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The distribution and relative abundance of ammonia-oxidizing bacteria in lakes of the McMurdo Dry Valley, Antarctica

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Abstract

Marked differences in the concentrations of major ions and cations, macronutrient chemistry and general trophic status exist among the lakes of the McMurdo dry valleys in Antarctica. These differences have been attributed to both variations in stream inputs and *in situ* lake processes (Priscu, 1995; Lizotte et al., 1996, Spigel and Priscu, 1996). This study examines the role of nitrifying bacteria in nitrogen transformations in these lakes. Applying two polymerase chain reaction (PCR) assays targeting the 16S rRNA genes of ammonia-oxidizing bacteria and the active site of the ammonia monooxygenase gene (amoA), the distribution of ammonia-oxidizers was examined in six Antarctic lakes: Lake Bonney, Lake Hoare, Lake Fryxell and Lake Joyce in the Taylor Valley, Lake Miers in the the Miers Valley and Lake Vanda in the Wright Valley. Using a two stage amplification procedure, ammonia-oxidizers from both the beta and gamma- subclasses of the Proteobacteria were detected and their relative abundances were determined in samples collected from all sites. Ammonia-oxidizers were detected in all lakes sampled. Members of the gamma subclass were only present in the saline lakes. In general, nitrifiers were most abundant at depths above the pycnocline and were usually associated with lower concentrations of NH_4 and elevated concentrations of NO_3 or NO_2. The distribution of nitrifiers suggests that the primary N_2O peak observed in most of the lakes was produced via nitrification. Preliminary data on the rate of nitrification (Priscu et al., 1996) support the occurrence of nitrification and the presence of nitrifiers at the depth intervals where nitrifiers were detected. In all lakes, except Lake Miers, the data indicate that nitrifying bacteria have an important role in the vertical distribution of nitrogen compounds in these systems.

Introduction

Biogeochemical processes in the nitrogen cycle of aquatic systems are controlled primarily by microorganisms. One of these processes, nitrification, the two-step oxidation of NH_4^+ through NO_2^- to NO_3^-, is a link between oxidized and reduced forms of nitrogen and is, therefore, of fundamental importance in all ecosystems. Nitrification has been investigated for its potential importance as an oxygen sink, as a source of nitrite and nitrate, substrates for denitrification, which results in the subsequent loss of fixed nitrogen from the system, and in aquatic systems as the supply of nitrate fueling surface primary productivity (Ward, 1986). Under low oxygen conditions, nitrification can also result in the production and release of trace gases which are not available for phytoplankton uptake and may contribute significantly to the distribution and flux of nitrous oxide (N_2O) and nitric oxide (NO) in aquatic environments and the atmosphere (Goreau et al., 1980; Poth & Focht, 1985; Downes, 1988; Priscu et al., 1996). Nitrification is often tightly coupled to denitrification (dissimilatory nitrate reduction) in environments containing oxygen gradients. Nitrification and denitrification are performed by phylogenetically and metabolically distinct groups of bacteria; denitrification is an anaerobic heterotrophic process, but shares many of the same substrates and intermediates

as nitrification. The spatial overlap of the distribution of nitrifying and denitrifying bacteria around environmental gradients and their metabolic overlap confound direct interpretation of rate measurements and features in nutrient profiles. As with nitrification, the significance of denitrification on global nitrogen budgets is still in dispute and varies considerably among systems.

Tracer methods have provided means to measure in-situ nitrification rates. Within marine aquatic environments, consistent patterns have emerged with respect to the magnitude and distribution of nitrifying activity. In general, higher nitrification rates are associated with more eutrophic regions or lower light environments (Ward, 1986). Minimum nitrification occurs in the surface photic zone where irradiance is high and substrates low; maximum rates are observed just below, in association with the nitrocline (Ward, 1986). Much less is known about water column nitrification in lakes and generalizations about the distribution of nitrification are not as straight forward (e.g. Hall, 1986). The environmental interpretation of these measurements often relies on laboratory studies examining the interaction of cultured nitrifying bacteria with their substrates and with environmental factors which may control their activity. A more appropriate analysis would employ studies of *in situ* populations using accurate methods for detecting and enumerating nitrifying bacteria. Immunofluorescent assays have been modified to study the serological diversity and distribution of nitrifying bacteria in soils (Schmidt, 1974), lake sediments (Smorczewski & Schmidt, 1991) and marine environments (Ward & Perry, 1982; Ward & Carlucci, 1985). However, this approach is limited by the difficulties associated with culturing representatives from the natural environment. Moreover, little is known about the diversity or genetic composition of lacustrine lithotrophic nitrifiers. The number of species that have been isolated and described from lakes is small even compared with the accepted low diversity of terrestrial and marine lithotrophic nitrifiers (Hall, 1986).

Studies based on the analyses of 16S rRNA and the use of the polymerase chain reaction have become common in the investigation of the spatial distribution of microbial taxa in the natural environment which have not been grown in culture (Giovanonni & Cary, 1993, Amann, 1995). Cultured ammonia-oxidizing bacteria belong to two phylogenetically distinct lineages within the Proteobacteria (Head et al., 1994; Teske et al., 1995); the gamma subclass line contains a single species and the beta subclass cluster contains several genera. Using a two stage amplification technique (Voytek & Ward 1995; Voytek, 1996), ammonia-oxidizing bacteria of the beta and gamma subclass can be detected and the relative abundance determined. There is some evidence that nitrifiers may be more phylogenetically diverse than we thought (i.e. not all nitrifying bacteria in natural systems belong to these two groups; Hovanec & DeLong, 1996). To try to capture the entire nitrifying population, techniques have been developed recently targetting a functional gene in the process of nitrification, ammonia monoxoygenase (AMO) (Holmes et al., 1995; Rotthauwe et al., 1997). AMO is the enzyme which catalyzes the oxidation of ammonia to hydroxylamine. The product of the amoA gene is the membrane bound active site polypeptide of AMO and primers designed to amplify this polypeptide have been developed as a means for analyzing indigenous ammonia-oxidizing bacteria (Rotthauwe et al., 1997).

In this study, we examined six permanently ice covered lakes in the McMurdo dry valleys, which is the largest relatively ice-free region on the Antarctic continent. The trophic status and chemistry of these lakes vary markedly and these differences are thought to be due to variations in meltwater input as well as in-situ biotic and abiotic lake processes (Priscu, 1995; Green et al., 1988). Using sensitive and specific molecular assays, we determined the distribution of ammonia-oxidizing bacteria to examine the role of nitrification in controlling the vertical distribution and magnitude of inorganic nitrogen compounds in these lakes.

Methods and materials

Chlorophyll *a*, ammonia, nitrite, nitrate and nitrous oxide concentrations were measured and the distribution of ammonia-oxidizers was examined in six Antarctic lakes: Lake Bonney (East and West Lobe), Lake Hoare, Lake Fryxell and Lake Joyce in the Taylor Valley, Lake Miers in the Miers Valley and from Lake Vanda in the Wright Valley.

Study sites

The McMurdo dry valleys ($\sim$ 4800 km^2, 77° 00′ S, 162° 52′ E) constitute the largest relatively ice-free region on the Antarctic continent and were formed by the advances and retreats of glaciers through the coastal ranges of the Transantarctic Mountains along

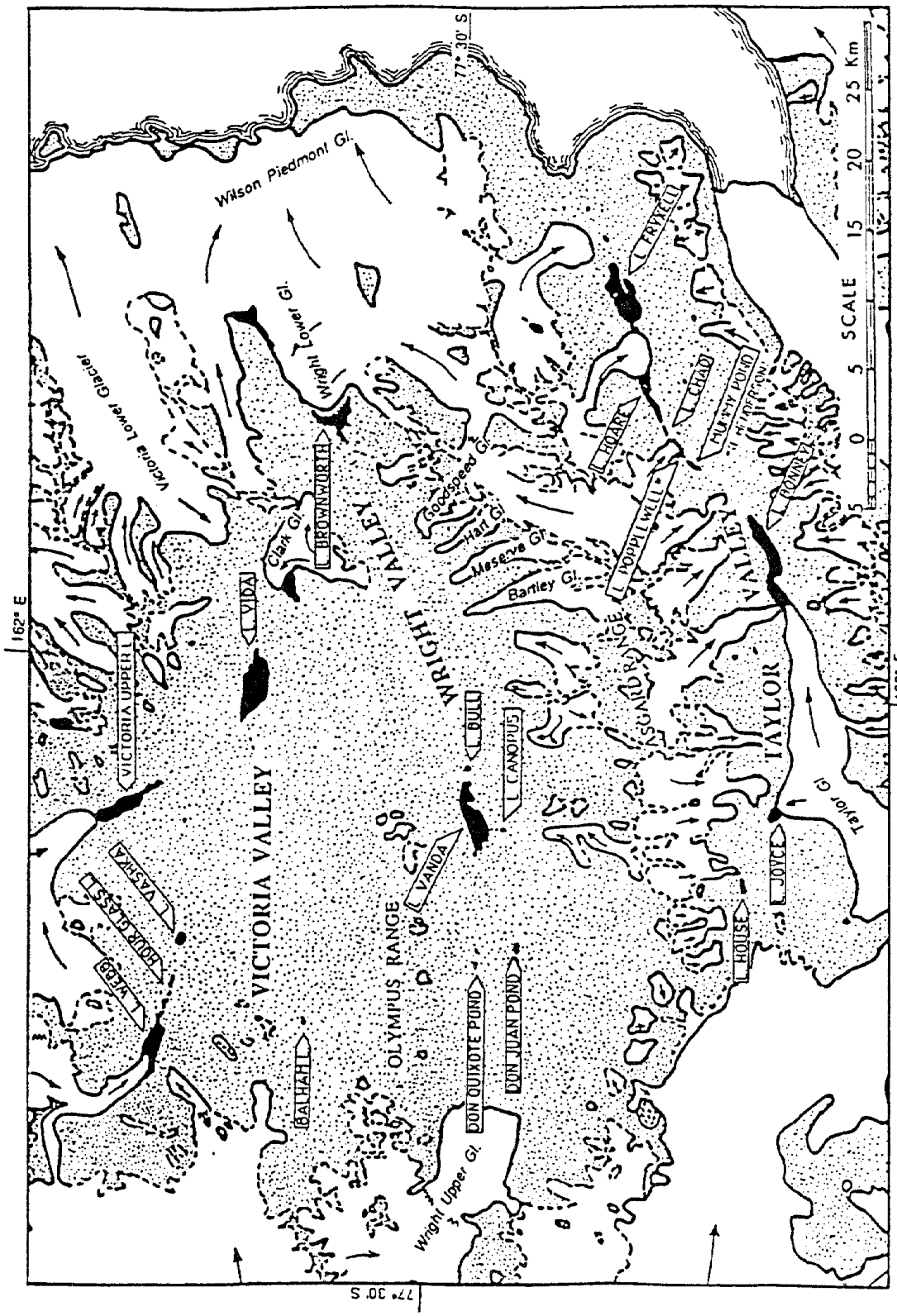

Figure 1. Map of the Antarctic Dry Valleys region (reprinted from Green et al., 1988).

the western coast of the Ross Sea. The valleys contain numerous closed basins (Figure 1) in which permanently ice-covered, chemically stratified lakes are found. The ice cover (3–6 m) has a profound effect on the chemical, biological and physical processes in the lakes. Specifically, it limits the transfer of mechanical energy from the wind and gas exchange with the atmosphere, modifies the quantity and spectral quality of solar radiation reaching the underlying water, and prevents the introduction of foreign species (Palmisano & Simmons, 1987; Lizotte & Priscu, 1992; Wharton et al., 1993; Priscu et al., 1996). Glacial meltwater and leaching from soils adjacent to streams provide freshwater and nutrients to the lakes.

Lake Fryxell is located near the mouth of the Taylor Valley proximate to McMurdo Sound (77° 37′ S, 163° 07′E, Figure 1). The total area of the lake is 7 km^2 and it is the shallowest lake in the study with a maximum depth of 18.5 m. Dissolved O_2 is supersaturated (>40 mg l^{-1}) above 9.5 m (Figure 2A). Water below 9.5 m is anoxic and the brackish, sulfidic bottom waters have salinities reaching 30% of seawater (Vincent, 1988). The temperature ranges from 0–4 °C. Inorganic nutrient concentrations are moderate, total organic carbon ranges from 1.4–29.1 (Matsumoto, 1989) and annual primary production is reported to be 7–50 g C $m^{-2}yr^{-1}$ (Simmons et al., 1993).

Lake Hoare is a wet-based lake ponded at the western margin of the piedmont lobe of the Canada Glacier (77° 38′ S, 163° 07′ E, Figure 1). Its total area is 2.9 $km^2$and it has a maximum depth of 34 m. Lake Hoare is relatively fresh and is oxygenated throughout the water column (> 50 mg l^{-1}) with isolated anoxic zones near the bottom (Figure 3A). The temperature is nearly uniform at ∼0 °C. Lake Hoare is oligotrophic with relatively low nutrient concentrations; annual production has been estimated to be 1.7 g C m^{-2} yr^{-1}(Simmons et al., 1993).

Lake Bonney (77° 43′ S, 162° 20′ E, Figure 1) is the largest lake in the Taylor Valley (area > 4 km^2). It is divided into two lobes by a sill (approximately 45 m wide and 13 m deep) which hydrographically isolates the deeper layers of each basin. Therefore, each lobe has been considered a separate lake system. The area of East Lobe Lake Bonney is 3.2 km^2 and the maximum depth is 40 m. Dissolved O_2 is supersaturated above 20 m (> 45 mg l^{-1}) and the water is fresh above 13 m (Figure 4A). Below the pycnocline, the water becomes anoxic and hypersaline reaching salinities of almost 10 times seawater. The temperature ranges from −2 to +6 °C (Priscu et al., 1993; Priscu, 1995). The West Lobe of Lake Bonney is a glacial margin lake at the advancing snout of the Taylor Glacier. It is smaller than the East Lobe with a total surface area of 0.8 km^2. The maximum depth is 40 m. Above 15 m the dissolved O_2 is supersaturated with values greater than 20 mg l^{-1}(Figure 5A). The deep water is anaerobic and salinities reach 6 times seawater (Priscu et al., 1993). The temperature ranges from −2 to +3 °C (Priscu et al., 1993; Priscu, unpublished).

Lake Joyce (77° 43′ S, 161° 33′ E, Figure 1) is an ice-dammed, proglacial lake against the marginal lobe of the Taylor Glacier in the Pearse Valley, an extension of the Taylor Valley. Its total surface area is 1 km^2 and the maximum depth is 35 m. The upper 20 m are well oxygenated and fresh (Figure 8A). Below 20 m, O_2 concentrations decrease and the salinity is 4‰. The temperature throughout the water column is nearly uniform and barely above freezing.

Lake Vanda (77° 32′ S, 161° 33′ E, Figure 1) is the largest lake in the Wright Valley with a total area of 5.2 km^2 and maximum depth of 80 m. The upper 65 m are oxic and relatively fresh (Figure 6A). The deep waters are warm (24 °C), hypersaline (9 times seawater) and sulfidic. Nutrient concentrations in the trophogenic zone of Lake Vanda are low and estimated annual primary production is low 0.34–2.5 g C m^{-2} yr^{-1} (Parker et al., 1982; Vincent & Vincent, 1982). Lake Vanda is relatively oligotrophic.

Lake Miers lies in the Miers Valley (78° 07′ S, 163° 54′ E, Figure 1). Its total area is 1.05 km^2 and maximum depth is 20 m. Glacial meltwater inflow from the Miers and Adams Glaciers is more significant at Lake Miers than at many of the other dry valley lakes (Andersen et al., 1993; Bell, 1967); it is the only lake we studied that has a surface outflow. The lake is relatively fresh and oxygen is present throughout the water column ranging from 4 mg l^{-1} to supersaturation (Figure 7A). The temperature range is 0–5 °C. Lake Miers is oligotrophic.

Sample collection

Each lake was sampled at a central site at least once during the 1993 austral summer. Sampling was done through a 10 inch diameter hole drilled through the ice cover. Depths are reported relative to the free water surface, i.e. the level to which water would rise in the sampling hole. Temperature and conductivity profiles were measured with a Seabird SBE 25 CTD system modified for use in these lakes (Spigel et al.,

1990). Oxygen was measured using a YSI oxygen probe. The YSI oxygen probe can measure a maximim oxygen concentrations of 20 mg 1^{-1}, values higher than 20 mg 1^{-1} were obtained by the Winkler method (see below). Water sampling was done with a 5 liter Niskin bottle at various depths based on the oxygen profile and CTD cast and encompassed significant hydrographic features. Samples were kept dark and on ice during transport back to the laboratory (<2 h). Samples for nutrient analysis were filtered (Whatman GF/F) and frozen until analysis. Bacterioplankton samples (approximately 4 liters) were concentrated 100 fold by ultrafiltration using a Filtron (Northborough, MA) open channel Ultrasette with a 300 kd nominal molecular weight cutoff membrane or a Pellicon tangential flow filtration system using GVLP, 0.22 μm ultrafilters (Millepore). The concentrate was filtered onto a 47 mm, 0.2 μm pore size Gelman Supor filter. Filters were stored frozen in EDTA (0.5 M, 0.5 ml) until total DNA was extracted.

Chlorophyll a *and nutrient analysis*

Chlorophyll *a* was measured on material collected on Whatman GF/F filters. Samples were extracted overnight in 90% acetone at -20 °C. Phaeophytin corrected chlorophyll *a* was quantified by comparing fluorescence of acidified and nonacidified samples measured on a Turner Designs AU10 fluorometer (Holm-Hansen et al., 1965). Ammonia, $NO_2{}^-$ and $NO_3{}^-$ were measured on 10 ml replicates of filtered (Whatman GF/F) lakewater samples following the protocols of Parsons et al. (1984). It was necessary to dilute samples exceeding seawater salinity with deionized water to yield salinities at or below seawater before analysis. Recovery of internal nutrient standards ranged between 90–110%. Samples for N_2O were collected following the procedure described in Priscu & Downes (1985) and Priscu et al. (1995) and was measured with a gas chromatograph fitted with a ^{63}Ni, electron capture detector (Priscu & Downes, 1985). Oxygen values obtained at Lakes Bonney, Hoare and Fryxell from the YSI oxygen probe were corroborated by Winkler titrations (Parson et al., 1984).

DNA extraction

High molecular weight DNA was extracted and purified from the frozen filters of concentrated bacteria following a standard protocol with slight modification (Ausubel et al., 1989; Kerkhoff & Ward, 1993). After ethanol precipitation the DNA was resuspended in TE2 (10 mM Tris and 0.1 mM EDTA).

Oligonucleotide primers

The universal eubacterial 16S rDNA primers EUB1 and EUB2 corresponding to sites at positions 9 through 27 and 1525 through 1542 respectively, of the Escherichia coli 16S rDNA (Liesack et al., 1991) were used to confirm the PCR quality of the DNA template. PCR primers corresponding to conserved sequences within the 5' and 3' regions of the 16S rDNAs of beta subclass (NITA and NITB; Voytek and Ward, 1995) and gamma-subclass (NOC1 and NOC2; Voytek, 1996) ammonia oxidizers were used to detect these groups. Amplification with these primers yields a 1080 bp NIT product and a 1130 bp NOC product, both internal to the EUB fragment. Primers designed to target the gene encoding the active site of the ammonia monoxygenase gene (amoA) were used to amplify an approximately 500 bp fragment (amoA-1F and amoA-2R; Rotthauwe et al., 1997). Primers were synthesized commercially (OPERON, Alameda, CA).

PCR amplification

PCR amplification was performed in a total volume of 100 μl using a DNA thermal cycler (Thermolyne Amplitron I, Barnstead) following the protocol described in Voytek & Ward (1995) and Voytek (1996) with slight modification. To reduce amplification of non-specific products the total number of cycles was reduced to 30. As discussed in Voytek & Ward (1995) a two stage PCR procedure is necessary for amplification of ammonia oxidizer template from the complex mixture of prokaryote and eukaryote DNA found in DNA extracts from natural samples. DNA templates were first amplified using the EUB primers. A 1 μl aliquot of the EUB amplification product mixture was added to a new reaction mixture and reamplified with either the NIT or the NOC primers. Amplification of the amoA product was performed following Rotthauwe et al. (1997). PCR-amplified products were resolved by electrophoresis.

Relative abundance of ammonia oxidizers

Direct quantification of the number of organisms in a natural sample based on specific PCR products amplified from DNA extracted from these samples is difficult. In all steps of the collection, extraction and

amplification procedures error and uncertainty are introduced. Some cells are more efficiently collected by ultrafiltration and not all cells readily lyse with standard DNA extraction protocols. Additionally, the template (e.g. rRNA operons) copy number per cell may vary almost ten-fold and some templates amplify more efficiently than others (Suzuki & Giovannoni, 1996). Farrelly et al. (1995) have shown that without foreknowledge of the genome size and gene copy number in all members, accurate estimations of the abundance of a particular species is impossible. The two-step amplification protocol used here reinforces these errors. For these reasons, an absolute determination of the abundance of ammonia oxidizers was not possible. In order to estimate the relative abundance of ammonia oxidizers in each natural sample, the EUB amplification product was diluted before amplification with the NOC or NIT primers. The dilution series included 5 dilutions (undiluted, 1:2, 1:5, 1:10; and 1:50). A number (1–5) was assigned to a sample based on the highest dilution in the series (e.g. 1=undiluted; 5= 1:50) that yielded the correct amplification product. This number should reflect the relative abundance of nitrifiers. The rationale for this was based on the assumption that the greater the dilution factor the higher the number of the original template and thus the higher the number of ammonia oxidizers present in the original sample.

Results and discussion

Lake Fryxell

The temperature in Lake Fryxell was 0 °C just under the ice and increased to a maximum of 3 °C at 11 m. Oxygen was supersaturated in the surface (Figure 2A) and decreased steeply to zero between 9 and 10 m. A deep chlorophyll *a* maximum existed at 9 m (12 μg l^{-1}) with a secondary peak at 11 m (6 μg l^{-1}). All nitrogenous compounds were low or undetectable in the trophogenic zone (Figure 2B). Below the oxycline, NH_4^+ increased to 400 μM. N_2O was low at the surface (0.2 μg-at N l^{-1}) and decreased to below detection with depth. Nitrate was undetectable throughout the water column. Nitrite had two small peaks, one at 8 m and the other just above the bottom.

In general, ammonia-oxidizers of the beta-subclass were more abundant than those of the gamma subclass (Figure 2B). Beta nitrifiers were most abundant at depths above the oxycline. Although the distribution of the two groups overlaps, gamma subclass nitrifiers were found at deeper depths and into the oxygen transition zone. The presence of ammonia oxidizers is consistent with a significant contribution from nitrification to the shallow nitrite peak and the N_2O which accumulates above the oxycline. However, the sharp drop off of N_2O and the low concentrations of NO_2^- and NO_3^- below the oxycline are evidence of the anaerobic process, denitrification. No nitrifiers from either subclass were detected in the sulfidic bottom waters of Lake Fryxell. This is not surprising since sulfide has been reported as an inhibitor of nitrification (Snra & Baggaley, 1975; Capone & Kiene, 1988) and nitrifiers are obligate aerobes. Indeed, Joye & Hollibaugh (1995) suggested that H_2S levels may control the spatial and temporal differences in nitrification rates observed in certain marine environments. Ammonia oxidizers would not be expected to persist in the presence of sulfide since they derive the energy necessary for all cellular metabolism from nitrification, the oxidation of ammonia using oxygen.

Lake Hoare

The vertical temperature distribution in Lake Hoare varied little (range 0.2 – 0.7 °C) and the water column was well oxygenated throughout (Figure 3A). There was a deep chlorophyll *a* peak at 14 m (4 μg l^{-1}). The overall concentration of nitrogenous compounds in Lake Hoare were generally lower than the other lakes examined (Figure 3B). NH_4^+ was very low, less than 0.5 μM, throughout the water column. Nitrate was undetectable in the trophogenic zone and accumulated (up to 8 μM) below 15 m suggesting that nitrification is occurring and that it surpasses biotic utilization in deeper waters. None of the intermediates in nitrification accumulated in this lake: nitrite was below detection and N_2O was between 0.3- and 0.6 μg-at N l^{-1} throughout the water column. Although not very abundant, beta nitrifiers were detected at all depths sampled and support the assumption of nitrifying activity (Figure 3B). Gamma nitrifiers were not found at any depth in this lake. Although pockets of anoxic waters have been observed near the sediment water interface (D. Anderson, pers. comm.), there was no evidence suggesting that denitrification is an important process controlling nutrient concentrations in the overall well oxygenated waters of Lake Hoare.

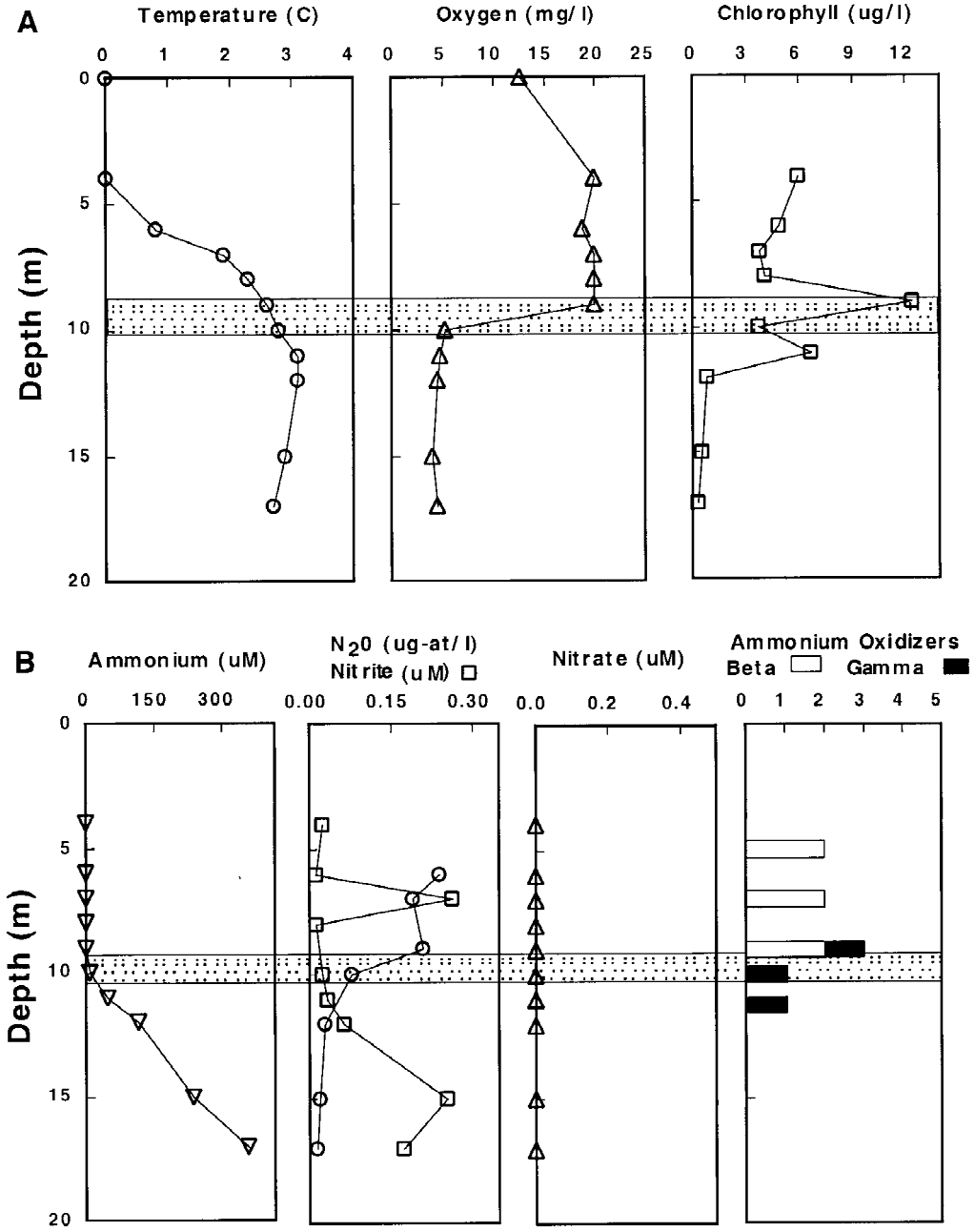

Figure 2. Temperature, oxygen, chlorophyll *a*, DIN, and beta and gamma ammonia oxidizer distributions in Lake Fryxell, November 1993. Stipled bar in panels A and B demarcates the oxic/anoxic transition.

Lake Bonney east lobe

Temperature increased in the east lobe of Lake Bonney from 0 °C just beneath the ice to 6.1 °C at 15 m and decreased to −2 °C near the bottom (Figure 4A). Chlorophyll *a* concentrations were highest just beneath the ice (2 μg l^{-1}) and decreased rapidly with depth. A strong chemocline occurred at about 17 m, above which conditions were aerobic and nutrient depleted. Below this region, O_2 dropped from supersaturated levels to nearly zero and dissolved inorganic N, including oxidized forms, increased rapidly, even though the deep waters (down to the bottom at

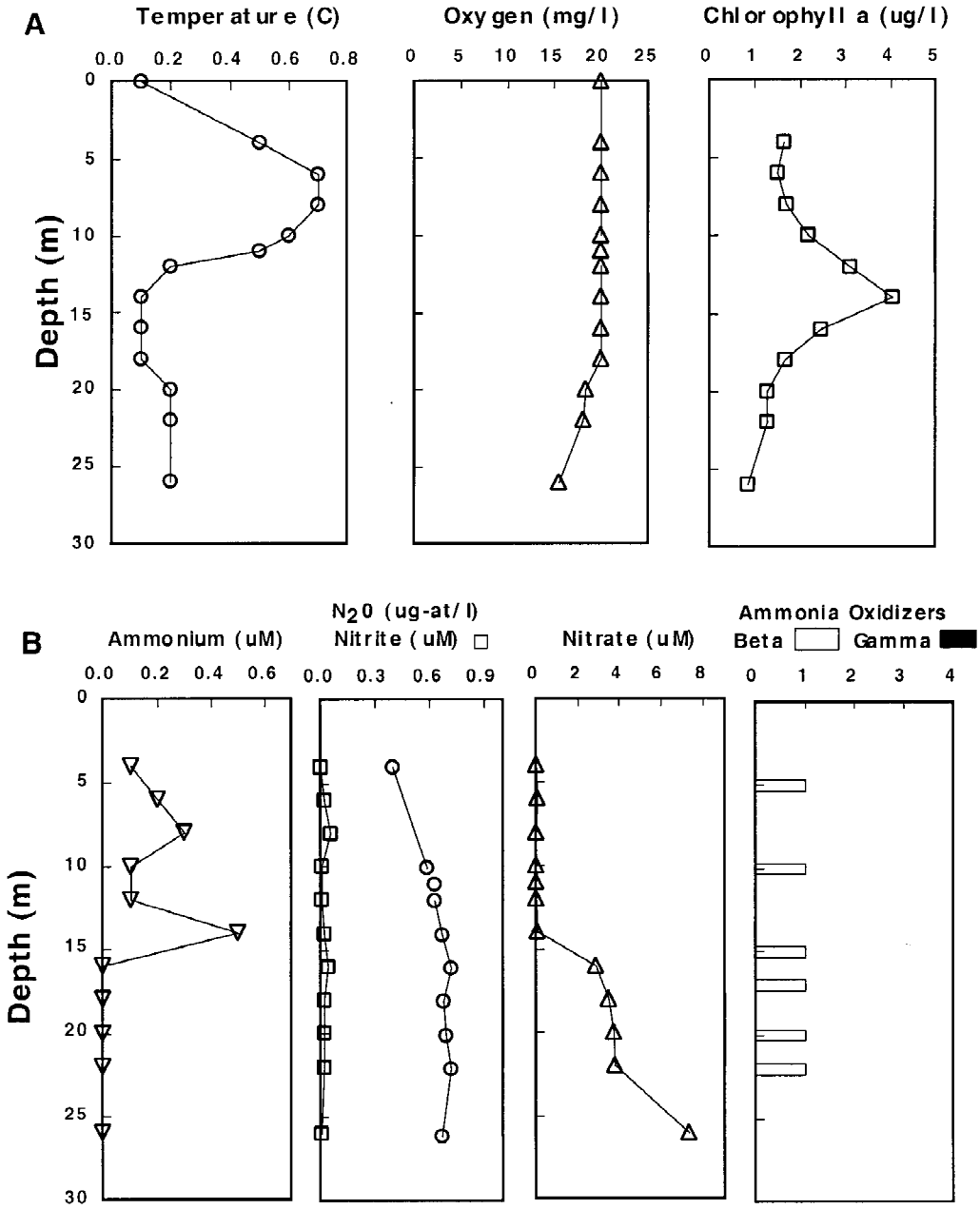

Figure 3. Temperature, oxygen, chlorophyll *a*, DIN, and beta and gamma ammonia oxidizer distributions in Lake Hoare, November 1993.

40 m) were virtually anoxic (Figure 4B). A broad N_2O peak occurred between 20–24 m reaching extremely high values of 40 μg-at N l^{-1}.

The presence of nitrifying bacteria was detected by the PCR assay from 7 depths above 25 m in the East Lobe of Lake Bonney (Figure 4B). Beta ammonia oxidizers were far more abundant than gamma ammonia oxidizers with relative abundances highest in the aerobic waters above the chemocline. Gamma nitrifiers had a small peak around 15 m. Despite the abundance of ammonia oxidizers in the surface waters, NO_2^- and NO_3^- do not accumulate presumably because these nutrients are readily used by phytoplankton and bacteria in the euphotic zone.

^{15}N natural abundance studies in Lake Bonney (Wada et al., 1984), indicate that the NO_3^- in the

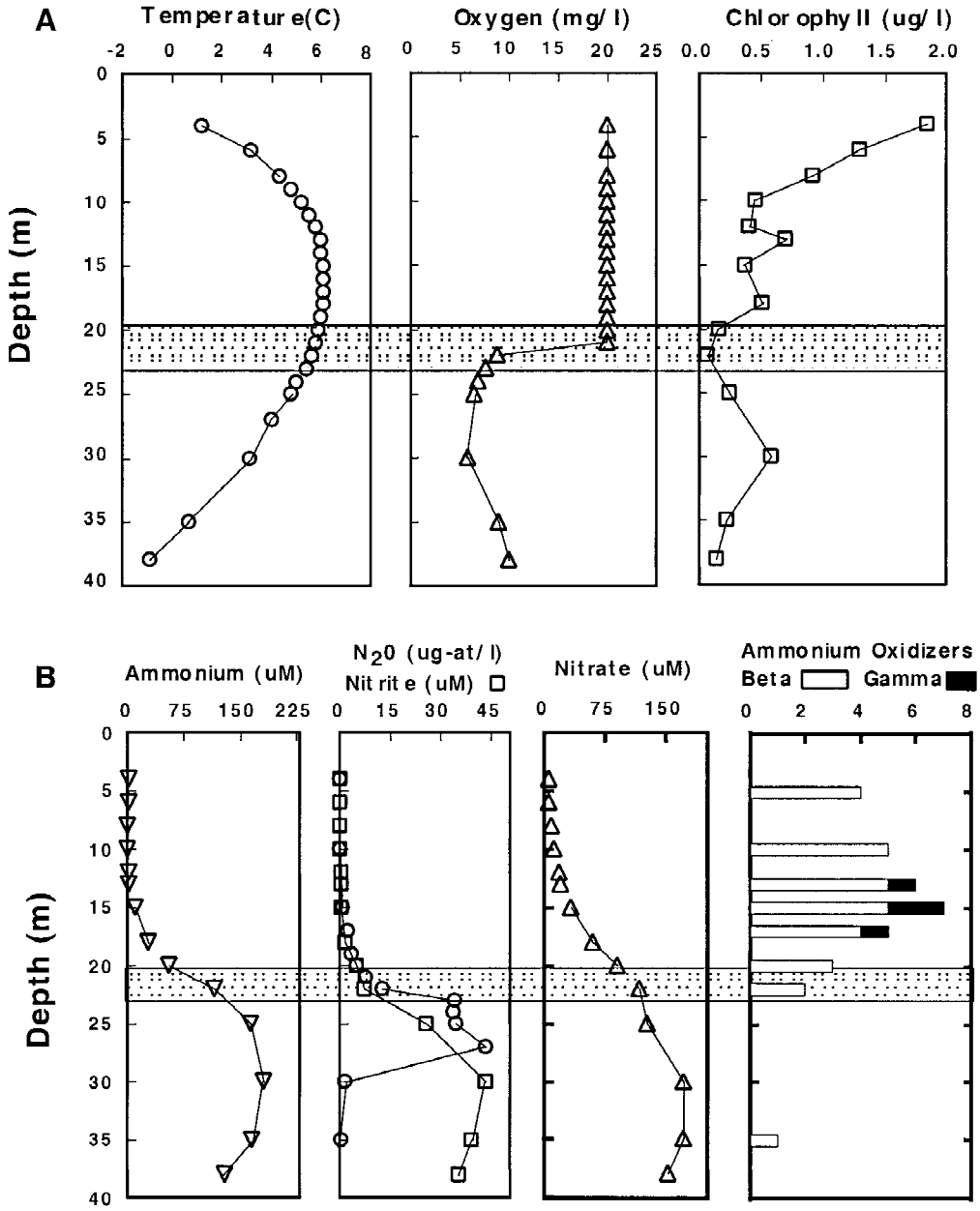

Figure 4. Temperature, oxygen, chlorophyll *a*, DIN, and beta and gamma ammonia oxidizer distributions in Lake Bonney (East Lobe), November 1993. Stipled bar in panels A and B demarcates the oxic/anoxic transition.

deep water of the east lobe is highly enriched in ^{15}N. Wada et al. (1984) proposed that microbial activity such as denitrification or nitrification may cause the observed variation in ^{15}N, as no other processes could produce the magnitude of enrichment observed. N_2O levels measured just below the chemocline exceeded 550 000% of air saturation for that depth and salinity, the highest recorded in any natural system (Priscu et al., 1996). Denitrification could be responsible for high levels of N_2O since it is an intermediate in the process; however, the NO_3^- and NO_2^- profiles show high levels ($>250\ \mu M$ and $>\ 40\ \mu M$ respectively) in the hypoxic deep water indicating bulk denitrification is not occurring. Moreover, although the denitrification substrates are available, oxygen is low and denitrifying bacteria have been detected and isolated from

these waters (Ward & Priscu, 1996), denitrification based on acetylene block assays could not be detected in this lobe (Priscu et al., 1993; Priscu et al., 1996).

It has been shown that cultures of ammonia oxidizing bacteria can produce N_2O at low oxygen tension (Goreau et al., 1980; Anderson et al., 1993) and nitrification has been suggested as the source of N_2O in many natural systems (Vincent et al., 1981; Kaplan & Wofsy, 1985; Priscu et al., 1986; Tortoso & Hutchinson, 1990). Using ^{14}C-bicarbonate based inhibitor experiments, ammonia oxidizer activity was detected at the top of the N_2O peak but not at 24 m (Priscu et al., 1996). The distribution of nitrifiers in the east lobe suggested that nitrification could be at least partially responsible for the shallower N_2O peak but does not support nitrification as the source of the lower peak. Priscu et al. (1996) have suggested that the peak we observe today is a 'fossil record'. They proposed that even small transformations of N are relatively easy to detect as chemical gradients within the water column of these ice covered lakes. Lack of turbulence allows chemical gradients to persist over long periods of time and to develop even if reaction rates are extremely low. Moreover, chemical gradients can persist even when there is no present day biological activity. Hence, the broad N_2O maximum observed in the East Lobe may be the result of past nitrification activity. There is no evidence of consumption within the peak and although diffusion may have dispersed the peak, the rates are not great enough to dissipate the signal substantially.

Lake Bonney west lobe

The chemistry and *in situ* processes shaping nutrient profiles in the west lobe of Lake Bonney appear to be very different from the adjacent east lobe. The temperature maximum (3 °C) occurred at 10 m and temperature decreased below the pycnocline to −2 °C (Figure 5A). The surface standing stock of biomass was higher in the west lobe than in the east lobe: chlorophyll *a* concentrations were 1–1.5 μg l^{-1} under the ice and peaked (6 μg l^{-1}) near the base of the trophogenic zone (13 m). The chemocline and oxycline began at 13 m and 15 m respectively, both shallower than in the east lobe (Figure 5 A & B). The reduced N substrate, NH_4^+ was undetectable in the trophogenic zone and steadily increased to 300 μM at the bottom. Nitrite, nitrate and N_2O peaked between 13–17 m, concentrations reaching 0.6 μM, 25 μM and 1.1 μg at N l^{-1}, respectively, and then decreased rapidly. Ammonia-oxidizing bacteria were detected throughout the water column but were less abundant here than in the east lobe (Figure 5B). Beta nitrifiers were most abundant above the oxycline and the distribution of the less abundant gamma nitrifiers was limited to the oxygen transition zone. Nitrification by these bacteria was probably responsible for the peaks of N_2O, NO_2^- and NO_3^-. Nitrifiers detected in the anoxic bottom waters were probably inactive.

Wada et al. (1984) found the highest $d^{15}N$ values for N_2 yet reported in an aquatic system (1.5–2.5‰) in the anoxic layers of the West Lobe of Lake Bonney. These authors suggest intense denitrification may be responsible for the high $d^{15}N$ observed. The concentrations of inorganic oxidized nitrogen, both NO_2^- and NO_3^-, decreased steeply in the deep water below the oxycline in this lobe, which supports this contention. Furthermore, Priscu et al. (1996) have measured significant rates of denitrification in the West Lobe and Ward & Priscu, (1997) isolated and enumerated (by immunofluorescence) denitrifying bacteria in this region.

Lake Vanda

The upper layer of Lake Vanda extending to 55 m depth was essentially fresh, well oxygenated and the temperature ranged between 0–6 °C (Figure 6A). Below 55 m, the temperature steadily increased to a maximum of 23 °C and the oxygen steeply decreased, reaching zero below 67 m. A deep chlorophyll *a* maximum of 0.45 μg l^{-1} occurred at 67 m. The concentrations of all nitrogenous compounds measured were low or undetectable in the trophogenic zone (Figure 6B). Below the oxycline, NH_4 increased to 1500 μM. N_2O began to accumulate at 55 m, peaked at 67 m (7.9 μg-at N l^{-1}) and rapidly decreased to zero below the oxycline. Nitrate and nitrite peaked at 67 (268 μM) and 70 m (1.65 μM), respectively and decreased below. In general, ammonia-oxidizers of the beta-subclass were more abundant than those of the gamma subclass (Figure 6B). Beta nitrifiers were detected at all depths sampled above the oxycline and were most abundant just above the oxycline. The less abundant gamma subclass nitrifiers were not found in the shallowest (5 and 10 m) samples but were present at deeper depths. Neither group was present in the oxygen transition zone at oxygen concentrations below 4 mg l^{-1} or in the anoxic zone. The nitrification activity of these ammonia oxidizers may be responsible for the peaks in N_2O and nitrate. The disappearance

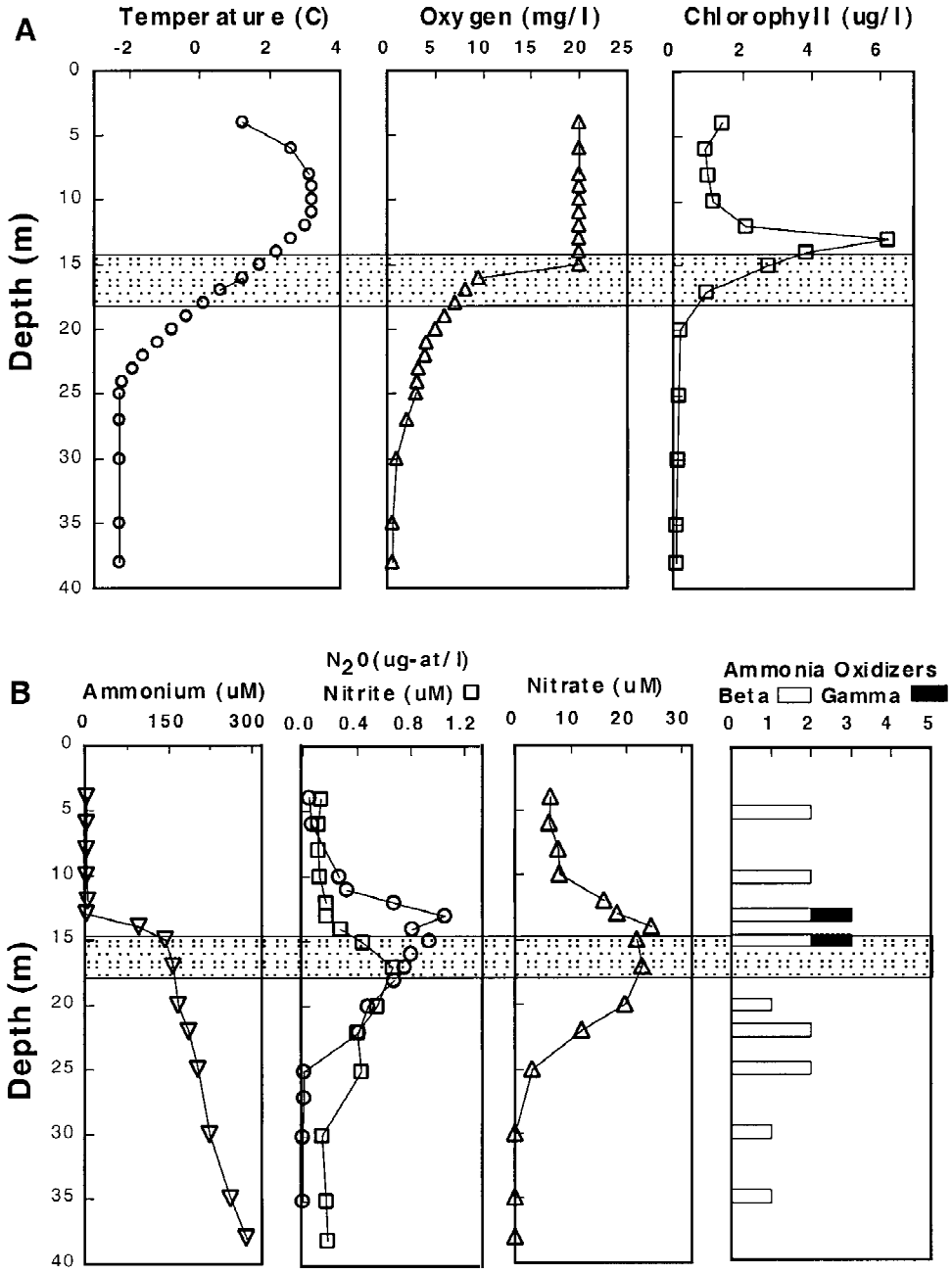

Figure 5. Temperature, oxygen, chlorophyll *a*, DIN, and beta and gamma ammonia oxidizer distributions in Lake Bonney (West Lobe), November 1993. Stipled bar in panels A and B demarcates the oxic/anoxic transition.

of N_2O, NO_2^- and NO_3^- below the oxycline are evidence of the anaerobic process denitrification. No nitrifiers were detected in the sulfidic bottom waters of Lake Vanda. As discussed above, it would be unlikely for nitrifiers to persist in the presence of sulfide which shuts down nitrification.

Vincent et al. (1981) examined N-fluxes in the Dry Valley Lakes. They found a region of intense N_2O cycling near the chemocline in Lake Vanda, mediated by nitrification, denitrification and diffusion. Maximum N_2O concentrations in Lake Vanda exceeded 20 000% of air saturation. They found the rate of

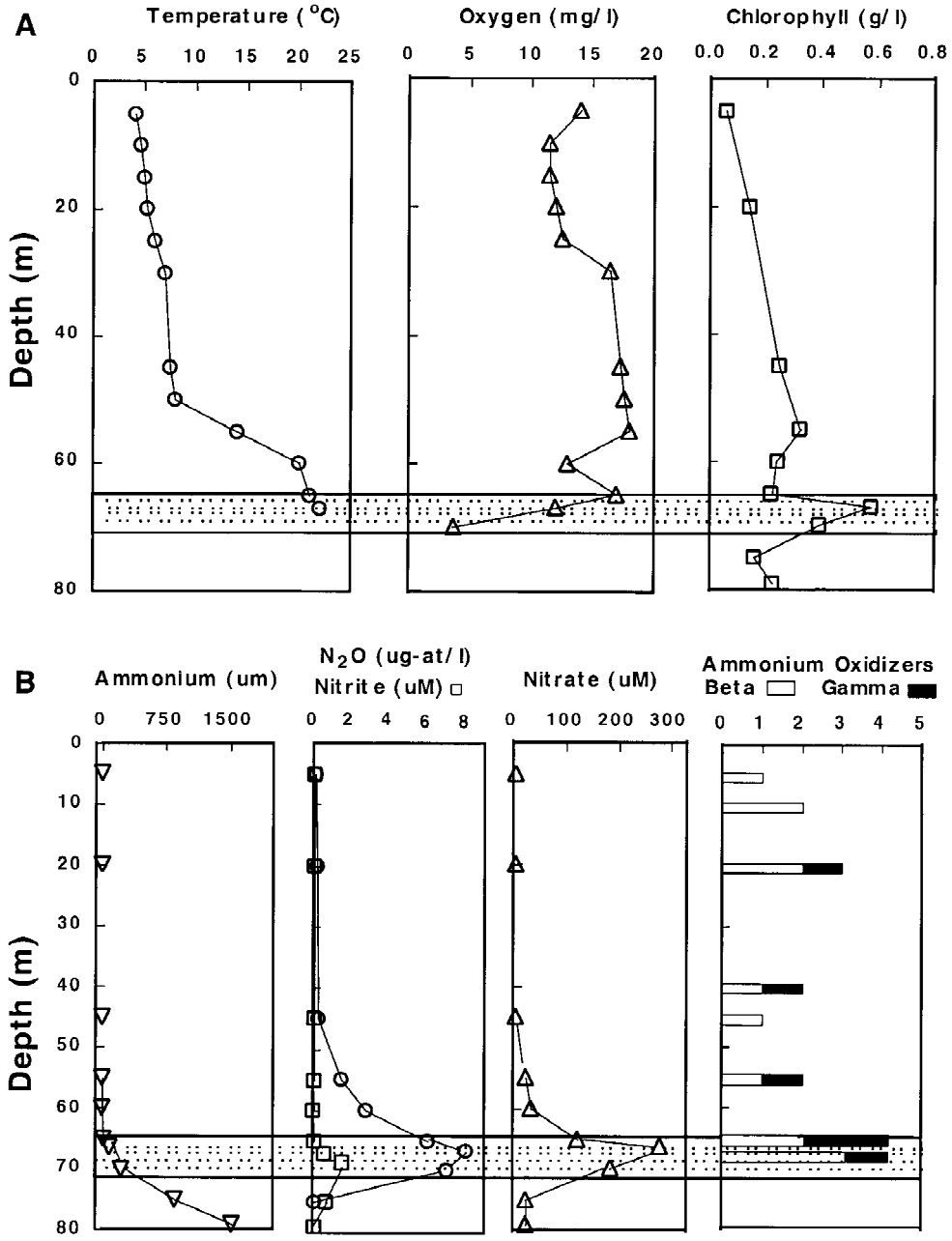

Figure 6. Temperature, oxygen, chlorophyll *a*, DIN, and beta and gamma ammonia oxidizer distributions in Lake Vanda, November 1993. Stipled bar in panels A and B demarcates the oxic/anoxic transition.

nitrification highest at a layer just above the deep chlorophyll maximum (50–55 m) and denitrification was poised between the DCM and the sulfate reducing community (61–65 m). Vincent (1987) suggested that the various forms of nitrogen in Lake Vanda regulated the discrete layering of organisms. The lake levels in the Dry Valleys have been rising, which should result in a transposition of the relative position of the populations, but the processes should continue in the same relative depth horizons. At present lake level, N_2O, NO_3^- and NO_2^- begin to accumulate and then peak at or just above the deep chlorophyll maximum, which occurs at 67 m. These chemical features and

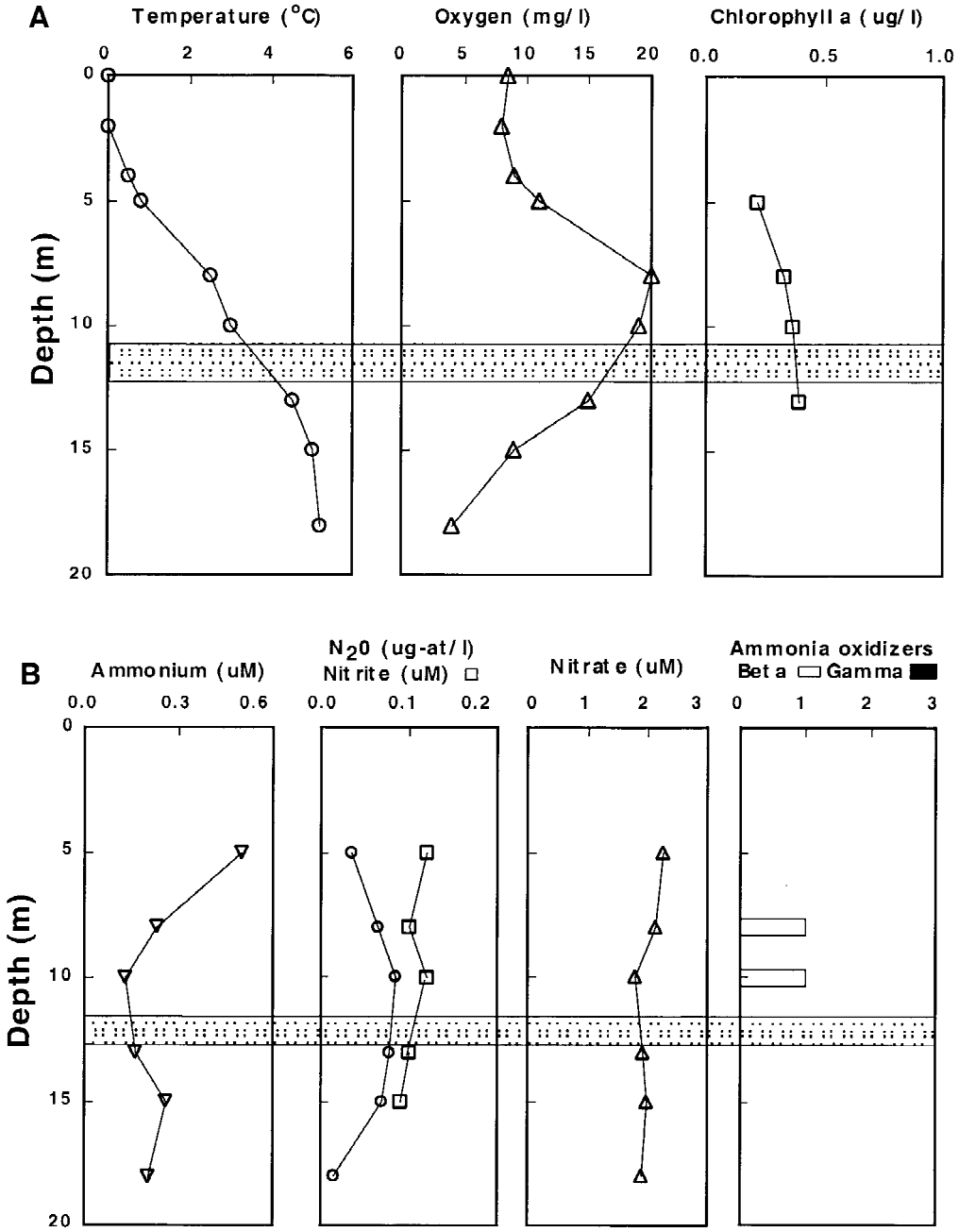

Figure 7. Temperature, oxygen, chlorophyll *a*, DIN, and beta and gamma ammonia oxidizer distributions in Lake Miers, November 1993. Stipled bar in panels A and B demarcates the oxic/anoxic transition.

the observed distribution of nitrifiers support Vincent's model.

Lake Miers

The temperature increased steadily from 0 °C at the surface to 5.2 °C at 18 m (Figure 7A). Oxygen concentrations were above 4 mg l^{-1} at all depths and oxygen was supersaturated at 15 m. Chlorophyll *a* was very low (less than 0.4 μg l^{-1}). Reported total heterotrophic bacterial abundances are very low (Simmons et al., 1993). The concentrations of all nitrogenous substrates, products and intermediates were low through-

out the water column and the profiles were nearly uniform (Figure 7B). NH_4 never exceeded 0.5 μM, nitrite <0.12 μM, nitrate <2.3 μM and N_2O was less than 0.09 μg-at N l^{-1}. Beta subclass nitrifiers were detected at two of the depths sampled and were not very abundant (Figure 7B). The low substrate and product levels observed here suggest that nitrification rates would be low and transformations due to nitrification would be insignificant in this ultra-oligotrophic lake. Of all the lakes sampled, the chemistry of Lake Miers most closely resembles the chemistry of the glaciers and meltwater that feed into the lake (Green et al., 1988) suggesting that there has been little change due to in-situ processes. A possible explanation may be that Lake Miers is relatively young and biogeochemical cycles are still evolving. Green et al. (1988) estimated the chloride age of Lake Miers to be 300 years. In addition, Lake Miers is the only lake with hydraulic through flow with a large annual influx of meltwater and substantial outflow.

Lake Joyce–There was a narrow temperature range in Lake Joyce (0-0.6°C) with a slight warming at 10 m (Figure 8A). Oxygen was supersaturated in the surface and decreased to zero below 20 m. The chlorophyll *a* maximum (5 μg l^{-1}) occurred at 10 m. With the exception of nitrate, the concentrations of nitrogenous compounds at the surface were low (Figure 8B). Below the oxycline, NH_4^+ was regenerated and increased to 12 μM. N_2O was low at the surface (0.2μg-at N l^{-1}), increased to a maximum of 0.97 μg-at N l^{-1} at 20 m and disappeared below the oxycline. Nitrate was relatively high in the upper water column, peaking at 15 m (130 μM) and disappeared rapidly below 15 m. Nitrite was 0.6 μM at the surface and decreased with depth. Beta nitrifiers were detected at depths above the oxycline and at the top of the transition (Figure 8B). Gamma subclass nitrifiers are found at deeper depths and through the oxygen transition zone. Ammonia oxidizers are probably responsible for the nitrate peak and the N_2O above the oxycline since their distribution overlaps with the distritbution of N_2O. The sharp drop off of N_2O and the low concentrations of NO_2^- and NO_3^- below the oxycline are evidence of the anaerobic process denitrification. The distribution of nitrifiers in this lake was also restricted to depths above the region of sulfate reduction (sulfide was detected in samples collected from 25 to 30 m). Detection and distribution of the amoA gene.

AmoA was detected in all lakes and followed the same distribution pattern as the nitrifier 16S rRNA genes (data not shown). Surprisingly, no additional information on the distribution of ammonia oxidizers was obtained from the use of amoA. Amplification of the amoA gene yielded the correct product in all samples where either the gamma or beta or both subclasses were detected and was not amplified in samples where the 16S rRNA genes were absent. Since none of the products from any of the amplifications were sequenced, we cannot determine if any additional nitrifier subclasses were present in our samples. If they were present and if we had used the amoA product to determine the relative abundance of nitrifiers, we may have observed different distribution patterns and depth profiles. Moreover, abundance estimates calculated from a functional gene may be less variable and more reliable. In general, the copy number of functional genes is 1–3 copies per genome (Norton et al., 1996) whereas, the number of rRNA genes can vary from 2 to 13.

Conclusion

Factors controlling ammonia oxidizer distributions

The distributions of ammonia-oxidizing bacteria observed in these Antarctic lakes are generally consistent with expectations based on available information on the factors that control nitrification rates and the growth of nitrifiers in culture. In this study nitrifiers were always found in the surface waters and in most lakes they were detected just beneath the ice. Nitrifiers have been reported to be light sensitive and in general, nitrifying activity is higher in low light environments, e.g. deeper in the water column (Olson, 1981; Ward, 1986). All of these lakes are permanently covered by 2.8–6 m of ice which limits penetration of light to between 0.8 and 14.7% (10–190 μE m^{-2} sec^{-1} PAR) incident irradiation (Palmisano & Simmons, 1987; Wharton et al., 1993). Therefore, light is not likely to limit the distribution of nitrifiers in surface waters. The rates of nitrification and growth of nitrifiers are also controlled by the concentration and/or supply of their energy substrate NH_4^+ (Ward, 1986; Koops & Möller, 1992). Although some of the lakes have well developed benthic microbial mats that could fix nitrogen (Wharton et al., 1983), the new nitrogen is primarily supplied by meltwater, usually in the form of nitrate. Lithoautotrophic bacteria would have to compete with primary producers and heterotrophic bacteria for these nutrients in the trophogenic zone. Rapid utilization in excess of inputs

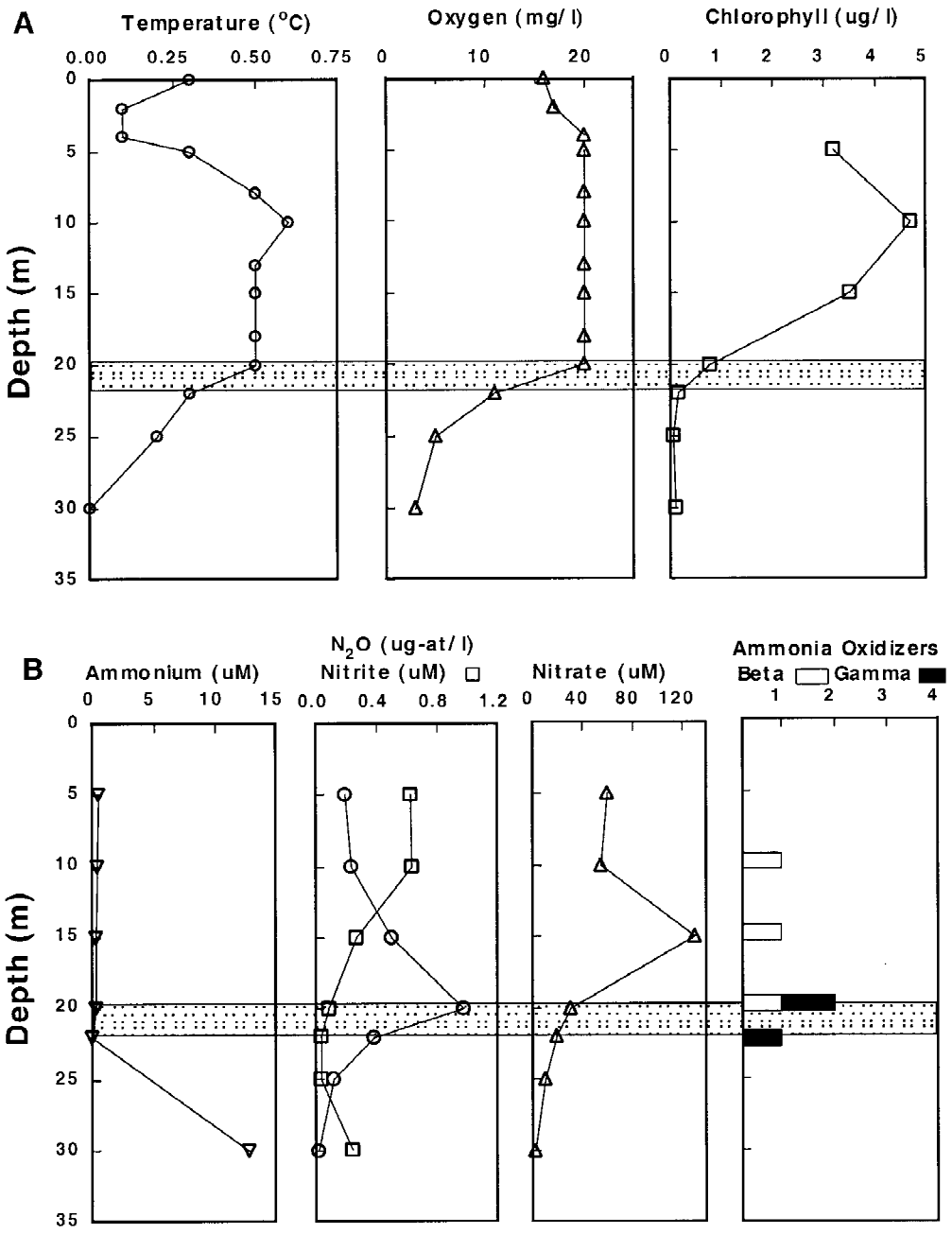

Figure 8. Temperature, oxygen, chlorophyll *a*, DIN, and beta and gamma ammonia oxidizer distributions in Lake Joyce, November 1993. Stipled bar in panels A and B demarcates the oxic/anoxic transition.

during the late austral summer when melt stream flow and primary production are highest, presumably results in the low concentrations observed there. Under these conditions the nutrient supply to nitrifiers may be low and we would expect low activity. In temperate marine systems, water column nitrification rates are usually lowest in the trophogenic zone (Ward, 1986). Although often present at surface depths in Antarctic lakes, the highest abundances of nitrifers were usually observed deeper in the water column.

As particulate organic matter containing fixed nitrogen settles, it undergoes microbial degradation, thus

releasing nitrogen (NH_4^+ and R-NH_2) which is then available to organisms at those depths or the reduced compounds can diffuse upward to fuel more production. In the hypersaline lakes, Lakes Bonney and Vanda for example, the sinking of organic detritus is severely retarded by the steep density gradients and the high density of deep waters, thus most of the material is degraded before reaching the sediments. High concentrations of refractory organic carbon are often observed in the anoxic bottom layers of these lakes, supporting this contention (Matsumoto et al., 1993). The regeneration and small, but significant, upward supply of NH_4^+ should favor nitrification and indeed, the highest relative abundances of both subclasses were found at depths near the nitracline. Rates of nitrification have been difficult to measure in some of these lakes since nitrifiers are slow growing and rates may be retarded by low temperatures. However, Vincent et al. (1981) and Priscu et al. (1996) were able to measure nitrification rates directly in Lake Vanda and Bonney, respectively. They found the rate of nitrification highest at depths just below the nitracline. In addition, at deeper depths in Lake Bonney, Priscu et al. (1996) postulated the possibility of 'fossil' nitrification activity, i.e. product accumulation from nitrification occurring hundreds to thousands of years ago.

A final environmental factor controlling nitrification and nitrifiers is oxygen (Koops & Möller, 1992). Peaks in nitrifier abundance were almost always found near the oxygen transition zone. Ammonia oxidizers are obligate aerobes but are often found in microaerophilic environments (Koops & Möller, 1992). Under low concentrations of oxygen N_2O is produced during nitrification (Goreau et al., 1980). N_2O is also a product and substrate in the anaerobic microbial process of denitrification. Within the transition zone, nitrification and denitrification compete for substrates and the resultant chemical profiles reflect the activity of organisms responsible for both processes. In anaerobic waters, denitrification becomes the primary process controlling nitrogen; denitrifiers use oxidized forms of nitrogen as alternative respiratory substrates. Although nitrifiers were detected in these waters, nitrification should not be occurring in the complete absence of oxygen. In the more extreme case where sulfur reduction has occurred, denitrification has completely removed the oxidized forms of nitrogen. Sulfide has been shown to inhibit nitrification (Joye & Hollibaugh, 1995) and therefore, should limit the distribution of nitrifiers. Indeed, the distribution of nitrifiers in Lakes Fryxell, Vanda and Joyce, where sulfide was detected, appears to be restricted to depths above the region of sulfate reduction.

Origin and relative abundances of beta and gamma subclass ammonia oxidizers

In general, there were more beta subclass than gamma subclass ammonia oxidizers present in these lakes and the vertical distribution pattern of the beta subclass nitrifiers was broader. One reason for this difference is simply that the beta subclass of ammonia oxidizers contains members from many genera which have been isolated from diverse environments: soils, marine and freshwater environments. The gamma subclass has one genetically confirmed member, thus far, and it is the marine species Nitrosococcus oceanus. It is not surprising that one species from a limited environmental niche may be less abundant and have a restricted distribution in a different environment compared to a group with environmentally diverse members. Alternatively, the distribution we observed for the gamma nitrifier may be a result of a difference in its response to environmental factors. The gamma nitrifier was found in deeper waters, through the transition zones and often below in anaerobic waters. It may be more light sensitive and more tolerant of sulfides and low oxygen than members of the beta group. Finally, the original mechanism of species seeding of these lakes when they were original formed may also account for some of the differences. The origin and precise mechanism of formation and development of these lakes is still controversial. Lake Fyxell, Bonney, Joyce and Vanda have ion signatures that indicate a marine source (Green et al., 1988; Chin, 1993). Chinn (1993) suggested that many of the lakes in the Dry Valleys were originally formed when the area lifted isostatically from the sea and sea water was trapped in basins formed by large outlet glaciers. However, Green et al., (1988) note that the major ionic components in Lakes Fryxell, Hoare, Joyce and Miers can be largely understood in terms of their present inflows without invoking trapped sea water. Others have suggested that Lakes Vanda, Bonney, Joyce and Fryxell have been influenced by trapped seawater in glacier or marine aerosols or that Lakes Fryxell and Vanda have had direct contact with McMurdo Sound in their recent past (Hendy et al., 1977; Wilson, 1981; Chinn, 1993). Thus the source of the gamma subclass nitrifier may be ultimately marine. No physical or chemical evidence

of a marine origin is found in Lake Miers or Hoare where only members of the beta subclass are found.

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