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Metabolic activity and diversity of cryoconites in the Taylor Valley, Antarctica

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[1] Metabolic activity and biogeochemical diversity within cryoconites from the Canada, Commonwealth, Howard, and Hughes glaciers in the McMurdo Dry Valleys revealed the presence of a productive microbial refuge in this polar desert ecosystem. Fluorescent in situ hybridization showed a high percentage of *Cytophaga-Flavobacteria* cells in cryoconite sediments (87.2%), while *β-Proteobacterial* cells dominated the ice overlying the sediment layer (54.2%). The biomass of bacterial cells in the sediments was also greater (4.82 μgC ml^{-1}) than that in the overlying ice (0.18 μgC ml^{-1}) and was related to bacterial productivity (on the basis of thymidine incorporation), which ranged from 36 ng C l^{-1} d^{-1} in the overlying ice to 3329 ng C l^{-1} d^{-1} in the sediment-containing layers. Bacteria within both the sediments and overlying ice were able to actively incorporate and respire radio-labeled glucose, as well as 17 other dissolved organic carbon compounds. The cryoconites in the Taylor Valley support an active, diverse assemblage of organisms despite the fact that they may remain sealed from the atmosphere for decades. Given the density of the cryoconites in the dry valleys (~4–6% of ablation zone surfaces), flushing of the cryoconites during warm years could provide a vital nutrient and organic carbon source to the surrounding polar desert.

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1. Introduction

[2] Cryoconites are cylindrical water and sediment filled depressions (Figure 1) found in the ablation zones of glaciers worldwide. The cryoconites are formed as low-albedo aeolian particles settle onto the glacier and melt to a depth of thermal equilibrium [*McIntyre*, 1984]. Hence the cryoconite consists of a distinct sediment layer at the bottom of the depression and the overlying ice; both undergo variable degrees of summer melting owing to the absorption of solar radiation by the dark sediments. The Swedish explorer A. E. Nordenskjöld first named these features, from "cryo" meaning ice and "conite" meaning dust, during his 1870 Greenland expedition [*Nordenskjöld*, 1875]. The windblown particles seed the cryoconites with microorganisms and mineral particles, the latter of which leach nutrients into the system [*Paul and Clark*, 1989]. These conditions provide a unique habitat for growth within these ice entombed environments. The organisms within cryoconites form a novel ecosystem with a unique structure and function.

[3] Cryoconites on glaciers in the McMurdo Dry Valleys, Antarctica are often frozen at the surface by ice up to 30 cm thick [*Mueller et al.*, 2001] particularly during the recent decadal period of cooling in this region [*Doran et al.*, 2002], but their tops can melt during relatively warm summer periods, (a common occurrence during the 1980s) exposing them to the atmosphere during a portion of the austral summer [*Wharton et al.*, 1985; J. Priscu, unpublished data, 1984, 1987, 1988]. Even when the tops remain frozen, solar energy absorbed by the underlying dark sediments produces subsurface liquid water, which may exist for 1–3 months during the austral summers (December–February), providing conditions that can support biological activity. The waters within the cryoconites are often isolated hydraulically from bulk glacial melt, resulting in chemistries that are distinct from the surrounding glacial ice [*Tranter et al.*, 2004]. When the surface ice melts in warmer years the contents of the cryoconites may flush (i.e., mix with bulk meltwater), supplying nutrients and carbon to the glacial melt streams and surrounding polar desert.

[4] Cryoconites in the McMurdo Dry Valleys support photosynthetic algae and cyanobacteria [*Wharton et al.*, 1981, 1985; *Mueller et al.*, 2001], as well as heterotrophic bacteria, rotifers, tardigrades and other microorganisms [*Porazinska et al.*, 2004]. A phylogenetic study of a cryoconite from the Canada Glacier, based on 16S rDNA and 18S rDNA gene sequences [*Christner et al.*, 2003],

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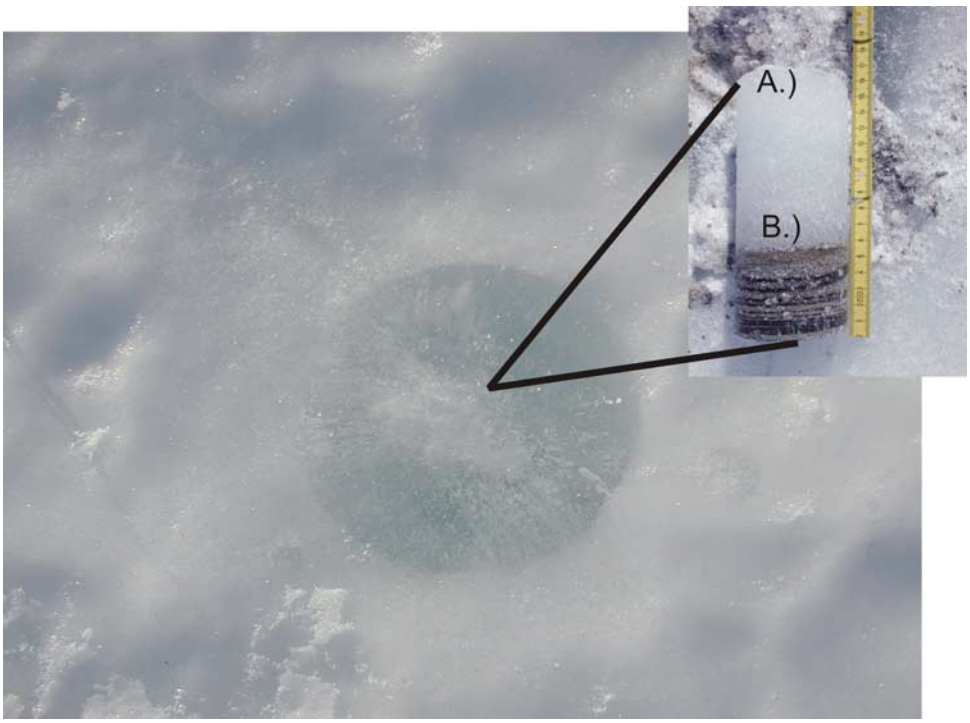

Figure 1. Photograph of a cryoconite hole on the glacier surface with a pullout showing an ice core with the sediment layer and overlying ice obtained from the hole.

identified eight different bacterial lineages as well as: nematodes, tardigrades, rotifers, truffles, ciliates and green algae. The DNA signatures presented by *Christner et al.* [2003] show a close similarity, (>91–99% 16S rDNA sequence similarity), to those in the sediments of permanent lake ice and streams in the region [*Priscu et al.*, 1998; *Gordon et al.*, 2000] suggesting a common microbial origin and regular mixing of the gene pools among these habitats. Despite the detection of DNA sequences from nematodes [*Christner et al.*, 2003], no nematodes have been visually observed in cryoconites examined in the dry valleys [*Porazinska et al.*, 2004].

[5] These seminal studies have revealed that cryoconites in the McMurdo Dry Valleys harbor a relatively diverse group of prokaryotic and eukaryotic organisms. Little is known about physiological biogeochemical processes within cryoconites, despite their importance to glacial and associated melt stream geochemistry [*Tranter et al.*, 2004]. Herein we present new data on metabolic activity and associated biogeochemical characteristics showing that cryoconites support thriving microbial populations that produce new organic carbon and transform nutrients within the polar desert of the Taylor Valley, Antarctica.

2. Methods

2.1. Site Description

[6] The McMurdo Dry Valleys form the largest ice-free area on the Antarctic continent, and represent the coldest and driest desert on Earth [*Doran et al.*, 2002]. The Taylor Valley (77°00′S, 162°52′E) runs east to west for 34 km from McMurdo Sound to the Taylor Glacier and is composed of a mosaic of glaciers, glacial streambeds, exposed soils, and permanently ice-covered lakes. The polar glaciers of the McMurdo Dry Valleys have interior ice temperatures below freezing, and their basal ice is frozen to the ground [*Fountain et al.*, 1998]. Glaciers are a dominant landscape feature in the Taylor Valley, covering approximately 35% of the surface [*Fountain et al.*, 1999]. Glacial melt during the summer is the primary source of liquid water to the surrounding environment, as any precipitation typically sublimates before it can melt [*Chin*, 1981]. Thus glaciers are an integral part of the landscape in the dry valleys and play a significant role in all ecosystem processes [*Priscu*, 1998].

2.2. Sample Collection

[7] Cryoconites were collected from the ablation zones of the Canada, Commonwealth, Howard, and Hughes glaciers in the Taylor Valley, Antarctica. During the austral summer of 2000, a 10 cm SIPRE ice corer was cleaned with ethanol and used to collect cores of frozen cryoconite (n = 33), encompassing both the overlying ice and the underlying sediment. In 2001 an ethanol cleaned ice axe was used to collect sediment from the bottom of selected cryoconites (23 cryoconite holes from various glaciers). The extracted ice cores were placed in sterile, high-density polyethylene bags and stored frozen until processing (within one week of collection). These ice cores were then cut into upper non-sediment and lower sediment-containing portions using an ethanol cleaned, deionized water (DIW) rinsed saw in a laminar flow hood. The cores were placed into sterile plastic containers and allowed to melt in the dark at 4°C. After

approximately 20% of the outer core had melted, the remaining ice from each core was transferred to a second set of sterile containers and allowed to melt completely. This inner portion of the core was used for all analyses reported.

2.3. Physical Properties

[8] The dry weight of the cryoconite sedimentary material was obtained following drying of the particles at 80°C for 48 h. The percentage of organic material was subsequently determined by weight loss after combustion at 450°C for 4 h. Samples for scanning electron microscopy were vacuum concentrated (~0.3 atm) onto 25 mm, 0.2 μm polycarbonate filters sputter coated with 10 nm Au-Pd. Particles were imaged and mineralogy determined using a scanning electron microscope (JEOL-6100 SEM) equipped with a NORAN X-ray energy dispersive system (EDS) and a cryogenic stage [see *Priscu et al.*, 1999]. Cryoconite morphology and sediment weight were reported in a parallel study [*Porazinska et al.*, 2004].

2.4. Chemical Measurements

[9] Measurements of reduction/oxidation (REDOX) potential (E_h) were made using an ORP combination electrode (Hach Company, Loveland, CO) connected to a Beckman F12 digital pH meter. The measured E_h potentials are reported relative to the standard hydrogen electrode following the manufacturer's instructions (Hach ORP manual). Major ions, inorganic N and P, and dissolved organic carbon (DOC) were determined with a Dionex DX-300 ion chromatograph, Latchat nutrient analyzer, and Shimadzu 5000 TOC analyzer, respectively, using methods described previously [*Priscu*, 1995; *Welch et al.*, 1996; *Takacs and Priscu*, 1998; J. C. Priscu and C. F. Wolf, *Limnological Methods for the McMurdo Dry Valleys Long Term Ecological Research Program*, 2000, available at http://www.homepage.montana.edu/~lkbonney/DOCS/Data.html].

[10] Chlorophyll-a was determined spectrophotometrically after extraction of material filtered onto Whatman GF/F filters in 90% acetone. The filter was sonicated in an ice water bath for 20 min, vortexed and allowed to extract overnight in the dark at −20°C before centrifugation and spectroscopic measurement. Pigment concentrations were based on scans of the visible wavelengths from 400 nm to 750 nm. The trichromatic equations of *Jeffrey and Humphrey* [1975] were used to calculate chlorophyll-a concentration.

2.5. Bacterial Abundance and Biomass

[11] Cryoconite meltwater (30 ml) was preserved with prefiltered (0.2 μm) formalin to a final concentration of 2% v/v. Bacteria were filtered onto polycarbonate filters (0.2 μm pore size) and stained with the fluorochrome DAPI (4′, 6-diamidino-2 phenylindole) at a final concentration of 0.2% v/v [*Porter and Feig*, 1980]. More than 400 bacteria per sample were counted in randomly selected fields with a Zeiss Axioplan epifluorescence microscope equipped with BP 365, FT 395, and LP 397 filter sets. Images were taken with a highly sensitive charge-coupled device camera (Optotronics ZVS-47EC) and processed with the image analysis software LUCIA_D, version 3.50 (Laboratory Imaging, Prague, Czech Republic). Bacterial biovolume was determined from length and width measurements according to *Posch et al.* [1997]. Bacterial carbon content was estimated as described by *Löferer-Krößbacher et al.* [1998].

2.6. Bacterial Activity

[12] Bacterial productivity was estimated as ^{3}H-thymidine incorporation into cold trichloroacetic acid (TCA) insoluble products [*Takacs and Priscu*, 1998] on the ice overlying the sediment layer and the sediment layer from three cryoconites on the Canada Glacier, sections of overlying ice from two cryoconites from the Hughes Glacier, and one from the Commonwealth Glacier. Samples were incubated with 20 nM thymidine for 20 h in the dark at 4°C. Thymidine incorporation was converted to carbon incorporation using conversion factors of 2.0×10^{18} cells mol thymidine^{-1} and 11 fg Carbon cell^{-1}, as outlined by *Takacs and Priscu*, 1998 and *Takacs et al.* [2001]. Incubations were carried out in the dark at 0.1°C; temperature was logged throughout the course of the experiments.

[13] Extracellular enzyme activity (EEA) assays followed the protocol presented by *Sinsabaugh et al.* [1997] and *Foreman et al.* [1998]. Cryoconite samples were assayed for the activity of three hydrolytic enzymes at 4°C, using substrates linked to methylumbelliferyl or coumarin residues. The substrates for the ß-1, 4-glucosidase (BG), leucine aminopeptidase (LAP), and alkaline phosphatase (AP), assays were 4-MUF-ß-D-glucoside (EC 3.2.1.21), L-leucine-7- amido-4-methyl-coumarin (EC 3.4.11.1), and 4-MUF-phosphate (EC 3.1.3.1) respectively. These enzymes are involved in the acquisition of carbon, nitrogen and phosphorous. BG is a carbohydrate degrading enzyme, LAP is a broad specificity enzyme involved in the final step of protein degradation and is used to assess organic nitrogen availability, and AP cleaves polyphosphate and orthophosphate monoesters resulting in the liberation of orthophosphorous. Activities are expressed as the rate of accumulation of methylumbelliferone or 7-amino-4-methyl coumarin, as appropriate, in units of nmol l^{-1} h^{-1}, using emission coefficients calculated from standards and correcting for quench effects. The reaction rate (V) for each enzyme was calculated under substrate saturating concentrations on eight analytical replicates.

[14] The BIOLOG system (BIOLOG, Incorporated, Hayward, California) was used to assess the degree to which organisms within the cryoconites were capable of metabolizing distinct carbon compounds. The Biolog GN microplates consist of 95 different organic carbon substrates and one carbon-free blank [*Garland*, 1996]. Each well contains a redox dye, tetrazolium violet, which is reduced to formazan by the respiratory activity of the microbes present in the samples. Readings of optical density (590 nm) were made after incubation at 10°C for 24 h using a BioTek (FL600) microplate fluorometer [*Sinsabaugh and Foreman*, 2001].

[15] Glucose uptake and respiration were determined on the sedimentary material from a cryoconite collected on the Canada Glacier. Melted sample (5 ml) was dispensed into eight replicate sterile sidearm flasks fitted with a plastic center well containing a folded Whatman 25 mm GF/F filter suspended above the aqueous sample [*Hobbie and Crawford*, 1969]. Trichloracetic acid (5% final concentration) was

Table 1. Fluorescently Monolabeled Oligonucleotide rRNA Probes

Probe	Specification	Sequence (5′ → 3′)	Position
EUB338	Eubacteria	GCTGCCTCCCGTAGGAGT	338–355
ALF 968	α-subclass of proteobacteria	GGTAAGGTTCTGCGCGTT	968–986
Bet42a	β-subclass of proteobacteria	GCCTTCCCACTTCGTTT	1027–1043
CF319a	Cytophaga-flavobacteria-cluster	TGGTCCGTGTCTCAGTAC	319–336

added to four of the vials to serve as killed controls; a single killed sample was processed along with duplicate samples at each time point. Uniformly labeled D^{-14}C-glucose (20 μM) (NEN, final concentration 4.4×10^6 dpm ml^{-1}) was added to each sample and all flasks were incubated in the dark at 9°C. Published data on glucose kinetics indicate that 20 μM should saturate the active sites of all enzyme systems involved with glucose metabolism [e.g., *Kirchman et al.*, 1990]. The reaction in replicate flasks was terminated by the addition of 5 ml ice cold trichloroacetic acid (TCA) at ~24 h intervals over 3 d. The addition of TCA reduced the pH to <3 converting all inorganic carbon to CO_2. The CO_2 absorber ß-pheneythylamine (150 μl) was then added to the filter and the flasks were placed on a shaker table for 24 h to ensure that all CO_2 released upon acidification was trapped by the ß-pheneythylamine on the wicks. The replicate samples, plus one of the kills, were then filtered through a 25 mm polycarbonate membrane filter (0.2 μm) to collect the cellular material. Radioactivity on the wicks and in the cellular material was counted using standard scintillation spectroscopy. Background treatments were included to ensure that chemiluminescence did not occur in the scintillation cocktail. Our final calculations assumed that ambient ^{12}C-glucose concentration in the cryoconite sample was insignificant relative to the 20 μM of ^{14}C-glucose added to each flask.

2.7. Flourescent In Situ Hybridization (FISH)

[16] Meltwater (100 ml) was filtered onto polycarbonate filters (Millipore, Type GTTP, 0.2 μm pore size, 47 mm diameter, white) and fixed with paraformaldehyde (4% final concentration) for 20 minutes, followed by a deionized water (DIW) rinse. FISH analysis used the fluorescently monolabeled oligonucleotide probes listed in Table 1 as described by *Alfreider et al.* [1996] and *Glöckner et al.* [1996]. Formamide concentration in the hybridization buffer was 20% for the α-Proteobacteria (α) and Cytophaga-Flavobacteria (CF) probes and 35% for the eubacterial (EUB) and β Proteobacteria (β) probes. The filter sections were counter-stained with DAPI (final concentration 1 μg l^{-1}) and examined with a Zeiss Axiophot epifluorescence microscope at a magnification of 1,600x. At least 600 DAPI-stained cells were counted per sample.

3. Results

3.1. Physical and Chemical Parameters

[17] Sediment weight in the cryoconites sampled ranged from 34–67 g per hole (Table 2). The sediment particles ranged in size from ~2 μm to ~200 μm. X-ray dispersion spectra collected from particle grains (Figure 2) showed the sediments to be composed of quartz grains, hornblende, and actinolite particles, with the majority being hornblende and actinolite [*Perkins*, 2002]. Several of the particles were covered with an iron hydroxide coating (grain C, Figure 2). The percentage of particulate organic matter as ash free dry weight (AFDW) in sediment from the Canada Glacier cryoconite was (~3%). The presence of particulate organic matter was verified on the basis of parallel evaluation of scanning electron and backscattered electron images. Organic particles are only visible on the scanning electron images, whereas mineral particles are visible on both and provide the means of discernment.

[18] Eh values in all samples were greater than 300 mV. When combined with the nutrient data (NO_2^- and NH_4^+) these redox potentials indicate a moderately oxidizing environment, with iron being present predominantly as Fe^{3+}. Cryoconite chemistry was variable both within individual glaciers, as well as between glaciers (Table 2; see also *Porazinska et al.*, 2004). Chemical measurements were not made on the Hughes Glacier samples during this study; however these can be found in the work of *Porazinska et al.* [2004]. The pH was near neutral or slightly alkaline in all samples ($\bar{X}$ = 7.6, SD = ±0.4), presumably because of photosynthetic activity within the cryoconites [*Tranter et al.*, 2004]. Dissolved inorganic nitrogen (DIN = NO_3^- + NO_2^- + NH_4^+) and soluble reactive phosphorus (SRP) ranged from 18–560 μg N l^{-1} ($\bar{X}$ = 136, SD = ±134) and <3 to 8.8 μg SRP l^{-1}($\bar{X}$ = 2.7, SD = ±2.2), respectively. The DIN:SRP ratio (range = 14.6–125.3, g:g) was lowest in samples from the Commonwealth Glacier and highest in those from the Canada Glacier ($\bar{X}$ = 61.7, SD = ±42.3). The ratios of DIN:SRP in these samples are above

Table 2. Water Chemistry Parameters From MCM Dry Valleys Cryoconites[a]

Glacier, *n*	Position	pH	Sediment, g	DIN, μg/L	SRP, μg/L	DOC, mg/L	Na^+, mg/L	Cl^-, mg/L	Mg^{2+}, mg/L	K^+, mg/L	SO_4^{-2}, mg/L
Howard (6)	lower west	8.1 (±0.2)	53.2 (±14.5)	81.7 (±53.9)	2.4 (±1.3)	2.1 (±1.1)	4.3 (±0.7)	8.7 (±1.6)	1.2 (±0.45)	1.0 (±0.24)	3.4 (±0.9)
C-wealth (6)	upper west	7.2 (±0.2)	66.8 (±32)	56.8 (±17.4)	3.9 (±4.2)	1.9 (±0.9)	8.2 (±3.6)	15.2 (±10.2)	2.5 (±1.2)	1.9 (±0.8)	3.7 (±1.9)
Canada (7)	upper west	7.6 (±0.4)	33.9 (±17.4)	261.6 (±181)	3.6 (±2.1)	2.2 (±0.9)	5.4 (±2.6)	10.3 (±5.5)	0.6 (±0.3)	1.4 (±0.6)	6.2 (±3.9)
Canada (7)	upper east	7.5 (±0.3)	57.2 (±22.6)	188 (±132)	1.5 (±0.4)	3.2 (±3.9)	5.1 (±1.3)	8.8 (±4.5)	0.9 (±0.4)	1.6 (±0.4)	6.2 (±3.9)
Canada (7)	lower west	7.5 (±0.3)	61.2 (±25.3)	74.6 (±110.3)	1.2 (±0.5)	2.7 (±1.1)	0.9 (±0.4)	6.2 (±2.4)	0.4 (±0.3)	0.9 (±0.2)	3.5 (±1.6)

[a]Values expressed as the mean ± the standard deviation.

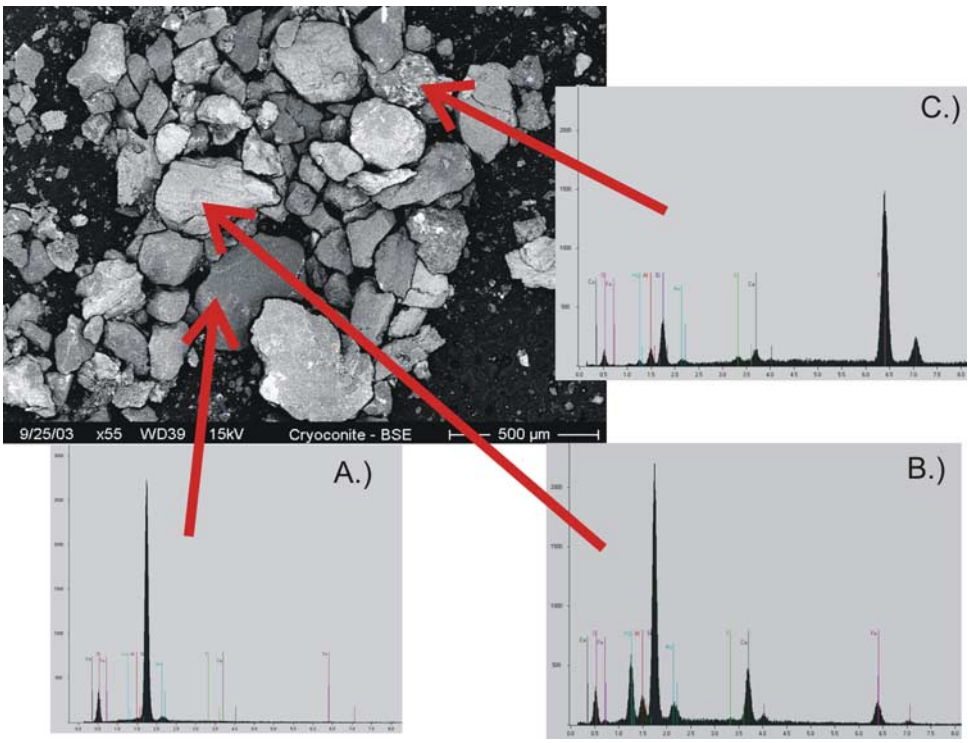

Figure 2. Scanning electron micrograph and X-ray spectra from a JEOL-6100 scanning electron microscope equipped with a NORAN X-ray energy dispersive system and a cryogenic stage. (a, b, c) X-ray spectra from select cryoconite particle grains showing the sediments to be composed of quartz grains, hornblende, and actinolite particles. Particle C also has an iron hydroxide coating. Scale bar = 500 μm.

those typically noted for balanced metabolism [*Redfield*, 1958], implying that phosphorus may limit microbial activity in the cryoconites, particularly in the Canada Glacier cryoconite. Sodium and chloride concentrations were greatest from cryoconites on the Commonwealth Glacier (Table 2), which is the glacier closest to the marine waters of McMurdo Sound. Chlorophyll-a measured from a Canada Glacier cryoconite was 1.5 μg l^{-1}, which is in the range of values found in the water columns of the dry valley lakes (e.g., east Lake Bonney 1993–2001 range = 0.05–3.37 μg l^{-1}).

3.2. Biological Abundance, Activity, and Diversity

[19] Bacterial cell numbers in the overlying ice layers ranged from 1.27×10^4 cells ml^{-1} on the Hughes Glacier to 7.94×10^4 cells ml^{-1} on the Canada Glacier. Abundances were always greater in the sediment-containing samples compared to the overlying ice (t = 2.315, p = 0.049, n = 23) (Figure 3). Bacterial cells in the sediment-containing layers were larger (see Table 3; range of length:width = 1.53 to 5.59; volume range = 0.104–0.549 μm^3 $cell^{-1}$; carbon content range = 19.45–27.62 fg C $cell^{-1}$) than those within the overlying ice (range of length:width = 1.50–2.37; volume range = 0.051–0.149 μm^3 $cell^{-1}$; carbon content range = 9.73–23.51 fg C $cell^{-1}$). Statistical analysis showed that the length (t = −2.32, p = 0.049, n = 23), biomass (t = −2.62, p = 0.03, n = 23) and associated carbon content (t = −2.51, p = 0.036, n = 23) were significantly greater in organisms associated with the sediment layer.

[20] Bacterial productivity in samples of the ice overlying the sediment layer were 95% lower, but owing to high variance in this quantity, were not significantly different from activity in the sediments (t = 0.21, p = 0.05, n = 6) when all of the glaciers are considered (Figure 4). Rates averaged 59.6 (SD = ±29.1) ng C l^{-1} d^{-1} and 1580 (SD =

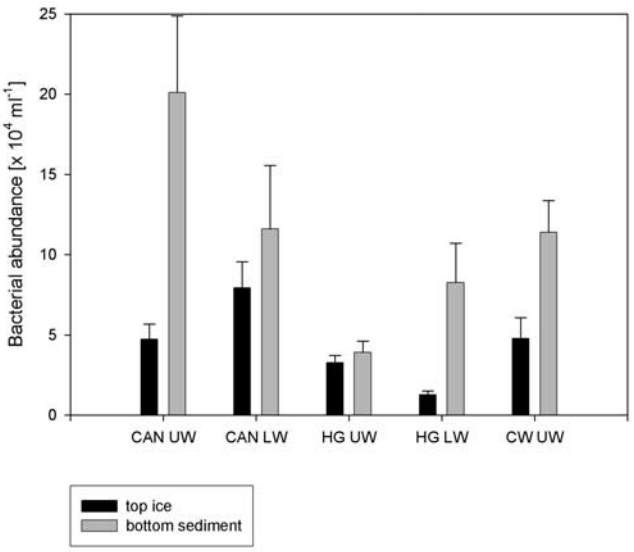

Figure 3. Bacterial abundance (direct epifluorescent microscopy counts) from the Canada (CAN), Commonwealth (CW), and Hughes (HG) glaciers. UW = upper west and LW = lower west represent locations on the glacier surface. Error bars are 1 standard deviation from the arithmetic mean.

Table 3. Bacterial Cell Volume, Carbon Content Per Cell, Biomass and Elongation From Taylor Valley Cryoconites[a]

Glacier, *n*	Cell Volume, μm^3 $cell^{-1}$	C-Content $cell^{-1}$, fg C $cell^{-1}$	Biomass, μg C l^{-1}	Elongation Ratio, Length:Width
		Canada Glacier		
CAN UW ice ($n = 5$)	0.053	12.48	0.59	2.37
CAN UW sed ($n = 5$)	0.133	23.99	4.82	5.59
CAN LW ice ($n = 6$)	0.124	23.51	1.87	1.90
CAN LW sed ($n = 6$)	0.104	19.45	2.26	1.53
		Hughes Glacier		
HG UW ice ($n = 5$)	0.149	21.36	0.70	1.50
HG UW sed ($n = 5$)	0.549	24.35	0.95	5.32
HG LW ice ($n = 4$)	0.082	13.28	0.18	2.17
HG LW sed ($n = 4$)	0.181	27.62	2.28	3.38
		Commonwealth Glacier		
CW UW ice ($n = 3$)	0.051	9.73	0.47	2.30
CW UW sed ($n = 3$)	0.129	22.52	2.56	3.27

[a]UW = upper west, LW = lower west. Values are expressed as the arithmetic mean of replicate samples ($n = x$).

±1554.9) ng C l^{-1} d^{-1} in the overlying ice and sediment layers, respectively.

[21] Enzyme activity varied considerably among the individual glaciers studied (Figure 5), as well as between the overlying ice and sediment portions of the cryoconites, but was always higher in the sediment than the overlying ice, a pattern shown by other biological parameters and the geochemical data. For example, leucine aminopeptidase (LAP) activity in a cryoconite from the Canada Glacier ranged from 0.001 nmol h^{-1} l^{-1} in the overlying ice to 66 nmol h^{-1} l^{-1} in the sediment. LAP was 99% and 93% greater in the overlying ice on the Commonwealth Glacier than on the Canada and Hughes glaciers, respectively. The microbial assemblage in the overlying ice from the Commonwealth Glacier also demonstrated increased alkaline phosphatase (AP) activity (86% and 10%) as compared to ice from cryoconites on the Canada and Hughes glaciers (Figure 5). ß-glucosidase activity was 8% and 54% higher in the overlying ice from the Hughes Glacier than in the Canada and Commonwealth glaciers (Figure 5). Enzyme activities in the cryoconites were less than those typically measured in temperate riverine systems [*Foreman et al.*, 1998; *Sinsabaugh and Foreman*, 2001], but greater than those from the water columns of the dry valley lakes (C. Foreman, unpublished data, 2002) or subglacial discharge from the Taylor Glacier at Blood Falls [*Mikucki et al.*, 2004].

[22] Biolog substrate utilization profiles from a Canada Glacier cryoconite showed that microbes within the cryoconites were capable of metabolizing 17 of the 95 available carbon substrates after 24 h. These 17 compounds (Table 4) were predominantly amino acids (29%), carbohydrates (35%) and carboxylic acids (24%). Glucose incorporation (I) and respiration (R) in a Canada Glacier sample increased linearly over a 3 d incubation period at 9°C (Figure 6). The rate of glucose incorporation and respiration (noted by the slope of activity over time) averaged 2.80 μM d^{-1} and 1.71 μM d^{-1}, respectively. Respiration exceeded incorporation (I:R = 0.002) directly following isotope addition (measured at 1.5 h). The I:R ratio remained relatively constant at 1.42 through the remainder of the experiment.

[23] The fraction of bacteria detectable with the eubacterial (EUB) probe used in the fluorescent in situ hybridization analysis of the cryoconite sediments ranged from 61.6% on the Canada Glacier up to 98.1% on the Hughes Glacier, with respect to the total DAPI counts (Table 5). The EUB probes hybridized to 52.5% and 79.3% of the DNA targets, relative to total DAPI counts, in the overlying ice from these same cryoconite samples. Relatively few bacteria (range = 1.6% in the Canada Glacier to 11.2% in the Hughes Glacier) were detected with the α-*Proteobacteria* (ALF968) probe in either the sediments or overlying ice. The bacterial assemblage varied between and within glaciers, but a higher number of bacteria of the ß-subclass were detected, with the highest percentages found in the overlying ice from the Canada Glacier (54.2%) and the lowest in the sediment layer from the Canada Glacier (2.5%). The *Cytophaga-Flavobacteria* (CF319a) probe hybridized to 87.2% of the total DAPI counts in cryoconite sediments from the Canada Glacier and 37.6 % of the total DAPI counts in the Hughes Glacier cryoconites. Hybridization was lower in the overlying ice from cryoconites on the Canada Glacier (17.2%) and the Hughes Glacier (37.6%).

4. Discussion

[24] The cryoconites in the Taylor Valley have typically been closed to the atmosphere and internal glacial drainage

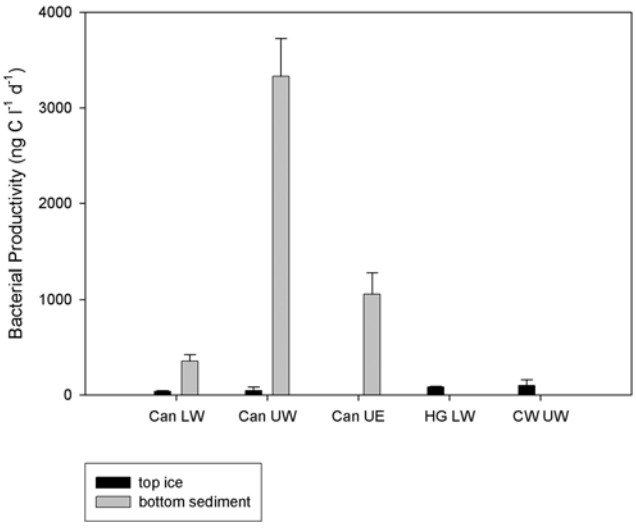

Figure 4. Bacterial production on cryoconites from the overlying ice and bottom sediment layers from the Canada (CAN), Commonwealth (CW), and Hughes (HG) glaciers. UW = upper west, LW = lower west, and UE = upper east represent locations on the glacier surface. Error bars are 1 standard deviation from the arithmetic mean.

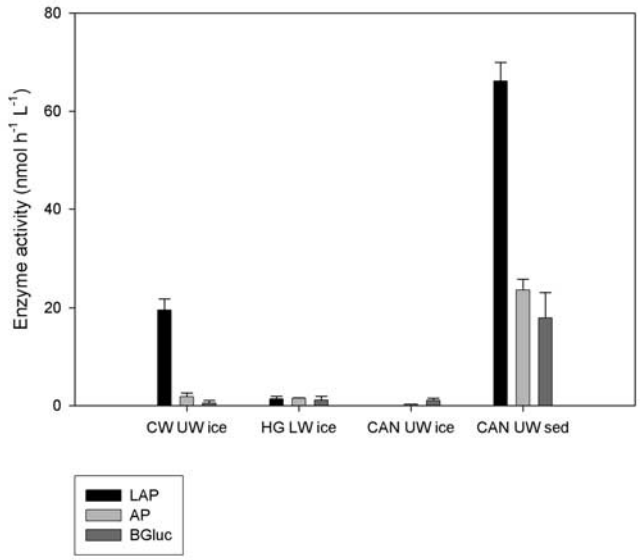

Figure 5. Activity of three extracellular enzymes: leucine aminopeptidase (LAP), alkaline phosphatase (AP), and β-glucosidase (BG) from the overlying ice and bottom sediment portions of cryoconites on the Canada (CAN), Commonwealth (CW), and Hughes (HG) glaciers. UW = upper west, LW = lower west, and UE = upper east represent locations on the glacier surface. Error bars are 1 standard deviation from the arithmetic mean.

over the past decade owing to their 30–40 cm thick ice covers. The situation in the dry valleys is in contrast to cryoconites examined in arctic and high-altitude temperate glaciers [*Mueller et al.*, 2001] as all other known cryoconites lose their lids on an annual or semiannual basis. Consequently, the dry valley cryoconites are not flushed annually and accumulate organic matter in the form of living organisms and detrital matter. *Fountain et al.* [2004] used chloride concentrations to estimate that cryoconites in the Taylor Valley have been closed to their surroundings from 2 to 11 years. The small area (4–6% of the ablation zone) occupied by cryoconites on the glaciers in the Taylor Valley makes them relatively insignificant features of these glaciers with respect to their geophysical dynamics [*Fountain et al.*, 2004]. However, owing to the low biomass and limited growing season that typifies the McMurdo Dry Valleys [e.g., *Freckman and Virginia*, 1998; *Moorhead and Priscu*, 1998; *Priscu*, 1998] any habitat that supports metabolic activity is important from an ecosystem perspective [e.g., *Priscu et al.*, 1998]. In addition, warm summers produce a ''meltdown'' that flushes the contents of the cryoconites into the surrounding polar desert. Hence the cryoconites provide a habitat that processes inorganic and organic matter during the colder years and releases these materials to the surrounding environment (e.g., soils, streams, lakes) providing nutrients that influence the structure (biodiversity) and function (biogeochemistry) of the entire ecosystem.

[25] Wind is believed to be the foremost process involved in redistributing organic matter in the dry valleys and provides integration between soils, lakes, streams, and glaciers [*Moorhead et al.*, 1999; *Lancaster*, 2002]. The absorption of solar energy by wind blown sediment is the dominant mechanism behind the formation of cryoconites [*Wharton et al.*, 1985]. The percentage of organic matter (relative to dry weight) in a cryoconite from the Canada Glacier (3%) was similar to that found within cryoconites from the Tyndall Glacier (range 0.6–2.7%), southern Patagonia [*Takeuchi et al.*, 2001]. Sediment particles examined from cryoconites in our study were rounded providing evidence of weathering and physical abrasion revealing their aeolian nature [*Perkins*, 2002]. We also noted an iron oxide coating on many particles further indicating chemical weathering of the cryoconite sedimentary matter, as iron bearing minerals weather to produce iron oxides. The majority of the particles in the Taylor Valley cryoconites consisted of relatively dense hornblende and actinolite, with much lower abundances of lighter quartz particles. Both hornblende and actinolite are amphiboles, which are common rock forming minerals and present in most metamorphic and many igneous rocks [*Veblen*, 1981]. Evidence of amphiboles in the dry valleys was first noted by geologists with the 1910–1913 *Terra Nova* Expedition [*Smith*, 1964]. The relative accumulation of hornblende and actinolites within the cryoconites we studied implies that these heavier minerals fell out and became entrapped within the cryoconites, while the lighter particles presumably remained airborne.

[26] [*Wharton et al.*, 1985; *Christner et al.*, 2003; *Porazinska et al.*, 2004] showed that many of the organisms present in the Taylor Valley cryoconites are similar to those found in the surrounding environment, underscoring the wind as the proximate source for these materials. The main nutrient sources on the glaciers may also be wind blown particles, with the chemical properties of the cryoconite sediments initially reflecting properties of the nearby aquatic and terrestrial environments. *Fortner et al.* [2005] recently showed that cryoconites, supraglacial, and proglacial streams in the Taylor Valley are enriched in major ions with respect to chloride, suggesting an abundance of terrestrial dust as opposed to marine or biogenically derived sources [*Lyons et al.*, 2003].

[27] *Fountain et al.* [2004] determined the aerial extent of cryoconites in the Taylor Valley glaciers within the ablation zones. On the basis of data from the Commonwealth, Canada, and Howard glaciers one can provide a rough estimate of the flux of organic carbon and organic nitrogen to downstream systems if all cryoconites were flushed, as occurs during warm summers. Given a fractional area of the ablation zones covered by cryoconites of 5%, the number of cryoconites m^{-2}, the amount of sediment per cryoconite and the average particulate organic carbon (POC) and nitrogen (PON) values (see *Fountain et al.* [2004] for the primary

Table 4. Carbon Substrates Utilized by Cryoconite Organisms in the Biolog Plates

Carbohydrates	Amino Acids	Carboxylic Acids
Glycogen	D-mannitol	Formic acid
L-fructose	D-serine	Succinic acid
Cellobiose	L-alanyl-glycine	Urocanic acid
i-erythritol	L-phenylalanine	Glycerol-L-glutamic acid
L-ornithine	D-mannose	α-hydroxy butyric acid
n-acetyl-D-glucosamine		D-glucuronic acid

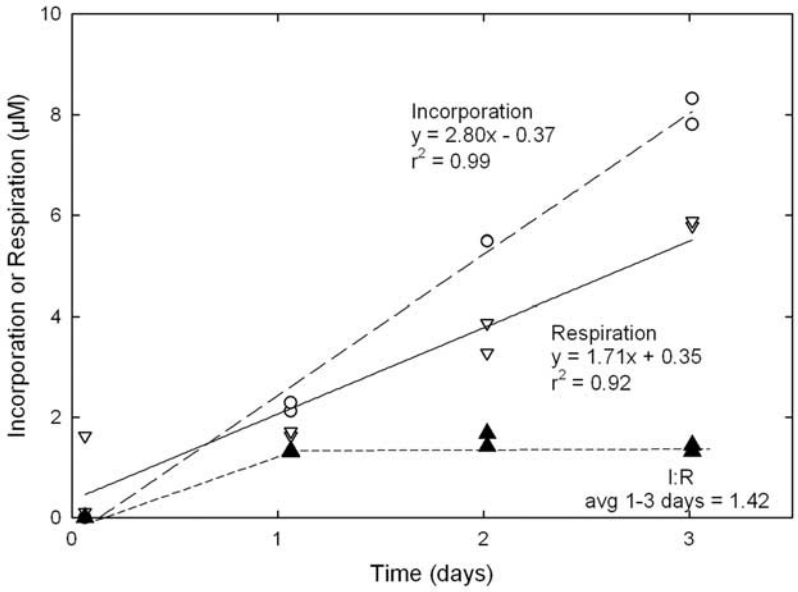

Figure 6. Time course for the incorporation (open circles) and respiration ($^{14}CO_2$ release) (inverted open triangles) and the ratio of incorporation to respiration (I:R) (solid triangles) of ^{14}C-D-glucose. Lines and equations for least squares fit through the incorporation and respiration data are included. Line for the I:R ratio was drawn by inspection; the average I:R (1.42) represents the average between 1 and 3 d of incubation.

data), we calculated the amount of POC and PON that can be flushed from the cryoconites. These calculations reveal that cryoconites on the Commonwealth, Canada, and Howard glaciers can yield 582 kg and 64 kg, 230 kg and 25 kg, and 50 kg and 5 kg of POC and PON, respectively to the surrounding environment, keeping in mind that we are extrapolating from only a few holes on each glacier. Because the soils are so depauperate (the POC and PON content of soils in the Taylor Valley ranges from 0.120 to 0.398 μg POC (mg sed)$^{-1}$ and 0.012–0.060 μg PON (mg sed)$^{-1}$ [*Fritsen et al.*, 2000]), the additional POC and PON from the cryoconites could prove to be a valuable component of the overall carbon and nutrient budgets in the McMurdo Dry Valleys ecosystem. The ratio of POC:PON released from the cryoconites (~9 by weight) is near the ratio found in microorganisms during balanced growth (~6, [*Redfield*, 1958]) indicating that cryoconite release should maintain the surrounding biotic systems in growth equilibrium, at least with respect to their C and N requirements. Given the P-deficient nature of the phytoplankton in the downstream lakes in the Taylor Valley [*Priscu*, 1995; *Dore and Priscu*, 2001], we recommend that future studies of cryoconites in the region include measurements of phosphate and particulate organic phosphorous.

[28] Viable microbial life is present within the dry valley cryoconites as shown by the activity and abundance measurements undertaken in this study, however it is important to

Table 5. Percentage of Oligonucleotide Probes Hybridizing to a Target[a]

Glacier, *n*	Probes, %, EUB	Probes, %, ALF968	Probes, %, Bet42a	Probes, %, CF319a
		Canada Glacier		
CAN UW top (n = 5)	71.5 (±15.4)	8.7 (±8.7)	17.2 (±3.6)	20.5 (±3.9)
CAN UW sed (n = 5)	97.9 (±16.2)	7.4 (±7.4)	2.5 (±0.9)	75.9 (±17.9)
CAN LW top (n = 6)	79.3 (±17.5)	1.6 (±1.6)	54.2 (±172)	17.2 (±4.7)
CAN LW sed (n = 6)	61.6 (±17.6)	4.6 (±4.6)	11.6 (±3.1)	87.2 (±20.3)
		Hughes Glacier		
HG UW top (n = 5)	54.5 (±16.2)	5.3 (±5.3)	34.2 (±8.3)	21.5 (±2.7)
HG UW sed (n = 5)	69.6 (±25.1)	6.2 (±6.2)	3.6 (±0.8)	42.6 (±9.9)
HG LW top (n = 4)	57.1 (±19.2)	11.2 (±11.2)	40.5 (±12.2)	37.6 (±4.6)
HG LW sed (n = 4)	98.1 (±26.8)	4.2 (±4.2)	6.2 (±0.9)	54.5 (±11.3)
		Commonwealth Glacier		
CW UW top (n = 3)	52.5 (±9.9)	3.2 (±3.2)	42.5 (±3.9)	28.5 (±4.3)
CW UW sed (n = 3)	82.5 (±18.5)	8.5 (±8.5)	5.2 (±1.7)	67.2 (±19.3)

[a]All Percentages are expressed relative to absolute bacterial DAPI counts. These percentages are expressed as the arithmetic mean of replicate samples (n = x), plus or minus the standard deviation.

note that the metabolic rates expressed in this study may not represent true in situ metabolic rates. During the summer months (December–February) production is not likely to be limited by solar radiation and temperatures; however it may be phosphorous limited especially within cryoconites on the Canada Glacier. Bacterial numbers in the Taylor Valley cryoconites are an order of magnitude lower than densities found in the lakes in the Taylor Valley [*Takacs and Priscu*, 1998], but comparable to those from alpine high mountain lakes [*Psenner and Sattler*, 1998]. Abundances, biomass and elongation of cells were higher in the sediment layers of the Antarctic cryoconites. This accumulation of larger cells may be because bacteria have a predilection for life on surfaces [*Costerton*, 1995], or because of an accumulation of nutrients as is found in alpine cryoconites (G. Fitz and B. Sattler, unpublished data, 2005). Moreover, the bottom sediments may undergo fewer freeze-thaw cycles than the overlying ice, providing a more constant habitat. If cryoconites are flushed during warmer periods, nutrients and biomass in the upper water layer may become diluted. This process has been observed in cryoconites of high-Arctic glaciers, where flushing during summer months reduces bacterial cells and nutrients, rapidly changing the cryoconite water and associated chemicals and biota so that it is comparable to the surrounding glacial ice [*Mindl et al.*, 2007].

[29] The microorganisms within the sediment layer of a Canada Glacier cryoconite incorporated and respired glucose in a linear fashion over a 3 d incubation period at rates of 2.80 and 1.71 μM d^{-1}, respectively. Incorporation rates of glucose over the incubation period were 64% higher than glucose respired as CO_2 indicating that the cells were, on average, actively retaining carbon within the cells. The microbial respiration rate in the cryoconite samples is more than an order of magnitude greater than the range of glucose respiration measured in melted glacial ice from a deep Antarctic ice core collected beneath Vostok Station; glucose incorporation was below the level of the kills in these glacial ice samples [*Christner et al.*, 2006]. This difference results from the lower cell densities within the Vostok ice ($\sim 10^2$ cell ml^{-1} compared to $\sim 10^4$ cell ml^{-1} in the cryoconites). However, even if these cell density differences are taken into consideration, the rates per unit cell for the cryoconites are still 2–3 orders of magnitude greater than that for the Vostok ice. This presumably reflects the general physiological state of the bacteria in these two environments, a contention corroborated by the Incorporation:Respiration (I:R) ratios. The I:R ratio in the cryoconite sample was 0.002 after 1.5 h (0.06 d) of incubation with the isotope and reached a plateau at 1.42 after 1 d of incubation, whereas that in the Vostok ice could not be computed owing to incorporation rates that were not significantly greater than the killed samples [*Christner et al.*, 2006]. The low initial I:R ratio in the cryoconite sample and the low average ratios in the Vostok ice presumably represent a period of resuscitation where most of the substrate is respired rather than being incorporated into macromolecules. The energy obtained during glucose respiration can then be used to repair cellular damage and prime biosynthetic pathways for active growth. Our contention is corroborated by *Dodd et al.* [1997] who showed that when nongrowing and sublethally injured cells are placed in a growth situation, metabolism must first be initiated to repair incurred cellular damage before growth and reproduction can occur.

[30] The Biolog results demonstrate that microbes in the cryoconites studied are capable of exploiting several different types of carbohydrates, amino acids, carboxylic acids and even aromatic compounds; suggesting the presence of metabolically diverse heterotrophic communities within the Taylor Valley cryoconites. Positive substrate induced respiration of several amino acids, including serine, alanylglycine, ornithine and phenylalanine provides a link to the measured extracellular enzyme activities. Aminopeptidases such as LAP, have a relatively broad specificity in their hydrolysis of amino acids. *McCarthy et al.* [1998] found that most dissolved organic nitrogen in marine systems was derived from peptidoglycan, a component of microbial cell walls, which may also be the primary source within cryoconites and why we see elevated levels of substrate induced respiration and EEA in the cryoconites.

[31] While the Biolog system presents 95 different carbon substrates, the substrate induced respiration is dependent upon growth under culture conditions, while profiles of EEA do not rely upon culture based responses. The extracellular enzyme activity measured in the cryoconites illustrates that a physiologically diverse range of microbial carbon, nitrogen and phosphorous transformations occur in the Taylor Valley cryoconites. The LAP:BG ratio for the sample from the Commonwealth Glacier was 37.4 indicating a greater reliance upon proteins than on carbohydrates for growth, whereas the LAP:BG ratio in the sample from the Hughes Glacier (1.2) showed a nearly equal reliance upon proteins and carbohydrates [*Christian and Karl*, 1995; *Foreman et al.*, 1998]. β-glucosidase activities in the overlying ice showed increasing activity down valley (i.e., heading from the Taylor Glacier toward McMurdo Sound), with the highest activities recorded from a cryoconite on the Hughes Glacier. Dissolved organic carbon concentration, invertebrate density, and cyanobacterial species richness were significantly lower ($p < 0.05$) on the Hughes Glacier than on the other glaciers [see *Porazinska et al.*, 2004]. Photosynthetically produced organic carbon is presumably the major energy source for the organisms entrapped within the cryoconites. If the available dissolved organic carbon is scarce the organisms must produce enzymes to acquire this carbon, a contention supported by the greater β-glucosidase activity on the Hughes Glacier. Extracellular enzymes mediate the cleavage of organic matter into hydrolyzable substrates through feedback mechanisms that are directly related to the availability of carbon and nutrients in their environment. The overlying ice from the Canada Glacier had very little LAP and AP activity, whereas the sediments from this same hole on the Canada Glacier exhibited high levels of all three enzymes indicating that these compounds were being hydrolyzed by the microbes.

[32] Combining the above activity measurements with bacterial production provides a snapshot into the trophic dynamics possible within the cryoconite system. The enzymes provide information about functional diversity and nutrient and energy cycling within the microbial community [*Sinsabaugh and Foreman*, 2001] while bacterial production (via tritiated thymidine incorporation) reflects the rate of DNA synthesis and microbial growth. Bacterial

production has often been shown to correlate with EEA [*Foreman et al.*, 1998], and in this study all three enzymes show positive correlations with bacterial production rates. Thymidine-based bacterial production in the cryoconites sampled during this study ranged from 36 ng C l^{-1} d^{-1} in the overlying ice, to 3329 ng C l^{-1} d^{-1} in the bottom sediments of cryoconites from the Canada Glacier. Bacterial production in the cryoconites is within the range of that found in the water columns of the dry valley lakes (>0–9 ng C ml^{-1} d^{-1}) [*Takacs and Priscu*, 1998]. Similar to the carbon and nitrogen flux calculations described above, if one assumes the same number of cryoconites per glacier, and bacterial production of 36 ng C l^{-1} d^{-1} (lowest measured in this study) for 30 d this yields conservative estimates of 4.5 g, 2.3 g and 0.5 g of bacterial carbon production on the Commonwealth, Canada and Howard glaciers, respectively. If the highest bacterial production values extrapolated in this study were used (3329 ng C d^{-1}), the bacterial carbon yield would increase to 415 g, 215 g, and 45 g for the Commonwealth, Canada and Howard glaciers, respectively for the same 30 d season.

[33] Fluorescent in situ hybridization analysis revealed distinct differences in the community structure between overlying ice and sediment layers in the cryoconites as well as differences among the glaciers in the Taylor Valley. Overall hybridization rates of Eubacteria were lower in the overlying ice layers, possibly because of smaller bacterial cell sizes and lower RNA content which could have decreased detection [*Alfreider et al.*, 1996]. *β-Proteobacteria* dominate in many alpine snow and ice samples [*Alfreider et al.*, 1996; *Battin et al.*, 2001] and were always found to be higher in the top ice layers of the cryoconites rather than the sediment layers in our study. Bacteria from the *Cytophaga-Flavobacteria* cluster were dominant in the sediment layers of all Taylor Valley cryoconites studied. According to Bergey's manual [*Reichenbach*, 1989], members of the *Cytophaga* genus are capable of degrading complex macromolecules, mainly proteins and polysaccharides, which correlates with our activity and substrate utilization measurements. Useful comparisons can be drawn between the dry valleys (high latitude) and frozen alpine environments (high altitude). In the alternating slush and ice layers of frozen high-alpine lakes [*Psenner et al.*, 1999] nutrients and debris accumulate within slush layers providing favourable conditions for bacteria. The ice layers act as a lid and a trap for aeolian material, which is then made available during rare melting events. The ice lids covering the dry valley cryoconites may play a similar function acting as solar greenhouses, protecting the inhabitants from the harsher environment outside of the holes [*Tranter et al.*, 2004] and allowing for complex communities to develop over time.

[34] The McMurdo Dry Valley cryoconites have previously been shown to harbor resident populations of eukaryotic and cyanobacterial phototrophic organisms and heterotrophic bacteria [*Mueller et al.*, 2001; *Christner et al.*, 2003; *Porazinska et al.*, 2004]. Although indirect evidence such as extreme values of $pCO2$ and O2 saturation [*Tranter et al.*, 2004] imply active metabolism, few studies have investigated the metabolic diversity within these microcosms. We show that there is both metabolic and phylogenetic diversity between cryoconites from various glaciers within the region and vertically (overlying ice vs. sediment layer) within individual cryoconites. We further contend that the organisms within cryoconites and their associated biogeochemical transformations may provide the surrounding desert environment with a diversity of microorganisms and essential nutrients to support their metabolism. By virtue of their seasonal liquid water production, cryoconites provide a refuge for organisms from the extremely dry and cold conditions present in near-surface environments within the MCM Dry Valleys. Consequently, cryoconites are similar to the liquid water inclusions that have been observed in the permanent ice covers of the lakes in the region [*Priscu et al.*, 1998; *Paerl and Priscu*, 1998] in that they serve as an oasis for life in what would otherwise appear to be an inhospitable environment.

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References

Alfreider, A., J. Pernthaler, R. Amann, B. Sattler, F. O. Glöckner, A. Wille, and R. Psenner (1996), Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by in situ hybridization, *Appl. Environ. Microbiol.*, *62*, 2138–2144.

Battin, T. J., A. Wille, B. Sattler, and R. Psenner (2001), Phylogenetic and functional heterogeneity of sediment biofilms along environmental gradients in a glacial stream, *Appl. Environ. Microbiol.*, *67*, 799–807.

Chin, T. J. (1981), Hydrology and climate in the Ross Sea area, *J. R. Soc. N. Z.*, *11*, 373–386.

Christian, J. R., and D. M. Karl (1995), Bacterial ecto-enzymes in marine waters: Activity ratios and temperature responses in three oceanographic provinces, *Limnol. Oceanogr.*, *40*, 1042–1049.

Christner, B. C., B. H. Kvitko, and J. N. Reeve (2003), Molecular identification of Bacteria and Eukarya inhabiting an Antarctic cryoconite hole, *Extremophiles*, *7*, 177–183.

Christner, B. C., G. Royston-Bishop, C. M. Foreman, B. R. Arnold, M. Tranter, K. A. Welch, W. B. Lyons, A. I. Tsapin, and J. C. Priscu (2006), Limnological conditions in subglacial Lake Vostok, Antarctica, *Limnol. Oceanogr.*, *51*, 2485–2501.

Costerton, J. W. (1995), Overview of microbial biofilms, *J. Ind. Microbiol.*, *15*, 137–140.

Dodd, C. E. R., R. L. Sharman, S. F. Bloomfield, I. R. Booth, and G. S. A. B. Stewart (1997), Inimical processes: Bacterial self-destruction and sublethal injury, *Trends Food Sci. Technol.*, *8*, 238–241.

Doran, P. T., et al. (2002), Antarctic climate cooling and terrestrial ecosystem response, *Nature*, *415*, 517–520.

Dore, J. E., and J. C. Priscu (2001), Phytoplankton phosphorus deficiency and alkaline phosphatase activity in the McMurdo Dry Valley lakes, Antarctica, *Limnol. Oceanogr.*, *46*, 1331–1346.

Foreman, C. M., P. Franchini, and R. L. Sinsabugh (1998), The trophic dynamics of riverine bacterioplankton: Relationships among substrate availability, ecto-enzyme kinetics, and growth, *Limnol. Oceanogr.*, *43*, 1344–1352.

Fortner, S. K., M. Tranter, A. Fountain, W. B. Lyons, and K. A. Welch (2005), The geochemistry of supraglacial streams of Canada Glacier, Taylor Valley (Antarctica) and their evolution into proglacial waters, *Aquat. Geochem.*, *11*, 391–412.

Fountain, A. G., G. L. Dana, K. J. Lewis, B. H. Vaughn, and D. M. McKnight (1998), Glaciers of the McMurdo Dry Valleys, southern Victoria Land, Antarctica, in *Ecosystem Dynamics in a Polar Desert, the McMurdo Dry Valleys, Antarctica*, edited by J. C. Priscu, pp. 65–76, *Antarct. Res. Ser.* vol. 72, AGU, Washington, D. C.

Fountain, A. G., et al. (1999), Physical controls on the Taylor Valley ecosystems, Antarctica, *Bioscience*, *49*, 961–971.

Fountain, A. G., M. Tranter, and T. H. Nylen (2004), Cryoconite holes of dry valley glaciers, Taylor Valley, Antarctica, *J. Glaciol.*, *50*, 35–45.

Freckman, D. W., and R. A. Virginia (1998), Soil biodiversity and community structure in the McMurdo Dry Valleys, Antarctica, in *Ecosystem Dynamics in a Polar Desert, the McMurdo Dry Valleys, Antarctica*, edited by J. C. Priscu, pp. 323–335, *Antarct. Res. Ser.*, vol. 72, AGU, Washington, D. C.

Fritsen, C. H., A. Grue, and J. C. Priscu (2000), Distribution of organic carbon and nitrogen in surface soils in the McMurdo Dry Valleys, Antarctica, *Polar Biol.*, *23*, 121–128.

Garland, J. L. (1996), Analytical approaches to the characterization of samples of microbial communities using patterns of potential carbon source utilization, *Soil Biol. Biochem.*, *28*, 213–221.

Glöckner, F. O., R. Amann, A. Alfreider, J. Pernthaler, R. Psenner, K. Trebesius, and K. H. Schleifer (1996), An in situ hybridization protocol for detection and identification of planktonic bacteria, *Syst. Appl. Microbiol.*, *19*, 403–406.

Gordon, D. A., J. C. Priscu, and S. Giovannoni (2000), Origin and phylogeny of microbes living in permanent Antarctic lake ice, *Microbial Ecol.*, *39*, 197–202.

Hobbie, J. E., and C. C. Crawford (1969), Bacterial uptake of organic substrates: New method of study and application to eutrophication, *Verh. Int. Limnol.*, *17*, 725–730.

Jeffrey, S. W., and G. F. Humphrey (1975), New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae, and natural phytoplankton, *Biochem. Physiol. Pflanzen*, *167*, 191–194.

Kirchman, D. L., R. G. Keil, and P. A. Wheeler (1990), Carbon limitation of ammonium uptake by heterotrophic bacteria in the sub-arctic Pacific, *Limnol. Oceanogr.*, *35*, 1258–1266.

Lancaster, N. (2002), Flux of eolian sediment in the McMurdo Dry Valleys, Antarctica: A preliminary assessment, *Arct. Antarct. Alpine Res.*, *34*, 318–323.

Löferer-Krößbacher, M., J. Klima, and R. Psenner (1998), Determination of bacterial cell dry mass by transmission and densitometric image analysis, *Appl. Environ. Microbiol.*, *64*, 688–694.

Lyons, W. B., K. A. Welch, A. G. Fountain, G. Dana, B. H. Vaughn, and D. M. McKnight (2003), Surface glaciochemistry of Taylor Valley, southern Victoria Land, Antarctica, and its relationship to stream chemistry, *Hydrol. Processes*, *17*, 115–130.

McCarthy, M. D., J. I. Hedges, and R. Benner (1998), Major bacterial contribution to marine dissolved organic nitrogen, *Science*, *281*, 231–234.

McIntyre, N. F. (1984), Cryoconite hole thermodynamics, *Can. J. Earth Sci.*, *21*, 152–156.

Mikucki, J. A., C. M. Foreman, J. C. Priscu, W. B. Lyons, B. Sattler, and K. Welch (2004), Geomicrobiology of Blood Falls: A saline, iron-rich sub-glacial feature of Taylor Glacier, Antarctica, *Aquat. Geochem.*, *10*, 199–220.

Mindl, B., A. Anesio, K. Meirer, A. Hodson, J. Laybourn-Parry, R. Sommaruga, and B. Sattler (2007), Factors influencing bacterial dynamics along a transect from supraglacial runoff to proglacial lakes of a high Arctic glacier, *FEMS Microbiol. Ecol.*, *59*, 307–317.

Moorhead, D. L., and J. C. Priscu (1998), The McMurdo Dry Valleys ecosystem: Organization, controls and linkages, in *Ecosystem Dynamics in a Polar Desert, the McMurdo Dry Valleys, Antarctica*, edited by J. C. Priscu, pp. 351–363, *Antarct. Res. Ser.* vol.72, AGU, Washington, D. C.

Moorhead, D. L., P. T. Doran, W. B. Lyons, D. M. McKnight, J. C. Priscu, R. A. Virginia, and D. H. Wall (1999), Ecological legacies: Impacts on ecosystem structure of the McMurdo Dry Valleys, *Bioscience*, *49*, 1009–1020.

Mueller, D. R., W. F. Vincent, W. H. Pollard, and C. H. Fritsen (2001), Glacial cryoconite ecosystems: A bipolar comparison of algal communities and habitats, *Nova Hedwigia*, *123*, 171–195.

Nordenskjöld, A. E. (1875), Cryoconite found 1870, July 19th-25th, on the inland ice, east of Auleitsivik Fjord, Disco Bay, Greenland, *Geol. Mag., Decade 2*, *2*, 157–162.

Paerl, H. W., and J. C. Priscu (1998), Microbial phototrophic, heterotrophic and diazotrophic activities associated with aggregates in the permanent ice cover of Lake Bonney, Antarctica, *Microbial Ecol.*, *36*, 221–230.

Paul, E. A., and F. E. Clark (1989), *Soil Microbiology and Biochemistry*, Elsevier, New York.

Perkins, D. (2002), *Mineralogy*, pp. 102–109, Prentice Hall, Upper Saddle River, N. J.

Porazinska, D. L., A. G. Fountain, T. H. Nylen, M. Tranter, R. A. Virginia, and D. H. Wall (2004), The biodiversity and biogeochemistry of cryoconite holes from McMurdo Dry Valley glaciers, Antarctic, *Arct. Antarct. Alpine Res.*, *36*, 84–91.

Porter, K. G., and Y. S. Feig (1980), The use of DAPI for identifying and counting aquatic microflora, *Limnol. Oceanogr.*, *25*, 943–948.

Posch, T., J. Pernthaler, A. Alfreider, and R. Psenner (1997), Cell-specific respiratoric activity of aquatic bacteria studied with the tetrazolium reduction method, cyto-clear slides, and image analysis, *Appl. Environ. Microbiol.*, *63*, 867–873.

Priscu, J. C. (1995), Phytoplankton nutrient deficiency in lakes of the McMurdo Dry Valleys, Antarctica, *Freshwater Biol.*, *34*, 215–227.

Priscu, J. C. (Ed.) (1998), *Ecosystem Dynamics in a Polar Desert, the McMurdo Dry Valleys, Antarctica*, *Antarct. Res. Ser.*, vol. 72, AGU, Washington, D. C.

Priscu, J., C. C. H. Fritsen, E. E. Adams, S. J. Giovannoni, H. W. Paerl, C. P. McKay, P. T. Doran, D. A. Gordon, B. D. Lanoil, and J. L. Pinckney (1998), Perennial Antarctic lake ice: An oasis for life in a polar desert, *Science*, *280*, 2095–2098.

Priscu, J. C., et al. (1999), Geomicrobiology of subglacial ice above Lake Vostok, Antarctica, *Science*, *286*, 2141–2144.

Psenner, R., and B. Sattler (1998), Microbial communities: Life at the freezing point, *Science*, *280*, 2073–2074.

Psenner, R., B. Sattler, A. Wille, C. H. Fritsen, J. C. Priscu, M. Felip, and J. Catalan (1999), Lake ice microbial communities in alpine and antarctic lakes, in *Cold Adapted Organisms, Ecology, Physiology, Enzymology, and Molecular Biology*, edited by R. Margesin and F. Schinner, pp. 17–31, Springer, New York.

Redfield, A. C. (1958), The biological control of chemical factors in the environment, *Am. Sci.*, *46*, 205–222.

Reichenbach, H. (1989), *Bergey's Manual of Systematic Bacteriology*, vol. 3, edited by J. T. Staley et al., pp. 2015–2021, Lippincott Williams & Wilkins, Baltimore, Md.

Sinsabaugh, R. L., and C. M. Foreman (2001), Activity profiles of bacterioplankton in a eutrophic river, *Freshwater Biol.*, *46*, 1239–1249.

Sinsabaugh, R. L., S. Findlay, P. Franchini, and D. Fisher (1997), Enzymatic analysis of riverine bacterioplankton production, *Limnol. Oceanogr.*, *42*, 29–38.

Smith, D. (1964), Antarctic geology, *Proceedings of the 1st International Symposium on Antarctic Geology*, *Cape Town*, edited by R. J. Adie, 758 pp., Elsevier, New York.

Takacs, C. T., and J. C. Priscu (1998), Bacterioplankton dynamics in the McMurdo Dry Valley lakes: Production and biomass loss over four seasons, *Microbial Ecol.*, *36*, 239–250.

Takacs, C. D., J. C. Priscu, and D. M. McKnight (2001), Bacterial dissolved organic carbon demand in McMurdo Dry Valley Lakes, Antarctica, *Limnol. Oceanogr.*, *46*, 1189–1194.

Takeuchi, N., S. Kohshima, T. Shiraiwa, and K. Kubota (2001), Characteristics of cryoconite (surface dust on glaciers) and surface albedo of a Patagonian glacier, Tyndall Glacier, southern Patagonia ice field, *Bull. Glaciol. Res.*, *18*, 65–69.

Tranter, M., A. G. Fountain, C. H. Fritsen, W. B. Lyons, J. C. Priscu, P. Statham, and K. Welch (2004), Extreme hydrochemical conditions in natural microcosms entombed within Antarctic ice, *Hydrol. Processes*, *18*, 379–387.

Veblen, D. R. (Ed.) (1981), *Amphiboles and other Hydrous Pyriboles–Mineralogy*, *Rev. Mineral.*, vol. 9A, 372 pp., Mineral. Soc. Am., Chantilly, Va.

Welch, K. A., W. B. Lyons, E. Graham, K. Neumann, K. M. Thomas, and D. Mikesell (1996), Determination of major element chemistry from Antarctica by ion chromatography, *J. Chromatogr. A*, *739*, 257–263.

Wharton, R. A., W. C. Vinyard, B. C. Parker, G. M. Simmons, and K. G. Seaburg (1981), Algae in cryoconite holes on Canada Glacier in southern Victoria land, Antarctica, *Phycologia*, *20*, 208–211.

Wharton, R. A., Jr., C. P. McKay, G. M. Simmons, and B. C. Parker (1985), Cryoconite holes on glaciers, *Bioscience*, *35*, 499–503.

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