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Dynamics of ammonium oxidizer activity and nitrous oxide (N_2O) within and beneath Antarctic sea ice

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ABSTRACT: Nitrapyrin, an inhibitor of NH_4^+ oxidizing bacteria, was used to estimate the activity of NH_4^+ oxidizing bacteria in the bottom 1 to 15 cm of annual sea ice and in the water column at various locations in McMurdo Sound and along the Ross Ice Shelf (RIS). Nitrapyrin significantly inhibited dark ^{14}C-HCO_3^- uptake in virtually all sea-ice samples, indicating the presence of NH_4^+ oxidizing bacteria. Inorganic carbon fixation by sea ice NH_4^+ oxidizers was only a small fraction of that fixed by sea-ice photoautotrophs, both on an hourly and annual basis. Despite their relative lack of importance to inorganic carbon fixation, NH_4^+ oxidizing bacteria may have an important role in the N dynamics within the biogenic layer of annual sea ice both in terms of NH_4^+ utilization and eventual NO_3^- production. Inorganic carbon fixation in the water column beneath sea ice was generally not inhibited significantly by nitrapyrin. NH_4^+ oxidizer activity was also not detectable in deep water flowing beneath (southward) or from under (northward) the RIS. N_2O (a by-product of NH_4^+ oxidation) levels in pelagic samples were always near 100 % of saturation with respect to the air above the sea surface corroborating the low levels of NH_4^+ oxidizer activity found in the water column.

INTRODUCTION

Micro- and macrofaunal densities in McMurdo Sound and under the Ross Ice Shelf (RIS), Antarctica, are surprisingly high despite little or no annual phytoplankton production (Littlepage & Pearse 1962, Dayton & Oliver 1977, Azam et al. 1979, Bruchhausen et al. 1979, Lipps et al. 1979, SooHoo et al. 1987). Autotrophic production by chemoautotrophic nitrifying bacteria has been implicated as a possible explanation for the persistence of observed macrofaunal populations under the RIS (Horrigan 1981). Specific chemoautotrophic nitrifying bacteria oxidize NH_4^+ to NO_2^- and NO_2^- to NO_3^-, respectively, the energy of which is used by the organisms to assimilate CO_2. Given adequate NH_4^+, nitrification can thus provide new reduced particulate carbon aphotically, and simultaneously influence nitrogen dynamics.

NH_4^+ concentration is relatively high (ca 1 µM) in the water immediately beneath the RIS both at the shelf's edge (Biggs et al. 1985, J. C. Priscu unpubl.) and at a site where a hole was melted through the 400 to 600 m thick RIS about 400 km from open water (Jacobs et al. 1979, see also Horrigan 1981). NH_4^+ levels of up to 5 µM have been measured in melt ice from the lower 5 cm of 2 m long ice cores in the land-fast ice of McMurdo Sound (J. C. Priscu unpubl., Sullivan & Buck unpubl.). In view of the relatively low half-saturation constants for NH_4^+ by NH_4^+ oxidizing bacteria (< 1 µM NH_4^+) shown by Olson (1981), the water under the RIS and the lower layers of land-fast sea ice would appear to be suitable environments to support growth of chemoautotrophic NH_4^+ oxidizing bacteria.

Our first objective was to quantify the rate of inorganic carbon fixation (primary production) by chemoautotrophic NH_4^+ oxidizing bacteria associated with (1) land-fast sea ice in McMurdo Sound, (2) water under the RIS and (3) water under fast ice in McMurdo Sound. Our second objective was to examine potential linkages between NH_4^+ oxidizer activity and N_2O (a by-

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product of chemoautotrophic NH_4^+ oxidation) levels in seawater below the RIS and beneath fast ice in McMurdo Sound. Appropriate pelagic sampling sites were selected on the basis of published information on current vectors to determine NH_4^+ oxidizer activity in bacterioplankton which are entrained in water flowing southward (under) and northward (from beneath) the RIS.

METHODS

Sampling. All but one sea-ice sample for NH_4^+ oxidation experiments were collected from the land-fast ice of McMurdo Sound south of 77°S latitude (Fig. 1)

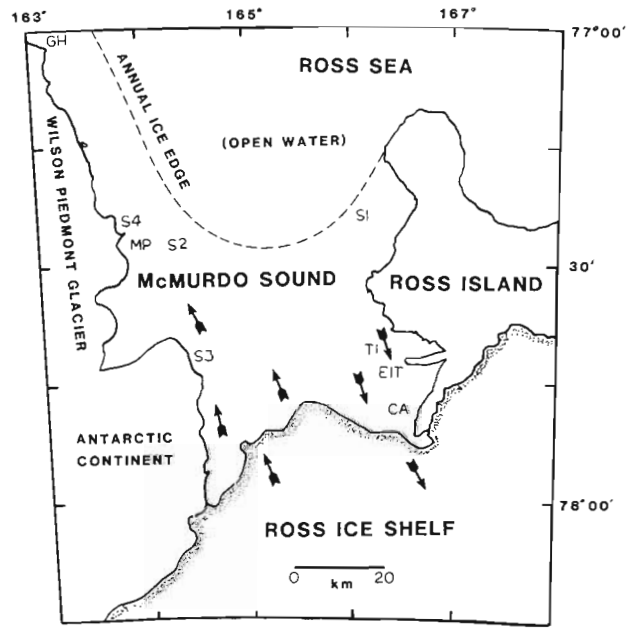

Fig. 1. McMurdo Sound (map modified from Dayton & Oliver 1977, Lewis & Perkin 1985, Palmisano et al. 1986, Barry & Dayton 1988) showing locations of sea ice and pelagic sampling sites. Net flow for the entire water column is denoted by arrows. GH: Granite Harbor; MP: Marble Point; TI: Tent Island; EIT: Erebus Ice Tongue; CA: Cape Armitage; S1 to S4 refer to sampling sites not associated with a geographical feature

during the austral summers (November to January) of 1985–1986 and 1986–1987. One ice sample was collected from pack ice at 64°22.80' S, 63°2.61' W during an austral winter (July 1987) cruise near the Bransfield Strait. Sea-ice cores of congelation ice were collected with a SIPRE coring device; platelet ice and pack-ice samples were obtained using SCUBA. The lower 10 cm of ca 2 m long cores and ice-algal slurries of platelet and pack-ice samples were melted slowly in 0°C, 0.2 µm-filtered seawater to minimize osmotic stress. The final salinity of the solution was kept between 29 and 33 ppt by appropriate additions of filtered seawater. All experiments were conducted on these cell suspensions.

Pelagic samples for NH_4^+ oxidizer activity and N_2O measurements were collected at several depths in the upper 50 m beneath the land-fast ice on the east and west sides of McMurdo Sound (Fig. 1). The water along the east side of the Sound is thought to be advected into the region from the north whereas the water along the west side is thought to issue from beneath the RIS (Dayton & Oliver 1977, Lewis & Perkin 1985, Palmisano et al. 1986, Barry & Dayton 1988). The average depth of the water in McMurdo Sound is about 550 m (see Barry & Dayton 1988, Fig. 1). Pelagic samples were also collected at 240 and 350 m for NH_4^+ oxidizing bacterial activity and N_2O measurements at 78°07.4' S, 175°58.4' W and 78°01.3' S, 179°01.6' W, respectively, within 2 km of the RIS (see Pillsbury & Jacobs 1988, Fig. 2, for site description). These depths represent water flowing under (southward) and from beneath (northward) the RIS (Pillsbury & Jacobs 1985). Two additional surface samples were collected within 2 km of the RIS at 77°28.2' S, 175°23.9' E (Site C) and 77°21.4' S, 175°0.00' E (Site D). All pelagic samples were obtained with a 9 l Niskin bottle. Care was taken throughout sampling and incubation to maintain in situ water temperatures which ranged from −1.0 to −2.2°C.

Measurement of NH_4^+ oxidizer activity. NH_4^+ oxidizer activity was determined by measuring dark ^{14}C-HCO_3^- uptake with and without nitrapyrin (2-chloro-6-trichloromethyl pyridine), a specific inhibitor of NH_4^+ oxidizing bacteria (Billen 1976, Somville 1978, Belser & Schmidt 1981). Nitrapyrin interferes with the initial biochemical step in NH_4^+ oxidation, catalyzed by NH_4^+ oxidase, where NH_4^+ is converted to hydroxylamine (Dua et al. 1979, Hooper & Terry 1979). Ten ml cell suspensions from melted cores or 130 ml seawater were preincubated in 20 ml scintillation vials or 130 ml borosilicate glass stoppered bottles, respectively, with nitrapyrin (10 mg l^{-1} final concentration) dissolved in 90% ethanol (0.08% final ethanol concentration) or with 0.08% ethanol controls for 1 h in the dark. The nitrapyrin was dissolved in ethanol to ensure its complete dissolution (Bremner et al. 1978). ^{14}C-HCO_3^- was then added to a final activity of 0.5 to 2 µCi ml^{-1}, and the incubations were continued for an additional 4 to 10 h in the dark at the temperature of collection. Replicates (4 or 5) were included for each treatment. The reaction was terminated by filtration of the entire sample through 0.2 µm pore size membrane filters (Nuclepore). The filters were rinsed with 15 ml of filtered seawater and fumed over hot concentrated HCl for 30 s before ^{14}C activity was determination by liquid scintillation spectrometry. NH_4^+ oxidizer activity was deemed significant when a t-test showed a significant

decrease (at the $p < 0.05$ level) in dark ^{14}C-HCO_3^- fixation by nitrapyrin relative to the controls.

N_2O measurement. N_2O was measured as described by Priscu & Downes (1985). Briefly, 15 ml seawater was removed without aeration from the hose of a Niskin bottle by a syringe and injected into 30 ml serum vials that had been sealed with neoprene stoppers and aluminum seals, and flushed with high purity N_2. These samples were preserved immediately by the addition of 0.7 ml formalin. On several occasions, subsamples were incubated in the dark at ambient sea temperature for 3 to 4 d before preservation to determine rates of N_2O change. A t-test was used to compare the significance of N_2O changes in the preserved vs the incubated sample. Following 1 h of vigorous shaking to equilibrate liquid and headspace gas, 1 ml of the headspace gas was analyzed for N_2O with a gas chromatograph fitted with an electron-capture detector. Ambient seawater concentrations of N_2O were determined after corrections for salinity, temperature and head-space volume (Weiss & Price 1980).

Culture experiments. To verify that the effect of nitrapyrin was specific for NH_4^+ oxidizing bacteria, nitrapyrin experiments were conducted in the laboratory with cultures of *Nitrosococcus oceanus,* a known NH_4^+ oxidizer isolated from the Southern California Bight by A. F. Carlucci and described by Watson (1965), and 2 strains of non-NH_4^+ oxidizing heterotrophic bacteria (HK11 and HK100) isolated from sea ice in McMurdo Sound by C. W. Sullivan (Kobori et al. 1984). *N. oceanus* was cultured at 20°C in the seawater media described by Ward (1987); heterotrophic bacteria were cultured at 0 to 4°C in DIFCO 2216E marine media (Kobori et al. 1984). All experiments were conducted when the organisms were in late exponential growth phase.

Inorganic carbon uptake experiments on *Nitrosococcus oceanus* were done in sterile, darkened glass scintillation vials (5 replicates per treatment) following the protocol described above for the sea-ice microorganisms, except that a separate set of replicates were killed at time zero by the addition of formalin (5% final conc.). Experiments were conducted at 20°C.

Thymidine incorporation by *Nitrosococcus oceanus* and the heterotrophic sea-ice bacteria was measured using ^3H-thymidine (Fuhrman & Azam 1982). Labeled [methyl-^3H] thymidine (concentration = 1 mCi ml^{-1}; sp. act. = 73 Ci $mmol^{-1}$) was added at a final concentration of 20 nM to sterile 20 ml scintillation vials (5 replicates per treatment) containing 10 ml of culture. The thymidine was initially taken to dryness under N_2 and rehydrated to the original volume to eliminate any volatile ^3H end-products that may have been formed by self radiolysis. Following 15 to 20 min of incubation at 20°C for *N. oceanus* and 0°C for the sea-ice bacteria, 10 ml of ice-cold 10% trichloroacetic acid (TCA) was added to each vial to stop the reaction and to precipitate nucleotides. Formalin (5% final concentration) was added at time zero to the killed samples. All samples were filtered onto 0.2 µm pore size polycarbonate filters and rinsed with 25 ml of ice-cold 5% TCA. Radioactivity on the filters was counted by standard liquid scintillation spectrometry calibrated by external standardization.

RESULTS

Significant NH_4^+ oxidizer activity occurred in virtually all sea-ice samples during December 1985 and in a Granite Harbor [GH (W)] sample examined during 1987 (Table 1). Rates ranged from 0.04 to 2.93 µmol C m^{-2} h^{-1} and showed no clear trends between samples collected from the east or west sides of McMurdo Sound [sides signified by (E) or (W) following station names]. NH_4^+ oxidizer activity in a single platelet ice sample was within the range of rates in congelation ice and was undetectable within an algal layer found at intermediate depths within multilayer ice collected near Marble Point [MP (W)] on the west side of McMurdo Sound.

Pelagic NH_4^+ oxidizer activity was generally undetectable in McMurdo Sound (Table 2). Based on the uncharacteristically high chlorophyll *a* concentration (7.73 µg l^{-1}) at 0 m at Stn S2 (W), the rate of 1.02 nmol C l^{-1} h^{-1} reflects possible sample contamination from the sea-ice community which occurred when the sampling hole was drilled through sea ice to gain access to the water column. Omitting surface water samples, pelagic chlorophyll *a* concentrations in McMurdo Sound during the sampling period were always below 0.8 µg l^{-1}. Measurable rates were obtained too infrequently to draw conclusions regarding spatial and temporal trends in NH_4^+ oxidizer activity.

NH_4^+ oxidizer activity was undetectable in the inflowing and outflowing deep water collected adjacent to the middle RIS (Table 3). The only site where significant NH_4^+ oxidizer activity occurred was the surface water at Site C where the rate (0.21 nmol C l^{-1} h^{-1}) was within the range of those measured in the pelagic waters of McMurdo Sound. Chlorophyll *a* values in the surface waters off the RIS were about 2-fold greater than the water collected from depth.

N_2O concentrations measured at various locations and depths in McMurdo Sound, and off the middle of the RIS, ranged between 30.9 and 33.9 nmol N_2O-N l^{-1} (Table 4). N_2O levels did not change significantly during dark incubations of up to 4.3 d. Based on an N_2O concentration of 318 ppbv at 20°C for the air above the sea surface, the pelagic N_2O values were not significantly different from air equilibrium.

Table 1. Average (±SD) dark ^{14}C-HCO_3^- uptake in control and nitrapyrin-amended samples, ammonium oxidizer activity (NH_4^+ ox) and chlorophyll *a* levels associated with the bottom of land-fast sea ice at various locations in McMurdo Sound. NH_4^+ ox represents the difference between control and nitrapyrin-amended samples. E, W: site on east or west side of Sound, respectively. C: congelation ice; P: platelet ice; IB: inner band of multi-year ice. Standard deviations on ammonium oxidizer activity were obtained by error propagation according to Parratt (1961) assuming random errors

Site	Ice type	Control	Nitrapyrin (μmol C m^{-2} h^{-1})	NH_4^+ ox	Chlorophyll (mg m^{-2})
1985					
12 Dec EIT (E)	C	11.11 ±1.75	8.18 ±0.79	2.93 ±1.92	128.6
16 Dec EIT (E)	C	2.47 ±0.28	2.03 ±0.28	0.44 ±0.40	40.3
18 Dec EIT (E)	P	10.16 ±0.76	7.89 ±0.67	2.27 ±0.98	36.7
20 Dec S1 (E)	C	0.21 ±0.04	0.20 ±0.03	0.01^{ns} ±0.05	0.8
20 Dec S2 (W)	C	3.26 ±0.19	1.69 ±0.17	1.57 ±0.26	38.3
27 Dec CA (E)	C	7.03 ±1.21	4.87 ±0.92	2.16 ±1.52	69.0
1986–87					
10 Dec MP (W)	IB	0.03 ±0.003	0.03 ±0.003	-0.004^{ns} ±0.004	0.5
11 Dec TI (E)	C	2.21 ±0.18	1.89 ±0.14	0.32^{ns} ±0.23	50.5
4 Feb GH (W)	C	0.20 ±0.02	0.15 ±0.02	0.04 ±0.02	no data
4 Jul PST[a]	C	0.01 ±0.001	0.01 ±0.001	0.003^{ns} ±0.001	no data

[a] PST: congelation ice sample collected from pack ice in the Bransfield Strait near Palmer Station. NH_4^+ ox rate given as μmol C l^{-1} h^{-1}

ns: nitrapyrin treatment had no significant effect ($p > 0.05$) on dark ^{14}C-HCO_3^- uptake, i.e. rate of dark ^{14}C-HCO_3^- uptake by NH_4^+ oxidizing bacteria was insignificant

To ensure that nitrapyrin did not inhibit heterotrophic sea-ice bacterial activity (i.e. to verify its specificity for NH_4^+ oxidizers), we examined the influence of nitrapyrin on ^3H-thymidine incorporation (a measure of DNA synthesis) on 2 strains of heterotrophic bacteria isolated from McMurdo Sound sea ice. Nitrapyrin had no significant effect on thymidine incorporation of either of the strains tested (Table 5).

We also tested the effect of nitrapyrin on dark ^{14}C-HCO_3^- uptake by the known marine NH_4^+ oxidizing bacterium *Nitrosococcus oceanus*. Dark ^{14}C-HCO_3^- uptake was completely eliminated (to the level of the controls) at the concentrations and incubation periods used (which were similar to those used in field studies). A further experiment to examine the influence of nitrapyrin on thymidine incorporation was inconclusive because *N. oceanus* showed no significant thymidine incorporation. These laboratory results indicate that nitrapyrin, used according to our protocol and in systems not conducive to carbon monoxide and methane oxidation (which are also sensitive to nitrapyrin; Topp & Knowles 1984, Ward 1987), is specific for dark ^{14}C-HCO_3^- fixation by NH_4^+ oxidizing bacteria. Because the environments we studied are generally well oxygenated (Jacobs & Haines 1982, Barry 1988), carbon monoxide and methane oxidation were presumed to be negligible.

A significant correlation ($r = 0.98$, $n = 8$) was found when areal rates from sea-ice core samples are converted to volumetric rates and plotted, along with the pelagic rates, as a function of chlorophyll *a* (Fig. 2). This correlation indicates that the activity of NH_4^+ oxidizing bacteria is closely coupled with microalgal standing stock.

DISCUSSION

The rates of dark ^{14}C-HCO_3^- fixation by ammonium oxidizing bacteria in the bottom layers of sea ice in McMurdo Sound ranged from below detection to 2.93 μmol C m^{-2} h^{-1}. On an hourly basis, new particulate organic carbon input via this process is less than 1.0%

Table 2. Average (±SD) dark ^{14}C-HCO_3^- uptake in control and nitrapyrin-amended samples, NH_4^+ oxidizer activity (NH_4^+ ox) and chlorophyll *a* levels from samples collected beneath annual fast-ice in McMurdo Sound. NH_4^+ ox represents the difference between control and nitrapyrin-amended samples. Standard deviations on NH_4^+ oxidizer activity were obtained by error propagation according to Parratt (1961) assuming random errors. E, W: site on east and west side of Sound, respectively

Site and depth	Control	Nitrapyrin (nmol C l^{-1} h^{-1})	NH_4^+ ox	Chlorophyll ($\mu g\ l^{-1}$)
1985				
20 Dec S1 (E) 0 m	3.51 ±4.25	2.71 ±2.43	0.80^{ns} ±4.90	1.04
20 Dec S1 (E) 25 m	0.47 ±0.24	0.42 ±0.29	0.05^{ns} ±0.37	0.71
20 Dec S1 (E) 50 m	0.29 ±0.03	0.24 ±0.015	0.05^{ns} ±0.04	0.69
20 Dec S2 (W) 0 m	1.72 ±0.19	0.70 ±0.13	1.02 ±0.23	7.73
20 Dec S2 (W) 25 m	0.21 ±0.03	0.26 ±0.07	−0.05^{ns} ±0.08	0.33
20 Dec S2 (W) 50 m	0.14 ±0.02	0.13 ±0.03	0.01^{ns} ±0.04	0.12
22 Dec EIT (E) 0 m	0.66 ±0.31	0.28 ±0.08	0.38 ±0.32	no data
22 Dec EIT (E) 12 m	0.24 ±0.04	0.20 ±0.02	0.04^{ns} ±0.05	0.64
22 Dec EIT (E) 25 m	0.28 ±0.02	0.18 ±0.04	0.10 ±0.05	0.77
1986				
10 Dec S3 (W) 1 m	0.13 ±0.02	0.11 ±0.07	0.02^{ns} ±0.07	0.30
10 Dec S3 (W) 25 m	0.02 ±0.01	0.03 ±0.02	−0.01^{ns} ±0.02	0.02
10 Dec S3 (W) 50 m	0.09 ±0.02	0.08 ±0.02	0.01^{ns} ±0.03	0.02
10 Dec S4 (W) 1 m	0.03 ±0.003	0.04 ±0.01	−0.01^{ns} ±0.01	0.02
10 Dec S4 (W) 25 m	0.02 ±0.01	0.03 ±0.01	−0.01^{ns} ±0.02	0.02
10 Dec S4 (W) 50 m	0.04 ±0.002	0.06 ±0.04	−0.02^{ns} ±0.04	0.01

ns: nitrapyrin treatment had no significant effect ($p > 0.05$) on dark ^{14}C-HCO_3^- uptake, i.e. rate of dark ^{14}C-HCO_3^- uptake by NH_4^+ oxidizing bacteria was insignificant

Table 3. Average (±SD) dark ^{14}C-HCO_3^- uptake in control and nitrapyrin-amended samples, NH_4^+ oxidizer activity (NH_4^+ ox) and chlorophyll *a* levels from samples collected in open water adjacent to the center of the RIS. NH_4^+ ox represents the difference between control and nitrapyrin-amended samples. Standard deviations on NH_4^+ oxidizer activity were obtained by error propagation according to Parratt (1961) assuming random errors. Coordinates for each station are given in 'Methods'

Site and depth	Control	Nitrapyrin (nmol C l^{-1} h^{-1})	NH_4^+ ox	Chlorophyll ($\mu g\ l^{-1}$)
1987				
10 Feb 350 m (Shelf outflow)	0.16 ±0.04	0.12 ±0.03	0.04^{ns} ±0.04	0.13
11 Feb 240 m (Shelf inflow)	0.15 ±0.03	0.16 ±0.03	0.01^{ns} ±0.05	0.65
14 Feb 0 m (Site C)	0.52 ±0.07	0.31 ±0.04	0.21 ±0.08	1.21
14 Feb 0 m (Site D)	4.66 ±0.38	4.53 ±0.17	0.13^{ns} ±0.42	1.39

ns: nitrapyrin treatment had no significant effect ($p > 0.05$) on dark ^{14}C-HCO_3^- uptake, i.e. rate of dark ^{14}C-HCO_3^- uptake by NH_4^+ oxidizing bacteria was insignificant

Table 4. Nitrous oxide (N_2O) concentrations under annual fast-ice in McMurdo Sound and in the deep inflow and outflow from the Ross Ice Shelf. % Saturation is based on an N_2O concentration of 318 ppbv for the air above the sea surface. The t_0 samples were preserved immediately after collection in 5 % formalin; the second set was incubated in the dark at the temperature of collection for 3.1 to 4.3 d. Mean (±SD) of 5 replicates is shown

Site and depth	nmol N_2O-N l^{-1}	% Saturation
McMurdo Sound		
20 Dec 1985		
S1 (E) 0 m t_0	30.9 ± 2.9	93.6 ± 8.8
+ 4.3 d	33.0 ± 0.9	100.2 ± 2.8
S1 (E) 25 m t_0	33.6 ± 1.1	102.0 ± 3.4
+ 4.3 d	33.1 ± 0.6	100.0 ± 1.9
S2 (W) 0 m t_0	33.3 ± 1.3	101.0 ± 4.3
+ 4.3 d	32.4 ± 1.6	98.1 ± 4.5
S2 (W) 25 m t_0	33.9 ± 0.9	103.2 ± 3.0
+ 4.3 d	34.4 ± 0.1	104.6 ± 2.8
Ross Ice Shelf		
(Polar Sea 1987)		
10 Feb 350 m (Shelf outflow)		
t_0	31.6 ± 1.3	96.2 ± 4.1
+ 3.5 d	31.9 ± 1.4	97.0 ± 4.5
11 Feb 240 m (Shelf inflow)		
t_0	33.5 ± 0.4	105.6 ± 1.1
+ 3.1 d	33.5 ± 0.4	102.6 ± 9.2

of photoautotrophic primary production rates measured for congelation ice microalgae (ca 3500 µmol C m^{-2} h^{-1}; Palmisano et al. 1985) during the austral summer. However, fixation of ^{14}C-HCO_3^- by NH_4^+ oxidizers can occur during the dark winter months in McMurdo Sound whereas photosynthetic production is essentially restricted to periods when the sun is above the horizon. Based on accumulation of chlorophyll *a* in the ice column, annual primary production by sea-ice

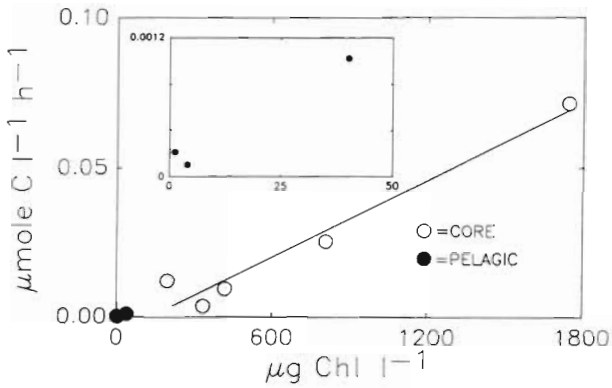

Fig. 2. Comparison of NH_4^+ oxidizer activity (µmol C l^{-1} h^{-1}) and chlorophyll *a* concentration (µg chl l^{-1}) in core and pelagic samples. Rates in core samples were converted to volume estimates for the comparison. Inset shows an expanded view of the pelagic relationship. A least squares fit through the data was significant ($r = 0.98$, $p < 0.05$)

microalgae in McMurdo Sound has been estimated to be 342 mmol C m^{-2} yr^{-1} (Palmisano & Sullivan 1983). Assuming (1) an average inorganic carbon fixation rate by NH_4^+ oxidizing bacteria in the ice column of 2 µmol C m^{-2} h^{-1}, and (2) that this rate is constant for 10 mo of ice cover, the annual estimate of new organic carbon production would be about 15 mmol m^{-2} yr^{-1}. This extrapolated rate is still less than 5 % of new organic carbon production by microalgal photosynthesis in sea ice and could be even lower if the relationship shown in Fig. 2 is considered; lower algal biomass during early and late periods of ice cover may result in diminished rates of NH_4^+ oxidation. It should be noted that, owing to generally negligible NO_2^- pools in the environments studied, additional new carbon is presumably fixed via NO_2^- oxidation to NO_3^-. Because about 65 % less CO_2 is fixed for every mole of NO_2^- oxidized with respect to an equivalent amount of NH_4^+ oxidized (Atlas 1984), we conclude that the total amount of new carbon fixed by

Table 5. Influence of nitrapyrin on (1) ^3H-thymidine incorporation in 2 strains of sea-ice bacteria, (2) ^{14}C-HCO_3^- incorporation in *Nitrosococcus oceanus* and (3) ^3H-thymidine incorporation in *N. oceanus*. Results are expressed as dpm (±SD) and are relative for each experiment, i.e. the same biomass, isotopic acitvity, incubation time and filtration volumes were used for each individual experiment. The p-levels are based on a t-test and denote the statistical probability that nitrapyrin-treated samples and controls will be the same. See 'Methods' for details

	Organism	dpm ± SD			p-level
		Kill	Control	Nitrapyrin	
(1) Thymidine incorp.	HK-31	1480 ±98	95268 ±6066	100029 ±12027	> 0.05
	HK-100	638 ±59	1534 ±248	1763 ±222	> 0.05
(2) ^{14}C-HCO_3^- incorp.	*N. oceanus*	2797 ±888	200673 ±32382	2161 ±493	< 0.01
(3) Thymidine incorp.	*N. oceanus*	436 ±82	479 ±37	461 ±17	> 0.05

complete nitrification is insignificant relative to photosynthetic carbon fixation.

Despite little relative importance with respect to new organic carbon input in sea ice, NH_4^+ oxidation may play a significant role in the N dynamics of the sea-ice microbial community. Assuming that NH_4^+ oxidizing bacteria fix 0.1 mole of HCO_3^- for each mole of NH_4^+ oxidized (see Priscu & Downes 1985), an NH_4^+ demand of up to 29 μmol NH_4^+ m^{-2} h^{-1} can be attributed to NH_4^+ oxidizers. Priscu et al. (1989) measured NH_4^+ incorporation rates into particulate organic matter in congelation ice of 0.61 nmol NH_4^+ (μg chlorophyll *a*)$^{-1}$ h^{-1}. Using the average chlorophyll *a* value for congelation ice presented in Tabel 1 (55 mg chlorophyll *a* m^{-2}), chlorophyll *a* specific NH_4^+ uptake can be converted to an areal rate of 33.6 μmol NH_4^+ m^{-2} h^{-1} which is similar to the NH_4^+ demand by NH_4^+ oxidizers. Applying a similar calculation to the NO_3^- uptake data presented by Priscu et al. (1989), sea-ice microalgal NO_3^- uptake can be estimated at 43.5 μmol m^{-2} h^{-1}. This value is near the amount of NO_3^- which can be produced by nitrification (assuming that NH_4^+ is nitrified completely to NO_3^-, up to 29 μmol NO_3^- m^{-2} h^{-1} can be produced). These estimates imply that (1) NH_4^+ oxidizers may compete with microalgae for available NH_4^+ and (2) NO_3^- is a regenerated nutrient which can supply almost 70 % of the NO_3^- requirements of the McMurdo Sound fast-ice microalgal community. Because pelagic NH_4^+ oxidizer activity was significant at only a few locations in McMurdo Sound and one surface site along the RIS, its importance on an annual basis cannot be estimated with any degree of certainty until techniques with greater sensitivity are applied to the measurement of NH_4^+ oxidizing bacteria activity in the oligotrophic waters found under fast-ice in McMurdo Sound and beneath the RIS.

Nitrous oxide in marine systems is generally thought to be a by-product of chemoautotrophic NH_4^+ oxidation (Kaplan & Wofsy 1985). The ratio of NH_4^+ oxidized to N_2O produced is a function of O_2 concentration; 1 atom of N appears in N_2O for every 300 to 1000 atoms of NH_4^+-N oxidized under well-oxygenated conditions (Kaplan & Wofsy 1985). That our pelagic N_2O data are close to equilibrium with the air above the sea surface implies that McMurdo Sound and water associated with the RIS are not major sources of N_2O, corroborating the low to non-detectable hourly NH_4^+ oxidation rates we found.

It might be expected that the sea-ice cover would hinder trace gases such as N_2O from escaping into the atmosphere. However, unlike freshwater ice, sea ice is more permeable to gases (Hemmingsen 1959, Gosink et al. 1976). Field studies showed that trace gases such as CH_4, CO, CO_2, H_2 and N_2O are exchanged at the ice-atmosphere interface in Arctic (Gosink et al. 1976, Gosink & Kelley 1977) and Antarctic regions (Gosink 1980). Consequently, the outward flux of N_2O produced at low rates in the water column under sea ice or within sea ice could balance production, making these environments appear to be neither a source or sink of N_2O. Without accurate knowledge of N_2O transfer rates through Antarctic fast ice, our bulk N_2O data can only be used to approximate N_2O dynamics in McMurdo Sound. Unlike sea ice, the ca 400 m thick RIS should form an effective barrier to atmospheric exchange. Unfortunately, little is known about the residence time of the water beneath the RIS (Jacobs et al. 1979, S. Jacobs pers. comm.) although Michel et al. (1979) have indicated that there has been a complete exchange of water with the Ross Sea since nuclear testing (ca 35 yr). Low NH_4^+ oxidizer activity, coupled with unknown water mass exchange, would make it difficult to use N_2O as a signature of NH_4^+ oxidizer activity under the RIS.

The observation that detectable N_2O production did not occur during short-term sealed experimental incubations is not surprising given the rates of NH_4^+ oxidizer activity. Based on the ratio of 700 moles NH_4^+ oxidized : mole N_2O-N produced, less than 0.5 nmol N_2O-N l^{-1} would have been produced during our 3 to 4 d incubations. Such a change would be undetectable given instrument sensitivity and the variability within our experiments.

Nitrous oxide is accumulating in the atmosphere at a rate of about 0.3 % per year (Weiss 1981, Rasmussen & Khalil 1986). There is concern that this build-up, thought to be due to anthropogenic input, may influence global temperatures and alter stratospheric ozone levels (Crutzen 1981, Rasmussen & Khalil 1986). Nitrous oxide concentrations in many areas of the ocean exceed atmospheric saturation by more than 200 % (Pierotti & Rasmussen 1980) making these systems important natural sources of atmospheric N_2O. Yoshinari (1976) and Pierotti & Rasmussen (1980) have reported mean global atmospheric N_2O levels of 328 and 332 ppbv, respectively; Pierotti & Rasmussen's value has recently been corrected downward to 302 ppbv (Rasmussen & Khalil 1986). Rasmussen & Khalil also reported mean atmospheric levels of 307.3 and 307.5 ppbv for January 1985 for sites in the Pacific Northwest and at the South Pole. The atmospheric value we used (318 ppbv) to compute seawater saturation values was measured over central North Island, New Zealand. This value is within 5 % of those given in the above reports and, in concert with the seawater N_2O values we measured, further implies that the McMurdo Sound and RIS regions of Antarctica are not major sources or sinks of N_2O.

The positive relationship we noted between NH_4^+ oxidizing bacterial activity and chlorophyll *a* concen-

tration is supported indirectly by other studies conducted in and under the sea ice of McMurdo Sound. A seasonal study by Grossi et al. (1984) presented information over the spring bloom of sea-ice microalgae which showed a strong positive correlation between bacterial and microalgal biomass over time. The significant positive correlation between bacterial biomass and production, and microalgal biomass and production in McMurdo Sound sea ice, led Kottmeier et al. (1987) to suggest a direct coupling between bacterial growth and microalgal photosynthesis. Pelagic bacterial activity in McMurdo Sound has also been shown to increase over time with *Phaeocystis pouchetii* cell number during a mid-December bloom of this phytoplankton species (Palmisano et al. 1986).

Through close association with microalgae and heterotrophic bacteria, NH_4^+ oxidizers can benefit from recycled NH_4^+, the presence of surfaces for attachment, and certain dissolved organic carbon compounds produced by microalgae and bacteria (Witzel & Overbeck 1979). $^{15}NH_4^+$ isotope dilution experiments (J. C. Priscu unpubl.) have shown internal regeneration to be an important source of NH_4^+ to the sea-ice community. Consequently, association with heterotrophic bacteria and other NH_4^+ remineralizers can supply the oxidizeable substrate required for nitrification. Sullivan & Palmisano (1984) concluded that ca 30% of the ice bacteria in McMurdo Sound were attached to living algae or associated detritus. Although these authors did not identify the attached organisms or measure their activity, laboratory studies have shown that nitrifying bacteria (both NH_4^+ oxidizers and NO_2^- oxidizers) grow better when attached to surfaces (Underhill & Prosser 1987, Diab & Shilo 1988, Keen & Prosser 1988). Diab & Shilo found that the nitrifying bacterial genera *Nitrosomonas* and *Nitrobacter* rapidly attached to a number of different surfaces which resulted in enhanced activity of attached bacteria relative to freely suspended cells. They further showed that enhancement in the nitrifying activity was rapid (within 1 h) following attachment and that attachment increased the survival of the cells relative to unattached cells. Assuming that surface attachment by NH_4^+ oxidizing bacteria is important in sea ice, our sea-ice NH_4^+ oxidation rates, which were conducted on melted samples, may underestimate actual rates in situ.

Previous results (Priscu & Downes 1985) indicate that the correlation between NH_4^+ oxidizer activity and chlorophyll *a* concentration was not due to inhibition of microalgal dark $^{14}C\text{-}HCO_3^-$ uptake by nitrapyrin. Furthermore, Sorokin (1971, 1973) and Peterson (1979) claimed that dark $^{14}C\text{-}HCO_3^-$ uptake by phytoplankton in certain marine waters was insignificant relative to bacterial uptake, and Taguchi (1983) found no statistically significant relationship between dark $^{14}C\text{-}HCO_3^-$ fixation and phytoplankton primary production (i.e. photosynthesis) in the ocean domain or shelf domain of the Weddell Sea. Total dark $^{14}C\text{-}HCO_3^-$ uptake (i.e. not amended with nitrapyrin) was also not significantly correlated with chlorophyll *a* for our pelagic samples ($r = 0.35$, $df = 16$, $p > 0.05$). We thus conclude that our method was specific for chemosynthetic NH_4^+ oxidizing bacteria and contend that nitrifying bacteria grow in close association with other microbes, particularly microalgae, present in Antarctic sea ice and pelagic waters.

The impetus for our study was provided by Horrigan (1981) who reported values of dark $^{14}C\text{-}HCO_3^-$ fixation under the RIS of about 0.1 mg C m^{-3} d^{-1} (ca 0.35 nmol C l^{-1} h^{-1}) leading her to suggest that chemoautotrophic bacteria such as nitrifiers might be an important source of new organic carbon to this system. Since Horrigan's study, Taguchi (1983) has measured dark $^{14}C\text{-}HCO_3^-$ fixation rates in the Weddell Sea of 0.16 to 0.26 mg C m^{-3} d^{-1} (ca 0.55 to 0.90 nmol C l^{-1} h^{-1}). The average pelagic dark $^{14}C\text{-}HCO_3^-$ uptake (i.e. without nitrapyrin addition) we measured was 0.70 nmol C l^{-1} h^{-1} which is near those reported by Horrigan and Taguchi. It should be noted that dark uptake rates exceeded 1 nmol C l^{-1} h^{-1} at 3 sites (20 Dec S1 (E) 0m; 20 Dec S2 (W) 0m; 14 Feb Site D 0m). As mentioned earlier, the 20 December samples appeared to be contaminated with sea-ice microorganisms during collection; we have no simple explanation for the uncharacteristically high rate at Site D. That nitrapyrin had relatively little effect on pelagic dark $^{14}C\text{-}HCO_3^-$ uptake in our study (most differences were not statistically significant), implies that much of the dark $^{14}C\text{-}HCO_3^-$ uptake is from heterotrophic and, perhaps in euphotic waters, microalgal anapleurotic carboxylation reactions rather than NH_4^+ oxidizing bacteria.

We emphasize that low *hourly* inorganic carbon fixation by NH_4^+ oxidizing bacteria, relative to microalgal production during the austral summer, does not conclusively demonstrate that annual chemoautotrophic new production via NH_4^+ oxidation is not an important source of new organic carbon to the foodweb under Antarctic sea ice in McMurdo Sound and under the RIS. Even if low NH_4^+ oxidation rates (i.e. below our limits of detection) persisted, the *annual,* integrated water-column contribution of new carbon by this process could be significant relative to microalgal primary production, the latter of which is either non-existent or very low owing to ice cover and lack of solar energy during the austral winter.

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