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Microbial Phototrophic, Heterotrophic, and Diazotrophic Activities Associated with Aggregates in the Permanent Ice Cover of Lake Bonney, Antarctica

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ABSTRACT

The McMurdo Dry Valley lakes, Antarctica, one of the Earth's southernmost ecosystems containing liquid water, harbor some of the most environmentally extreme (cold, nutrient-deprived) conditions on the planet. Lake Bonney has a permanent ice cover that supports a unique microbial habitat, provided by soil particles blown onto the lake surface from the surrounding, ice-free valley floor. During continuous sunlight summers (Nov.–Feb.), the dark soil particles are heated by solar radiation and melt their way into the ice matrix. Layers and patches of aggregates and liquid water are formed. Aggregates contain a complex cyanobacterial–bacterial community, concurrently conducting photosynthesis (CO_2 fixation), nitrogen (N_2) fixation, decomposition, and biogeochemical zonation needed to complete essential nutrient cycles. Aggregate-associated CO_2- and N_2-fixation rates were low and confined to liquid water (i.e., no detectable activities in the ice phase). CO_2 fixation was mediated by cyanobacteria; both cyanobacteria and eubacteria appeared responsible for N_2 fixation. CO_2 fixation was stimulated primarily by nitrogen (NO_3^-), but also by phosphorus (PO_4^{3-}). PO_4^{3-} and iron ($FeCl_3$ + EDTA) enrichment stimulated of N_2 fixation. Microautoradiographic and physiological studies indicate a morphologically and metabolically diverse microbial community, exhibiting different cell-specific photosynthetic and heterotrophic activities. The microbial community is involved in physical (particle aggregation) and chemical (establishing redox gradients) modification of a nutrient- and organic matter-enriched microbial ''oasis,'' embedded in the desertlike (i.e., nutrient depleted) lake ice cover. Aggregate-associated production and nutrient cycling represent microbial self-sustenance in a microenvironment supporting ''life at the edge,'' as it is known on Earth.

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Introduction

The McMurdo Dry Valley, southern Victoria Land, Antarctica, is one of the most extreme microbial habitats on Earth. It is one of the highest, driest, and coldest deserts on the planet [27]. These glaciated valleys are located within the Transantarctic mountain range and are the largest ice-free expanse of land on the continent. The valleys are surrounded by 3,000+ m peaks that shield the region from appreciable precipitation (exclusively snowfall), imparting a year-round ''snow shadow,'' and leaving much of the valley floor soils exposed. Because air temperatures remain well below 0°C most of the year (avg. ~−20°C), the glacier-fed lakes comprising much of the valley floor have permanent ice covers [25]. Strong and persistent katabatic winds deposit soils on the ice cover. Because of differential ablation rates and cracking, the surface of the lake ice is uneven. Soil accumulates in ridges, crevices, and small holes in the ice surface. During mid- to late summer (Nov.–Feb.), air temperatures can be near 0°C. Continuous solar radiation promotes localized warming of dark soil aggregates. This allows water to associate with aggregates for up to 150 days per year [1, 8, 27]. Aggregates are able to melt their way into the ice cover (Fig. 1). They form distinct layers as they settle into the ice matrix [1]. The depths of layers depend on solar warming, snow deposition, ice ablation and ice surface morphology. Radiant-heated soil particles form interstitial water and aggregate as they become embedded in the ice matrix [1, 8, 28] (Fig. 1), providing a mineral-rich, aqueous microbial habitat.

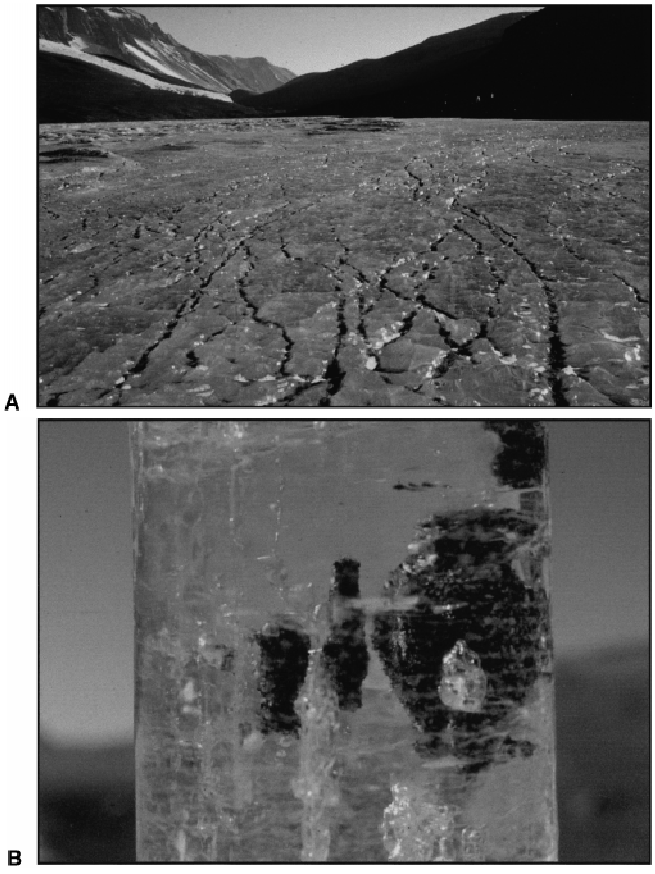

Fig. 1. **(A)** The permanent ice cover on Lake Bonney, McMurdo Dry Valley Lakes Region, Antarctica. Note ridges and depressions in the ice cover, which trap wind-blown soil particles as aggregates. **(B)** Side view of an ice core taken from 1.5 m depth in Lake Bonney's (East Lobe) ice cover. Soil aggregates (1–2 cm across) can be seen embedded in the core.

Microscopic examinations of freshly collected (in ice cores) aggregates reveal a morphologically diverse microbial community intimately associated with the aggregates [20, 27]. Molecular and photopigment analyses indicate that this community is phylogenetically diverse [9, 20, Olson et al., in press]. Both cyanobacterial phototrophs and bacterial heterotrophs have been identified, and the microbial community is most likely of soil origin [7, 9]. In addition, some protozoan and invertebrate grazers, including nematodes and tardigrades, have been observed.

On the surrounding dry valley floor, the primary life forms associated with soils include epilithic and endolithic prokaryotic (bacterial, actinomycete, cyanobacterial) communities [7]; nematodes and mosses [6]; and ubiquitously distributed cyanobacteria-dominated (*Nostoc* spp., *Phormidium* spp., *Scytonema* spp.) mats, associated with dry stream beds and patches of snow [11, 24, 25]. These mats remain desiccated (i.e., inactive), except for a few weeks of snowmelt in mid- to late summer [11, 25]. As a result, soils contain little organic matter [4]. Combined nitrogen, phosphorus, and organic matter are nutrients that potentially limit autotrophic and heterotrophic microbial production, respectively, in these lakes [10, 21, 23, 26]. Moreover, liquid water is essential for metabolic activity and growth [8, 24, 25]; its availability may be the overriding factor limiting life in the valleys.

These studies indicate that the *potential* for active microbial life exists with soil-based aggregates embedded in the ice cover. However, little is known about how the basic processes that synthesize ''new'' organic matter, namely CO_2 and N_2 fixation, function in this permanently frozen ecosystem. The overall objective of this study was to characterize soil-based aggregates as loci of microbial activity and production in the permanent ice cover. Specifically, (1) factors affecting rates of CO_2 and N_2 fixation in ice aggregate com-

munities were determined; (2) microbial organic matter utilization associated with aggregates was examined; (3) aggregate-associated microbial autotrophy and heterotrophy were localized; and (4) environmental controls on, and limitations of, the above processes were identified and assessed.

Materials and Methods

Study Site

Lake Bonney is a narrow (7 km long) glacial lake located at the head of Taylor Valley, southern Victoria Land, Antarctica (77° 43′ S, 162° 23′ E). The lake comprises eastern and western lobes, both of which have a permanent ice cover. The thickness of the cover is approximately 4 m; it varies spatially and seasonally from 3.5 to 4.5 m [1, 3, 8, 28].

Ice core samples, collected from 0.5 to 2 m depths, were analyzed for phototrophic, heterotrophic, and diazotrophic activities during two consecutive early austral spring periods, September–October 1995 and October–November 1996.

Microautoradiography

Microautoradiography was used to examine CO_2 fixation and heterotrophic utilization of organic matter on scales ranging from individual microorganisms (μm) to aggregates (mm). This technique is complementary to [^{14}C]CO_2 fixation assessments of primary production and ^{3}H- or ^{14}C-labeled organic matter uptake studies. The method is noninvasive, allowing for microscopic observation of intact aggregates and associated microorganisms, following incubation with radioisotopes. Details concerning its general utility and application in aquatic microbial productivity, heterotrophy, and nutrient-cycling studies are provided elsewhere [15, 16, 18].

We examined [^{14}C]CO_2 and ^{3}H dissolved organic matter (DOM; D-glucose, L-amino acid mixture) uptake in parallel with rate measurement studies on intact aggregates. Small ice blocks (200–500 cm^3) containing soil aggregates were cored from approximately 2 m depth in Lake Bonney's ice cover. Ice blocks were melted in the dark at 4°C, in 500 ml beakers. Meltwater (18 ml) samples containing aggregates were transferred, in triplicate, to 20 ml clear and opaque (foil-wrapped) glass liquid scintillation vials that had been rinsed with acid (5% HCl) and deionized water. Radioisotopes were then added at trace concentrations. In individual experiments, we added 10 μCi [^{14}C]$NaHCO_3$ (58 mCi mmol^{-1}; ICN Inc.), 15 μCi of a uniformly labeled L-[^{3}H]amino acid mixture (240 mCi mmol^{-1}; ICN Inc.), 12 μCi of uniformly labeled D-[^{3}H]glucose (220 mCi mmol^{-1}; ICN Inc.), and 10 μCi of [^{3}H]thymidine (20 Ci mmol^{-1}; New England Nuclear). Samples were incubated under either illuminated (80 μmol photons m^{-2}sec^{-1} photosynthetically active radiation = PAR) or dark conditions at 4°C, from 2 to 12 h, in an incubator with cool white fluorescent lighting. Borate-buffered (pH 7.5) formalin (2%) was added to terminate the incubations. Incorporation of either [^{14}C]CO_2 or ^{3}H-labeled organics was monitored by liquid scintillation spectrometry, using a Packard Tri Carb liquid scintillation counter. Counting efficiencies for ^{14}C ranged from 91 to 96%, and for ^{3}H from 42 to 45%.

The potential stimulatory effects of nutrients on aggregate-associated [^{14}C]CO_2 fixation were examined by conducting several nutrient enrichment experiments, which were run in parallel with the radioisotope uptake assays described above. Nutrients added included nitrate, as KNO_3 (10 μM); phosphate (PO_4^{3-}), as KH_2PO_4 (5 μM); and iron as $FeCl_3$ + EDTA (as a chelator), each at 2 μM. Nutrients were added 24 to 72 h before [^{14}C]CO_2 uptake assays.

For preparing microautoradiographs, formalin-preserved meltwater subsamples, containing radiolabeled aggregates, were gently gravity-filtered onto 0.45 μm porosity HA Millipore filters. Filters were rinsed several times with unlabeled, 0.2 μm prefiltered meltwater, to remove excess radioisotope. Filters were then air dried, optically cleared on microscope slides under fuming acetone, and prepared for microautoradiography [15, 16, 18]. A liquid nuclear track emulsion (Kodak NTB-2) was used as the radiosensitive emulsion [18]. Exposed (for 1–3 weeks under complete darkness) slides containing radioactive samples were processed, washed, air dried, and viewed by phase contrast light microscopy. Microphotographs were recorded on Ilford Pan F 35 mm film.

Nitrogenase Activity (Acetylene Reduction Assays)

Ice cores containing aggregates were melted, as described for microautoradiography. For each treatment, samples containing 8 g (wet weight) sediment were placed in triplicate 36 ml serum bottles. Filtered (0.45 μm HA Millipore) meltwater (20 ml) was added to each bottle; the bottles were sealed with serum stoppers. In some instances, freshly collected aggregates were maintained in a frozen state and incubated alongside melted samples. The following treatments were assayed for N_2 fixation rates (nitrogenase activity), using the acetylene reduction technique [1]; (1) illuminated, (2) dark, and (3) illuminated + 3(3,4 dichlorophenyl)-1,1-dimethylurea (DCMU; 2×10^{-5} M). Serum bottles containing only 20 ml of deionized water served as blanks. They were incubated in parallel with samples. Each bottle was injected with 4 ml acetylene (generated by the addition of deionized H_2O to calcium carbide). The bottles were incubated at 4°C for 4–12 h. Constant illumination was provided by cool-white fluorescent lights at ~80 μmol photons m^{-2}sec^{-1}. Following incubation, 2 ml aliquots of the headspace gas were removed and injected into evacuated serum vials for gas chromatographic analysis of ethylene. Any ethylene contamination in blanks was subtracted from incubated samples. We utilized either Carle AGC 311 or Shimadzu GC 9 gas chromatographs, equipped with 2-m Poropak Q columns and FID detectors, for gas analysis [2].

On several occasions, samples were preincubated with nutrients previously shown to help regulate N_2 fixation in a variety of marine and freshwater environments [17], including the liquid water columns of Lake Bonney and other dry valley lakes [21, 23, 24]. These included: (1) phosphate (PO_4^{3-}), as KH_2PO_4 (5 μM); (2) iron as $FeCl_3$ + EDTA (as a chelator), each at 0.5 μM; and (3) mannitol at 2 mM. Samples were preincubated with nutrients for 24 to 72 h before acetylene reduction assays. Nutrients were administered in

Table 1. Relative proportions of $^{14}CO_2$ ($NaH^{14}CO_3$) fixation, [^{3}H]glucose, and amino acid mixture uptake associated with ice aggregates vs surrounding ice matrix in Lake Bonney's ice cover[a]

Substrate type	Incubation conditions	Radioactivity (DPM × 10^3) and % of total incorporation			
		Sediment		Aqueous supernatant	
$^{14}CO_2$ ($NaH^{14}CO_3$)	Illuminated	25.3 ± 0.13	98%	0.29 ± 0.05	2%
$^{14}CO_2$ ($NaH^{14}CO_3$)	Dark	0.02 ± 0.004	71%	0.008 ± 0.003	29%
[^{3}H]Glucose	Illuminated	18.7 ± 0.84	96%	0.79 ± 0.13	4%
[^{3}H]Glucose	Dark	16.5 ± 1.34	97%	0.54 ± 0.25	3%
^{3}H-labeled amino acid mix	Illuminated	14.6 ± 2.29	94%	0.91 ± 0.54	6%
^{3}H-labeled amino acid mix	Dark	13.9 ± 1.46	95%	0.66 ± 0.23	5%

[a] Results are from melted 2 m-deep ice core samples incubated, in triplicate 20 ml vials, with individual radiolabeled substrates for 12 h, followed by separation of aggregates (by sedimentation), determinations of radioactivity (DPM × 10^3) and percentage of total uptake in sediment and aqueous fractions.

triplicate and compared to untreated controls. Responses were examined under illumination.

Tetrazolium Reduction

The reduction of 2,3,5-triphenyltetrazolium chloride (TTC) was examined as an indication of localized respiratory O_2 consumption, leading to the development of O_2 depletion. Because of its relatively high redox potential (−0.40 V), TTC is converted to bright red formazan crystals under fully reduced conditions. These conditions are suitable for O_2-sensitive processes such as N_2 fixation [16, 17, 19]. TTC is also nontoxic to a wide range of phototrophic and heterotrophic microorganisms [16]. Localized TTC reduction was micro- and macroscopically examined, in order to delineate microaerophilic and anoxic regions in aggregates. TTC was added, at 0.01% w/v, to a variety of melted aggregate samples on which parallel radioisotope uptake and nitrogenase activity was measured. Following 12- to 36-h incubations, TTC reduction was stopped by adding 2% formalin. This also preserved samples for subsequent microscopic observations.

Results and Discussion

Phototrophic Activity

Sediment–meltwater separation and autoradiographic studies indicated that a vast majority of photosynthetic [^{14}C]CO_2 incorporation was associated with aggregates, as opposed to individual microorganisms embedded in the ice matrix. When soil aggregates were separated from meltwater by settling (after incubation with [^{14}C]CO_2), at least 95% of phototrophy was confined to aggregates (Table 1). Microautoradiographs confirmed this result. Figure 2 illustrates the radioexposures obtained from ^{14}C incubations. A variety of exposures illustrates the morphologies and sizes of phototrophs associated with aggregates. These included fairly abundant, small (1–2 μm), individual and colonial coccoid cells, a few rod-shaped cells ranging in length from 1 to 3 μm, and a high abundance of filaments ranging in length from 10 μm to over several hundred micrometers (Fig. 2). Substantial differences in cell-specific ^{14}C labeling were observed, indicating varying activities among phototrophs. In addition, some filaments and rods were devoid of labeling, indicating that these cells were either inactive or nonviable. Very few freely suspended, active phototrophic cells were observed; some suspended cells may have been dislodged from aggregates during sample preparation.

Photosynthetic activity appeared confined to the liquid phase. When melted aggregates received [^{14}C]bicarbonate, were rapidly refrozen, and incubated (frozen) alongside melted samples, no net ^{14}C uptake was detectable (i.e., not significantly different from deionized water blanks; $P > 0.05$), under either illuminated or dark conditions. Our aggregate-based results agree with previous studies of constraints on dry valley stream microbial mat communities [23–25], which showed no net photosynthetic activities when frozen; however, photosynthesis commenced immediately after thawing or wetting.

Detailed autoradiographic and fluorescence microscopic observations indicated that a majority of the phototrophs exhibiting ^{14}C labeling were cyanobacteria. Cyanobacterial taxa associated with aggregates included solitary and colonial coccoid cells (filamentous, non-heterocystous forms, including relatively thin (0.5–1 μm) *Phormidium* sp. and wider (2–4 μm) *Oscillatoria* sp. filaments). In addition, short filaments of a heterocystous cyanobacterium resembling *Nostoc* sp. were found. All cell types revealed red autofluorescence associated with chlorophyll *a* [22]. This indicated they were cyanobacteria, as opposed to other photosynthetic prokaryotes (i.e., photosynthetic bacteria). Molecular (16S rRNA) characterization of microbial taxa associated with the aggre-

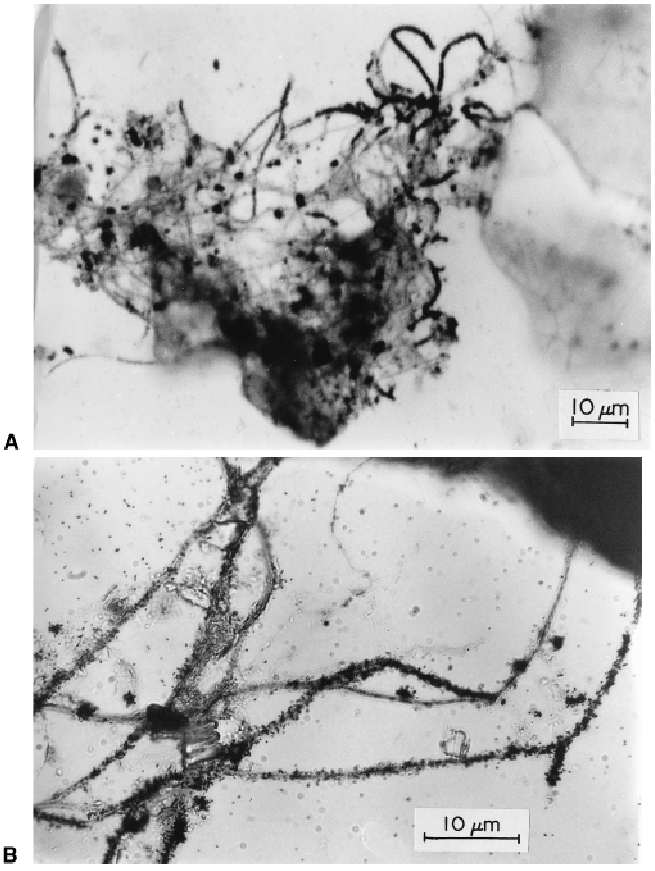

Fig. 2. **(A)** Low magnification (originally ×200) microautoradiograph of photosynthetic $^{14}CO_2$ incorporation, shown as deposition of opaque (black) silver grains, in aggregate-associated cyanobacteria. Sample was obtained from 1.5 m depth in the ice cover. Both filamentous and coccoid cyanobacteria show radiolabeling. Radiolabeling varied among actively photosynthesizing cyanobacteria. All cyanobacteria showed chlorophyll *a*-associated autofluorescence. **(B)** High magnification (originally ×400) microautoradiograph, showing photosynthetic $^{14}CO_2$ incorporation associated with individual cyanobacterial filaments and coccoid cells. Some unexposed regions of filaments, showing the stained filaments themselves, can be seen, while coccoid cells are entirely covered by silver grains.

gates has revealed substantial cyanobacterial and eubacterial diversity [9, 22]. *Phormidium*, *Scytonema*, *Synechoccus*, and *Nostoc* were among the cyanobacterial genera identified. Independent characterization of N_2-fixing genera, utilizing PCR amplification of the structural gene for the N_2-fixing enzyme subunit dinitrogenase (*nifH*), identified *Nostoc* sp. as a cyanobacterial N_2 fixer in aggregates (Olson et al., in press). Very few eukaryotic algae were associated with aggregates or suspended in the ice matrix. This sharply contrasts with the underlying water column, which is dominated by chrysophytes, cryptophytes, and chlorophytes [13, 14].

Cyanobacterial phototrophs were tightly bound to and, at times, densely covered soil particles constituting the aggregate cores (Fig. 3). Filamentous and coccoid cyanobacteria were often found adhering to several particles simultaneously (Fig. 3), indicating their possible involvement in the sediment aggregation process. This observation is reminiscent of detrital aggregation in freshwater and marine ecosystems, where bacteria and microalgae associated with suspended and settled detrital particles excrete adhesive polymers involved in adhesion and aggregation [15, 16]. Ice aggregate-associated microbiota formed weblike extracellular matrices in which a variety of microorganisms and small amorphous mineral and organic particles were trapped. This suggested a role in the aggregation process, at least during the liquid water growth phase, when growth could commence.

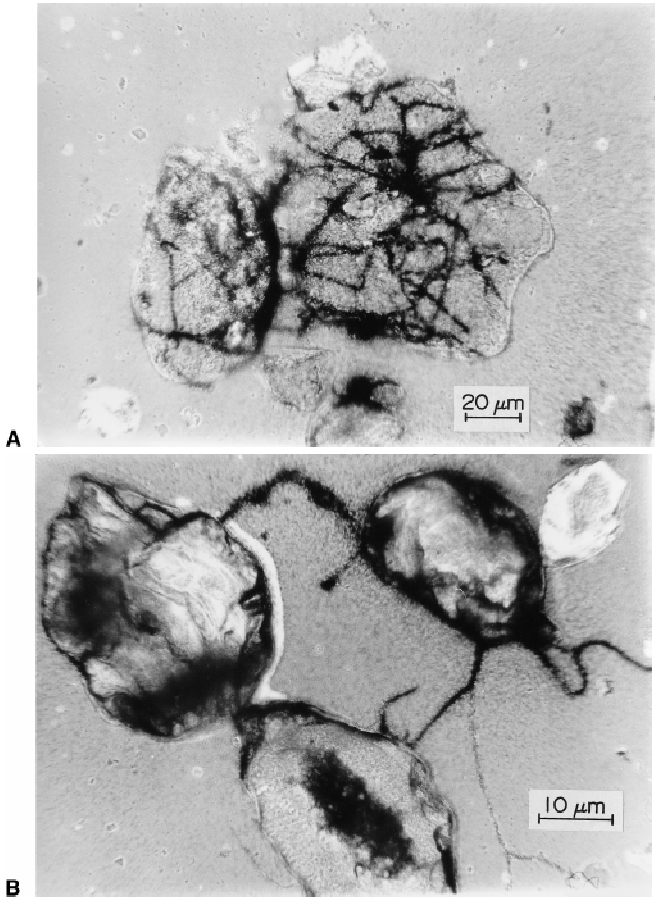

Fig. 3. **(A)** Microautoradiograph (original magnification ×200), showing photosynthetically active (light-mediated $^{14}CO_2$ incorporation) filamentous cyanobacteria firmly attached to soil particles embedded in the ice cover (2 m depth). **(B)** Photosynthetically active (^{14}C labeled) filamentous cyanobacteria simultaneously attached to several soil particles. Same sample as shown in Fig. 1A.

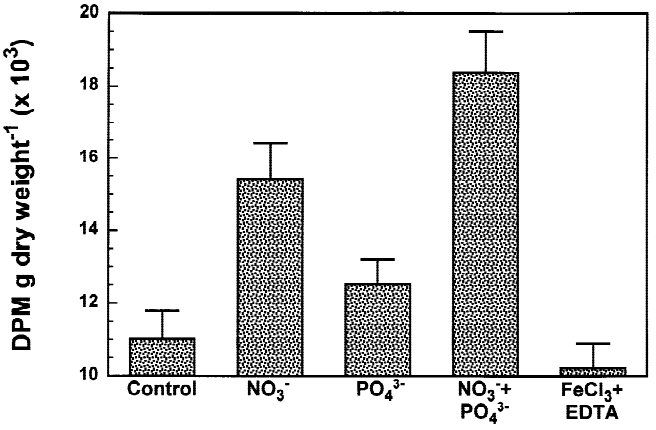

Fig. 4. Results from a $^{14}CO_2$ fixation bioassay, in which melted Lake Bonney ice aggregates (1 m depth) were enriched with various nutrients, as indicated. Results are expressed as DPM ^{14}C incorporation g^{-1} aggregate dry weight. Standard error bars among triplicate samples are shown.

Bioassay experiments indicated that light-mediated [^{14}C]CO_2 fixation was stimulated in response to nitrogen (added as NO_3^-) and nitrogen-plus-phosphorus (added as PO_4^{-3}) enrichment (Fig. 4). When added individually, N led to the greatest degree of stimulation. N and P together elicited maximal stimulation. No stimulation in response to iron (either $FeCl_3$ or $FeCl_3$ + EDTA) was observed. Observations of microautoradiographs with epifluorescence microscopy showed the autofluorescing cyanobacterial community to be the source of enhanced ^{14}C uptake in response to N and P enrichment. These results suggested that CO_2 fixation may be limited primarily by N, and secondarily by P availability, substantiating earlier observations of nutrient limitation in stream [10] and water column phytoplankton communities in the dry valley lakes region [21, 23, 26]. Microautoradiographs indicated that N additions enhanced cellular [^{14}C]CO_2 fixation rates among aggregate-associated cyanobacteria (Fig. 5). Within and among aggregates, differences in cellular [^{14}C]CO_2 fixing activities were observed among filamentous and coccoid/rod-shaped cyanobacteria. These differences possibly indicated microscale differences in growth potential, physiological state, and/or nutrient supplies.

Heterotrophic Activity

Microbial utilization of ^3H-labeled glucose, amino acids (mixture), and thymidine were examined by uptake studies and microautoradiography. All aggregate samples exhibited metabolically mediated uptake of these organic compounds (Table 1). Formalin-treated abiotic controls revealed no significant uptake of any of these compounds (i.e., ^3H incorporation), relative to prefiltered controls. Biologically mediated uptake was at least tenfold higher (in terms of DPM uptake) than in either controls or formalin-treated samples. There was often no significant difference between light- and dark-mediated uptake of these compounds. Uptake of glucose and amino acids was slightly (but not significantly; $P > 0.05$) higher in light than dark samples, in 3 out of 15 occasions.

Microautoradiographs indicated that virtually all the organic matter uptake was mediated by nonfluorescent, bacterial-size rods (0.5–1 μm length) and filaments (0.5 μm width) closely associated with aggregates (Fig. 6). Heterotrophic bacteria were attached to soil particles and associated

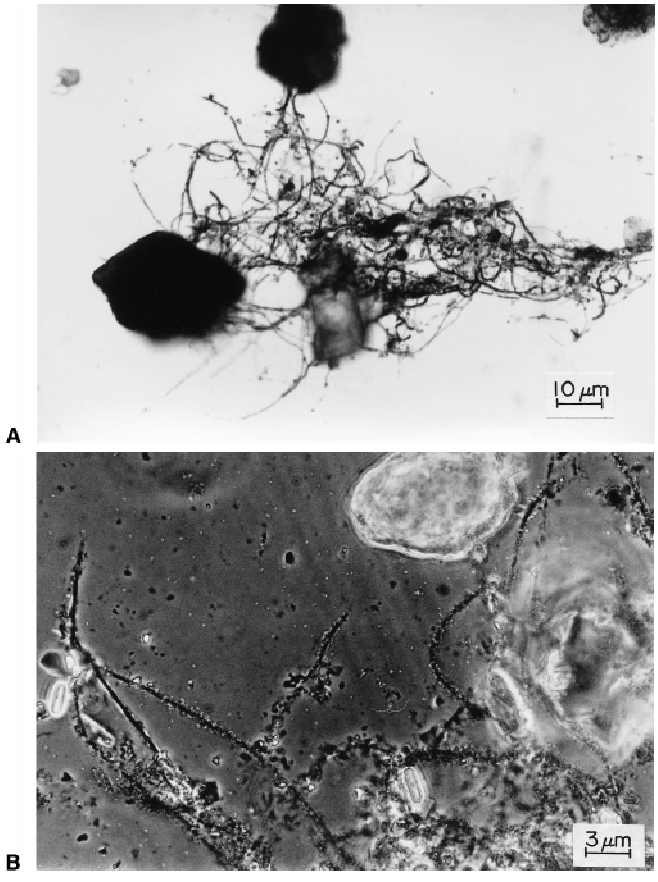

Fig. 5. **(A)** Low-magnification microautoradiograph, showing heavily labeled (^{14}C), aggregate-associated, filamentous (*Phormidium*-like), autofluorescent cyanobacteria, in response to N (as NO_3^-) enrichment (sample from bioassay shown in Fig. 4). **(B)** High-magnification microautoradiograph, showing individual ^{14}C labeled *Phormidium*-like cyanobacterial filaments, in response to N enrichment.

Fig. 6. Microautoradiograph (original magnification ×400), showing heterotrophic uptake of [^{3}H]glucose by nonautofluorescent bacterial filaments attached to soil particles embedded in the ice cover (1.5 m depth). Note that some filaments were heavily labeled (black), while others (light gray) were virtually devoid of labeling.

with cyanobacterial colonies and aggregates. These observations are similar to those reported for temperate and tropical cyanobacteria-dominated surficial communities, such as aggregates, biofilms, and mats, where heterotrophic bacteria are closely associated with cyanobacterial phototrophs. In these communities, microheterotrophs form metabolite-exchanging consortial associations with their phototrophic counterparts, including colonization of mucilagenous sheaths, heterocysts, and akinetes [19]. Metabolite exchange may be mediated by consortial interactions in ice aggregate-associated microbial communities, but is confined to the relatively brief summer period when localized melting occurs.

The uptake of all the organics administered here was almost solely mediated by bacteria-like cells, as judged by size (Fig. 6) and lack of autofluorescence (not shown). Cyanobacterial cells and filaments revealed only marginal glucose uptake, and no significant amino acid or thymidine uptake. These results confirm that the uptake of [^{3}H]thymidine, which has been used to measure bacterial production in ice aggregate communities [22], is exclusively mediated by heterotrophic bacterial cells.

Cell-specific uptake of all organic substrates administered among microheterotrophic communities varied substantially. Differential cell-specific uptake may reflect metabolic diversity, contrasting growth rates, senescence or dormancy, or biogeochemical gradients in aggregates.

Diazotrophy

Aggregate microbial communities were capable of N_2 fixation, as confirmed by nitrogenase activity (acetylene reduction) measurements (Fig. 7). Rates of nitrogenase activity were low when compared to temperate and tropical microbial assemblages [17]. Detectable activity was only observed in liquid water; when ice aggregates were kept frozen and assayed by acetylene reduction, no significant differences ($P > 0.05$) were observed. This proved to be the case for all incubation periods. In their examinations of N_2-fixing activities of dry valley stream microbial mat communities, Vincent et al. [10, 24, 25] also reported no detectable nitrogenase activity in the absence of liquid water. In melted aggregates, activity occurred in both light and dark conditions, with activity being highest in light (Fig. 7). The addition of DCMU reduced nitrogenase activity under light to the level of dark samples (Fig. 7). Light-driven nitrogenase activity was stimulated by P and Fe (Fig. 8), evidence that availability of these nutrients may be restricted. These results indicate that phototrophs are, at least in part, responsible for N_2-fixing activity associated with aggregates. Since cyanobacteria are numerically dominant phototrophs, they are the most likely source of light-mediated fixation. A few filamentous, heterocystous cyanobacteria resembling the genus *Nostoc* were found associated with aggregates. Since heterocystous cyanobacteria are diagnostic of N_2 fixation [5], this is direct evidence that cyanobacteria play a role in aggregate N_2 fixation dynamics. Other, nonheterocystous, cyanobacterial genera found associated with aggregates (i.e., *Lyngbya*, *Oscillatoria*, *Phormidium*) may also be diazotrophic; however,

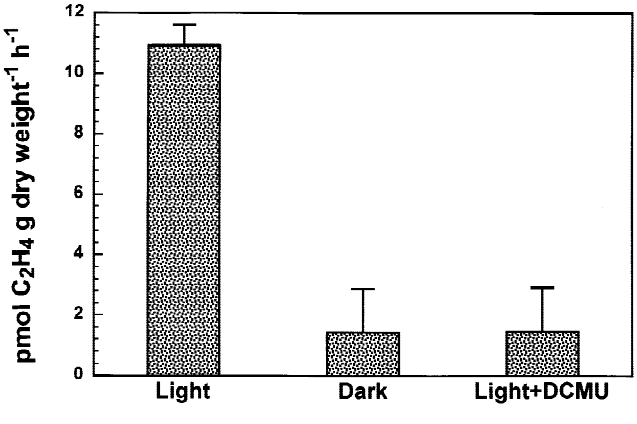

Fig. 7. Nitrogenase activity associated with ice aggregates (1.5 m depth). Note that the activity is normalized for dry weight of the aggregates. Standard error bars for triplicate samples are shown.

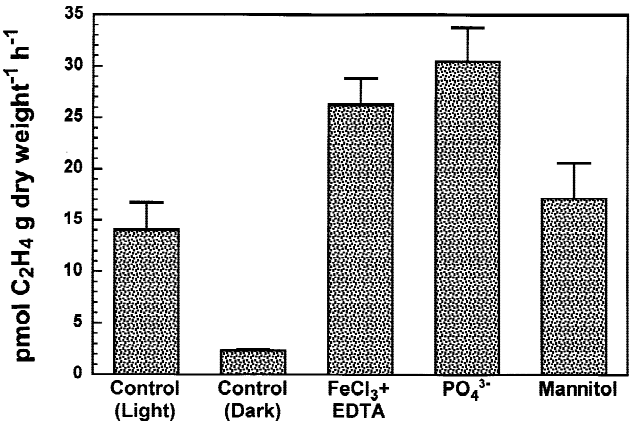

Fig. 8. Results from a nitrogenase activity assay, in which nutrients were added to ice aggregates sampled from 1.5 m depth. Results are normalized for dry weight of the aggregates. Standard error bars for triplicate samples are shown.

evidence for this is far less certain, since there are no definitive structural features to confirm N_2 fixing capabilities. PCR-based (*nifH*) screening of aggregates for N_2-fixing taxa has thus far revealed a *Nostoc*-like sequence, as well as evidence for microaerophilic bacterial (*Klebsiella, Vibrio*) contributions to N_2 fixation (Olson et al., in press).

Tetrazolium Reduction as an Indicator of Biogeochemical Zonation

Tetrazolium salt (TTC) reduction assays revealed that, when melting occurs, localized O_2 consumption associated with aggregates is sufficient for creating reduced microzones (Fig. 9). These microzones were associated with regions colonized by bacteria and cyanobacteria, suggesting they may be potential sites of O_2-sensitive processes such as N_2 fixation. In previous microscale aggregate studies where the reduction of the tetrazolium salt 2,3,5-triphenyltetrazolium chloride (TTC; $E' = -0.4$ V) was observed microscopically, Paerl and Pinckney [19] noted microscale gradients in O_2 tension (i.e., redox potentials). Microalgal and bacterial biomass and activities were heterogeneously distributed among aggregates [20], promoting the development of O_2 and, possibly, other biogeochemical (nutrients, pH, S^{-2}) gradients. Patchiness, resulting from microscale differences in microbial metabolic activities (i.e., photosynthesis, respiration), is the likely cause of biogeochemical zonation and diffusional O_2 and nutrient concentration gradients. These gradients, in turn, promote metabolic diversity and differential photosynthetic and heterotrophic growth rates.

Consortial Microbial Interactions in Ice Aggregates: Linkage to Survival and Growth

Phototrophy, heterotrophy, and diazotrophy can occur simultaneously in ice aggregate microbial communities. Key environmental factors controlling the rates and biogeochemical significance of these processes include: (1) the presence of liquid water; (2) N, P, and trace metals sufficient for phototrophy; (3) adequate organic matter (for meeting C and energy requirements) for heterotrophy; and (4) an energy source (light or organic C), P, Fe, and other trace metals sufficient for diazotrophy. Soil particles comprising the aggregate core are the sources of minerals (P, Fe, trace metals) needed to sustain these processes. Release of mineral constituents may be facilitated by microbial metabolic activity associated with particles, especially mineralization of organic matter, which consumes O_2 (respiration) and liberates CO_2, thus creating reduced, relatively low pH conditions well-suited for solubilization of a variety of mineral elements.

Mineralization is highly dependent on organic matter availability, the main source of which is photosynthesis. Therefore, close proximity of heterotrophs to phototrophs is essential for completion of carbon cycling, specifically the production and respiration of organic C. The paucity of higher trophic level heterotrophs (e.g., protozoans) magnifies the importance of microbial mineralization of photosynthetically fixed C and nutrients. Net photosynthetic production is confined to the illuminated, aqueous phase, sum-

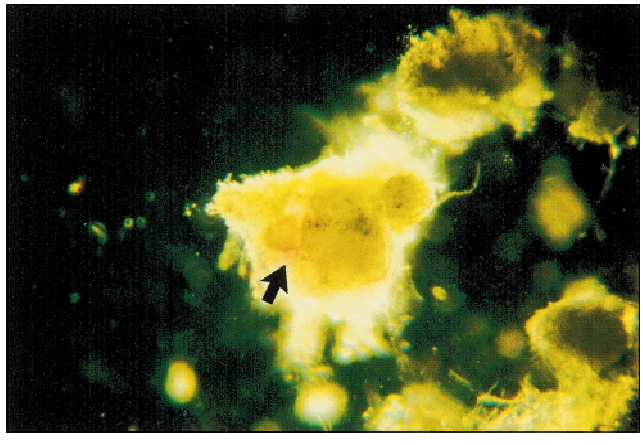

Fig. 9. Dark-field light micrograph, showing the deposition of reduced 2,3,5-triphenyltetrazolium chloride (TTC) in melted aggregates from 2 m depth in Lake Bonney's ice cover (W. Lobe). Reduced TTC deposition appears as accumulations of red formazan crystals (arrow).

mer period (up to ~150 days). Because the ice cover is highly transparent to photosynthetically active radiation (2–6% of incident radiation at the base of the ice cover) [1, 12], photosynthesis is possible throughout the ice cover during this period. Under aqueous conditions, the key environmental factors controlling photosynthesis are CO_2, inorganic nutrients, and temperature. CO_2 is supplied by the atmosphere, by leaching of carbonate-containing sediments, and by mineralization of previously formed organic matter. Phosphorus, Fe, and other trace metals must be largely derived from soils and, to a lesser extent, from the ice meltwater, through which the aggregates move. The remaining limiting factor is N. In light of the fact that there is preciously little N associated with soils [3, 4], snow, and ice [8], atmospheric N_2 appears to be an important alternative N source. This situation places a crucial demand on diazotrophy, the biological ''bottleneck'' process capable of potentially meeting microbial community N demands [17]. Nutrient addition bioassays indicate that the rates of P and Fe supplied from soils may control N_2 fixation potential. The reliance on biologically fixed N puts a premium on optimizing N_2 fixation in close proximity to phototrophy and heterotrophy. Conversely, heterotrophic regeneration and solubilization of P, Fe, and other potentially limiting factors, as well as localized O_2 consumption, may play key roles in sustaining N_2-fixing activity.

Clearly, the ability to spatially and temporally coordinate and complement phototrophy, heterotrophy, and diazotrophy along biogeochemical gradients in aggregates is of fundamental importance for initiating, maintaining, and optimizing these essential life-sustaining production and nutrient-cycling processes. Close spatial and temporal coupling of metabolite exchange among producers and consumers of organic matter would appear to be *the* limiting ecological factors enabling microbial processes to coexist in what may best be characterized as ''the edge of life.'' To accomplish this feat, microbial communities have had to evolve in a highly cooperative and efficient manner, where, functionally, ''one organism's trash is another's treasure'' [19].

If extraterrestrial life exists on bodies known to contain water in its various forms (i.e., Martian ice caps, Europa), it may well resemble the microbial community dynamics thus far identified with ice aggregates. Current findings indicate that metabolic complementation among functionally diverse, but structurally simple, prokaryotic consortia along microscale biogeochemical gradients is a unique and effective strategy for meeting the requirements of life in one of the most extreme environments on Earth.

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