl_2 , 240 mM KCL) and incubated at 37°C for 10 minutes. To correct for adenylate adsorption to and luciferase inhibition by the sediment in the sample extracts, two samples were prepared in parallel for each measurement. Following incubation at 37°C, 10 μL of TAB supplemented with 10 μM ATP was added to one of the samples to serve as an internal standard for each measurement. All samples were kept on ice during preparation but were equilibrated to room temperature (22 °C) prior to the addition of luciferase and measurement of luminescence.

Weighted linear regression was used to generate calibration curves using concentrations of ATP (in TAB) from 10 μM to 1 pM. The luciferase-luciferin cocktail was

prepared fresh for each series of measurements and consisted of 100 U ml⁻¹ luciferase and 0.140 mM D-luciferin. Luminescence was quantified with a 20/20n luminometer (Promega) using auto-injection of 100 µL of the luciferase-luciferin cocktail. Relative luminescence units (RLU) were integrated for three seconds immediately after injection. Sample RLU values were corrected for adsorption and inhibition using the following formula:

$$\frac{1}{\left(\frac{RLU_{\text{sample+std}} - RLU_{\text{sample}}}{RLU_{\text{blank+std}}}\right)} \times RLU_{\text{sample}} = RLU_{\text{corrected}}$$

The corrected RLU values were used together with the standard calibration curve to determine the ATP concentration in the extractant. Final ATP concentrations were corrected for dilution and normalized to the mass of the ice sampled. All values reported are the means of replicate samples (n = 3 to 5).

2.4 Nucleic acid extraction

After decontamination, basal ice samples were placed in a sterilized container, and melted at 4°C. For samples from sediment-rich basal ice (i.e. banded, solid, and dispersed ice), DNA was extracted directly from the sediment recovered after melting using MoBio PowerMax DNA Isolation kits. To increase DNA extraction yields, two 10g (wet weight) extractions were combined onto a single silica spin column for each sample. For samples from sediment-poor basal ice (i.e. clean ice), meltwater was filtered onto 47 mm, 0.22 µm pore size Supor PES filter membranes (Pall) with ≤20 cm Hg of vacuum. DNA was extracted from the filters using MoBio PowerWater DNA Isolation kits as per the manufacturer's instructions with one exception: the mechanical lysis step was performed on a BioSpec

MiniBeadbeater-8 for 2 minutes at maximum speed. All DNA samples were stored at -80°C until PCR amplification.

Due to the low biomass we expected in the basal ice ($\leq 10^4$ cells g⁻¹; (Doyle *et al.*, 2013)), very large samples (~5 kg of ice) were processed to recover enough RNA for reverse transcription PCR (Table S1). To facilitate filtration of such large sample sizes, coarse sediment was removed from sediment-rich basal ice samples after melting using low speed centrifugation (700×g, 10 min, 4°C). The resulting supernatant containing fine clay and silt-like particles was then concentrated onto 90 mm, 0.22 µm pore size Supor PES filter membranes. Meltwater from clean ice samples did not require this centrifugation step and were directly filtered onto 90mm, 0.22 µm pore size Supor PES filters. After filtration, all filters were immediately processed for RNA extraction using a modified phenol-chloroform extraction protocol (Dieser *et al.*, 2014). Briefly, filters were sliced into small pieces using a sterile scalpel, immersed in 3 mL of TE buffer (1 mM EDTA, 10 mM Tris; pH 6.3) containing lysozyme (15 mg mL⁻¹), and vortexed for 30 minutes at room temperature. Samples were then homogenized in a BioSpec Mini-Beadbeater for 2 minutes at maximum speed using ~1 g of sterilized 0.1 mm diameter zirconia/silica beads. After bead beating, crude extracts were amended with two volumes of chilled (4°C) denaturing buffer (4M guanidine thiocyanate, 50 mM Tris, 10 mM EDTA, 1% w/v N-lauroylsarcosine, 1% β-mercaptoethanol), the resulting insoluble material was pelleted via centrifugation (4500×g for 5 min at 4°C), and the supernatant was collected. The pellet was washed with 3 mL of chilled denaturing buffer, centrifuged again, and the resulting supernatant pooled with the first. This pooled lysate was then extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1, pH 6.6), followed by a second extraction with

chloroform:isoamyl alcohol (24:1). Nucleic acids were purified from these extracts via an overnight ethanol precipitation with 0.3 M sodium acetate (pH 6.0). Linear acrylamide (20 $\mu\text{g mL}^{-1}$; Ambion) was used as a coprecipitant to aid recovery. Genomic DNA was eliminated from RNA extracts by digesting for one hour at 37°C with 4 U of TURBO DNAase (Ambion) followed by purification with MEGAclear Transcription Clean-Up kits (Ambion). Extracted DNA and RNA concentrations were measured using Quant-it Picogreen and Ribogreen kits (Life Technologies), respectively, per the manufacturer's instructions.

2.5 16S rRNA amplicon sequencing

From the RNA extracted, complementary DNA (cDNA) was reverse transcribed from ~1 ng of total RNA using SuperScript II reverse transcriptase with the 806R primer (5'-GGACTACVSGGTATCTAAT-3') following the manufacturer's protocol. Negative control reactions lacking RNA template or reverse transcriptase were conducted to monitor for contaminating RNA and DNA molecules, respectively.

The hyper-variable V4 region of the 16S rRNA gene was PCR amplified from the DNA extracts and cDNA libraries using a barcoded 515F-806R primer pair (Caporaso *et al.*, 2012). Each 50 μL PCR contained ~100 pg of DNA or cDNA, 2.5 U of AmpliTaq Gold DNA polymerase LD (Invitrogen), 1 $\times$ Gold Buffer, 2.5 mM MgCl_2 , 0.2 μM of each primer, and 0.2 μM dNTPs. Amplification conditions included at 95°C for 9 min, followed by 30 to 40 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 30 s, and extension at 72°C for 30 s. A final extension was performed at 72°C for 10 min. Following amplification, amplicons were separated by electrophoresis on a 2% agarose gel to assess relative band intensity and size. The amplicons were quantified with Quant-it fluorometry and pooled at equimolar concentrations. Two extraction blanks were included in the pooled libraries to

serve as procedural controls (Salter *et al.*, 2014). Pooled libraries were purified with a MoBio UltraClean PCR Cleanup Kit and sequenced on the Illumina MiSeq platform (v2 chemistry, 2× 250 bp; Georgia Genomics Facility).

2.6 16S rRNA amplicon analysis

Sequence read curation and processing was performed using DADA2 (Callahan *et al.*, 2016) with the following filtering parameters (maxN=0, trunQ=2, rm.phix=TRUE, maxEE=2, R1 truncLen=240, R2 truncLen=200). Error rates for the filtered and trimmed R1 and R2 reads were calculated using the *learnErrors* function and subsequently used to denoise reads using the DADA2 sample inference algorithm. The denoised reads were merged into amplicon sequence variants (ASV) using a global ends-free alignment. Paired reads containing mismatches in the overlapping region were removed from the dataset. Chimeric ASVs were identified and removed by using the consensus method within the *removeBimeraDenovo* function. A consensus taxonomy for each ASV was then assigned using a naïve Bayesian classifier (Wang *et al.*, 2007) trained on release 132 of the SILVA reference database (Quast *et al.*, 2013). The dataset was then subsampled to an even read depth across all samples (59,577 sequences). Any ASV classified as a chloroplast, mitochondria, or from which $\geq 0.1\%$ of its reads were from a procedural blank were removed. Any ASV that was observed exclusively in an RNA library was flagged as a potential contaminant.

2.7 Meta-analyses of microbiomes of permanently frozen ecosystems

Publicly available 16S rRNA amplicon datasets from similar permanently frozen environments were identified using a search of the NCBI Sequence Read Archive and downloaded using the SRA Toolbox *fastq-dump* function. Run tables were aggregated and

studies based on Sanger sequencing were excluded; only datasets based on Illumina, 454, or IonTorrent sequencing were considered in the analysis. Datasets wherein the sequence reads were not demultiplexed before being uploaded to the SRA were also excluded. A list of datasets that met these criteria and were included in the meta-analysis are detailed in Table S2.

Each dataset was individually processed using DADA2 to infer ASV sequences and produce an ASV table. For each study, the filtering parameters were adjusted to account for variation in fragment length and the 16S rRNA variable region sequenced. Due to higher rates of homopolymer and indel errors with 454 and IonTorrent sequencing, datasets using these platforms used a band size parameter of 32 and homopolymer region (≥ 3 repeated bases) gap cost of -1 during the sample inference algorithm. For Illumina datasets, the band size parameter was set to 16 and gaps in homopolymer regions were treated as normal gaps (gap cost of -8). Nine datasets had to be removed from the meta-analysis after these steps due to the loss of too many sequences during quality control steps within the DADA2 pipeline. Complete parameter details and throughput statistics for each dataset in the final meta-analysis are available in Table S3 and S4.

ASV sequences from each study were merged and inserted into the 99% Greengenes reference tree (McDonald *et al.*, 2012) using the SATé-enabled phylogenetic placement (SEPP) technique (Mirarab *et al.*, 2012) within the QIIME2 *fragment-insertion* plugin (Janssen *et al.*, 2018). The merged ASV tables from each study were filtered to remove ASVs rejected from tree insertion by SEPP.

2.8 Data availability

Sequence read data generated in this study is available on NCBI SRA under BioProject PRJNA282540.

3. RESULTS

3.1 Description of Basal Ice Samples

Based on the nomenclature of (Hubbard *et al.*, 2009), we identified three distinct types of basal ice in the basal ice profiles sampled from Taylor Glacier: clean ice, banded ice, and solid ice (Table 1; Figure S3). All three types were collected during the 2007 expedition, while only clean ice and banded ice horizons were targeted during the 2009 expedition. The basal ice recovered from Matanuska Glacier was identified as dispersed ice and was the only type observed at the location of sampling.

Table 1. Description of basal ice types collected in this study

Type	Glacier	Temp (°C)	Description
Clean ice	Taylor	-15	debris-free ice
Banded ice	Taylor	-15	finely stratified layers of debris and ice, layers range in thickness from a few mm to several cm
Solid ice	Taylor	-15	composed primarily of frozen debris with only interstitial ice, no visible layering
Dispersed ice	Matanuska	~0	contains scattered debris aggregated into small clusters, no visible layering

3.2 Nucleic acids in sediment-rich and sediment-poor basal ice

The amount of DNA and RNA extracted from each sample was normalized to sample mass to assess the relative amounts of microbial biomass present within each type of basal ice. Using this approach, we measured approximately 10,000-fold more DNA per gram of basal ice in the sediment-rich basal ice types (i.e. banded, solid, and dispersed) than in the

sediment-poor clean ice (Table 2). Between the sediment-rich basal ice types, the temperate dispersed basal ice from Matanuska Glacier contained approximately 6-fold more DNA per gram of ice than the banded and solid basal ices from the cold-based (-15°C) Taylor Glacier. RNA concentrations were very low in all basal ice types, with the highest concentration—though only marginally—observed in Matanuska’s dispersed basal ice.

Table 2. Cell counts, biomass estimates, and yield of DNA and RNA extracted from basal ice samples. Sediment-rich basal ice facies are highlighted in gray.

Sample	Cell Counts*	Yield (pg g ⁻¹ ice)		Biomass Estimates	
	cells g ⁻¹ ice (×10 ³)	DNA	RNA	estimated genomes† g ⁻¹ ice (×10 ³)	ATP (pg g ⁻¹ ice)
Clean07	0.3 - 0.5	4.7	0.7	1.9	<0.01 - 0.04
Clean09	-	1.4	-	0.6	-
Banded07	1.8 - 18.2	16994.3	2.3	6797.7	-
Banded09	-	16140.1	0.8	6456.0	0.24 - 2.8
Solid	6.7	14366.2	1.2	5746.5	<0.01
Dispersed	-	98860.2	3.2	39544.1	0.06 - 0.14

*Doyle et al. 2014. †Estimated number of microbial genomes estimated using 2.5 fg of DNA per genome

3.3 Composition of microbial assemblages in different types of basal ice

A total of 4,227,678 paired-end reads with an average read length of 253bp were obtained from MiSeq sequencing. After filtration and denoising, 2,883,221 non-chimeric sequences representing 3310 ASVs remained. Subsampling and culling of ASVs classified as plastids or flagged as potential contaminants produced a final, curated dataset composed of 2021 ASVs. Based on average-neighbor clustering of Bray-Curtis distances, the basal ice samples clustered into three significantly different (AMOVA; F[2,3]=5.23, p=0.017) groups: (1) Taylor Glacier sediment-poor basal ice, (2) Taylor Glacier sediment-rich basal ice, and (3) Matanuska Glacier dispersed ice. The sediment-poor clean ice samples contained

microbial assemblages overwhelmingly composed of *Firmicutes* (68%) and *Proteobacteria* (25%), with smaller proportions of *Bacteroidetes* (5%) and *Actinobacteria* (1%). The genus *Tumebacillus* was especially abundant in the clean ice samples (Figure 1A). Assemblages within the banded and solid ice samples were comparatively more diverse than those found in clean ice (Figure 2), with a wider range of abundant phyla represented: *Actinobacteria* (26%), *Firmicutes* (23%), *Bacteroidetes* (19%), *Proteobacteria* (11%), *Atribacteria* (9%), *Tenericutes* (7%), and *Planctomycetes* (1%). Notably, ASVs related to the class *Clostridia* composed about 20% of the communities in these samples. Lastly, the microbial assemblage within Matanuska's dispersed basal ice was distinct from those observed from Taylor Glacier. Here we observed significant amounts of *Nitrospirae* (39%) and *Betaproteobacteria* (30%), of which several of the most abundant ASVs were related to sulfur and/or iron cycling members of the genera *Thermodesulfovibrio*, *Rhodoferrax*, and *Thiobacillus*.

3.4 Assessing metabolic status of bacterial taxa within different types of basal ice

With the RNA libraries, we identified 141 potentially metabolically active ASVs (7% of total) taxa within the basal ice. Of these ASVs, 80 had RNA/DNA abundance log-ratios >1 (Table S5) and thus we focused on these as having the highest potential for metabolic activity. The vast majority (63 ASVs) of these taxa were observed in the dispersed ice from Matanuska Glacier (Figure 1B), the warmest and youngest basal ice sampled in this study. Here, many of these ASVs were members of the *Chloroflexi* or *Deltaproteobacteria*, with some of the largest RNA/DNA abundance log-ratios belonging to *Desulfocapsa*, *Syntrophus*, and unassigned members of the family *Anaerolineaceae*. Within the banded basal ice from Taylor Glacier, over half of potentially active ASVs instead belonged to the *Firmicutes*.

Among this phylum, members of the *Paenisporosarcina*, *Paenibacillaceae*, *Desulfosporosinus*, *Caldicoprobacter*, and *Virgibacillus* had by far the highest activity potential and represented >90% of the RNA reads in these samples. In comparison, the clean and solid basal ice had the least number of potentially active ASVs—10 altogether comprising only 472 RNA reads in total, only 2 of which had RNA/DNA abundance log-ratios >1—suggesting very little to no metabolic activity.

3.5 Meta-Analysis: Microbial populations of various permanently frozen environments

For the meta-analysis, we analyzed 33.6 million 16S rRNA sequences across 146 individual samples from 15 publicly available datasets that represented five different types of permanently frozen environments: (1) supraglacial ice, (2) englacial ice, (3) basal ice, (4) perennial cave ice, and (6) permafrost. After filtering and curation, the final meta-analysis dataset contained 17.5 million non-chimeric sequences representing 14,114 unique ASVs.

We calculated Shannon Diversity indices to compare microbial alpha diversity between these frozen environments. Of the basal ice assemblages characterized in this study, alpha diversity was higher in sediment-rich basal ice types than in the sediment-poor clean ice ($t(4)=3.34$, $p=0.03$) (Figure 2). Compared to the other frozen environments in this meta-analysis, alpha diversity within our sediment-rich basal ice samples from Antarctica were most similar to that observed in permafrost sampled from the Storflaket peat bog in northern Sweden (Monteux *et al.*, 2018). Conversely, alpha diversity within Taylor Glacier's clean ice was most similar to that found in englacial ice samples from the GISP2D, NEEM, and WAIS Divide ice cores (Buford Price *et al.*, 2015).

To visualize microbial β -diversity between samples, we used a hierarchical cluster analysis of weighted UniFrac distances (wUF). Microbial communities largely clustered by the type of permanently frozen environment they inhabited, though some environments displayed larger variation in community structures than others. For example, communities inhabiting englacial ice were the most similar (wUF: 0.47 ± 0.15 ; mean $\pm$ s.d). In contrast, perennial cave ice samples from a single location (Itcus *et al.*, 2018) harbored the widest range of microbial communities (wUF: 0.81 ± 0.14). β -diversity of permafrost microbiomes were more intermediate: relatively tight clustering was observed in permafrost from the Storflaket peat bog (northern Sweden) with wider variation in samples of permafrost from University Valley (Antarctica) (Goordial *et al.*, 2016; Monteux *et al.*, 2018). The microbial communities within the Taylor Glacier's banded and solid basal ice facies clustered closely with the Sweden permafrost samples, while those within the clean basal ice and Matanuska's dispersed basal ice were most similar to those observed in englacial ice from the NEEM ice core in Greenland (Miteva *et al.*, 2015, 2016)(Figure 3).

We used an RDA model to determine how microbial lineages were distributed across the different types of frozen environments (Figure 4, left). The final RDA model explained 24.9% of the variability between samples and effectively separated the samples into three clusters, like that seen in Figure 3. Of the 89 microbial lineages included in this analysis, we identified 14 had loading vectors greater than the equilibrium contribution—the proportion of variance which would be explained by a random constrained axis—indicating they were differentially abundant across the five types of frozen environments.

Scalar projections of these lineages onto the centroid factor of each type of frozen environment allowed us to estimate how strongly these lineages were associated with each

environment (Figure 4, right). As one would expect, supraglacial ice environments harbored comparatively higher proportions of *Cyanobacteria* and plastid-related taxa than the other frozen environments. Englacial ice environments were instead enriched with members of the *Pseudomonas*, *Janthinobacterium*, *Methylobacterium*, and *Oxalobacteraceae*. In contrast, basal ice, permafrost, and perennial cave ice environments all contained higher populations *Firmicutes* related lineages: *Clostridium*, *Ruminococcaceae*, *Desulfosporosinus*, and *Paenibacillus*.

4. DISCUSSION

4.1 Microbial communities vary in size and composition across basal ice types

Of the four different types of basal ice analyzed in this study, we found that those containing large quantities of sediment or debris contained more extractable nucleic acids, consistent with higher microbial abundances than those containing little or no subglacial sediments. This is consistent with previous microscopic investigations of the GISP2 ice core that found higher cell concentrations in deeper sections of the core where clay particles were embedded in the ice (Tung *et al.*, 2006). In addition to higher numbers of cells and biomass, microbial diversity was also notably higher in the sediment-rich basal ice facies relative to clean basal ice or englacial ice, indicating that subglacial debris is the primary source of microorganisms in the basal ice.

Our estimates of bacterial cell abundance based on extracted DNA yields ($\sim 10^6$ cells g^{-1} ice) were approximately 100-fold higher than those based on direct microscopic counts ($\sim 10^4$ cells g^{-1} ice), but only in the sediment-rich banded and solid basal ice. In the clean

basal ice, these estimates were comparable. This suggests that Taylor Glacier's debris-rich basal ice may contain significant amounts of microbial necromass.

We found microbial assemblages within basal ices of the cold-based (-15°C) Taylor Glacier contain high abundances of *Firmicutes* and *Actinobacteria* relative to the other frozen habitats examined. Bacteria within these two phyla have been commonly observed as the dominant taxa in a wide-range of frozen environments such as ground ice, permafrost, polar saline springs, cryopegs, and sea ice (Kochkina *et al.*, 2001; Steven *et al.*, 2007, 2008; Perreault *et al.*, 2008; Lacelle *et al.*, 2011; Boetius *et al.*, 2015; Spirina *et al.*, 2017). One explanation for these observations is that the physiochemical stresses faced by cells under frozen conditions (Doyle *et al.*, 2012) favor the prevalence of microorganisms that can form highly resistant endospores (*Firmicutes*) or spores (*Actinobacteria*). Although this is consistent with the notion that extreme environmental conditions select for microbial lineages with robust survival strategies (Filippidou *et al.*, 2016), multiple lines of evidence indicate this hypothesis does not adequately explain the abundance of these taxa in our basal ice samples. First, ASVs related to known endospore-forming genera such as *Paenisporosarcina*, *Virgibacillus*, *Clostridium*, and *Desulfosporosinus* were abundant in all our RNA libraries. Because the rRNA content of endospores decreases relatively quickly after sporulation—on the order of days to weeks (Segev *et al.*, 2012; Korza *et al.*, 2016)—their detection in ≥10,000 year-old basal ice strongly implies these taxa existed as vegetative cells within the ice. Second, we found cultured isolates of *Paenisporosarcina* sp. recovered directly from Taylor Glacier banded ice would not sporulate after being refrozen in basal meltwater at in situ temperatures (-15°C), but instead continuously incorporate radiolabeled substrates into macromolecules (Doyle *et al.*, 2013). Third, these isolates were

heat-killed if the banded basal ice meltwater was pasteurized prior to cultivation experiments, indicating we were not recovering these isolates from ancient endospores. Finally, these findings are consistent with recent a study that performed 16S rRNA gene sequencing of ancient permafrost samples after endospore enrichment and selective depletion of DNA from dead cells and found many bacteria capable of forming endospores, especially members of the *Clostridia*, were in a nondormant state (Burkert *et al.*, 2019). Collectively, these results suggest the high abundances of *Firmicutes* and *Actinobacteria* in Taylor Glacier's basal ice is because they had the ability to remain active while frozen as opposed to simply enduring in a durable, yet dormant state.

4.2 Young and warm versus old and cold basal ice

Due to the chemical lability of RNA, paired RNA/DNA 16S rRNA amplicon libraries are a useful tool for discerning live organisms in a microbial community from those that are long-dead or dormant. One should note there are limitations to this analysis and these ratios are more reliable indicators of potential activity rather than real-time activity (Blazewicz *et al.*, 2013).

On average, only about 2% of the total ASVs in each sample were also detected in the paired RNA library. This low proportion of potentially active ASVs suggests only a small fraction of the microorganisms entrained into basal ice are suited to remain metabolically active inside ice. Despite this small fraction, a pattern does emerge: the dispersed basal ice from Matanuska Glacier contained a much larger population of potentially active ASVs than any of the basal ice cryofacies recovered from Taylor Glacier.

One plausible explanation for this observation is the substantial difference in geology and age of basal ice between these two glaciers. Assuming a non-zero rate of

microbial attrition over time in ice due to, for example, the accumulation of cellular damage or the depletion of resources, older ice would be expected to harbor smaller populations of potentially active microorganisms than younger ice. Analysis of the δD and $\delta^{18}O$ composition of meltwater from Taylor Glacier surface ice samples estimated they are between 11,500 and 65,000 years old (Aciego *et al.*, 2007), while more recent radiometric ^{81}Kr dating efforts near the glacial terminus expanded this estimate to nearly 123,500 years old (Buizert *et al.*, 2014). In comparison, measurements of oxygen isotopes in Matanuska Glacier's dispersed basal ice layer indicated the basal ice originated in the accumulation area (Lawson and Kulla, 1978). Based on the velocity of Matanuska Glacier ($\sim 110 \text{ m yr}^{-1}$), this would represent an approximate time of 250 years between deposition and terminal ablation. Trace amounts of anthropogenic tritium produced by atmospheric thermonuclear weapon tests in the 1950s and 1960s have also been detected in Matanuska Glacier's basal ice zone (Strasser *et al.*, 1996), indicating portions of this ice were formed even more recently by subglacial freeze-on of surface-derived meltwater (Lawson, 1979). Taken together, this indicates the dispersed ice we sampled from Matanuska is substantially younger than any of the basal ice types we collected from Taylor Glacier.

In addition to ice age, the large difference in ice temperature between a temperate and cold-based glacier should also affect the potential for metabolic activity in basal ice environments. Taylor Glacier is primarily a cold-based glacier with a measured basal ice temperature of $-15^{\circ}C$ (Montross *et al.*, 2014). In contrast, Matanuska Glacier is a temperate glacier with a basal ice zone temperature just below $0^{\circ}C$ (Lawson *et al.*, 1998). As such, cells frozen in Taylor Glacier's basal ice are under considerably higher physiochemical stress than ice near the melting point. For example, the predicted ionic strength of the

briny liquid at ice crystal boundaries—where the microorganisms also exist— is nearly 4.6M at -15°C. Comparatively, this is 11.5-fold higher than the unfrozen water in ice at -1°C (Doyle *et al.*, 2012). Hence, the wider diversity and increased number of ASVs with a high potential for metabolic activity in the dispersed basal ice may reflect the more favorable conditions for metabolism in temperate versus cold-based glacial ice.

4.3 Debris-rich basal ice types are hot spots for microbial activity

Previous analyses of the gasses entrapped within Taylor Glacier banded basal ice found that that O₂ was depleted to as low as 4% in horizons where CO₂ concentrations were concurrently enriched as high as 20,000-fold relative to atmospheric concentrations (Montross *et al.*, 2014). Isotopic analyses of $\delta^{13}\text{C}$ -CO₂ revealed this CO₂ is isotopically depleted (-24‰), and therefore, was likely produced by *in situ* microbial respiration of organic matter (Montross, 2012). This data supports our RNA-based 16S rRNA analyses, which found the largest read counts and number of potentially active ASVs in the same banded basal ice samples. Together, these observations strongly suggest Taylor Glacier's banded basal ice horizons harbor metabolically active microbiomes that are altering the ice gas chemistry at temperature of -15°C.

We also observed an enrichment of anaerobic taxa (e.g. *Clostridia*) within the same banded basal ice samples where O₂ concentrations were depleted. This raises the intriguing possibility that, given sufficient time, the microbiomes of debris-rich basal ice may shift to members with anoxic and anaerobic metabolisms (e.g. methanogenesis). In support of this, multiple investigations have found large excesses of CH₄ in debris-rich basal ice samples from Greenland and Antarctica (Tung *et al.*, 2006; Stibal *et al.*, 2012; Wadham *et al.*, 2012; Rhodes *et al.*, 2013; Lamarche-Gagnon *et al.*, 2019; Lee *et al.*, 2020). This hypothesis is also

supported by a recent laboratory experiment that suggests cryoconite hole meltwater will become enriched with anaerobic taxa after it is transported to a subglacial environment (Zdanowski *et al.*, 2017). However, it should be noted that this study was not performed in ice.

Previous chemical analysis of Taylor Glacier's basal ice revealed dissolved organic carbon (DOC) concentrations are much higher in the debris-rich basal ice types ($\sim 63 \text{ mg L}^{-1}$) than in the debris-poor types ($\sim 0.8 \text{ mg L}^{-1}$) (Montross, 2012; Montross *et al.*, 2014). This is also consistent with our microbiological results. Increased microbial activity in debris-rich basal ice horizons may simply be a consequence of there being more substrates available to consume. However, our investigation of multiple basal ice types indicates there are likely additional, unidentified factors besides sediment content controlling whether basal ice harbors an active microbiome. For example, although the banded and solid basal ice samples harbored very similar microbial communities in our DNA-based libraries, we found very little evidence for active ASVs within the solid basal ice. This suggests—at least in polar glaciers containing basal ice horizons well below 0°C —the presence of debris and/or DOC alone is not sufficient to support a metabolically active microbiome.

4.4. Phylogenetically similar communities in debris-rich basal ice and permafrost

Our meta-analysis indicates that that microbial communities within the banded and solid basal ice from Taylor Glacier were phylogenetically most like those in permafrost and were more distantly related to those in englacial ices and supraglacial ecosystems. This suggests the ecophysiological stresses experienced by cells frozen in permafrost and basal ice of a cold-based glacier are comparable and select for similar taxa. Both subsurface environments contain high mineral debris and may remain frozen for many thousands of

years. In fact, there is debate on whether the physical differences between cold, debris-rich basal ice and permafrost are only terminological (Dobinski, 2006; Dąbski, 2019). Our results support this contention, adding a microbiological dimension that provides additional evidence for the parallels between these cryospheric environments. New data from other cold-based glaciers and regions are needed to test if these similarities in microbial composition with permafrost are robust across multiple locales. If so, permafrost environments may be a useful analog for gaining valuable insight into the microbiology of unexplored basal ice environments which are logistically more difficult to access.

4.5 Meta-analysis limitations

Comparing 16S amplicon datasets from different studies is often hindered by inconsistencies in sequencing platform used and which variable region of the 16S rRNA gene was amplified. Using SEPP to insert heterogeneous ASVs from different studies into the same reference tree creates stable phylogenetic placements, enabling integration and meta-analysis of amplicon data from multiple variable regions of the 16S rRNA gene (Janssen *et al.*, 2018). Despite this, it is important to note the limitations of this approach. First, integrating previously published 16S rRNA amplicon datasets based on Sanger sequencing of clone libraries or DGGE profiling remains difficult as these older approaches lack the read depth or taxonomic resolution to make meaningful comparisons with those datasets prepared with more modern approaches. As a result, our meta-analysis unfortunately excluded many early works which investigated the microbial ecology of glacial ice using these approaches. Second, there are some potential biases in our meta-analysis due to low coverage: despite growing interest in the microbial ecology of the cryosphere, high-throughput surveys of permanently frozen ecosystems are still

uncommon. For example, the Matanuska dispersed basal ice most resembled englacial ice samples in our meta-analysis. However, many of the englacial ice samples in the meta-analysis are also from Matanuska Glacier. Indeed, there are exceedingly few microbiome analyses of true englacial ice samples unaffected by either supraglacial or subglacial processes, largely due to both the logistical difficulty of accessing and sampling these environments.

4.6 Conclusions and Implications

Our findings show that certain microbes do not persist in a state of dormancy while frozen and, coupled with other biogeochemical data (Doyle *et al.*, 2013; Montross *et al.*, 2014), indicate that some sediment-rich basal ice horizons host active microbial communities that participate in biogeochemical cycling. Although we have limited observations, our data further implies that a larger diversity of bacteria may remain metabolically active in the relatively warm basal ice found beneath temperate glaciers and ice sheet interiors (Lawson *et al.*, 1998; Bell *et al.*, 2011). Indeed, since the basal temperature of the Antarctic and Greenland ice sheets are near or at the pressure-melting point (Gow *et al.*, 1968), this raises the intriguing possibility that these regions of the cryosphere are more biogeochemically active than previously thought. We also found that microorganisms in the basal ice of cold-based glaciers may be important biogeochemical actors as well. Although rates of microbial activity in these environments are certainly slower than those of warmer environments (Christner, 2002; Panikov *et al.*, 2006; Amato *et al.*, 2009, 2010; Doyle *et al.*, 2013), this may be offset by the long residence time of most basal ice environments, providing ample time for biogeochemical processing. As climate change continues to shrink the cryosphere and warm more basal ice environments, this

may be an important consideration for high latitude land- and marine-based coastal environments that receive regular discharges of meltwater derived from ancient basal ice horizons, an important source of dissolved organic carbon, nutrients, and trace metals (Rignot and Jacobs, 2002; Jung *et al.*, 2019; Vick-Majors *et al.*, 2020).

5. ACKNOWLEDGEMENTS

This study was funded by a Division of Polar Programs grant (ANT-0636828) from the National Science Foundation.

6. REFERENCES

- Aciego, S.M., Cuffey, K.M., Kavanaugh, J.L., Morse, D.L., and Severinghaus, J.P. (2007) Pleistocene ice and paleo-strain rates at Taylor Glacier, Antarctica. *Quaternary Research* **68**: 303–313.
- Amato, P. and Christner, B.C. (2009) Energy Metabolism Response to Low-Temperature and Frozen Conditions in Psychrobacter cryohalolentis. *Applied and Environmental Microbiology* **75**: 711–718.
- Amato, P., Doyle, S., and Christner, B.C. (2009) Macromolecular synthesis by yeasts under frozen conditions. *Environmental Microbiology* **11**: 589–596.
- Amato, P., Doyle, S.M., Battista, J.R., and Christner, B.C. (2010) Implications of Subzero Metabolic Activity on Long-Term Microbial Survival in Terrestrial and Extraterrestrial Permafrost. *Astrobiology* **10**: 789–798.
- Barker, J.D., Grottoli, A.G., and Lyons, W.B. (2018) Stable isotope evidence for the biogeochemical transformation of ancient organic matter beneath Suess Glacier, Antarctica. *Arctic, Antarctic, and Alpine Research* **50**: e1448643.
- Batjes, N.H. (2014) Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* **65**: 10–21.
- Bell, R.E., Ferraccioli, F., Creyts, T.T., Braaten, D., Corr, H., Das, I., et al. (2011) Widespread Persistent Thickening of the East Antarctic Ice Sheet by Freezing from the Base. *Science* **331**: 1592–1595.
- Bhatia, M.P., Das, S.B., Xu, L., Charette, M.A., Wadham, J.L., and Kujawinski, E.B. (2013) Organic carbon export from the Greenland ice sheet. *Geochimica et Cosmochimica Acta* **109**: 329–344.
- Blazewicz, S.J., Barnard, R.L., Daly, R.A., and Firestone, M.K. (2013) Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. *The ISME Journal* **7**: 2061–2068.

- Boetius, A., Anesio, A.M., Deming, J.W., Mikucki, J.A., and Rapp, J.Z. (2015) Microbial ecology of the cryosphere: sea ice and glacial habitats. *Nature Reviews Microbiology* **13**: 677–690.
- Boyd, E.S., Skidmore, M., Mitchell, A.C., Bakermans, C., and Peters, J.W. (2010) Methanogenesis in subglacial sediments: Subglacial methanogenesis. *Environmental Microbiology Reports* **2**: 685–692.
- Buford Price, P., Jeffrey Morris, J., Bay, R.C., Adhikari, A., Giovannoni, S.J., and Vergin, K.L. (2015) Molecular Fossils from Microorganisms Preserved in Glacial Ice. *bioRxiv*.
- Buizert, C., Baggenstos, D., Jiang, W., Purtschert, R., Petrenko, V.V., Lu, Z.-T., et al. (2014) Radiometric ⁸¹Kr dating identifies 120,000-year-old ice at Taylor Glacier, Antarctica. *Proceedings of the National Academy of Sciences* **111**: 6876–6881.
- Burkert, A., Douglas, T.A., Waldrop, M.P., and Mackelprang, R. (2019) Changes in the Active, Dead, and Dormant Microbial Community Structure across a Pleistocene Permafrost Chronosequence. *Appl Environ Microbiol* **85**: e02646-18.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., et al. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME journal* **6**: 1621–1624.
- Christner, B.C. (2002) Incorporation of DNA and Protein Precursors into Macromolecules by Bacteria at -15°C. *Applied and Environmental Microbiology* **68**: 6435–6438.
- Christner, B.C., Mikucki, J.A., Foreman, C.M., Denson, J., and Priscu, J.C. (2005) Glacial ice cores: A model system for developing extraterrestrial decontamination protocols. *Icarus* **174**: 572–584.
- Dąbski, M. (2019) Should Glaciers Be Considered Permafrost? *Geosciences* **9**: 517.
- Dieser, M., Broemsen, E.L.J.E., Cameron, K.A., King, G.M., Achberger, A., Choquette, K., et al. (2014) Molecular and biogeochemical evidence for methane cycling beneath the western margin of the Greenland Ice Sheet. *The ISME Journal* **8**: 2305–2316.
- Dobinski, W. (2006) Ice and environment: A terminological discussion. *Earth-Science Reviews* **79**: 229–240.
- Doyle, S., Dieser, M., Broemsen, E., and Christner, B. (2012) General characteristics of cold-adapted microorganisms. In *Polar Microbiology: Life in a Deep Freeze*. American Society of Microbiology, pp. 103–125.
- Doyle, S., Montross, S., Skidmore, M., and Christner, B. (2013) Characterizing Microbial Diversity and the Potential for Metabolic Function at -15 °C in the Basal Ice of Taylor Glacier, Antarctica. *Biology* **2**: 1034–1053.
- Filippidou, S., Wunderlin, T., Junier, T., Jeanneret, N., Dorador, C., Molina, V., et al. (2016) A Combination of Extreme Environmental Conditions Favor the Prevalence of Endospore-Forming Firmicutes. *Front Microbiol* **7**.
- Goordial, J., Davila, A., Lacelle, D., Pollard, W., Marinova, M.M., Greer, C.W., et al. (2016) Nearing the cold-arid limits of microbial life in permafrost of an upper dry valley, Antarctica. *The ISME Journal* **10**: 1613–1624.
- Gow, A.J., Ueda, H.T., and Garfield, D.E. (1968) Antarctic Ice Sheet: Preliminary Results of First Core Hole to Bedrock. *Science* **161**: 1011–1013.

- Hood, E., Fellman, J., Spencer, R.G.M., Hernes, P.J., Edwards, R., D'Amore, D., and Scott, D. (2009) Glaciers as a source of ancient and labile organic matter to the marine environment. *Nature* **462**: 1044–1047.
- Hopwood, M.J., Carroll, D., Dunse, T., Hodson, A., Holding, J.M., Iriarte, J.L., et al. (2019) Review Article: How does glacier discharge affect marine biogeochemistry and primary production in the Arctic? *The Cryosphere Discussions* 1–51.
- Hubbard, B., Cook, S., and Coulson, H. (2009) Basal ice facies: a review and unifying approach. *Quaternary Science Reviews* **28**: 1956–1969.
- Itcus, C., Pascu, M.D., Lavin, P., Perşoiu, A., Iancu, L., and Purcarea, C. (2018) Bacterial and archaeal community structures in perennial cave ice. *Sci Rep* **8**.
- Janssen, S., McDonald, D., Gonzalez, A., Navas-Molina, J.A., Jiang, L., Xu, Z.Z., et al. (2018) Phylogenetic Placement of Exact Amplicon Sequences Improves Associations with Clinical Information. *mSystems* **3**: e00021-18.
- Jung, J., Yoo, K.-C., Rosenheim, B.E., Conway, T.M., Lee, J.I., Yoon, H.I., et al. (2019) Microbial Fe(III) reduction as a potential iron source from Holocene sediments beneath Larsen Ice Shelf. *Nat Commun* **10**: 1–10.
- Knight, P.G. (1997) The basal ice layer of glaciers and ice sheets. *Quaternary Science Reviews* **16**: 975–993.
- Kochkina, G., Ivanushkina, N., Karasev, S., Gavrish, E.Y., Gurina, L., Evtushenko, L., et al. (2001) Survival of micromycetes and actinobacteria under conditions of long-term natural cryopreservation. *Microbiology* **70**: 356–364.
- Korza, G., Setlow, B., Rao, L., Li, Q., and Setlow, P. (2016) Changes in Bacillus Spore Small Molecules, rRNA, Germination, and Outgrowth after Extended Sublethal Exposure to Various Temperatures: Evidence that Protein Synthesis Is Not Essential for Spore Germination. *J Bacteriol* **198**: 3254–3264.
- Lacelle, D., Radtke, K., Clark, I.D., Fisher, D., Lauriol, B., Utting, N., and Whyte, L.G. (2011) Geomicrobiology and occluded O₂–CO₂–Ar gas analyses provide evidence of microbial respiration in ancient terrestrial ground ice. *Earth and Planetary Science Letters* **306**: 46–54.
- Lamarche-Gagnon, G., Wadham, J.L., Sherwood Lollar, B., Arndt, S., Fietzek, P., Beaton, A.D., et al. (2019) Greenland melt drives continuous export of methane from the ice-sheet bed. *Nature* **565**: 73–77.
- Lawson, D. (1979) Sedimentological Analysis of the Western Terminus Region of the Matanuska Glacier, Alaska. 132.
- Lawson, D.E. and Kulla, J.B. (1978) An Oxygen Isotope Investigation of the Origin of the Basal Zone of the Matanuska Glacier, Alaska. *The Journal of Geology* **86**: 673–685.
- Lawson, D.E., Strasser, J.C., Evenson, E.B., Alley, R.B., Larson, G.J., and Arcone, S.A. (1998) Glaciohydraulic supercooling: a freeze-on mechanism to create stratified, debris-rich basal ice: I. Field evidence. *Journal of Glaciology* **44**: 547–562.
- Lee, J.E., Edwards, J.S., Schmitt, J., Fischer, H., Bock, M., and Brook, E.J. (2020) Excess methane in Greenland ice cores associated with high dust concentrations. *Geochimica et Cosmochimica Acta* **270**: 409–430.
- McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., et al. (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME journal* **6**: 610.

- Michaud, A.B., Dore, J.E., Achberger, A.M., Christner, B.C., Mitchell, A.C., Skidmore, M.L., et al. (2017) Microbial oxidation as a methane sink beneath the West Antarctic Ice Sheet. *Nature Geoscience* **10**: 582–586.
- Mirarab, S., Nguyen, N., and Warnow, T. (2012) SEPP: SATé-enabled phylogenetic placement. In *Biocomputing 2012*. World Scientific, pp. 247–258.
- Miteva, V., Rinehold, K., Sowers, T., Sebastian, A., and Brenchley, J. (2015) Abundance, viability and diversity of the indigenous microbial populations at different depths of the NEEM Greenland ice core. *Polar Research* **34**: 25057.
- Miteva, V., Sowers, T., Schüpbach, S., Fischer, H., and Brenchley, J. (2016) Geochemical and Microbiological Studies of Nitrous Oxide Variations within the New NEEM Greenland Ice Core during the Last Glacial Period. *Geomicrobiology Journal* **33**: 647–660.
- Miteva, V.I., Sheridan, P.P., and Brenchley, J.E. (2004) Phylogenetic and Physiological Diversity of Microorganisms Isolated from a Deep Greenland Glacier Ice Core. *Applied and Environmental Microbiology* **70**: 202–213.
- Monteux, S., Weedon, J.T., Blume-Werry, G., Gavazov, K., Jassey, V.E.J., Johansson, M., et al. (2018) Long-term in situ permafrost thaw effects on bacterial communities and potential aerobic respiration. *The ISME Journal* **12**: 2129–2141.
- Montross, S., Skidmore, M., Christner, B., Samyn, D., Tison, J.-L., Lorrain, R., et al. (2014) Debris-Rich Basal Ice as a Microbial Habitat, Taylor Glacier, Antarctica. *Geomicrobiology Journal* **31**: 76–81.
- Montross, S.N. (2012) Biogeochemistry of basal ice from Taylor Glacier, Antarctica, Montana State University.
- Montross, S.N., Skidmore, M., Tranter, M., Kivimäki, A.-L., and Parkes, R.J. (2013) A microbial driver of chemical weathering in glaciated systems. *Geology* **41**: 215–218.
- Panikov, N.S., Flanagan, P.W., Oechel, W.C., Mastepanov, M.A., and Christensen, T.R. (2006) Microbial activity in soils frozen to below –39°C. *Soil Biology and Biochemistry* **38**: 785–794.
- Perreault, N.N., Greer, C.W., Andersen, D.T., Tille, S., Lacrampe-Couloume, G., Lollar, B.S., and Whyte, L.G. (2008) Heterotrophic and Autotrophic Microbial Populations in Cold Perennial Springs of the High Arctic. *Appl Environ Microbiol* **74**: 6898–6907.
- Priscu, J.C., Tulaczyk, S., Studinger, M., Kennicutt, M., Christner, B.C., and Foreman, C.M. (2008) Antarctic subglacial water: origin, evolution and ecology. *Polar lakes and rivers: limnology of Arctic and Antarctic aquatic ecosystems Oxford University Press, Oxford* 119–135.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**: D590–D596.
- Rhodes, R.H., Fain, X., Stowasser, C., Blunier, T., Chappellaz, J., McConnell, J.R., et al. (2013) Continuous methane measurements from a late Holocene Greenland ice core: Atmospheric and in-situ signals. *Earth and Planetary Science Letters* **368**: 9–19.
- Rignot, E. and Jacobs, S.S. (2002) Rapid Bottom Melting Widespread near Antarctic Ice Sheet Grounding Lines. *Science* **296**: 2020–2023.
- Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., et al. (2014) Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC biology* **12**: 87.

- Segev, E., Smith, Y., and Ben-Yehuda, S. (2012) RNA Dynamics in Aging Bacterial Spores. *Cell* **148**: 139–149.
- Sharp, M., Parkes, J., Cragg, B., Fairchild, I.J., Lamb, H., and Tranter, M. (1999) Widespread bacterial populations at glacier beds and their relationship to rock weathering and carbon cycling. *Geology* **27**: 107.
- Sheridan, P.P., Miteva, V.I., and Brenchley, J.E. (2003) Phylogenetic Analysis of Anaerobic Psychrophilic Enrichment Cultures Obtained from a Greenland Glacier Ice Core. *Applied and Environmental Microbiology* **69**: 2153–2160.
- Skidmore, M.L., Foght, J.M., and Sharp, M.J. (2000) Microbial Life beneath a High Arctic Glacier. *Appl Environ Microbiol* **66**: 3214–3220.
- Spirina, E., Durdenko, E., Demidov, N., Abramov, A., Romanovsky, V., and Rivkina, E. (2017) Halophilic-psychrotrophic bacteria of an Alaskan cryopeg—a model for astrobiology. *Paleontological Journal* **51**: 1440–1452.
- Steven, B., Briggs, G., McKay, C.P., Pollard, W.H., Greer, C.W., and Whyte, L.G. (2007) Characterization of the microbial diversity in a permafrost sample from the Canadian high Arctic using culture-dependent and culture-independent methods: Microbial diversity in Canadian high Arctic permafrost. *FEMS Microbiology Ecology* **59**: 513–523.
- Steven, B., Pollard, W.H., Greer, C.W., and Whyte, L.G. (2008) Microbial diversity and activity through a permafrost/ground ice core profile from the Canadian high Arctic. *Environmental Microbiology* **10**: 3388–3403.
- Stibal, M., Wadham, J.L., Lis, G.P., Telling, J., Pancost, R.D., Dubnick, A., et al. (2012) Methanogenic potential of Arctic and Antarctic subglacial environments with contrasting organic carbon sources. *Global Change Biology* **18**: 3332–3345.
- Strasser, J.C., Lawson, D.E., Larson, G.J., Evenson, E.B., and Alley, R.B. (1996) Preliminary results of tritium analyses in basal ice, Matanuska Glacier, Alaska, U.S.A.: evidence for subglacial ice accretion. *Annals of Glaciology* **22**: 126–133.
- Tranter, M., Sharp, M.J., Lamb, H.R., Brown, G.H., Hubbard, B.P., and Willis, I.C. (2002) Geochemical weathering at the bed of Haut Glacier d’Arolla, Switzerland? a new model. *Hydrological Processes* **16**: 959–993.
- Tranter, M., Skidmore, M., and Wadham, J. (2005) Hydrological controls on microbial communities in subglacial environments. *Hydrological Processes* **19**: 995–998.
- Tung, H. c., Price, P. b., Bramall, N. e., and Vrdoljak, G. (2006) Microorganisms Metabolizing on Clay Grains in 3-Km-Deep Greenland Basal Ice. *Astrobiology* **6**: 69–86.
- Vick-Majors, T.J., Michaud, A.B., Skidmore, M.L., Turetta, C., Barbante, C., Christner, B.C., et al. (2020) Biogeochemical Connectivity Between Freshwater Ecosystems beneath the West Antarctic Ice Sheet and the Sub-Ice Marine Environment. *Global Biogeochemical Cycles* **34**: e2019GB006446.
- Wadham, J.L., Arndt, S., Tulaczyk, S., Stibal, M., Tranter, M., Telling, J., et al. (2012) Potential methane reservoirs beneath Antarctica. *Nature* **488**: 633–637.
- Wadham, J.L., Bottrell, S., Tranter, M., and Raiswell, R. (2004) Stable isotope evidence for microbial sulphate reduction at the bed of a polythermal high Arctic glacier. *Earth and Planetary Science Letters* **219**: 341–355.
- Wadham, J.L., Tranter, M., Skidmore, M., Hodson, A.J., Priscu, J., Lyons, W.B., et al. (2010) Biogeochemical weathering under ice: Size matters: GLACIAL BIOGEOCHEMICAL WEATHERING. *Global Biogeochemical Cycles* **24**: n/a-n/a.

- Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. (2007) Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Appl Environ Microbiol* **73**: 5261–5267.
- Yde, J.C., Finster, K.W., Raiswell, R., Steffensen, J.P., Heinemeier, J., Olsen, J., et al. (2010) Basal ice microbiology at the margin of the Greenland ice sheet. *Annals of Glaciology* **51**: 71–79.
- Yung, P.T., Shafaat, H.S., Connon, S.A., and Ponce, A. (2007) Quantification of viable endospores from a Greenland ice core: Quantification of viable endospores from a Greenland ice core. *FEMS Microbiology Ecology* **59**: 300–306.
- Zdanowski, M.K., Bogdanowicz, A., Gawor, J., Gromadka, R., Wolicka, D., and Grzesiak, J. (2017) Enrichment of Cryoconite Hole Anaerobes: Implications for the Subglacial Microbiome. *Microb Ecol* **73**: 532–538.

A

Composition of Basal Ice Microbial Communities

B

Figure 1. [A] Relative abundances of microbial lineages observed in basal ice using both DNA and RNA for detecting 16S rRNA sequences. The graph displays the highest resolution classification for the most abundant taxa and was constructed as follows: First, ASVs were clustered by genus and any having a relative abundance $\geq 20\%$ in at least one of the samples were plotted. This procedure was subsequently repeated with the remaining unplotted ASVs at the rank of family, order, class, and finally phylum. Any remaining rare taxa left after this procedure were not plotted. The bottom plot was scaled to reduce inflated relative abundance biases introduced during curation. **[B]** Scatter plots of potentially active ASVs within four types of basal ice. Each point represents an ASV. Points falling on the dotted-line are equally abundant in both the DNA- and RNA-based 16S rRNA library. Gray points denote those ASVs observed only in the DNA-based libraries. In the banded basal ice plot, circles denote ASVs from the Banded07 sample while squares denote ASVs from the Banded09 sample. The # of active ASVs in each plot denotes the number of ASVs falling above the $x=y$ line.

Figure 2. Microbial alpha diversity (Shannon) within various permanently frozen environments.

Figure 3. Hierarchical cluster analysis of wUF distances between samples using Ward's minimum variance method.

Figure 4. [Left] RDA triplot of the distribution of 89 microbial lineages across five different permanently frozen environments. Colored circles represent individual samples. Centroids of each sample type are indicated with an “x”. Plus signs (+) represent microbial lineages. The purple circle denotes the equilibrium contribution of the overall model and delineates lineages which substantially constrained the overall ordination. Those lineages are highlighted in red. **[Right]** Magnitude of the scalar projections of these 14 lineages onto the centroids for each ice types approximates that lineage’s association with that frozen environment. Scalar projections smaller than the equilibrium contribution were considered inconclusive and marked gray. Positive and negative associations are purple and orange, respectively.

Figure S1. **[A]** A subglacial tunnel was excavated into the basal zone of Taylor Glacier's northern lateral margin. **[B]** View from inside one of the excavation tunnels. **[C]** During the 2007 expedition, a vertical shaft was excavated and stratigraphic sequence of multiple basal ice types was sampled. **[D]** During the 2009 expedition, a large chamber was excavated to sample a horizon of debris-rich banded basal ice.

Figure S2. An exposed horizon debris-rich basal ice near the terminus of Matanuska Glacier, Alaska. The sampled area, collected using an electric chainsaw, is highlighted inside the yellow-box.

Clean Ice

Banded Ice

Solid Ice

Dispersed Ice

Figure S3. Examples of each type of basal ice analyzed in this study. Clean ice, banded ice, and solid ice were collected from Taylor Glacier, Antarctica. Dispersed ice was collected from Matanuska Glacier, Alaska.