Terrestrial biology

Natural abundance of carbon and nitrogen isotopes in potential sources of organic matter to soils of Taylor Valley, Antarctica

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The McMurdo Dry Valleys of southern Victoria Land, Antarctica, represent a unique environment where climatic extremes limit the development of complex and diverse soil communities. In these soils, the microbial feeding nematode is the most abundant and widespread invertebrate. The distribution of this nematode is highly patchy and is related to soil properties such as salinity and pH (Freckman and Virginia in press). The primary source of soil organic matter (SOM) sustaining these low-diversity dry valley soil ecosystems is not obvious given the virtual absence of above-ground plant biomass. The photosynthetic capacity of the soils may be inadequate to account for observed levels of SOM, implicating other sources of organic matter such as windborne particulates from biologically richer lakes and streams, cryptoendolithic communities, and the Ross Sea (Wynn-Williams 1990, pp. 71–146). An understanding of dry valley soil communities and the cycling of carbon and other nutrients in these ecosystems requires information about the sources, quality, and distribution of SOM. In this article, we report first-year results of a study to examine the sources of organic matter to Taylor Valley using stable isotopes of carbon and nitrogen. This work is part of a larger effort to determine the factors controlling the distribution, diversity, and function of soil biota in the McMurdo Dry Valleys (Freckman and Virginia in press; Powers, Freckman, and Virginia 1995).

In austral summer 1994, we began an intensive stable isotopic study of Taylor Dry Valley soils. Because the isotopic fractionation of soil nitrogen reactions in Antarctica is known to be large (Wada, Shibata, and Torii 1981), we hypothesized that potential organic matter sources to dry valley soils would have distinct nitrogen isotopic abundance [ratio of nitrogen-15/nitrogen-14 ($^{15}N/^{14}N$)]. This information coupled with data on the carbon-13/carbon-12 ($^{13}C/^{12}C$) ratio of organic matter, also known to vary between marine and terrestrial sources, might allow us to distinguish between potential sources of organic matter to soil by, first, determining the carbon and nitrogen isotopes of sources and, second, examining isotopes and SOM concentrations along gradients (distance, elevation) from potential sources of organic matter to study mixing of sources and their influence on the isotopic signature of SOM.

Potential organic matter sources (marine and lake algae, rock infected with cryptoendolithic organisms, penguin rookery soil, and bird remains) from dry valley lakes to Ross Island penguin rookeries were sampled. Anthracite coal from the surrounding Beacon Supergroup lithologies was also sampled because of its high organic carbon content and its possible dissemination in the glacial tills upon which most dry valley soils form (Campbell and Claridge 1987). To characterize the potential range of isotopic variation in the SOM of dry valley soils themselves, 41 soil sites were systematically sampled throughout Taylor Dry Valley. Samples were taken along six elevational transects perpendicular to the length of the valley, from the head of the valley to the Ross Sea, areas roughly corresponding to the three major drainage basins for Lakes Bonney, Hoare, and Fryxell, respectively. The relative abundance of ^{13}C and ^{15}N of organic matter in all samples was analyzed using combustion and cryogenic purification at the Dartmouth Light Isotope Tracers in the Environment Laboratory. Isotopic measurements are expressed in parts per thousand difference from a standard using the equation:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample} - R_{standard})/(R_{standard})] \times 1000$$

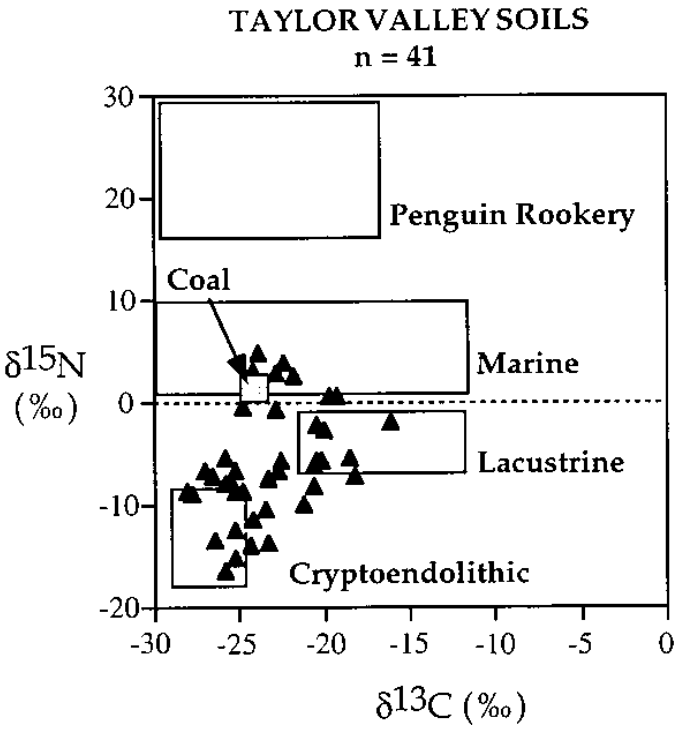
where $R = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$. The standard for carbon is the PeeDee Belemnite (PDB) and, for nitrogen, atmospheric N_2.

Based on the samples of source materials collected in our study and on literature reports, organic matter derived from penguin rookeries, marine algae, lacustrine, and cryptoendolithic sources have sufficiently distinct carbon and nitrogen isotope signatures for measurements of SOM to provide data on the source or sources of organic matter to a particular location (figure). The range of variation for the 41 soils was about 22 $\delta^{15}N$ and 10 $\delta^{13}C$ units. The data show that distant Ross Island penguin rookeries are not a likely source of SOM to Taylor Valley because the characteristic ^{15}N-enriched isotopic signature of penguin rookeries (Mizutani and Wada 1988) was not found. The soil isotopic data do not rule out the other hypothe-

sized sources, however, so sources of SOM to Taylor Valley may be multiple. Isotopically, SOM in Taylor Valley was shown to share isotopic signatures with anthracite coal, marine-derived organic matter, lacustrine-derived organic matter, and cryptoendolithically derived organic matter. Coal is probably not an important source of carbon to the Taylor Valley soils we sampled because samples falling within the isotopic range of both coal- and marine-derived organic matter (see stippled area, figure) have an organic carbon-to-nitrogen ratio of 11.6±4.6. In contrast, soils with significant coal content would be expected to have organic carbon-to-nitrogen ratios greater than 100 (see Campbell and Claridge 1987). Petrographic and SEM research is currently being done to verify this result.

The pattern of the soil isotope data also suggests a mixing of sources in Taylor Valley SOM. Sites at elevations more than 150 meters above sea level contained organic matter isotopically most similar to cryptoendolithic systems, whereas sites at the valley floor contained organic matter more similar to that of lacustrine and/or marine systems. Our early results indicate measurements of carbon and nitrogen isotopes hold considerable promise for organic matter source identification in antarctic soils.

We thank the McMurdo Dry Valley Long-Term Ecological Research (LTER) project and Jim Raymond for lacustrine and marine samples. We are also grateful for the excellent logistic support given by the VXE-6 Squadron of the United States Navy, and the Royal New Zealand Air Force helicopter crews. The help of L.E. Powers and M. Ho, as well as E. Courtright, R. Alward, M. Roberts, and E. Marlies was invaluable. This work is supported by National Science Foundation grant OPP 91-20123 to R.A. Virginia and D.W. Freckman and is a contribution to the National Science Foundation McMurdo LTER Program.



The isotopic signature of SOM in Taylor Valley soils (triangles) overlain by the expected range of isotopic values for possible sources of organic matter to these soils. Isotopic ranges for organic sources represent data collected in this study and, in the case of rookery and marine organic matter, our data combined with that collected from previous studies (Mizutani and Wada, 1988).

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The role of phytoplankton extracellular release in bacterioplankton growth of Taylor Valley Lakes, Antarctica

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Because of their high numbers, large surface-area-to-biovolume ratios, and transport systems efficient at low-substrate concentrations, heterotrophic bacteria are largely responsible for the turnover of dissolved organic carbon (DOC) in aquatic ecosystems (Moran and Hodson 1990). DOC concentrations in these lakes vary little over the season, whereas heterotrophic activity increases (Priscu, unpublished data). Previous experiments showed that bacterial activity at the primary productivity maxima of Lakes Bonney, Hoare, and Fryxell is not glucose deficient, though DOC concentrations were typically lowest in these margins (Takacs and Priscu 1995). DOC availability in these lakes is believed to be regulated by upward diffusion and internal production due to the lack of mixing because ice cover and stream input is limited to several weeks per year.

Healthy algal cells have been shown to excrete soluble products of photosynthesis into the surrounding medium in both the laboratory and the natural environment (Fogg 1983). Estimates of percentage of phytoplankton extracellular release for Lake Bonney range from 6 to 30 percent as measured by Sharp (1993) and as great as 95 percent by Parker et al. (1977, pp. 859–872). Analysis of the DOC pool of Lakes Hoare and Fryxell revealed that 16–20 percent of the fulvic acids are microbially produced (McKnight, Aiken, and Smith 1991).

Natural bacterial assemblages from the primary productivity maximum of Lakes Bonney, Hoare, and Fryxell were incubated in the presence of concentrated phytoplankton from the same depth, isolated in dialysis tubing, to determine the effect of phytoplankton extracellular release on bacterial activity and numbers. Twenty liters of lake water were collected from just below the ice cover in November and December 1995. Ten liters were filtered onto a 90-millimeter (mm) 3.0-micron (µm) polycarbonate filter under low vacuum. The filter was then placed into 1 liter of 0.2-µm filtered water and gently mixed to resuspend the microorganisms. Lake water [900 milliliters (mL)] was incubated in glass flasks (duplicate) for 4 days at 4°C at 60–72 micromolar per square meter per second irradiance and in the dark. Dialysis tubing (12,000–14,000 molecular weight cutoff) with 100 mL of the concentrated phytoplankton or 0.2-µm filtered water (control) was suspended in flasks. The dialysis tubing was soaked in 0.2-µm filtered lake water for at least an hour to reduce leaching.

Thymidine uptake rate (bacterial activity) and cell numbers were measured daily using methods outlined previously (Takacs and Priscu 1995). Acridine-orange-stained bacteria were counted (more than 200 cells) by epifluorescent microscopy.

Chlorophyll-*a* in dialysis tubing and dissolved organic carbon in flasks were measured initially and at the end of the 4-day incubation. Chlorophyll-*a* was measured fluorometrically after 20–100 mL of phytoplankton concentrate was filtered onto glass fiber filters and extracted overnight in 90 percent acetone. Lake water for DOC analysis was filtered through precombusted glass fiber filters into precombusted glass amber bottles and fixed with 1 mL of concentrated phosphoric acid. DOC was analyzed using an Oceanographic International model 700 carbon analyzer.

Three-way mixed factors analysis of variance was used to determine differences in thymidine uptake rate (TdR), TdR per cell (specific activity), and cell numbers. Differences among the treatments compared to the control were tested using Dunnet's test. A log transformation was used when necessary to satisfy the assumption of normality. Data were analyzed for differences daily, over the 4-day incubation period, and between days.

Significant increase in log cell numbers for both the light and dark flasks relative to the control was found in the east lobe of Lake Bonney ($p<0.05$). The log of TdR and specific activity of both the light and dark flask increased significantly compared to the control in the west lobe of Lake Bonney ($p<0.05$). The log of TdR for the dark flask, specific activity in the dark flask, and the log of cell numbers for both the light and dark flasks of Lake Hoare were significantly increased relative to the control ($p<0.05$). Dark flask TdR and specific activity in Lake Fryxell increased significantly ($p<0.05$) relative to the control (*see* table). Chlorophyll-*a* in all flasks, except the controls (which had no measurable chlorophyll-*a*), decreased over the incubation period and DOC increased in all flasks.

Although TdR, specific activity, or cell numbers increased significantly relative to the control in all lakes, this rarely occurred until the third or fourth day of the incubation (figure). One explanation for this may be that differences observed are due to a bottle effect. Data suggest though, that bacteria grown in the presence of phytoplankton extracellular release may have a delayed increased growth response (Nalewajko, Dunstall, and Shear 1976). The means by which phytoplankton were concentrated in these experiments may have caused cell rupture and contributed to the decrease in chlorophyll-*a* and increase in DOC. Though concentration was achieved by filtering under low vacuum and gently resuspended as opposed to brushing cells back into filtered lake water, it is likely that the cellular integrity of the flagellates that are common in these lakes was diminished.

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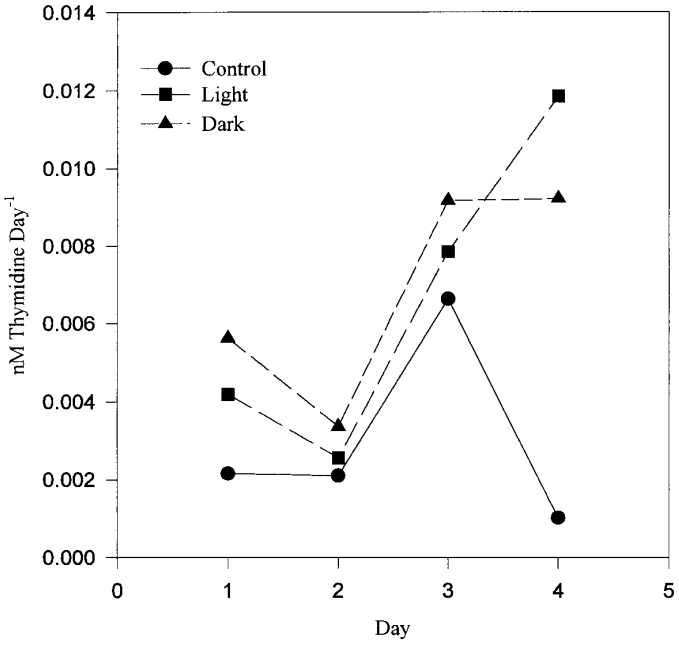

^{3}H-thymidine incorporation for the west lobe of Lake Bonney experiment showing the delayed bacterial stimulation generally observed in this study. (nM denotes nanomolar.)

Summary of significant stimulation of bacterial activity and cell numbers in light and dark flasks relative to control measured as thymidine uptake (TdR), specific activity, or cell numbers, analyzed for differences daily, over the 4-day incubation period, and between days

Lake	Growth parameter	Treatment	Difference	*p*
Bonney, east lobe	Log cells per milliliter	Light	Day 3-1	<0.01
			Day 4-1	<0.01
			Day 4-2	<0.01
			Day 3	<0.05
		Dark	Day 4-1	<0.05
			Day 4-2	<0.05
			Day 4	<0.05
Bonney, west lobe	Log TdR	Light	Day 2	<0.05
		Dark	Day 2	<0.05
	Log specific activity	Light	Day 2	<0.05
Hoare	Log TdR	Dark	Day 4	<0.05
	Specific activity	Dark	Day 4-2	<0.05
	Log cell per milliliter	Light	Day 2-1	<0.05
			Day 3-1	<0.01
			Day 4-1	<0.01
			Day 4-2	<0.01
			Day 3	<0.01
			Day 4	<0.05
		Dark	Day 2-1	<0.05
			Day 3-1	<0.01
			Day 4-1	<0.01
			Day 4-2	<0.01
			Day 3	<0.01
Fryxell	TdR	Dark	Day 4-1	<0.01
			Day 4	<0.01
	Specific activity	Dark	Day 4-1	<0.01
			Day 4	<0.01

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Microbially mediated transformations of manganese in Lake Vanda

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The most commonly observed forms of manganese in aquatic environments are particulate manganese oxides and soluble manganous ions. Although manganese is generally a minor chemical constituent of fresh and marine waters, the remarkable surface area and charge distribution of manganese oxides make them a potentially rich reservoir of metals adsorbed to their surfaces. These adsorbed metals are subsequently released during the reduction of the manganese oxide to manganous ion, and in this way, redox transformations involving manganese may influence the geochemical cycles of numerous metals in aquatic ecosystems.

Microorganisms are known both to oxidize and reduce manganese and are believed to have a major impact on manganese cycles in both terrestrial and aquatic ecosystems (Lovley 1993). The potential role of manganese oxides in the cycling of many metals and the capacity of microorganisms to catalyze the redox transformations of manganese, led us to explore the biogeochemical cycling of manganese in Antarctica's Lake Vanda. This lake's permanent ice cover, calcium chloride ($CaCl_2$) brine in the deep waters, and limited, seasonal surface inflow result in an extremely stable water column amenable to studies of chemical and microbial fine structure.

The water column of Lake Vanda contains both soluble manganous ion (Mn^{2+}) and particulate manganese oxides (Green et al. 1993, pp. 145–163). Two peaks of reduced manganese were detected: one just below the oxic/anoxic interface, as is found in many stratified lakes, and a second peak in oxic waters approximately 5 meters above the interface (figure 1). The occurrence of two peaks of soluble manganese, one within oxic waters, is uncommon and was used to divide the water column into four vertical zones where microbially mediated manganese reduction or oxidation may be favored (figure 1).

The major peak of soluble manganese was just below the oxic-anoxic interface (zone 4), a possible habitat for anaerobes that derive energy for growth solely from the direct reduction of manganese (Nealson and Myers 1992; Lovley 1993). Although this zone may support the growth of anaerobic manganese-reducing bacteria, experiments conducted during the 1994 field season suggest that manganese is reduced primarily by sulfide (Bratina, Green, and Schmidt 1995).

The second manganese peak was a minor peak approximately 5 meters above the interface (zone 2). Aerobic microbes are more likely to inhabit this zone of the lake and reduce manganese indirectly via extracellular metabolites, although some cases of aerobic enzymatic reduction are known (Gounot 1994). We have isolated, from around this second manganese peak, several bacteria that reduce manganese oxides aerobically. The vertical distribution of aerobic manganese reducers, mechanism of reduction, and potential competitive advantages derived from aerobic manganese reduction are currently under investigation.

Bordering the areas of highly soluble manganese are zones where manganese oxidation is likely to occur (zones 1 and 3). Both of these zones are below pH 7 and 20°C, conditions where chemical oxidation of manganese is known to

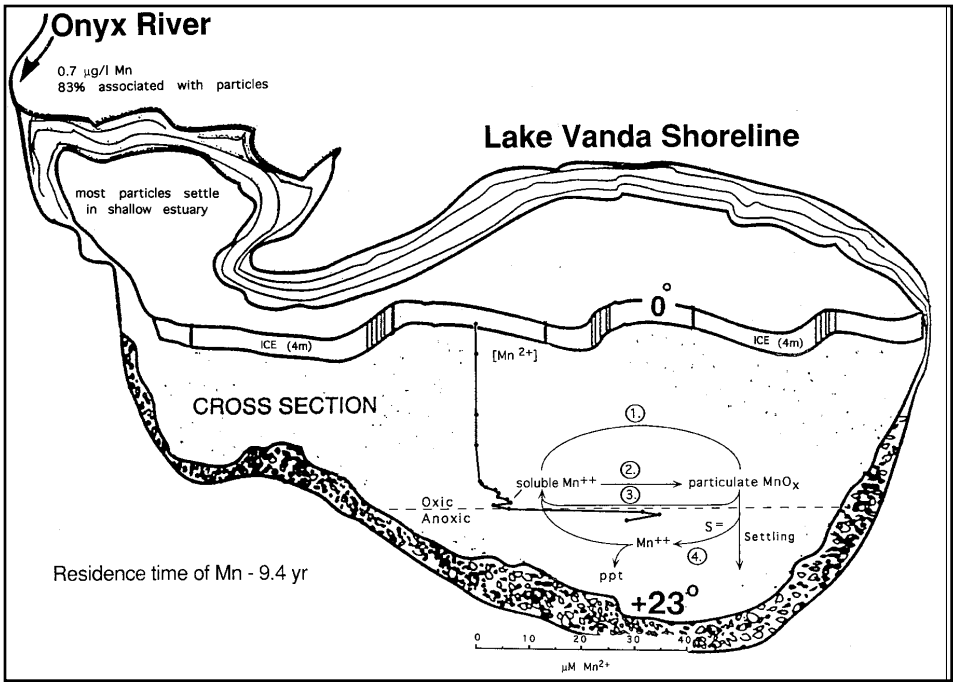

Figure 1. The manganese cycle in Lake Vanda has several zones with potential for microbially mediated manganese oxidation and reduction. Zone 1 (located just above the oxic Mn^{2+} peak) and 3 (located between the oxic Mn^{2+} peak and the interface) are both below pH 7 and 20°C, conditions where chemical oxidation of manganese is known to occur very slowly. Zones 2 (oxic) and 4 (anoxic) have peaks in Mn^{2+} which is indicative of regions where manganese is being reduced. (µg/l denotes micrograms per liter. ppt denotes precipitate. m denotes meter. µ*M* denotes micromolar.)

occur very slowly (Morgan 1967, pp. 561–624). Microbial manganese oxidation has been demonstrated in numerous aquatic environments, particularly just above oxic-anoxic interfaces similar to zone 3 (Ghiorse 1984).

The results for manganese oxidation measurements are summarized in figure 2. Manganese oxides were detected with both the leucocrystal violet (LCV) and leucoberbelin blue (LBB) assays in bins 3 and 5 (zone 1). Manganese oxidation was detected only when samples were amended with nutrients, so whether the activity occurs *in situ* in this oligotrophic lake is not known. Manganese oxidation in zone 3 (bin 7) was detected with the LCV assay but was not corroborated with the LBB assay. Because all of the assays were performed in the dark, no light-dependent metabolism would have been detected. The major phototroph peak in Lake Vanda is in zone 3 and coincides with a decrease in soluble manganese. Phototrophs, which are known to oxidize manganese (Richardson, Aguilar, and Nealson 1988), could therefore be responsible for manganese oxidation in the area just above the oxic-anoxic interface.

Besides being found in lakes, reduced manganous ions have been detected in the Earth's major ocean basins and numerous groundwater systems. The potential interactions between microbes and metals in Lake Vanda could serve as a useful model for other aquatic systems that are less amenable to study due to the confounding effects of rapid mixing. Special thanks go to field party members Bradley Stevenson and Dave Harris. This research was supported by National Science Foundation grant OPP 93-19708.

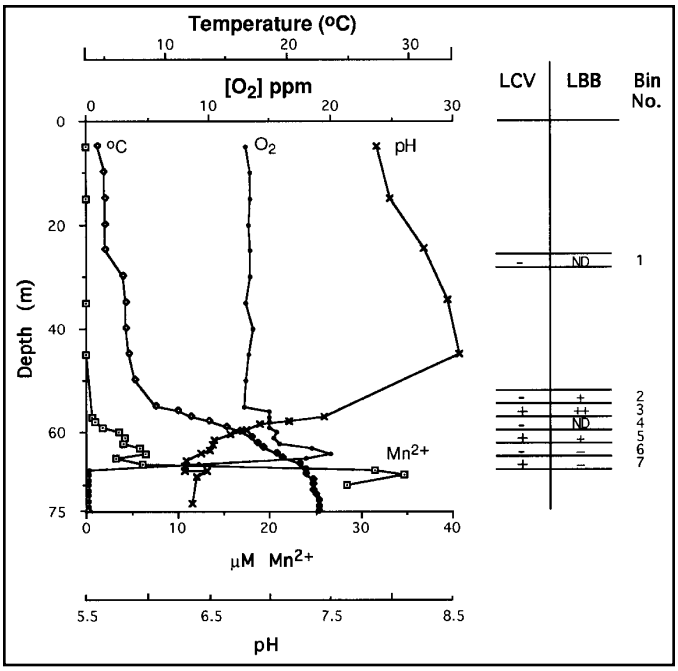

Figure 2. Lake Vanda depth profiles for several key parameters compared to the manganese oxidation assay results. Manganese oxidation was measured in water samples collected from seven different 2.5-meter regions of the water column ("bins") with a Beta Bottle sampler. Assays were set up with 500 milliliters (mL) from each bin and were amended with manganese chloride. The assays were incubated, stationary, in the dark at 15°C. Samples (1 mL) were removed at various timepoints and checked for Mn oxides using the LCV spectrophotometric assay (Spratt, Siekmann, and Hodson 1994). At the end of the incubation, portions (up to 500 mL) from each bottle were passed through a GA filter which was sprayed with leucoberbelin blue (LBB) reagent which reacts with manganese oxides to produce a blue color (Richardson et al. 1988). (m denotes meter. ppm denotes parts per million. ND denotes no data.)

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Lake-ice algal phototroph community composition and growth rates, Lake Bonney, dry valley lakes, Antarctica

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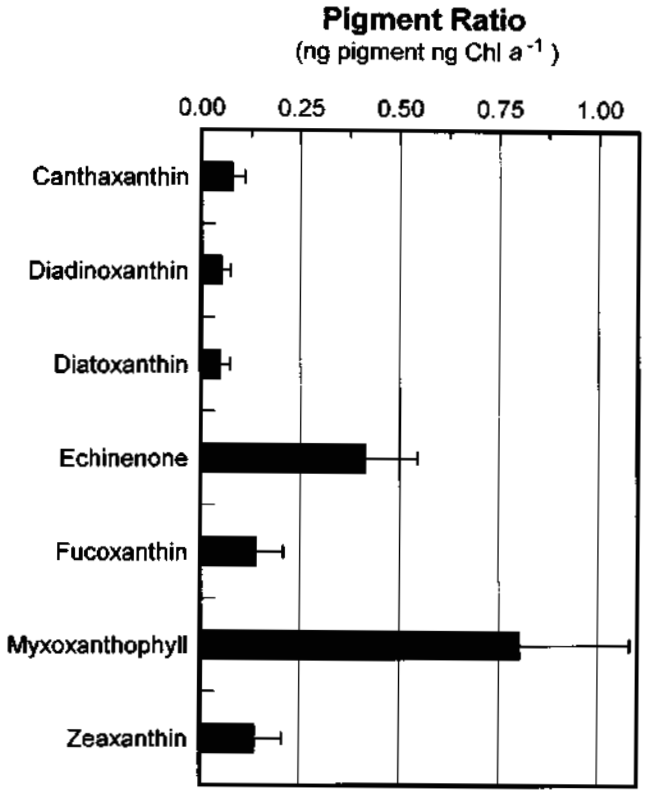
Lake Bonney (77.70°S 162.33°E), one of several lakes in the Dry Valley Lakes Long-Term Ecological Research Program, is a permanently ice-covered lake in the dry valley region of Antarctica near McMurdo Sound. Preliminary studies of lake ice cores by Wing and Priscu (1993) revealed a layer of terrigenous aggregate material (sand and small stones) permanently embedded within the lake ice. Ice aggregates are a common feature in Lake Bonney and other ice-covered dry valley lakes. Terrigenous soil particles and attached microbial communities (phototrophic and heterotrophic prokaryotes and eukaryotes) are wind-blown onto the lake and accumulate in cracks and depressions on the uneven ice surface. During the semi-solid ice melting phase in the summer months, aggregates sink into the ice. When the ice resolidifies, ice aggregates and associated microbial communities become imbedded in the ice matrix.

An interdisciplinary study was initiated in October 1995 to clarify the structure (species composition and abundance) and function (carbon and nitrogen fixation) of ice aggregate microbial communities in Lake Bonney. The purpose of this component of the study was to characterize the community composition (using diagnostic photopigments) and measure the carbon-specific growth rates of phototrophic microbes associated with ice aggregates in Lake Bonney.

Ice aggregate samples were collected from ice blocks removed from Lake Bonney during the excavation of a large pit within the ice cover. Small chunks of ice (approximately 50 milliliters) containing aggregates were slowly melted (3°C), and the meltwater was used for incubations. Subsamples of ice aggregates were collected for microalgal photopigment analysis using high-performance liquid chromatography (HPLC) (Van Heukelem et al. 1992; Pinckney et al. in press). Microalgal growth rates were determined using the carbon-14 (^{14}C) photopigment radiolabeling method (Goericke and Welschmeyer 1993; Pinckney et al. in press). Ice aggregates were placed in 20-milliliter glass vials, filled with meltwater, inoculated with sodium bicarbonate, and incubated in an environmental chamber for 24 hours (3°C; 50 microeinsteins per square meter per second). The ^{14}C-specific activity of individual photopigments was quantified using a flow scintillation counter inline with the HPLC system (Pinckney et al. in press).

Photopigment analyses of ice aggregates revealed relatively high concentrations of chlorophyll-*a* (994±702 nanograms per gram of sediment, mean ±1 standard deviation, *n*=18). The concentrations of diagnostic accessory photopigments (carotenoids) were normalized to chlorophyll-*a* to compensate for the high variability (range = 283–2,317) in microalgal biomass among the samples. The chlorophyll-*a*–normalized photopigment profiles provided a measure of the relative contributions of different microalgal groups within the community. Myxoxanthophyll, echinenone, zeaxanthin, and canthaxanthin, which are all markers for cyanobacteria, were the most abundant photopigments (figure). Fucoxanthin, diadinoxanthin, and diatoxanthin, indicative of diatoms, were also present in low concentrations relative to cyanobacterial pigments.

The microalgal component of ice aggregates consisted primarily of cyanobacteria. Photopigment profiles were more similar to communities inhabiting the terrestrial margins of the lake than to the phytoplankton community beneath the lake ice. Some accessory photopigments (e.g., myxoxanthophyll, echinenone, diatoxanthin) may function as "sunscreen" pigments that protect the photosynthetic apparatus from high irradiances during austral summer months (November to February).



Photopigment profile for Lake Bonney ice aggregate phototrophs. Values are the mean ±1 standard deviation (*n*=18). (ng pigment ng Chl a^{-1} denotes nanograms of pigment per nanogram of chlorophyll-*a*.)

Microalgal carbon-specific growth rates were below the detection limits for the analytical and incubation techniques used in this study (i.e., less than 0.10 per day). Very low rates of photosynthetic ^{14}C uptake were measurable, however, (data not shown) and indicated that the microalgal community was photosynthetically active. The growth rates of ice aggregate phototrophs appear to be very low within the first 24 hours of thawing. Longer exposure to aqueous conditions may be necessary for the synthesis of enzymes needed for cell division and proliferation of native populations. Collectively, our results suggest that the ice aggregates in Lake Bonney contain a diverse and relatively abundant prokaryote-dominated microbial community that exhibits very low metabolic activity within the first few days following exposure to liquid water and light. Future research will examine community metabolic responses in longer term (weeks) bioassays to gain a better understanding of the role of these communities in ecosystem-level production and biogeochemical cycling processes in Lake Bonney.

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Photosynthetic characteristics of cyanobacteria in permanent ice covers on lakes in the McMurdo Dry Valleys, Antarctica

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Lakes in the McMurdo Dry Valleys contain 3- to 20-meter-thick ice covers which do not melt entirely during the austral summer. Several ice covers were sampled during the austral winter and spring of 1995 to determine the distribution of photosynthetic microorganisms (primarily cyanobacteria) within several different lake settings. Most lakes (six out of seven) contained accumulations of sediments and associated cyanobacterial assemblages (the exception being Lake Vanda in the Wright Valley) within the ice. Enhanced concentrations of cyanobacteria (figure 1) (and sediments; data not shown) were found within 50 centimeters of the ice surface at Lake Fryxell to an average depth of approximately 2.0 meters at other lakes. The discovery of viable microorganisms in this environment is relatively new (Wing and Priscu 1993) and knowledge of their ecological role in the terrestrial and lake ecosystems has yet to be determined.

This report describes results from fundamental experiments designed to determine if the microbes within the ice environments are photosynthetically competent upon exposure to liquid water and, if so, their photosynthetic response to variable irradiances. Results from these experiments can be used to determine their importance to the ecosystem's energy budgets and biogeochemical cycles.

Ice samples (collected with a SIPRE corer) were melted at 2–4°C in the dark for 8 to 16 hours (depending on the volume of ice). Upon complete melting, a subsample of water was

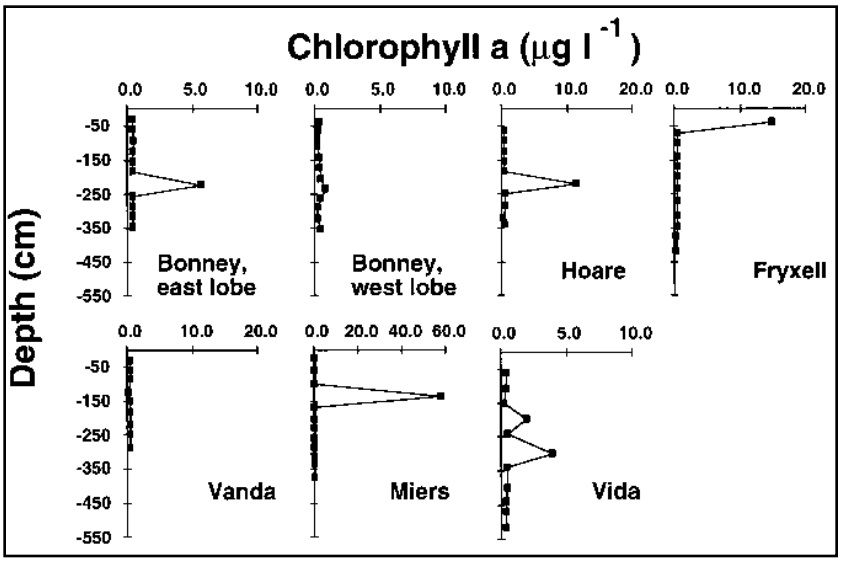

Figure 1. Profiles of chlorophyll-*a* concentrations in the ice covers from seven lakes throughout the McMurdo Dry Valleys. Profiles of sediments (data not shown) show the same vertical distributions as chlorophyll-*a*. (μg l^{-1} denotes micrograms per liter.)

inoculated with carbon-14 (^{14}C) sodium bicarbonate (final activity approximately 1 microcurie per milliliter) in the dark. Ten milliliters were then dispensed into borosilicate scintillation vials and placed under variable irradiances (10 irradiances achieved by neutral density screening of a cool-white fluorescent light source) at 0–4 °C. After the incubation period, samples were acidified and dried; the radioactivity remaining in the vials was determined via standard liquid scintillation spectrometry. Chlorophyll-*a* concentrations were determined via standard fluorescence procedures on extracts of GF/F filters (50:50 dimethyl sulfoxide and 90 percent acetone) through which 10 to 100 milliliters of water had been filtered. Dissolved inorganic carbon, required for photosynthetic rate calculations, was determined on acidified and nitrogen-sparged water samples by infrared gas analysis.

A time-series of ^{14}C incorporation showed detectable ^{14}C uptake in both light and dark bottles within 4 hours of exposure to liquid water; uptake increased linearly over the following 4 to 78 hours (figure 2). Dark fixation of ^{14}C was several orders of magnitude lower than in the light, showing that these algae have the potential for photosynthetic fixation of carbon upon exposure to liquid water and light.

Photosynthetic-irradiance relationships of the various lakes' cyanobacterial assemblages are illustrated in figure 3; the coefficients describing the hyperbolic-tangent fit (Jassby and Platt 1976) determined via Marquardt's algorithm are given in the table. Assemblages showed low biomass-specific photosynthetic rates at saturating irradiances ($P^b m$, range = 0.004 to 0.041 milligrams of carbon per milligram of chlorophyll-*a* per hour). The lowest rates were about tenfold lower than those reported for phytoplankton in the upper water column of Lake Bonney at 0°C (Lizotte and Priscu 1992), and their associated alphas (the slopes of the light-limited portion of the photosynthetic versus irradiance curves) were similarly low.

Low maximum rates of photosynthesis are typically found in algae from cold environments with low irradiances and high vertical stability (Lizotte and Priscu 1992), features characteristic of the habitats in the lake ice. These low rates may also be attributed to the accumulation of "inactive" chlorophyll-*a* within the cold-ice environment as a result of low degradation rates as has been suggested for mat consortia (Vincent and Howard-Williams 1989). Experiments designed to assess the photophysiological response after extended exposures to water and light are planned to determine if the cyanobacteria "shift-up" their photosynthetic rates once environmental conditions become more favorable during the austral spring and summers.

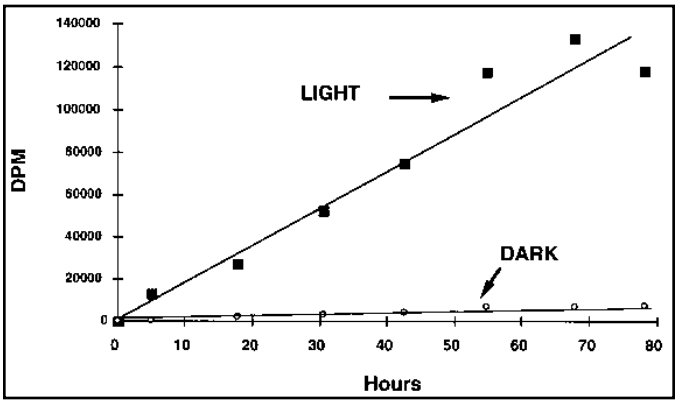

Figure 2. Time-course of light and dark uptake of ^{14}C-labeled bicarbonate (expressed as disintegrations per minute, DPM) by ice-cyanobacteria in their associated meltwater and exposed to irradiances of approximately 100 micromoles of photons per square meter per second.

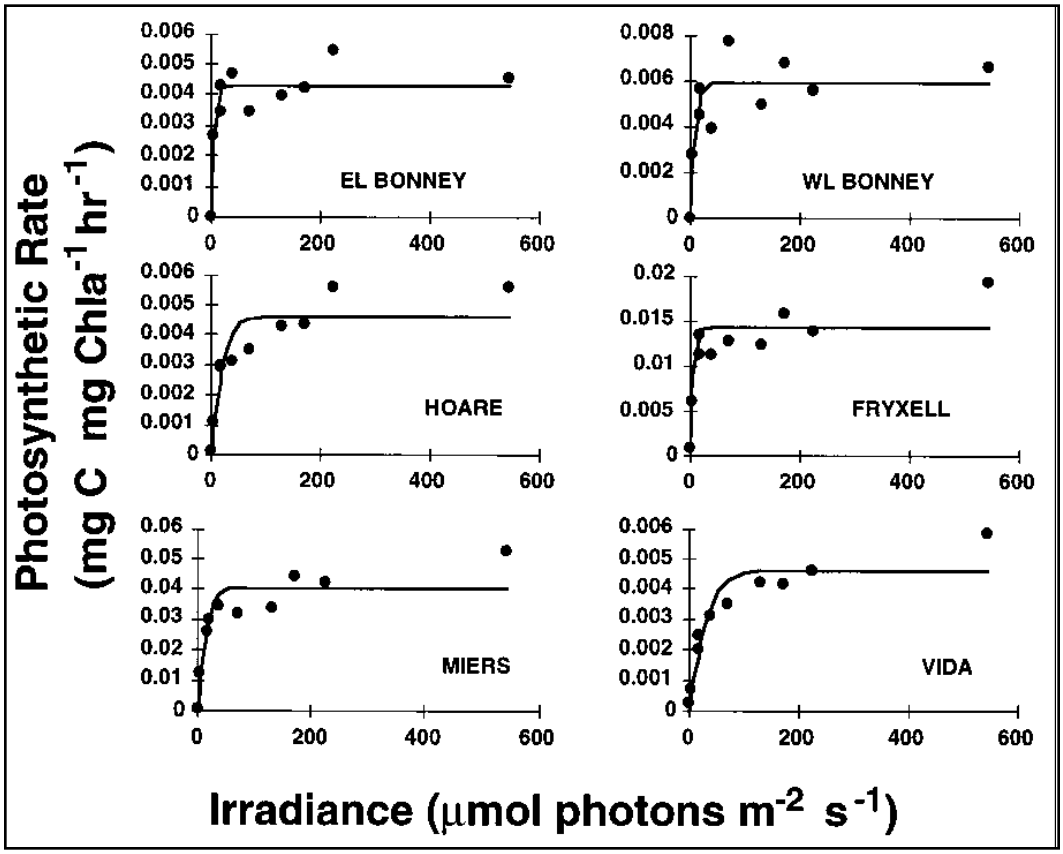

Figure 3. Photosynthesis versus irradiance relationships for cyanobacterial consortia collected from ice covers on lakes throughout the McMurdo Dry Valleys. (mg C mg $Chla^{-1}$ hr^{-1} denotes milligrams of carbon per milligram of chlorophyll-*a* per hour. EL denotes east lobe. WL denotes west lobe. μmol photons m^{-2} s^{-1} denotes micromoles of photons per square meter per second.)

Coefficients describing the hyperbolic-tangent curve fit to the photosynthesis-versus-irradiance (P vs. I) data for lake-ice cyanobacterial consortia

Lake	$P^b m$	α	I_k
Bonney, east lobe	0.0043	0.0005	8.65
Bonney, west lobe	0.0059	0.0005	13.0
Hoare	0.0046	0.0002	29.4
Fryxell	0.0142	0.0011	13.2
Miers	0.0405	0.0018	25.1
Vida	0.0046	0.0001	41.8

Note: Maximum biomass specific photosynthetic rates, $P^b m$ (in milligrams of carbon per milligram of chlorophyll-a per hour); slope of the light-limited portion of the P vs. I curves, α (in milligrams of carbon per milligram of chlorophyll-a per hour per micromole of photons per square meter per second); irradiances at which photosynthetic rates are maximized, I_k (in micromoles of photons per square meter per second).

We thank Ed Adams, Tom Meuwissen, Doug Gordon, Jay Pinckney, Hans Paerl, and Steve Giovannoni for assistance in the field and informative discussions. This research was supported by National Science Foundation grant OPP 94-19413.

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Nitrogen fixation within permanent ice covers on lakes in the McMurdo Dry Valleys, Antarctica

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Ice cyanobacterial assemblages have been documented inside the permanent ice covers on lakes throughout the McMurdo Dry Valleys (Fritsen and Priscu 1996; Priscu and Fritsen 1996; Wing and Priscu 1993). The majority of the ice microbes coincide with pockets and layers of sand and gravel in the ice covers, which are often located at approximately 2 meters depth. We believe these sediment layers within the ice matrices have accumulated from migration of sand and gravel into the ice interior during several summer melting periods. The annual downward movement of the sediment is approximately balanced by the net upward movement of the ice; the upward movement of the ice results from ablation at the ice surface and new ice growth at the bottom of the ice cover over an annual cycle. This dynamic relative movement of the ice cover and sediment migration is believed to maintain an ice-resident microbial population (dominated by cyanobacterial biomass) within the ice covers over periods of years.

Nitrogen fixation is a fundamental process whereby "new" atmospheric nitrogen (*sensu* Dugdale and Goeing 1967) can be made available to an ecosystem. Cyanobacteria assemblages are primary sources of nitrogen fixation in aquatic systems; therefore, they are key elements of an aquatic ecosystem's biogeochemical cycles. In this article, we report initial estimates of the rates of atmospheric nitrogen fixation by the ice cyanobacterial assemblages.

Ice cores and sediment samples were collected from lakes in the McMurdo Dry Valleys during late winter/early spring (August to November) 1995. Rates of atmospheric nitrogen fixation were estimated by the acetylene-reduction assay for nitrogenase activity (Flett, Hamilton, and Campbell 1976) on ice meltwater at 0–2°C.

Rates of ethylene (C_2H_4) production were converted to rates of nitrogen fixation assuming a conversion factor of 1 mole of fixed nitrogen to 0.33 • moles C_2H_4 produced (Flett et al. 1976).

Time-course of nitrogen fixation

Initial experiments on samples from Lake Fryxell were carried out over a time course of approximately 54 hours to determine whether ethylene production was linear over the period. During this time-course experiment, approximately 24 hours was needed to detect ethylene production above background levels. When nitrogenase activities were normalized to both chlorophyll-*a* and sediment weights, they generally increased over time (figure). The trend for increasing nitrogen fixation when normalized to sediments (figure, block *B*) was greater than when normalized to chlorophyll-*a* (figure, block *A*), although slopes were not significantly different from 0 (p=0.8, normalized to chlorophyll-*a*; p=0.1, normalized to sediment weight). During the time-course, chlorophyll-*a* in the incubation bottles increased at a rate of 0.046 micrograms

of chlorophyll-*a* per gram of sediment per hour (data not shown), thereby partially explaining why rates normalized to chlorophyll-*a* did not seem to increase as they did when normalized to sediment weights.

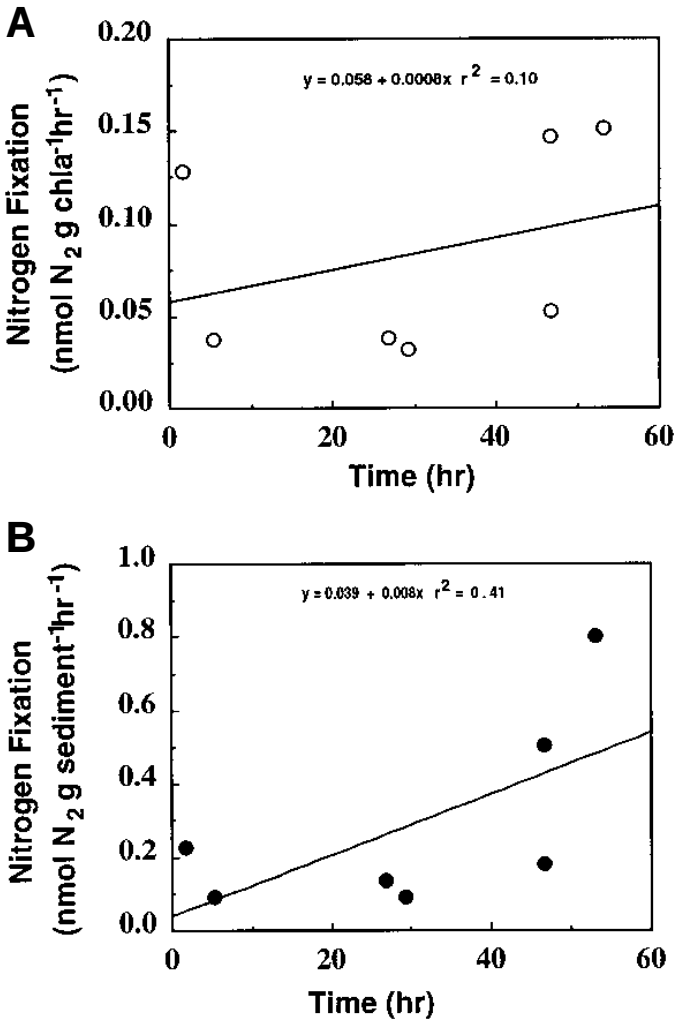
The apparent increase in nitrogen fixation rates over time could be attributed to several factors. First, the increase in chlorophyll-*a* per unit sediment weight over time suggests that the cyanobacteria were either synthesizing new photosynthetic pigments or were experiencing net growth during the incubation. If new growth was occurring, then nitrogen fixation would increase as biomass increased. Second, nitrogen fixation requires metabolic energy and intact/functioning cellular membranes. The assays were performed within hours of sediments being exposed to liquid water and irradiances, which were saturating to photosynthesis (Fritsen and Priscu 1996). Before melting, the cyanobacteria were frozen *in situ* at –20°C to –30°C and experienced 3 to 4 months of antarctic winter. During the antarctic winter, they may have experienced cellular damage and/or depletion of their cellular energy reserve. Upon melting, cellular repair and/or increasing cellular energy levels may have contributed to an acceleration in the rate of nitrogen fixation. Third, oxygen is an inhibitor of nitrogen fixation. Because the samples were removed from their *in situ* condition and the ice-sediment aggregates were distributed, existing microzones of oxygen depletion may also have been disturbed. During the incubations, however, oxygen-depleted microzones may have been reestablished, therefore, allowing nitrogen fixation to resume over time.

Nitrogen fixation in ice sediments

Sediments from the surface of the ice and from the lake's shores showed no detectable ethylene production even when 4–5 grams of sediments were assayed over 40 hours under irradiances of 200 micromoles per square meter per second. Rates of nitrogen fixation by ice assemblages ranged from below levels of detection for the sediments tested from the east lobe of Lake Bonney to 91.3 nanomoles of nitrogen per milligram of chlorophyll-*a* per hour at Lake Miers (table).

Annual nitrogen fixation estimates were extrapolated from the number of days the ice aggregates are believed to be exposed to liquid water in each ice cover (Fryxell 80 days; Hoare 130 days; Miers 100 days) (derived from temperature profiles at the east lobe of Lake Bonney over several seasons) in concert with standing stocks of chlorophyll-*a* in each of the ice covers assayed (table). Estimates of annual rates of nitrogen fixation ranged from 0 (east lobe Lake Bonney) to 2.7 millimoles of nitrogen per square meter at Lake Miers (table).

Rates of nitrogen fixation for Nostoc communities from Vestfold Hills, Antarctica, ranged from 0.27 to 69.7 nanomoles of nitrogen per milligram of chlorophyll-*a* per hour (assuming conversion factor listed above). Rates from nitorgen-fixing cyanobacteria at the two southern Victoria Land streams show a range from 9 to 939 milligrams of nitrogen per square meter per year (Howard-Williams, Priscu, and Vincent 1989). These rates are comparable to those produced in the ice covers of Lakes Miers and Hoare, indicating that the amount of nitrogen fixed by the ice microbial consortia may be important local sources of fixed



Time-series of nitrogen fixation normalized to chlorophyll-*a* (*upper panel*) and sediment weight (*lower panel*).

Nitrogen fixation rates, standing stocks of chlorophyll-a, and estimates of annual nitrogen fixation in McMurdo Dry Valley lakes

Lake	Temperature (°C)	Nitrogen fixation rate[a]	Chlorophyll-*a*[b]	Annual ice nitrogen fixation[c]
Bonney, east lobe	0–2	Below level of detection	1.9–15.5	No data
Fryxell	0–2	9.4–13.1	14.3	0.3–0.4
Hoare	0–2	91.3	9.1	2.6
Miers	0–2	19.9	56.0	2.7

[a]In nanomoles per milligram of chlorophyll-*a* per hour.
[b]In milligrams per square meter.
[c]In millimoles of nitrogen per square meter.

nitrogen. Estimating the total contributions of new nitrogen to the desert ecosystems will rely on better knowledge of the spatial distributions of these consortia in the ice covers and their seasonal dynamics.

We thank Doug Gordon, Jay Pinckney, Tom Meuwissen, Ed Adams, Steve Giovannoni, and Hans Paerl for field assistance and technical discussions. This research was supported by the National Science Foundation grant OPP 94-19423.

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Ice aggregates as a microbial habitat in Lake Bonney, dry valley lakes, Antarctica: Nutrient-rich microzones in an oligotrophic ecosystem

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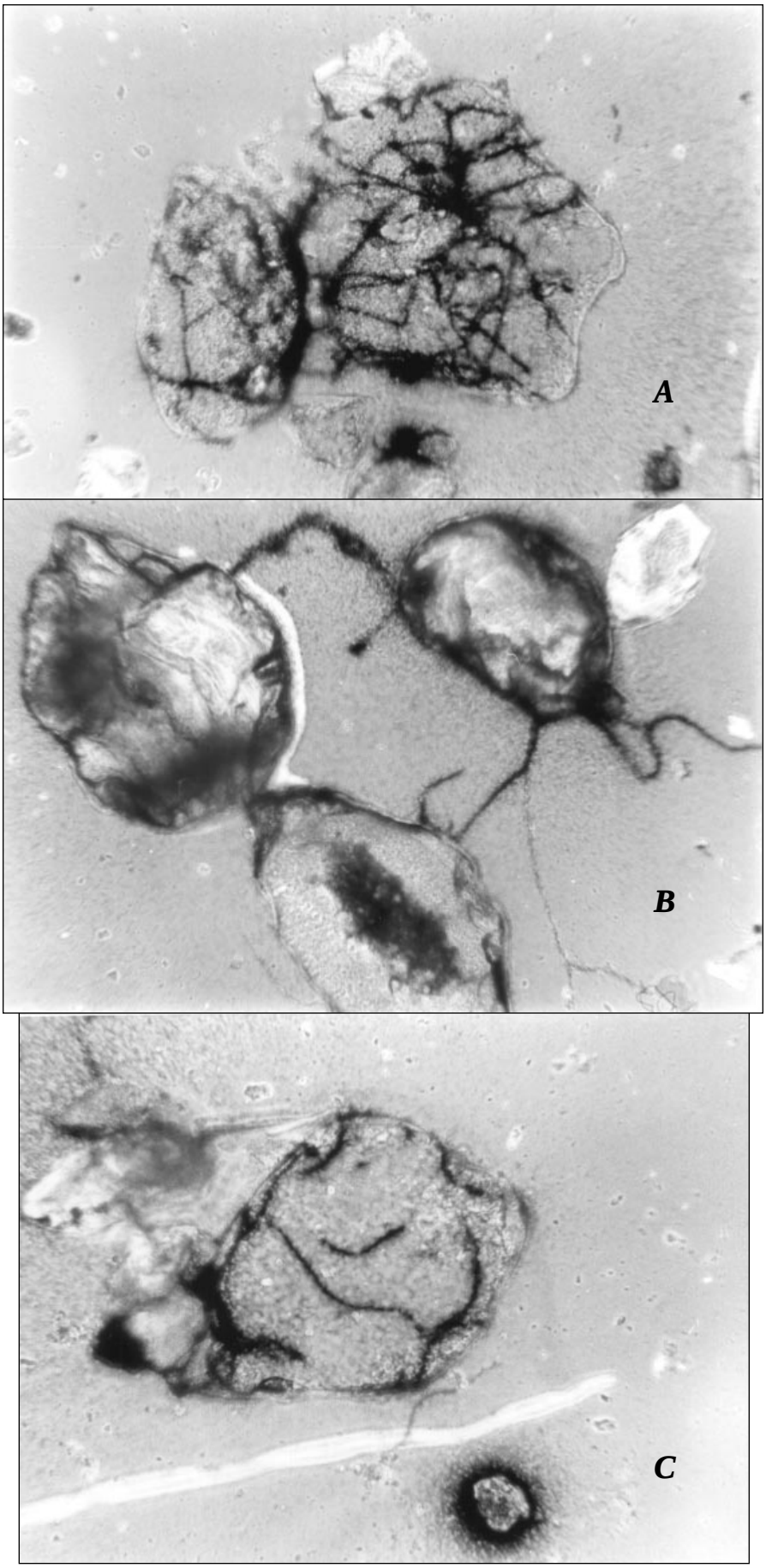
Previous research by Wing and Priscu (1993) and Pinckney and Paerl (*Antarctic Journal*, in this issue) identified a diverse photoautrophic microbial community associated with terrigenous aggregate material (sand and small stones) permanently embedded within the lake ice at Lake Bonney (77.70°S 162.33°E, dry valley region of Antarctica near McMurdo Sound). Aggregate material is wind deposited onto the surface of the lake ice and concentrated in depressions and fissures within the ice. In the summer months, solar heating of the darker particles results in the downward movement of these aggregates into the lake ice. Aggregates, and associated microbial communities, become embedded in the ice matrix when the semisolid ice resolidifies in the colder months.

An examination of photopigments (chlorophylls and carotenoids) indicated a photosynthetically active community composed of cyanobacteria and diatoms (Pinckney and Paerl, *Antarctic Journal*, in this issue). The microscale spatial arrangement and metabolic activities of individual species was considered here to construct a process-based understanding of community dynamics.

The soil-based aggregates embedded in lake ice cover represent a relatively nutrient-rich microenvironment, or microzone, in which microbial production and material cycling can be localized and optimized. Given the nutrient-poor conditions (i.e., scarcity of organic and inorganic substrates) and lack of attachment sites in the ice cover, aggregates provide an attractive microhabitat. This microhabitat supports photosynthetic production, thus providing food and energy for microheterotrophs and higher ranked consumers in the lake ecosystem.

Characterization of ice aggregates as loci of production and nutrient cycling was done under natural (i.e., *in situ*) conditions without disturbing structural and chemical integrity. Carbon dioxide (CO_2) fixation (primary production) and organic matter utilization (heterotrophic activity) were localized and examined at relevant (microbial) scales (microns to millimeters). Microautoradiography, a microscopic technique for measuring the microscale utilization and cycling of radiolabeled inorganic and organic substrates, was used to examine the uptake and fate of carbon-14 (^{14}C) labeled CO_2 administered as ^{14}C sodium bicarbonate ($NaH^{14}CO_3$) as well as

Figure 1. Light microscopy observations of ^{14}C and ^{3}H microautoradiographs of ice aggregates. *A.* Microautoradiograph showing attached photosynthetic (light-mediated) incorporation of $^{14}CO_2$ by filamentous cyanobacteria. ^{14}C exposure is indicated as dark patterns of reduced silver grains in the radiosensitive emulsion covering aggregate-associated microorganisms. Nonheterocystous filamentous and coccoid cyanobacteria dominated light-mediated $^{14}CO_2$ incorporation. Dark-incubated samples showed no detectable radioexposure. *B.* Microautoradiograph illustrating aggregate binding mediated by ^{14}C-labeled (photosynthetically assimilated $^{14}CO_2$) filamentous cyanobacteria. *C.* Microautoradiograph showing dark-incubated incorporation of ^{3}H-labeled D glucose by heterotrophic bacteria associated with ice aggregates.



hydrogen-3 (^3H) labeled organic substrates (glucose, amino acids) known to be readily assimilated by heterotrophs in oligotrophic environments (Paerl 1984a,b).

Small ice blocks (200–500 cubic centimeters) containing aggregates were obtained from various depths in Lake Bonney's ice cover melted at 4°C and 18 milliliters (mL) of aggregate containing meltwater was transferred in triplicate to 20-mL clear and opaque (foil-wrapped) glass liquid scintillation vials. In separate experiments, we added

- 10 microcuries (μCi) ^{14}C sodium bicarbonate ($NaHCO_3$) [58 millicuries per millimole (mCi $mmol^{-1}$); ICN Inc.],
- 15 μCi of a uniformly labeled ^3H L-amino acid mixture (240 mCi $mmol^{-1}$; ICN Inc.), and
- 12 μCi of uniformly labeled ^3H D-glucose (220 mCi $mmol^{-1}$; ICN Inc.).

Isotopic substrates were added at trace (nanomolar or less) concentrations. Samples were incubated under either illuminated (80 microeinsteins per square meter per second photosynthetically active radiation, or PAR) or dark conditions at 4°C from 2 to 8 hours in an incubator. Borate-buffered (2 percent, pH 7.5) formalin was added to terminate the incubations. Water subsamples containing aggregates were gently gravity filtered onto 0.45-micron porosity HA Millipore filters and rinsed several times with unlabeled, prefiltered meltwater to remove excess radioisotope. Filters were then air dried, optically cleared on microscope slides under fuming acetone, and prepared for microautoradiography according to the protocol of Paerl (1984a). A liquid emulsion (Kodak NTB-2) was used as the radiosensitive emulsion (Paerl and Stull 1979).

Microautoradiographs revealed that filamentous cyanobacteria closely associated with soil particles forming the aggregate "core" were largely responsible for photosynthetic incorporation of $^{14}CO_2$ (figure 1*A*). These cyanobacteria dominated the phototrophic biomass and were responsible for binding individual soil particles, thus enhancing the aggregation process (figure 1*B*). Aggregate accumulations were frequently observed inside ice samples. Cyanobacterial photosynthetic production and associated carbon and nutrient (nitrogen, phosphorus, and trace metals) metabolism are localized to these aggregates.

Microautoradiographs also revealed thin (<0.5 micron) filamentous, as well as rod and coccoid-shaped, bacteria and cyanobacteria to be responsible for the uptake of radiolabeled glucose (figure 1*C*). Uptake of radiolabeled amino acids was largely confined to the bacterial community. On occasions, gas bubbles, resulting from thawing and freezing of water associated with the aggregate as it sinks through the ice, can be seen entrapped in the ice blocks (figure 2). Relatively few free-living microorganisms were observed in the ice matrix.

Results obtained thus far indicate a potentially active (during the liquid phase) phototrophic and heterotrophic, microbial community, closely associated with soil-based aggregates. Virtually all the photosynthetic activity was attributable to filamentous and coccoid cyanobacteria, underscoring the important roles these prokaryotic phototrophs play in primary production and in carbon- and nitrogen-cycling dynamics in the dry valley lakes region of Antarctica. In conjunction with parallel molecular analyses of total microbial community diversity based on 16S rRNA sequence analysis (Gordon et al., *Antarctic Journal*, in this issue), and the functional diversity of nitrogen-fixing microorganisms based on *nifH* sequence analysis (Paerl et al. unpublished data), our knowledge of the structural and functional roles of aggregate-based microbial consortia is expanding.

Aggregates are considered an "oasis" of relatively high nutrient supply and biogeochemical gradients (Paerl 1984b), essential ingredients for supporting key metabolic processes (i.e., photosynthesis, respiration, nitrogen fixation), growth, and reproduction at the "edge of life" that this extreme environment represents.

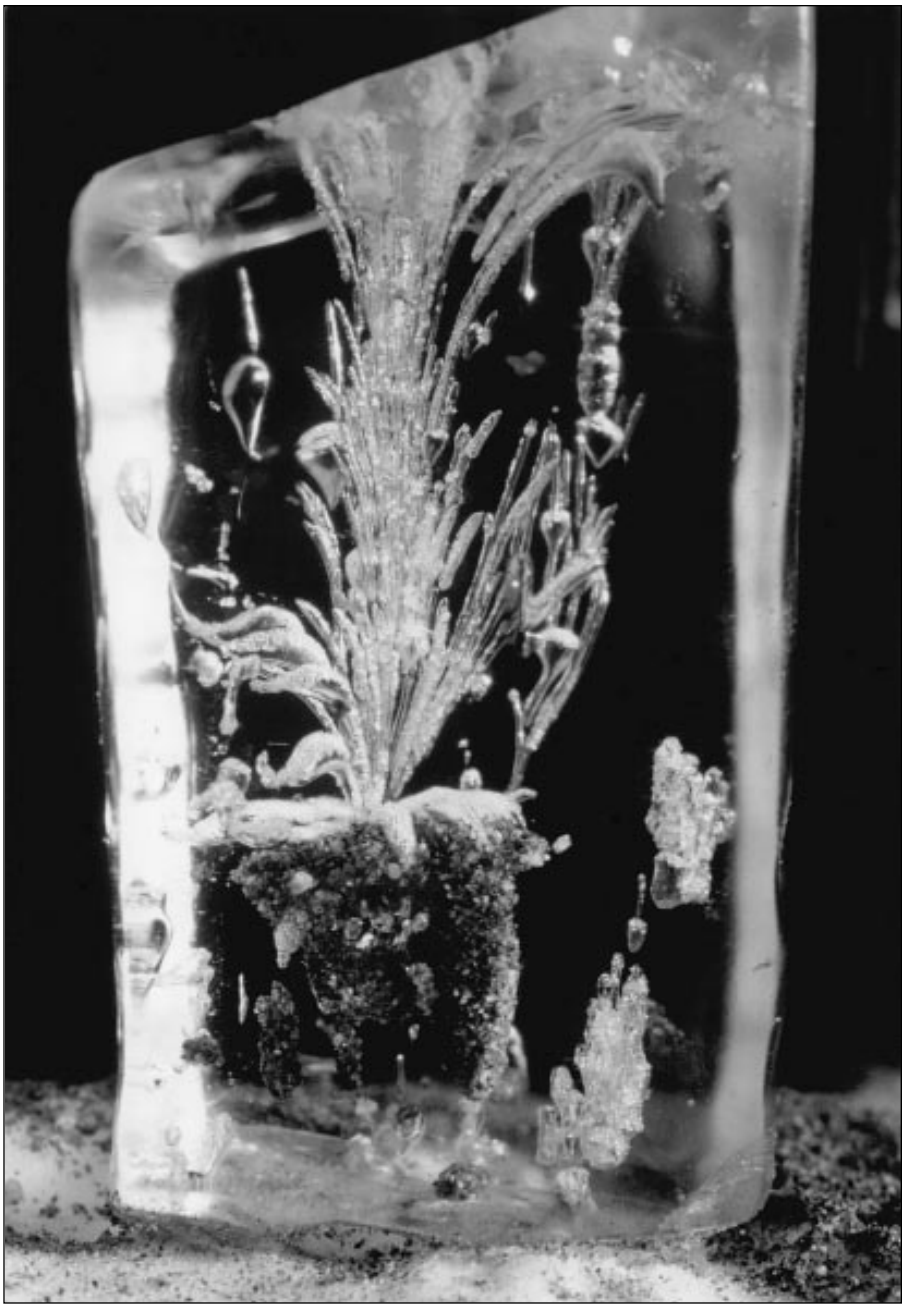


Figure 2. Example of a large ice aggregate embedded in a block of Lake Bonney ice (approximately 12 × 17 centimeters). Gas bubbles are associated with the freezing front of the ice as the liquid water lens refreezes during winter.

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Antarctic lake-ice microbial consortia: Origin, distribution, and growth physiology

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The 3- to 6-meter-thick permanent ice covers of most lakes of the McMurdo Dry Valleys contain a sand and gravel layer of aeolian origin often located about 2 meters below the surface of the ice. The vertical location of this layer represents a dynamic equilibrium between downward melting of sediments during the summer and the combination of about 30 centimeters per year upward movement of ice from ablation at the surface and new ice formation at the bottom. A liquid water lens exists in this layer during the summer (about 150 days) when measurable solar radiation persists for 24 hours per day. We discovered and previously reported (Wing and Priscu 1993) that the liquid water, which is a rare commodity at these latitudes, supports a viable microbial assemblage consisting primarily of cyanobacteria and bacteria in Lake Bonney. The general objectives of our current 3-year study are to

- determine the geographical distribution of lake-ice microbial assemblages in lakes of the McMurdo Dry Valleys;
- characterize phylogenetically terrestrial, benthic, pelagic, and lake-ice assemblages using 16S rRNA gene cloning and sequencing;
- estimate primary production and growth rates of cyanobacteria and bacteria within the ice;
- use molecular probes for nitrogenase in concert with acetylene-reduction assays to determine genotypic potential and enzymatic and physiological expression of atmospheric nitrogen fixation; and
- measure the influence of products of cyanobacterial photosynthesis on bacterial activity.

These objectives are addressed in a series of manuscripts in this issue (*see* Fritsen and Priscu; Gordon, Lanoil, and Giovannoni; Grue, Fritsen, and Priscu; Paerl and Pinckney; Pinckney and Paerl). This article presents an initial budget for particulate organic carbon (POC) developed from data collected between August and October 1995.

Wind-blown POC and associated sand and gravel particles deposited on the surface of the lake ice were collected during early September in polyethylene bags from an areally defined grid. We assume that this material represents most of the deposition over the year because the highest winds occur during the austral winter and lack of sunlight does not force melting into the ice. POC within the ice was collected from SIPRE cores; POC lost through the bottom of the ice was collected with moored sediment traps. POC was measured on acidified samples using a Carlo-Erba elemental analyzer.

A growth rate of 0.5 per year was estimated for photosynthetic organisms (primarily cyanobacteria) from photosynthesis-irradiance relationships (Fritsen and Priscu, *Antarctic Journal*, in this issue) and a carbon-to-chlorophyll-*a* ratio of 50 (gram per gram). This low rate was corroborated by Pinckney and Paerl (*Antarctic Journal*, in this issue).

A conceptual model of sediment and associated organic matter dynamics within the ice covers of permanently ice-covered antarctic lakes is presented in the figure. This model depicts wind-blown (aeolian) transport as the primary source of POC to the lake ice. The POC and associated sediment melts to a depth within the ice that is in equilibrium with solar-forced downward melting and the upward movement of the ice via ablation. Meltwater in this region, which exists between November and February for about 150 days per year (Fritsen and Priscu, unpublished data), supports photosynthetic carbon production during this period. POC losses from the ice cover through meltwater conduits and cracks are depicted as the primary loss from the ice column.

The concepts presented in the figure were quantified as follows:

$$\frac{\delta C_i}{\delta t} = (\mu \cdot C_i) + Q_a - Q_W \quad (1)$$

Where:

$\frac{\delta C_i}{\delta t}$ = change of particulate organic carbon (POC) per area per time (milligram carbon per square meter per year; mg C m^{-2} y^{-1})

μ = biological growth rate (0.5 per year)

C_i = standing stock of POC in the ice [375 milligram carbon per square meter (mg C m^{-2})]

Q_a = aeolian flux of POC (43 mg C m^{-2} y^{-1})

Q_w = sinking flux of POC from the ice (2.3 mg C m^{-2} y^{-1})

Note that the term ($\mu \cdot C_i$) represents biological processes whereas ($Q_a - Q_w$) represents physical processes affecting POC in the model.

Hence:

$$\frac{\delta C_i}{\delta t} = \left(\frac{0.5}{y} \cdot \frac{375 mgC}{m^2} + \frac{43 mgC}{m^2 \cdot y}\right) - \frac{2.3 mgC}{m^2 \cdot y} \quad (2)$$

$$\frac{\delta C_i}{\delta t} = 229 mgC \cdot m^{-2} \cdot y^{-1}$$

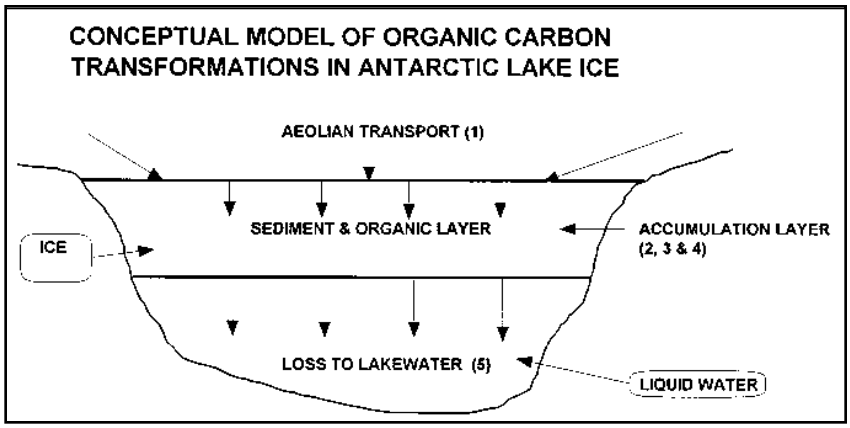
(represents accumulation in the ice)

Based on our conceptual idea of POC dynamics within the ice covers, numerical analysis shows a net accumulation of organic carbon within the ice. Over 80 percent of this estimated accumulation results from carbon produced by cyanobacterial photosynthesis despite low growth rates (0.5 per year).

Our current study shows that the permanent lake ice provides novel habitats for the existence and growth of life in Antarctica. Specifically, we are showing how physical processes

(e.g., sediment deposition and liquid-water formation) are tightly coupled with the presence of microbial life in this extreme environment. Other studies by our group are showing the following:

- Bubbles associated with lithic material and microorganisms within the ice covers can significantly alter light attenuation within the ice, directly affecting the rate of photosynthesis (Adams, Priscu, and Sato 1995).
- A close spatial association occurs between cyanobacteria and bacteria, inferring that the latter depends on photosynthetically produced organic carbon (*see* Paerl and Pinckney, *Antarctic Journal*, in this issue).
- A portion of the nitrogen demands can be met by atmospheric nitrogen fixation (Grue et al., *Antarctic Journal*, in this issue).
- Positive relationships exist between bacteria and cyanobacteria with respect to their biomass and activity. Biological activity is also related to sediment mass (see Gordon et al., *Antarctic Journal*, in this issue; Paerl and Pinckney, *Antarctic Journal*, in this issue).



Pathways of organic carbon thought to occur in the permanent ice covers of lakes in the McMurdo Dry Valleys. (1) Aeolian deposition of inorganic material and associated microorganisms on the ice surface. (2) Material from the surface reaches an equilibrium level in the ice. (3) The ice environment provides liquid water and nutrients supporting microbial growth during the summer. (4) Microorganisms double at least once during the period when liquid water is available (grazing and decomposition losses are thought to be low). (5) Sediment and organic matter are lost from the ice to the lakewater through conduits in the ice.

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Cyanobacterial communities associated with mineral particles in antarctic lake ice

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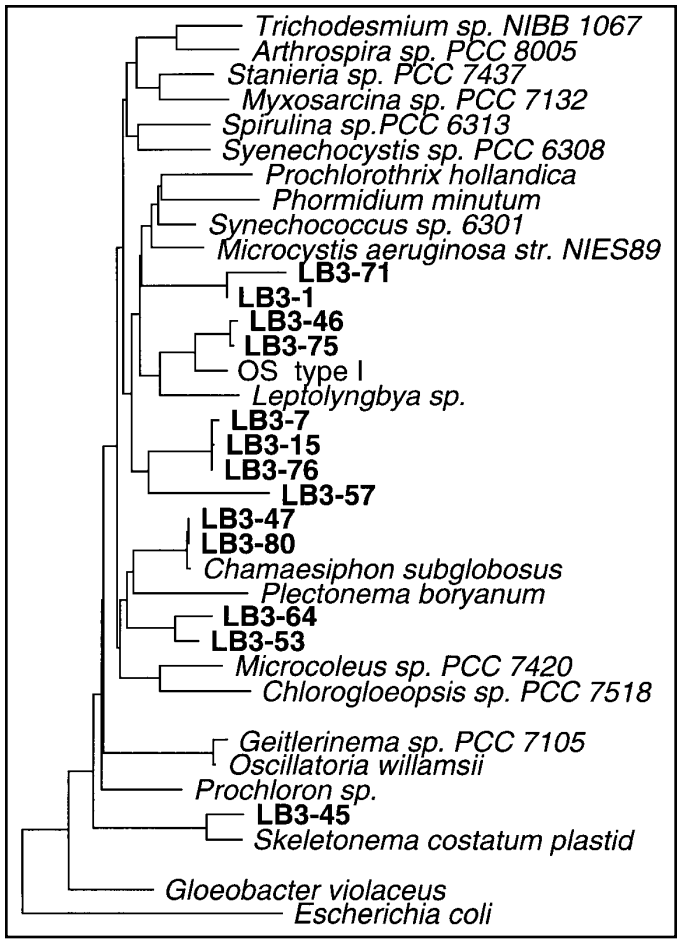
We report on the molecular taxonomy of the unique microbial assemblage within the permanent ice cover of Lake Bonney in the McMurdo Dry Valleys of Antarctica. The existence of this assemblage has been described previously by Wing and Priscu (1993). Although we will focus on the molecular taxonomy of Lake Bonney, microscopic examination revealed that the cyanobacterial component of the assemblage is of similar morphology to that of Lakes Hoare, Fryxell, and Vida. Consequently, we believe that our results for cyanobacteria can be extrapolated to these other lakes.



Phylogenetic tree illustrating relationships among selected 16S rDNA clones (shown in bold) and representative cyanobacterial sequences. This tree was derived using the Desoete algorithm from a mask of approximately 350 nucleotides.

Other aspects of this assemblage are presented elsewhere in this volume (Priscu and Fritsen, Fritsen and Priscu, *Antarctic Journal*, in this issue).

DNA was extracted from mineral particles collected from the permanent ice cover of Lake Bonney and used to construct a 16S rDNA gene clone library. Partial sequences were obtained from 88 clones selected at random. The remaining 132 clones have not yet been characterized.

Phylogenetic analyses indicate that the ice contains a complex community of microorganisms that includes a wide variety of cyanobacteria. Preliminary results suggest that, although many of the cyanobacterial 16S rDNAs are similar to previously characterized sequences, the library also contains cyanobacterial 16S rDNA genes that cannot be identified by reference to existing sequence databases. Analysis of the same sediment via confocal microscopy showed diverse morphological types of cyanobacteria tightly associated with the sediment.

Cyanobacterial 16S genes accounted for 17 of the genes sequenced in this study (figure). The remaining 71 clones include rDNA genes that fall into recognized phylogenetic groupings (e.g., β-proteobacteria or planctomycetes) as well as clones that may represent novel lineages. The primers used in the construction of this library were designed to amplify bacterial 16S rDNA genes. Efforts to amplify archaeal genes from the same DNA samples were unsuccessful.

It is clear even from this preliminary analysis that the lake ice harbors a complex microbial assemblage. We anticipate that the analysis of the remaining gene clones will provide a more refined picture of the phylogenetic structure of this community. These data will be used to design oligonucleotide probes that will be used in future ecological experiments aimed at clarifying the ecological roles and origins of key microorganisms in the ice community. "Species"-specific probes will make it possible to determine if the ice microbiota is also found in the surrounding environment or, alternatively, if it is a distinct community comprising microorganisms specifically adapted to the ice mineral layer habitat.

This ice ecosystem provides a model for evaluating the potential for life to exist in ice elsewhere in the solar system, for example, on the polar caps of Mars or the ice-covered Jovian moon Europa.

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Impacts of climate change on antarctic vascular plants: Warming and ultraviolet-B radiation

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Strong evidence indicates that the climate of the Antarctic Peninsula has changed appreciably during this century. Weather records show that mean summer air temperatures have risen more than 1°C over the past 45 years at some peninsula locations (Smith 1994; Smith, Stammerjohn, and Baker 1996). In addition to this warming trend, springtime ozone depletion events have resulted in well-documented increases in ultraviolet-B (UV-B) radiation (280–320 nanometers) levels (Booth et al. 1994; Madronich et al. 1995). These rapid changes in regional climate provide a unique opportunity to assess the impacts of climate change on vascular plants. The presence of only two native vascular plant species (*Deschampsia antarctica* and *Colobanthus quitensis*) and their sparse distribution in Antarctica attest to the severe conditions for plant survival. Recently, there are indications that climate change is beginning to exert a strong influence on these species. Regional warming may be leading to rapid increases in populations of these species, based on censuses along the peninsula (Smith 1994). The influence of enhanced UV-B levels on these species is less clear.

During the 1995–1996 field season (November to March), we established a field experiment on the east island of Stepping Stones, near Palmer Station, Antarctic Peninsula. We used filters to manipulate UV levels and temperatures around naturally growing vascular plants (*Deschampsia antarctica* and *Colobanthus quitensis*). The experiment consisted of 90 UV radiation-exclusion/warming frames, representing 9 treatments with 10 replicates per treatment. The treatments involved excluding different components of UV radiation (UV-B and/or UV-A) in combination with increasing the temperatures around plants. Additionally, in some treatments, we supplemented soil nutrients or water. Monitoring of microclimate in our treatments over the growing season confirmed that they were providing the desired manipulations. We assessed the performance of *Deschampsia* and *Colobanthus* under each frame by examining photosynthetic responses as well as more integrated measures such as growth rate and reproductive success. Field net photosynthetic rates (Pn) of both species were usually higher under warming treatments than under partial or open treatments. The notable exception was on warm, sunny days (canopy air temperature higher than 20°) when Pn of plants under all treatments was close to 0. In contrast to warming, UV-exclusion did not have any consistent effects on Pn of either species, although Pn tended to be higher when UV-B was excluded, particularly early in the season. By examining the photosynthetic temperature and light responses of plants brought into the laboratory, we found that high temperatures, not high levels of visible light, appeared to be responsible for depressions in Pn on warmer days (which were nearly always sunny) in the field. The temperature optima for Pn in both species was about 12°, above which Pn levels declined rapidly. Net photosynthesis of both species was very sensitive to temperature. We did not find any warming or UV-treatment effects on chlorophyll concentrations. UV-B–absorbing compound concentrations were higher under the warming treatments but were not influenced by UV-exclusion. Compared to plants of temperate regions, concentrations of these screening compounds were very high in both species under all treatments (Day 1993), a finding that may stem from the high UV-B to photosynthetically available radiation ratios these plants are exposed to early in the growing season during ozone hole events.

Our warming treatments had very strong effects on sexual reproduction of both species. Under warming, the reproductive structures of both species were more developed or mature on each of three census dates, and warming led to more reproductive structures in *Colobanthus*. Although warming appears to improve reproductive success of plants, we suspected that enhanced UV-B may counteract this by damaging pollen. We developed a protocol for examining the effects of UV-B on pollen (using two annual plant species) and found that exposure to enhanced UV-B lead to large reductions (more than 40 percent) in the production of viable pollen per plant (Demchik and Day 1996). We are preparing to employ these techniques with *Deschampsia* and *Colobanthus* at our field site.

We found no evidence that performance of either species was improved with the addition of soil nutrients or water. Not surprisingly, soil analysis indicated that levels of carbon are relatively low whereas levels of nutrients such as nitrogen are generally high. (Carbon-to-nitrogen ratios are less than 10.) We suspect that soil elemental cycling processes are strongly limited by carbon. Hence, warming could have a strong positive feedback on nutrient cycling and plant performance since warming will likely improve plant productivity, and ensuing carbon additions (via litter) will enhance nutrient decomposition rates and provide additional nutrients for plant growth.

In addition to our main experiment on Stepping Stones, we discovered a new (undocumented) population of *Colobanthus* on Gamage Point, near Palmer Station. Using allometric relationships developed between cushion diameter and age for this species, we reconstructed age distributions and estimated population recruitment each year; we estimate this population became established in 1982. We found strong correlations between recruitment and mean summer temper-

atures (Grobe, Ruhland, and Day in press), which corroborates our findings that warmer temperatures improve plant performance.

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