

Oxidation of ammonia and methane in an alkaline, saline lake

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Abstract

The oxidation of ammonia (NH_3) and methane (CH_4) was investigated in an alkaline saline lake, Mono Lake, California (U.S.A.). Ammonia oxidation was examined in April and July 1995 by comparing dark $^{14}\text{CO}_2$ fixation rates in the presence or absence of methyl fluoride (MeF), an inhibitor of NH_3 oxidation. Ammonia oxidizer-mediated dark $^{14}\text{CO}_2$ fixation rates were similar in surface (5–7 m) and oxycline (11–15 m) waters, ranging between 70–340 and 89–186 nM d^{-1} , respectively, or 1–7% of primary production by phytoplankton. Ammonia oxidation rates ranged between 580–2,830 nM d^{-1} in surface waters and 732–1,548 nM d^{-1} in oxycline waters. Methane oxidation was examined using a $^{14}\text{CH}_4$ tracer technique in July 1994, April 1995, and July 1995. Methane oxidation rates were consistently higher in July, and rates in oxycline and anaerobic bottom waters (0.5–37 and 7–48 nM d^{-1} , respectively) were 10-fold higher than those in aerobic surface waters (0.04–3.8 nM d^{-1}). The majority of CH_4 oxidation, in terms of integrated activity, occurred within anoxic bottom waters. Water column oxidation reduced the potential lake-atmosphere CH_4 flux by a factor of two to three. Measured oxidation rates and water column concentrations were used to estimate the biological turnover times of NH_3 and CH_4 . The NH_3 pool turns over rapidly, on time scales of 0.8 d in surface waters and 10 d within the oxycline, while CH_4 is cycled on 10^3 -d time scales in surface waters and 10^2 -d time scales within oxycline and bottom waters. Our data suggest an important role for NH_3 oxidation in alkaline, saline lakes since the process converts volatile NH_3 to soluble NO_2^- , thereby reducing loss via lake-atmosphere exchange and maintaining nitrogen in a form that is readily available to phytoplankton.

Lakes occupying closed basins are often characterized by extreme geochemical and physicochemical parameters, including alkaline pH (>9), elevated salinity, and persistent or frequent meromixis (Melack 1983; Hammer 1986). Alkaline, saline lakes are present around the globe and frequently exhibit high rates of primary production despite the harsh environmental conditions found there (Jellison and Melack 1993a). Mono Lake is an alkaline, saline lake located in the Eastern Sierra Nevada region of California, U.S.A. (38°N, 119°W). Recently, the response of primary production, vertical mixing, and biogeochemical cycling to prolonged meromixis was examined. Persistent stratification resulted in decreased primary production; increased inventories of CH_4 , NH_3 , and hydrogen sulfide (H_2S) in the monimolimnion; and increased lake-atmosphere fluxes of CH_4 , NH_3 , and H_2S during overturn (Jellison and Melack 1993b; Jellison et al. 1993; Miller et al. 1993b). In terms of primary nutrients, dissolved inorganic phosphate (DIP) is abundant in Mono Lake water (>400 μM ; Jellison et al. 1993), and the ratio

of dissolved inorganic nitrogen (= $\text{NH}_3 + \text{NO}_2^- + \text{NO}_3^-$) to DIP is extremely low (<<0.5), suggesting that Mono Lake is a chronically nitrogen-limited system.

Uptake of NH_3 by phytoplankton and regeneration of NH_3 by zooplankton (and probably bacteria) are important processes in Mono Lake (Jellison and Melack 1988, 1993b). Because external nitrogen sources are limited, rapid internal cycling is required to provide inorganic nitrogen sources to primary producers (Jellison et al. 1993). $\text{NO}_2^- + \text{NO}_3^-$ concentrations are low in the lake (<1 μM ; Jellison and Melack 1988); thus, NH_3 is the most abundant fixed nitrogen species. At the lake's alkaline pH (>9), NH_3 is more abundant than the ammonium ion (NH_4^+), and lake-atmosphere exchange of NH_3 has been suggested to be an important sink for nitrogen in this system (Jellison et al. 1993). Ammonia-oxidizing bacteria convert NH_3 to NO_2^- , and this process could reduce NH_3 loss via lake-atmosphere exchange and maintain inorganic nitrogen in a form that is stable (not volatile) and bioavailable.

The present study was designed to estimate the oxidation rates of NH_3 and CH_4 in Mono Lake. NH_3 oxidation is an obligate aerobic process and plays several important roles in closed-basin lake biogeochemistry. In addition to reducing or eliminating atmospheric NH_3 loss, NH_3 oxidation connects the O_2 and N cycles since O_2 is incorporated into NO_2^- during the oxidation of NH_3 ; the process contributes to total lake primary production since NH_3 -oxidizing bacteria

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are chemoautotrophs; and it could influence primary production by supplying a regenerated nitrogen source (NO₂⁻ or NO₃⁻) to phytoplankton.

Physiologically, aerobic CH₄ oxidation and NH₃ oxidation are similar processes. Both methanotrophs and nitrifiers are capable of oxidizing each other's substrate in addition to the primary substrate (Bedard and Knowles 1989). Methanotrophic bacteria oxidize CH₄ to carbon dioxide (CO₂) under aerobic conditions. Methane oxidation may also occur under anaerobic conditions, but the organisms responsible for, and the biochemistry of, this process are not currently known (Zehnder and Brock 1979; Hoehler et al. 1994). Both aerobic and anaerobic CH₄ oxidation occur in lakes (Rudd et al. 1974, 1976; Reeburgh and Heggie 1977; Rudd and Hamilton 1978). Oxidation lowers the CH₄ concentration and alters the stable carbon isotope ratio (Oremland and Desmarais 1983; Oremland et al. 1987). Aerobic CH₄ oxidation influences the lake-wide O₂ budget and, because of co-metabolism, could also impact nitrogen cycling (Bedard and Knowles 1989).

Methods

Site description—The phytoplankton community of Mono Lake is comprised of coccoid green algae, cyanobacteria, and diatoms (Lovejoy and Dana 1977; Melack 1983). Primary production averages 554 g C m⁻² yr⁻¹ (ca. 1.5 g C m⁻² d⁻¹), with highest rates observed during early spring and late fall, while lowest rates occur during winter and summer (Jellison and Melack 1993a). The major zooplankton species is the brine shrimp, *Artemia monica* (Lenz 1980, 1982). Bacterial abundances between 1.4 and 2.0 × 10⁷ bacteria ml⁻¹ have been observed in the water column of Mono Lake (Oremland et al. 1993). Methanogenesis produces CH₄ within the bottom waters and sediments of Mono Lake via the anaerobic bacterial consumption of (fossil) organic matter (Oremland et al. 1987). Nitrogen fixation in the littoral zone has been reported (Oremland 1990), and sulfate reduction occurs in pelagic sediments (Oremland et al. 1993).

Limnological and geochemical methods—Water samples from discrete depths were collected near a permanently moored buoy at Sta. 9 (28-m depth) using a 5-liter polyvinyl chloride (PVC) sampling bottle (Jellison and Melack 1993a,b; Miller et al. 1993b). Care was taken to prevent introduction of air into the sampling containers during transfer from the PVC sampler. Standard curves for NH₃ and H₂S were prepared in 0.5 M NaCl. For NH₃ concentration determination (note that we use "NH₃" to refer to NH₃ + NH₄⁺), water samples were filtered (0.4-μm Nuclepore) directly into dark polyethylene bottles and kept cool until analysis (within 4 h) using the method of Soloranzo (1969). The detection limit was 0.5 μM. Samples for determining H₂S concentration were filtered, immediately fixed with 0.5% (wt:vol) zinc acetate and were analyzed later according to Cline (1969). The detection limit was 2 μM. Dissolved CH₄ concentration was determined using a headspace extraction-gas chromatography technique (Oremland and Desmarais 1983), and the detection limit was ca. 0.5 μM.

Dissolved oxygen (O₂) concentration was determined using a Martek Mark XV sensor (Model XMS) in July 1994

and April 1995 and a YSI O₂ temperature probe in July 1995. The detection limit for O₂ was 10 μM. Depth distributions of photosynthetically active radiation (PAR) and temperature (°C) were obtained using a Sea-Bird SEACAT Model 19 profiler equipped with a 4π PAR sensor and a temperature sensor. Chlorophyll *a* (Chl *a*) concentration was determined as described in Jellison et al. (1993). Rates of primary production were estimated using previously described regressions, which consider the variables of temperature, light, and pigment concentration in the calculation (Jellison and Melack 1993a).

Ammonia oxidation—In April and July 1995, NH₃ oxidation rates were determined using a ¹⁴C tracer (Billen 1976; Enoksson 1986; Owens 1986; Berounsky and Nixon 1993) and an inhibitor, MeF (Miller et al. 1993a). The addition of MeF allowed us to distinguish dark CO₂ fixation due to NH₃ oxidation from other autotrophic processes that are not sensitive to MeF (e.g., phytoplankton dark CO₂ fixation). Serum bottles (78 ml) were rinsed (two times) and overfilled with water from the PVC sampling bottle. Bottles were sealed without introducing a gas phase by slowly inserting a black butyl rubber stopper containing a vent needle into the bottle. To minimize the amount of isotope required and maximize replication for samples where microbial activity was expected (i.e., "live" samples), we included one time-zero, one filter-sterilized ("dead"), and three experimental samples per treatment group. One treatment group received no amendment (control, nitrification occurring) and the other was amended with pure MeF gas (inhibited, no nitrification). Filter-sterilized samples were passed through 0.2-μm Nuclepore membrane filters to remove phytoplankton and bacteria. Isotope and inhibitor (MeF) amendments were made after water samples were collected from all depths.

Isotope stocks (1 mCi ml⁻¹) were diluted with pH 10 NaCl solution (0.35 M) and chilled in an ice bath prior to addition, which assured that the isotope sank to the bottom of the bottle as it was added. Fifty microliters of an NaH¹⁴CO₃ stock solution (40 μCi per bottle; ~0.5 μCi ml⁻¹) was added to each bottle, and 1 ml of pure MeF (Matheson) was added to appropriate bottles (final concentration = ~1.5% vol:vol). The added MeF dissolved into the water phase within minutes. After isotope and inhibitor addition, activity in time-zero bottles was terminated by opening each bottle and adding an NaOH pellet to increase the pH to ca. 14. After experimental samples were deployed in the lake, time-zero bottles were filtered (*see below*). All experimental samples were wrapped twice in Al-foil and placed into nylon bags, which were attached at the appropriate depth on the mooring to incubate in situ for ca. 48 h. After incubation, all bottles were removed and placed in a cooler. Upon return to the laboratory, all samples were killed by adding an NaOH pellet.

The total ¹⁴CO₂ activity (DPM ml⁻¹) was determined for each bottle by collecting a 100-μl aliquot and transferring it to a 7-ml scintillation vial containing Scintiverse II cocktail (Fisher SX12-4). Samples were counted on a Beckman LSC 6000 scintillation spectrometer. Because the pH (9.7) and ΣCO₂ concentrations (0.4 M) of Mono Lake water are very stable, the ratio of HCO₃⁻:CO₃²⁻ is relatively constant (Miller

et al. 1993; Oremland and Miller 1994). Thermodynamic calculations of CO₂ speciation under the experimental conditions indicate that ca. 20% of the total activity (H¹⁴CO₃⁻ + ¹⁴CO₃²⁻) was available to NH₃-oxidizing bacteria (i.e., H¹⁴CO₃⁻) during the experiment.

Samples were filtered at low vacuum through 0.2- μ m Nuclepore filters to determine the incorporation of ¹⁴CO₂ into biomass. Filters were rinsed with NaCl (0.5 M) solution and placed in 7-ml scintillation vials (Cloern et al. 1983). Inorganic ¹⁴CO₂ was removed via soaking with 200 μ l of 6N H₂SO₄ for 12 h, after which 7 ml of scintillation cocktail was added. The bottles were then counted on a Beckman LSC 6000 scintillation spectrometer. The counting efficiency was ca. 85% as determined by external ¹⁴C standards and internal ¹⁴C controls (¹⁴C-glucose and H¹⁴CO₃⁻) treated as samples.

Ammonia oxidizer-mediated dark HCO₃⁻ fixation rates were estimated using the following equation:

$$R = [(a/A) \times C]/T \quad (1)$$

where R = HCO₃⁻ fixation rate in nmol C liter⁻¹ h⁻¹; a = ¹⁴C DPM fixed by NH₃ oxidizers; A = ¹⁴HCO₃⁻ DPM available; C = HCO₃⁻ concentration; and T = incubation time (hours). The a term was obtained by subtracting the average activity in the presence of MeF (no NH₃ oxidization) from the average control activity (NH₃ oxidization). The treatment means ($n = 3$) were compared using a t -test, and only significant ($p < 0.05$) differences were used to calculate HCO₃⁻ fixation rates. To estimate NH₃ oxidation, we used an average conversion factor of 8.3 moles of N oxidized per mole of C fixed (Billen 1976, 1978; Owens 1986). Reported conversion factors vary by a factor of 10 or more. We chose 8.3 because it represents the mean of the literature values and is relatively conservative. Daily rates were obtained by multiplying hourly rates by 12 within the upper 10 m (euphotic zone), based on the consideration that NH₃ oxidation rates may occur primarily under low-light (night) conditions (Olson 1981). Daily rates below the euphotic zone (>10 m) were obtained by multiplying hourly rates by 24. We realize that light vs. dark conditions vary with season, but in order to be constant in our calculations, we used the 12-h activity period for the euphotic zone NH₃ oxidizers in April and July. Areal integrations of rates were obtained by trapezoidal integration of the rate vs. depth data. Fluxes across the oxycline were estimated using the measured concentration gradients and the eddy diffusivity (K_z) values given by Jellison et al. (1993).

Methane oxidation—Water samples ($n =$ six per depth) collected with the PVC sampling bottle were used to rinse (three times) 10-cc glass syringes to overflowing. Syringes were sealed by inserting a plunger and closing (without introducing bubbles) the hub end with a modified luer fitting containing two butyl rubber septa (Iversen et al. 1987). Abiological controls ($n = 3$) were obtained by filter sterilization via passage through a 0.2- μ m Nuclepore membrane filter. All syringes were kept cool and dark prior to isotope addition (1–2 h).

Carbon-14 labeled CH₄ (¹⁴CH₄) was prepared using methanogens and stored in crimp-sealed 25-ml glass tubes con-

taining 1 ml of 5N NaOH (Daniels and Zeikus 1982). Prior to the in-lake experiments, ¹⁴CH₄ tracer was checked for trace contamination by ¹⁴CO₂ or ¹⁴CO using gas proportional counting (GPC); only ¹⁴CH₄ was detected (GPC limit of detection is ~ 0.5 nCi per 0.5 ml injection). A 50- μ l volume of tracer (¹⁴CH₄ + He) was added to each syringe by inserting a needle through the luer hub containing the butyl rubber insert. The specific activity of the tracer was 55 mCi mmol⁻¹, and the total activity added was 1,850 nCi (37 nCi μ l⁻¹); thus, tracer addition resulted in a 3.4- μ M increase in CH₄ concentration. The syringes were gently mixed and kept cool until initial (time zero) samples were collected (<2 h). Time-zero aliquots were collected by withdrawing 2 ml of water from each syringe and dispensing it into a crimp-sealed, 25-ml serum bottle containing 1 ml of 5N NaOH and 0.2 ml of 37% buffered formalin, which fixed ¹⁴CO₂ in the liquid phase and stopped biological activity.

The syringes were placed into nylon mesh bags, attached at the appropriate depth on the moored array, and incubated in the lake for 24–72 h. Subsamples were collected daily to generate time courses. Most of the tracer ¹⁴CH₄ had dissolved into the water phase after 24 h. After sample collection, ¹⁴CH₄ and H¹⁴CO₃⁻ were quantified as follows. The activity of ¹⁴CH₄ was determined using GPC (Culbertson et al. 1981). Fixed samples were equilibrated via shaking for at least 12 h to assure transfer of ¹⁴CH₄ from the aqueous to gaseous phase. Then, a 0.5-ml headspace sample was withdrawn and injected into the GPC, and the activity (DPM) was determined by comparison to ¹⁴CH₄ standards. Tracer activity was essentially constant over time.

Activity of H¹⁴CO₃⁻ was determined by first opening the serum bottles and placing them on a shaker table (50 rpm) overnight under a hood to vent ¹⁴CH₄. Experiments with separate bottles containing Mono Lake water, buffered formalin, and H¹⁴CO₃⁻ confirmed that no H¹⁴CO₃⁻ was lost during this procedure (<5%; data not shown). The next morning, each sample was transferred to a 125-ml Erlenmeyer flask. The serum bottle was rinsed three times with 4 ml of pH-10 deionized water; the rinse water was also transferred to the Erlenmeyer flask. Flasks were sealed with a butyl rubber stopper, to which a 7-ml scintillation vial containing a CO₂ trap (phenethylamine-soaked GF/F filterwick) was attached. One milliliter of 12N H₂SO₄ was injected down the side of the flask to convert H¹⁴CO₃⁻ to ¹⁴CO₂, and the flasks were placed on a shaker table (200 rpm) to collect ¹⁴CO₂ for 90 min. Standards ($n = 2$) of known activity were run with each set of samples ($n = 10$) so that the ¹⁴CO₂ capture efficiency could be calculated. After shaking, the scintillation vials were retrieved, 7 ml of Scintiverse II scintillation cocktail (Fisher SX12-4) was added, and the samples were counted on a Beckman LSC 6000 liquid scintillation spectrometer. The ¹⁴CO₂ trapping efficiency was always >85% and averaged >93%. Any residual ¹⁴CH₄ would not have been trapped on the filter since the binding mechanism for ¹⁴CO₂ is the formation of a carbamide bond with phenethylamine. The CH₄ oxidation rate was calculated from the accumulation of ¹⁴CO₂ DPM over time and the specific activity of ¹⁴CH₄ using an equation similar to Eq. 1.

MeF inhibition and time-series experiments were performed under controlled laboratory conditions in July 1994

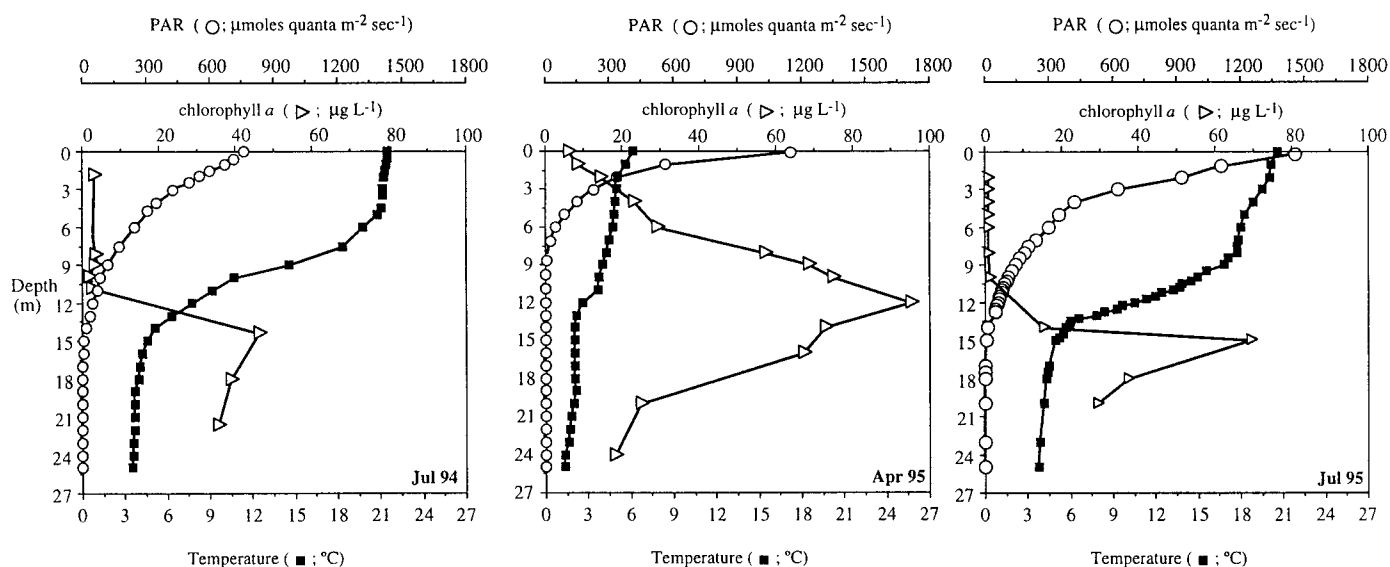


Fig. 1. Depth profiles of temperature (°C, ■), photosynthetically active radiation (PAR, ○), and chlorophyll *a* (▷) during July 1994, April 1995, and July 1995.

and 1995 using samples collected from 10-m (oxic) and 15-m (oxycline) depths. MeF is a potent inhibitor of aerobic methane oxidation (Oremland and Culbertson 1992). Additionally, selected samples were amended with 300 μM ammonium (NH₄⁺), which is a competitive substrate for CH₄ monooxygenase (Bedard and Knowles 1989). All experiments were conducted under constant temperature conditions (24 and 22°C in July 1994 and 1995, respectively).

Results

Limnology and geochemistry—During July 1994, the lake was thermally stratified, with aerobic surface waters having a temperature of 22°C and anoxic bottom waters having a temperature between 4 and 9°C. The thermocline was locat-

ed between 7 and 13 m (Fig. 1). PAR penetrated to 15 m. The presence of a microbial (phytoplankton) plate was evident between 13 and 15 m, based on the Chl *a* distribution (Fig. 1). The oxycline was located between 9 and 12 m, and no O₂ was detected beneath 13 m. Concentrations of CH₄ increased linearly between 7 and 15 m and increased only slightly between 15 and 25 m. Concentrations of NH₃ were low in aerobic surface waters but increased below the oxycline (Fig. 2). H₂S concentrations increased rapidly below the oxycline (peak of 40 μM at 20 m) but approached detection limits in the aerobic water column (data not shown).

The lake mixed completely (vertically) during the winter of 1994–1995 but had begun to stratify by April 1995 (Jelison unpubl. data). Again, a microbial plate was present between 7 and 15 m (Fig. 1). Rapid decreases of PAR with

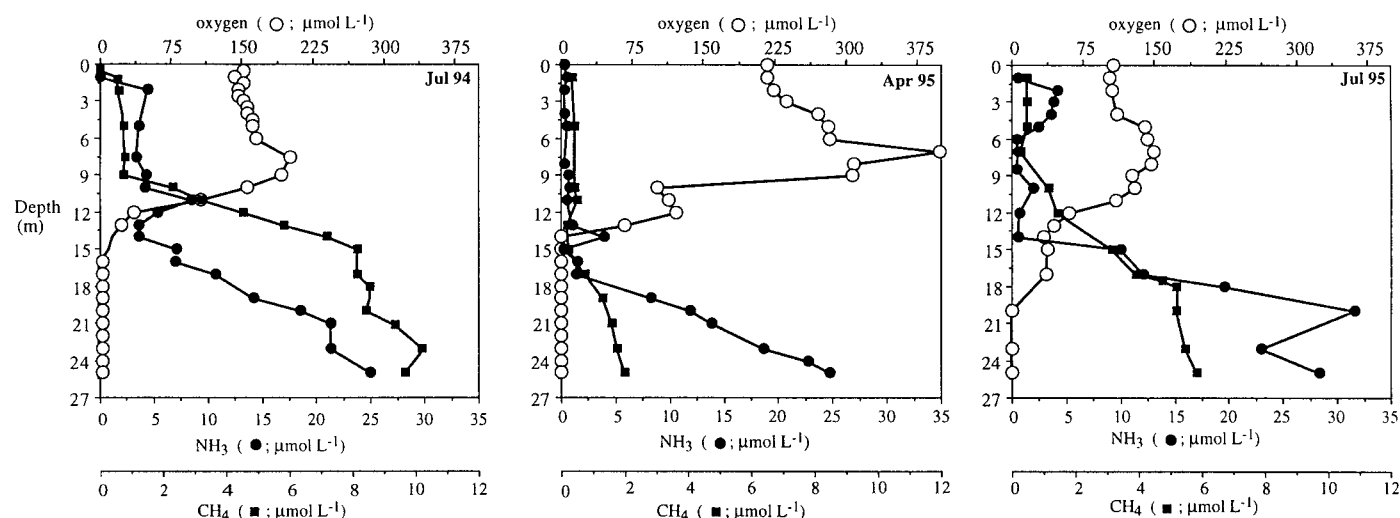


Fig. 2. Depth profiles of the concentration of oxygen (O₂, ○), methane (CH₄, ■), and ammonia (NH₃, ●) during July 1994, April 1995, and July 1995. All concentrations are in μmol L⁻¹.

Table 1. Methyl fluoride (MeF)-sensitive dark HCO_3^- fixation and NH_3 oxidation rates.

Depth (m)	Carbon	Fixation*	Ammonia	Oxidation†
	April	July	April	July
5	148 (48)‡	264 (108)	1,236 (372)	2,184 (780)
7	ND§	340 (151)	ND	2,830 (1,248)
10	70 (18)	145 (65)	581 (144)	1,202 (540)
11	89 (22)	ND	732 (180)	ND
12	98 (24)	ND	814 (204)	ND
13	0	0	0	0
15	ND	186 (120)	ND	1,548 (996)
17	ND	125 (36)	ND	1,032 (300)
20	ND	0	ND	0

* Carbon fixation units = $\text{nmol C liter}^{-1} \text{ d}^{-1}$.† Ammonia oxidation units = $\text{nmol N liter}^{-1} \text{ d}^{-1}$; equal to C fixation rate $\times 8.3$ (8.3 = C:N molar conversion factor; see text for explanation).‡ Number in parentheses = SD of the mean; $n = 3$.

§ ND, no data.

|| 0 = no significant difference between control and MeF treatments.

depth were observed, with no PAR detected beneath 15 m. The lake exhibited a pronounced oxycline between 7 and 14 m. NH_3 concentrations were low in the upper water column but increased in bottom waters. H_2S concentration was variable, with highest concentrations observed in the anoxic hypolimnion ($5 \mu\text{M}$; data not shown). Methane concentrations were low, with $<5 \mu\text{M}$ present in bottom waters (Fig. 2).

In July 1995, the lake was thermally and chemically stratified. A peak in Chl *a* was observed at the base of the thermocline, which was located between 8 and 14 m (Fig. 1). The O_2 concentration was uniform throughout the upper 10 m, and a steep oxycline was located between 10 and 12 m; O_2 gradually decreased to zero between 12 and 18 m (Fig. 2). Ammonium and CH_4 concentrations increased sharply through the oxycline (Fig. 2). H_2S concentrations increased below the oxycline to a maximum of $40 \mu\text{M}$ at 20 m (data not shown).

Ammonia oxidation—Rates of dark $\text{H}^{14}\text{CO}_3^-$ fixation in control (no MeF) treatments were approximately 30–50% higher than rates observed in MeF-amended treatments (data not shown). Rates of MeF-sensitive $\text{H}^{14}\text{CO}_3^-$ fixation were greater in July, and similar activity was observed in aerobic surface waters (5–10 m; 70–340 nM d^{-1}) and within the oxycline (89–186 nM d^{-1} ; Table 1). Rates reported as zero represent depths where high variability among replicates prevented the calculation of rates associated with NH_3 oxidation (e.g., 13 m) or where MeF-sensitive HCO_3^- activity was not detected (e.g., 20 m). During this study, we did not perform experiments to determine the appropriate factor to convert HCO_3^- fixation rates to NH_3 oxidation rates. The estimated NH_3 oxidation rates obtained using the 8.3 conversion factor (see Methods) ranged between 581–2,830 and 740–1,550 nM NH_3 oxidized d^{-1} in April and July, respectively.

Integrating NH_3 oxidizer-mediated chemoautotrophic C fixation yielded rates of 1.2 and 3.2 $\text{mmol C fixed m}^{-2} \text{ d}^{-1}$ in April and July, respectively. Phytoplankton production in the lake was estimated to be 158 and 42 $\text{mmol C m}^{-2} \text{ d}^{-1}$ in April and July 1995, respectively. In terms of N, integrated NH_3 oxidation rates were quite large, being 10 and

Table 2. Spatiotemporal variation in $(\text{NH}_4^+ + \text{NH}_3)$ turnover.

Depth/Time	$[\text{NH}_4 + \text{NH}_3]^*$	Oxidation†	Turnover time‡
Surface water§			
Apr 95	0.7	0.9	0.7
Jul 95	1.3	1.7	0.8
Oxycline			
Apr 95	2.1	0.8	2.6
Jul 95	24	0.9	27
Bottom water¶			
Apr 95	15	0	#
Jul 95	20	1.0	20

* Concentration in μM .† NH_3 oxidation rate in $\mu\text{mol liter}^{-1} \text{ d}^{-1}$.‡ Turnover time is the NH_3 turnover time (in days) via NH_3 oxidation, calculated from concentration/oxidation rate.

§ Surface water = aerobic waters between 5 and 10 m.

|| Oxycline = O_2 depletion zone between 12 and 13 m.

¶ Bottom water = 17-m microaerophilic waters.

unable to calculate.

27 $\text{mmol N m}^{-2} \text{ d}^{-1}$ in April and July, respectively. Considering only nitrification, biological turnover times for NH_3 were short (Table 2). The most rapid turnover was observed in the surface waters (~ 1 d), with longer turnover times observed in oxycline waters (10 d).

Methane oxidation—Methane oxidation activity was linear over 48 h in both aerobic (10 m) and microaerophilic (15 m) Mono Lake water (Fig. 3). Activity in filter-sterilized or MeF-inhibited samples was significantly lower than that in live, uninhibited samples, illustrating that CH_4 oxidation was biologically mediated. Measured CH_4 oxidation activity was lowest in samples receiving 10 μl of $^{14}\text{C-CH}_4$ but was similar in samples receiving 25 or 50 μl of $^{14}\text{C-CH}_4$ (Fig. 3). The similarity in DPMS recovered in samples receiving 25 or 50 μl of tracer may have resulted from an incomplete solution of tracer. We measured the tracer activity in all samples to correct for variability in tracer solution. In July 1995, filter sterilization and MeF addition alleviated CH_4 oxidation $>80\%$ at 10 m and $>99\%$ at 15 m relative to control rates (data not shown). The addition of 300 $\mu\text{M NH}_3$ strongly inhibited CH_4 oxidation, decreasing activity by 87% (10 m) and 96% (15 m) relative to activity measured in controls receiving no NH_3 (data not shown).

Rates and rate constants were highest in July 1994 (Figs. 4, 5). Highest CH_4 oxidation rates were observed in lower oxycline and anoxic bottom waters (Fig. 4). Methane oxidation rate constants were similar in bottom waters but varied in surface waters (Fig. 5). Despite variations in oxidation rates and pool sizes, the residence time of CH_4 in the hypolimnion was relatively constant (<200 d; Table 3), while residence times in the epilimnion and oxycline regions were longer— $\sim 3,000$ and ~ 450 d, respectively.

Discussion

Our NH_3 and CH_4 oxidation rate measurements in Mono Lake support three general conclusions. First, NH_3 oxidation

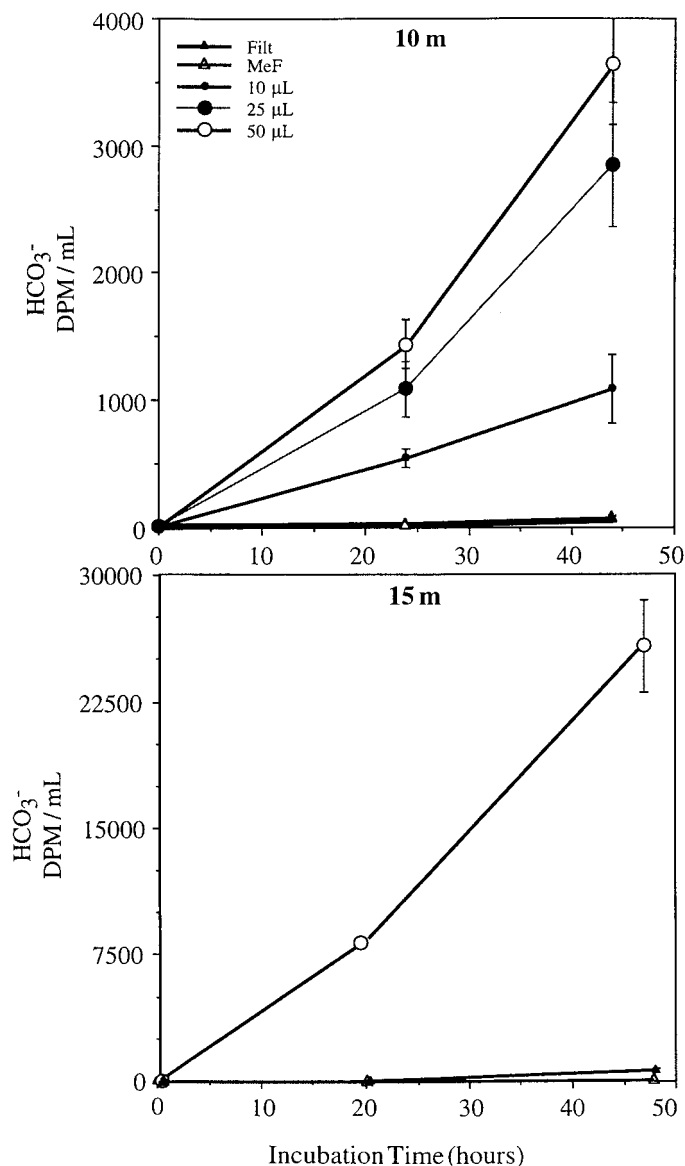


Fig. 3. Time-series data for $^{14}CH_4$ oxidation in 10 m, aerobic, and 15 m, microaerophilic Mono Lake water. Each point represents the average of $n = 3$ samples, and the bars represent the SD of the mean. If there is no error bar, the SD is smaller than the symbol. Treatment definitions: Filt, filter-sterilized control (\blacktriangle); MeF, methyl fluoride-amended sample (\triangle); 50 μ L, 50 μ L of tracer $^{14}C-CH_4$ added (\circ); 25 μ L, 25 μ L of tracer $^{14}C-CH_4$ added (\bullet); and 10 μ L, 10 μ L of tracer $^{14}C-CH_4$ added (\circ).

appears to be an important process in Mono Lake. Comparing NH_3 oxidation rates to rates of other processes (phytoplankton nitrogen uptake and zooplankton NH_3 excretion) suggests a possible link between NH_3 oxidation, ammonification (by bacteria and zooplankton), and perhaps the activity of phytoplankton. Since NH_3 oxidation is active in the surface waters of the lake, this process could play a central role in the lake nitrogen budget by reducing lake-atmosphere losses of NH_3 . Second, most CH_4 oxidation is anaerobically mediated, with rates in oxic surface waters being extremely low. Nonetheless, CH_4 oxidation reduces the potential lake-

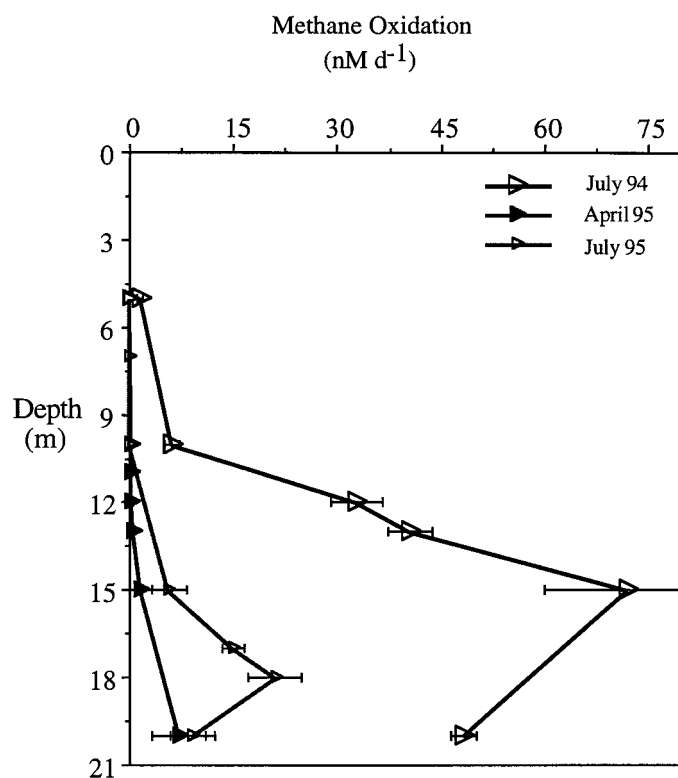


Fig. 4. Depth profiles of CH_4 oxidation rates ($nM d^{-1}$) in July 1994 (large \triangleright), April 1995 (\blacktriangleright), and July 1995 (small \triangleright). Error bars reflect the SD of the mean.

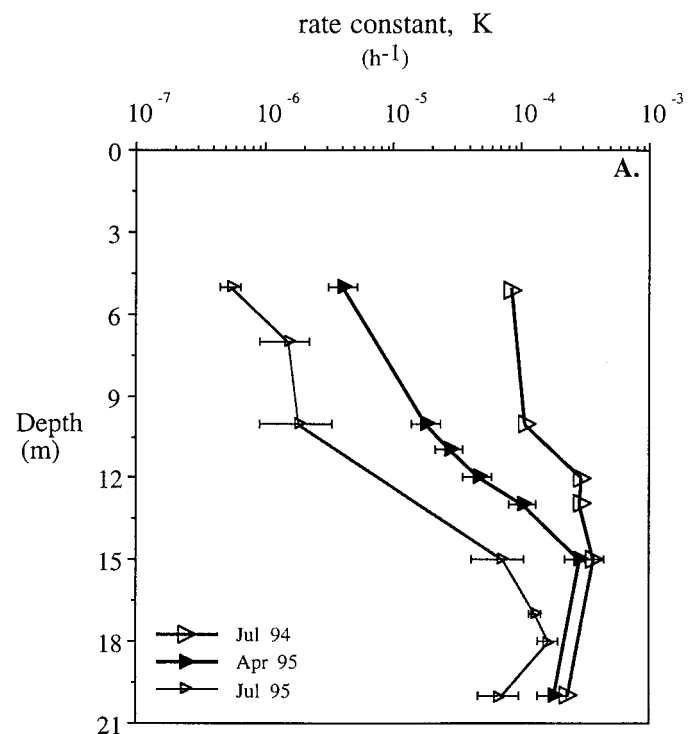


Fig. 5. Depth profiles of the CH_4 oxidation rate constant in July 1994 (large \triangleright), April 1995 (\blacktriangleright), and July 1995 (small \triangleright). Error bars reflect the SD of the mean.

Table 3. Spatiotemporal variation in CH₄ turnover time.

Depth/Time	CH ₄ *	Oxidation†	Turnover time‡
Surface water§			
Jul 94	1.5	3.8	395
Apr 95	0.4	0.3	1,333
Jul 95	0.3	0.04	7,500
Oxycline			
Jul 94	5.2	36.7	142
Apr 95	0.3	0.5	600
Jul 95	3.2	5.7	561
Bottom water¶			
Jul 94	8.4	48	175
Apr 95	1.6	7.1	225
Jul 95	5.2	9.1	571
		21#	247

* Concentration in μM .

† Oxidation = CH₄ oxidation rate in $\text{nmol liter}^{-1} \text{d}^{-1}$.

‡ CH₄ turnover time (in days) calculated from concentration/oxidation rate.

§ Surface water = aerobic waters between 5 and 10 m.

|| Oxycline = O₂ depletion zone between 12 and 13 m in July 1994 and April 1995 and between 13 and 17 m in July 1995.

¶ Bottom water = anaerobic waters at 20 m and below.

Using rate from 18 m instead of 20 m.

atmosphere flux by a factor of two to three. And third, interactions between CH₄ and NH₃ oxidation occur at certain depths in the lake.

Ammonia oxidation—MeF-sensitive, dark CO₂ fixation was documented throughout the surface waters and within the oxycline of Mono Lake in April and July 1995 (Table 1). Since both aerobic CH₄ oxidation (Oremland and Culbertson 1992) and NH₃ oxidation (Miller et al. 1993a) are inhibited by MeF, each process may have contributed to the measured ¹⁴CO₂ fixation. However, our concurrent measurements of CH₄ oxidation suggest that NH₃ oxidation was the dominant process in the upper water column and within the oxycline. Methane oxidation rates were at a maximum in lower oxycline and anoxic deep waters; the lowest rates were observed in oxic surface waters. NH₃ oxidation rates exhibited the opposite pattern, with no detectable activity present in anoxic waters and highest activity observed in oxic surface and oxycline waters. Furthermore, NH₃ (300 μM) was a potent inhibitor of CH₄ oxidation in surface and oxycline waters, suggesting that NH₃ was preferentially oxidized.

In oxic surface waters in July 1995, the CH₄ oxidation rate was on ca. 0.05 nM d^{-1} (Table 3). Assuming that CO₂ assimilation into biomass is equal to CO₂ production via CH₄ oxidation, methanotroph activity could account for 0.05 nM d^{-1} of ¹⁴CO₂ fixation. The measured ¹⁴CO₂ fixation rate was ca. 264 nM d^{-1} (Table 1). Thus, CH₄ oxidation could have accounted for <0.1% of the total ¹⁴CO₂ fixation. Within the oxycline, CH₄ oxidation could have supported $\sim 5 \text{ nM d}^{-1}$ of ¹⁴CO₂ fixation (Table 3), a mere 3% of the measured ¹⁴CO₂ fixation activity (180 nM d^{-1} ; Table 1). Furthermore, molecular biological data support our contention that MeF-sensitive ¹⁴CO₂ fixation rates reflect NH₃ oxidation: deoxyribonucleic acid (DNA) from NH₃-oxidizing bacteria was found

at every depth where NH₃ oxidation was measured (Ward unpubl. data). A molecular probe to distinguish between the DNA of CH₄- and NH₃-oxidizing bacteria is not available at this time (Holmes et al. 1995); thus, we cannot rule out the presence of both groups in our samples. However, the rates of CH₄ oxidation we measured were much lower than the observed rates of ¹⁴CO₂ fixation, so we conclude that the measured MeF-sensitive ¹⁴CO₂ activity represents chemoautotrophic ¹⁴CO₂ fixation mediated by NH₃-oxidizing bacteria.

In Big Soda Lake, chemoautotrophic dark ¹⁴CO₂ fixation (411 $\text{mg C m}^{-2} \text{d}^{-1}$) accounted for ca. 30% of the lake-wide annual primary production (1.37 $\text{g C m}^{-2} \text{d}^{-1}$), and 45–80% of chemoautotrophic activity was attributed to nitrifying bacteria (184–329 $\text{mg C m}^{-2} \text{d}^{-1}$; Cloern et al. 1983). Our estimated phototrophic primary production rates for April and July 1995 were 1.9 and 0.5 $\text{g C m}^{-2} \text{d}^{-1}$, respectively. Integrated NH₃ oxidizer-mediated C fixation accounted for 12 and 31.2 $\text{mg C m}^{-2} \text{d}^{-1}$ in April and July, respectively. Thus, in contrast to Big Soda Lake, chemoautotrophic C fixation in Mono Lake is a minor contributor to lake-wide primary production (1–7%).

Ammonia concentrations in the anoxic bottom waters of Big Soda Lake were 2.6 mM , whereas concentrations in the anoxic bottom waters of Mono Lake ranged from <2 to 30 μM during our study. This difference in NH₃ concentration, and thus in NH₃ flux across the thermocline, may explain the lower rates of NH₃ oxidation in Mono Lake compared to Big Soda Lake. NH₃ concentrations in Mono Lake bottom waters reach much higher levels during meromixis (Miller et al. 1993b) and could regulate the magnitude of NH₃ oxidation activity. Given the existing data, we were unable to determine whether the summer increase in NH₃ oxidation activity resulted from increased temperature (16°C increase in surface-water temperature between April and July) as opposed to increases in NH₃ concentrations and gradient-driven NH₃ fluxes across the thermocline (6.4 vs. 9 $\text{mmol N m}^{-2} \text{d}^{-1}$ in April and July, respectively; more discussion follows) and decreased phytoplankton N demand (phytoplankton production decreased during summer, possibly because of increased grazing pressure). All of these factors probably contributed to the summer increase in NH₃ oxidation rates.

Converting NH₃ oxidizer-mediated C fixation rates to N units yielded daily N oxidation rates between 580 and 2,800 nM d^{-1} (~ 0.5 – $2.8 \mu\text{M d}^{-1}$; Table 1). If a more conservative factor (than our 8.3) were used (e.g., 5.95; Owens 1986), the NH₃ oxidation rates would be reduced by 28%. The NH₃ oxidation rates we documented are comparable to those reported in other aquatic habitats. For example, water column NH₃ oxidation in estuarine and freshwater environments determined with either ¹⁴C or ¹⁵N methods vary between 0.05 and 32 $\mu\text{M d}^{-1}$ (Enoksson 1986; Berounsky and Nixon 1993). Rates in pelagic marine systems are lower, ranging from 0.05 to 0.3 $\mu\text{M d}^{-1}$ (Ward 1987; Ward et al. 1989; Lipshultz et al. 1990; Ward and Kilpatrick 1991). Comparing our measured NH₃ oxidation rates with estimates of other nitrogen fluxes suggests that this process plays a central role in the nitrogen cycle of Mono Lake (see below).

Light, i.e., irradiance, is often cited as a regulatory agent of NH₃ oxidation in pelagic environments (Olson 1981; Ward 1985, 1987). Typically, NH₃ oxidation becomes im-

portant in terms of the total nitrogen flux at depths where the light level is ca. 5–10% of the surface irradiance, I_0 (Olson 1981; Ward 1985, 1986). In the present study, high NH₃ oxidation rates were observed in surface (5–7 m) waters where the irradiance levels were 12–18% of I_0 (Fig. 1; Table 1). Light may play a regulatory role in determining when, during day or night, NH₃ oxidizers are active, but it does not appear to preclude their activity within the photic zone of Mono Lake. Perhaps Mono Lake NH₃ oxidizers are not as light sensitive as those in other environments. An alternative hypothesis is that NH₃ oxidation commonly occurs in the euphotic zone during certain parts of the day when light inhibition is naturally alleviated (e.g., at night).

One potentially troubling aspect of our data set is that the calculated NH₃ turnover times in surface waters (0.8 d; Table 2) were shorter than our incubation times (2 d). This could imply that the NH₃ pool in the serum bottles was exhausted during the incubation, thus underestimating activity. However, if active NH₃ regeneration occurred in the incubation bottles, this in situ source of NH₃ could have supported the measured NH₃ oxidation. The concentration of dissolved organic nitrogen (DON) in Mono Lake is ca. 145 μM , while the particulate nitrogen (PN) concentration is ca. 11 μM (Jellison et al. 1993). The 78-ml serum bottles we used for surface-water (5 m) NH₃ oxidation assays in July contained ca. 12.2 μmol of total nitrogen (12.1 μmol as DON + PN and 0.08 μmol as NH₃). At this time, the surface-water NH₃ oxidation rate was 180 nmol liter⁻¹ h⁻¹, corresponding to an NH₃ demand of 14.2 nmol N h⁻¹ in a 78-ml incubation bottle. Thus, during a 48-h incubation, remineralization of about 5% of the PN + DON could provide the substrate demands of NH₃-oxidizing bacteria.

The required NH₃ remineralization rate of 180 nmol liter⁻¹ h⁻¹ lies well within the range reported for aquatic environments. For example, NH₃ remineralization rates between 100 and 700 nmol liter⁻¹ h⁻¹ have been reported for a tropical lake (Fisher et al. 1988; Morrissey and Fisher 1988). Estuarine NH₃ remineralization rates between 100 and 400 nmol liter⁻¹ h⁻¹ have been reported (Hanson et al. 1990), as have turnover times <1 d for specific fractions of the nitrogen pool. For example, Price and Harrison (1988) report turnover times of 0.5–0.8 d for the urea pool in the Atlantic Ocean. Given the concentrations of the various nitrogen pools in Mono Lake and considering the probable importance of zooplankton NH₃ excretion and diffusive delivery of NH₃ from deep waters across the thermocline, meeting the N requirements of NH₃-oxidizing bacteria does not appear to pose a significant problem. If NH₃-oxidizing bacteria were extremely NH₃ limited, they might be expected to play a more important role in aerobic CH₄ oxidation. Yet the CH₄ oxidation rate in surface waters is very low, and the residence time for CH₄ is very long. This suggests that NH₃-oxidizing bacteria are not NH₃ starved, and, as we have shown above, N recycling processes occurring within the lake can sufficiently meet the demands of NH₃ oxidizers.

Though the epilimnetic NH₃ concentration is low (<1–5 μM), the relative distribution between NH₃ and NH₄⁺ favors NH₃ oxidizers. Using the equilibrium expression $K_a = \{[\text{NH}_3][\text{H}^+]/[\text{NH}_4^+]\}$, we calculated speciation of NH₃ in Mono Lake waters. The K_a for NH₄⁺ is 10^{-9.3}, so at pH 9.7,

the speciation is shifted toward NH₃, making this species approximately 3.6 times more abundant than NH₄⁺ (Jellison et al. 1993). Since the substrate used by NH₃-oxidizing bacteria is NH₃, not NH₄⁺ (Suzuki et al. 1974), alkaline pH increases substrate availability (NH₃) relative to the total pool (NH₃ + NH₄⁺) and could promote NH₃ oxidation. High NH₃ oxidation rates do not contradict the observed low substrate concentration. Rather, rapid NH₃ oxidation implies and requires efficient cycling of nitrogen by the Mono Lake plankton community.

Our estimates of phytoplankton primary production for April and July 1995 were 158 and 42 mmol C m⁻² d⁻¹, respectively. Using an average molar C:N ratio of 7 for phytoplankton in this system (Jellison and Melack 1988; Jellison et al. 1993), the daily phytoplankton N demand would have been 22 and 6 mmol N m⁻² d⁻¹. Integrated NH₃ oxidation rates were 10 and 27 mmol N m⁻² d⁻¹ in April and July, respectively. If a more conservative factor of 5.95 is used to convert CO₂ fixation rates to NH₃ oxidation rates (Owens 1986; Berounsky and Nixon 1993), the integrated NH₃ oxidation rates (7 and 19 mmol N m⁻² d⁻¹ in April and July, respectively) are still similar in magnitude to the phytoplankton N demands.

Since the phytoplankton N demand is similar to the NH₃ oxidation-N demand, these two groups may compete for NH₃. Alternatively, phytoplankton may use NO₂⁻ or NO₃⁻, the products of NH₃ and NO₂⁻ oxidation, respectively, as the primary inorganic nitrogen source. Though we did not quantify NO₂⁻ oxidation, it is usually coupled closely with NH₃ oxidation, as has been illustrated in other pelagic environments (Ward et al. 1989; Lipshultz et al. 1990). NH₃ oxidation is typically the rate-limiting step of the nitrification process, so the intermediate (NO₂⁻) does not usually accumulate; rather, it is consumed by NO₂⁻ oxidizers as quickly as it is produced. We have measured NO₂⁻ accumulation in sediment pore water when the process of NO₂⁻ oxidation is inhibited (by H₂S; Joye and Hollibaugh in prep.). However, since H₂S was not abundant in the aerobic water column of Mono Lake, we expect that NH₃ and NO₂⁻ oxidations were closely coupled.

The nitrogen nutrient preference of Mono Lake phytoplankton is not currently known. However, NH₃ oxidation rates of this magnitude suggest a close coupling between NH₃ oxidation, primary production, and zooplankton (and possibly bacterial) NH₃ regeneration. During summer, NH₃ regeneration associated with grazing is probably an important link between autotrophic primary production and NH₃-oxidizing bacteria. During winter and spring, deep mixing and the intrusion of NH₃-rich bottom water into the surface zone may serve that role. Energetically, a coupling between NH₃ oxidizers and phytoplankton is inefficient because the energy gained by NH₃ oxidizers by oxidizing NH₃ to NO₂⁻ would have to be replaced by the phytoplankton when they reduce NO₂⁻-N back to the -3 valence to incorporate it into cellular components. Competition between phytoplankton and NH₃ oxidizers for NH₃ could be very important.

In a box model of Mono Lake nitrogen dynamics, Jellison et al. (1993) proposed that nitrogen was cycled efficiently by phytoplankton and zooplankton within the mixolimnion. NH₃ oxidation could effectively couple phytoplankton and

zooplankton dynamics. During winter, NH_3 oxidation ($10 \pm 3 \text{ mmol N m}^{-2} \text{ d}^{-1}$) is similar to the NH_3 flux across the chemocline ($6.4 \pm 2 \text{ mmol N m}^{-2} \text{ d}^{-1}$; calculated from data in Fig. 2 and K_z given in Jellison et al. 1993). During summer, the NH_3 oxidation rate ($22 \pm 9 \text{ mmol N m}^{-2} \text{ d}^{-1}$) exceeds the flux across the chemocline ($9 \text{ mmol N m}^{-2} \text{ d}^{-1}$), but an additional source of NH_3 , zooplankton excretion ($20\text{--}30 \text{ mmol N m}^{-2} \text{ d}^{-1}$; Jellison et al. 1993; Jellison and Melack 1993b), is present at this time. Jellison et al. (1993) proposed that the flux across the chemocline was balanced by losses (volatilization) to the atmosphere. Volatilization rates were calculated to be highest following turnover and during well-mixed periods ($10 \text{ mmol m}^{-2} \text{ d}^{-1}$) but were substantial during stratified periods as well ($1\text{--}4 \text{ mmol m}^{-2} \text{ d}^{-1}$; Jellison et al. 1993). Losses of this magnitude represent a tremendous sink for nitrogen and are made possible by the fact that volatile NH_3 is the more stable form of the $\text{NH}_3/\text{NH}_4^+$ couple under alkaline conditions. Given that new nitrogen inputs to Mono Lake are small (atmospheric + runoff + nitrogen fixation = $<0.1 \text{ mmol m}^{-2} \text{ d}^{-1}$; Jellison et al. 1993) compared to internal inventories ($\sim 10^6 \text{ mol N}$; Jellison et al. 1993) and fluxes ($5\text{--}30 \text{ mmol m}^{-2} \text{ d}^{-1}$; Jellison et al. 1993), sustained volatilization losses on the order of $1\text{--}10 \text{ mmol m}^{-2} \text{ d}^{-1}$ could drive the system toward chronic nitrogen limitation within very short time scales. For example, the lake surface area is approximately 162 km^2 , and the NH_3 inventory during our study averaged 22×10^6 moles $\text{NH}_3\text{-N}$. Given the range of volatilization rates noted above, the entire N inventory (neglecting inputs from sediments) could be exhausted in $10\text{--}100 \text{ d}$. The assumption that volatilization-related losses balanced the flux across the thermocline (which originates from the sediments) suggests that the sediment flux is decoupled from water column processes, because it would require that the sediment flux be independent of the particulate (PON) flux and regeneration, otherwise, the sediment flux would shunt the NH_3 from the sediments to the water column and, finally, to the atmosphere. Such high rates of volatilization would therefore not be expected, nor could they be maintained for more than very brief periods (days), if the system were to remain at steady-state conditions.

Ammonia oxidation plays an important role in the nitrogen economy of Mono Lake and perhaps in other alkaline lake environments by transforming NH_3 -nitrogen to a stable (NO_2^-) form, thereby reducing losses that might otherwise intensify nitrogen limitation. We propose that NH_3 oxidation efficiently captures NH_3 diffusing through the thermocline and converts NH_3 to NO_2^- . Our data indicate that internal cycling, with NH_3 oxidation serving a central role, could maintain and cycle nitrogen very efficiently within the surface mixed zone. Interactions between phyto- and zooplankton and NH_3 -oxidizing bacteria could retain nitrogen diffusing across the thermocline within the system and reduce volatilization-related losses.

Methane oxidation—Methane oxidation varied between the major depth zones of the lake and is similar to that observed in other alkaline, saline lakes (Iversen et al. 1987). Though CH_4 oxidation rates and rate constants varied throughout this study, the CH_4 turnover time in the hypolimnion was fairly consistent, being ca. 200 d (Figs. 4, 5; Table

3). During this study, the upper 10-m waters of Mono Lake were generally aerobic, the oxycline varied between 10 and 17 m, and waters beneath 18 m were generally anaerobic. Thus, the majority of CH_4 occurred in anoxic waters ($\sim 65\%$), where high CH_4 oxidation activity was consistently observed. The dominance of the anoxic bottom water in terms of total integrated activity occurs because activity in the oxycline takes place within a restricted depth zone; thus, the integrated oxycline rates represent a smaller fraction of the total volume-weighted activity. Thus, anaerobic CH_4 oxidation was the primary sink for CH_4 on all sampling dates, and this process appeared to be an efficient control on CH_4 concentration and on the potential CH_4 efflux from Mono Lake. Our integrated aerobic oxidation rates could underestimate activity because of limited sampling along the upper oxycline; that is, an intense maximum present over a restricted depth range within oxic waters could have been missed with our sampling protocol. However, the available data show that the turnover times in aerobic waters are much longer ($400\text{--}7,500 \text{ d}$) than those in anaerobic waters ($\sim 200 \text{ d}$; Table 3), so any CH_4 diffusing into aerobic waters is likely to escape to the atmosphere.

Slow removal rates (i.e., long turnover times) in surface waters suggest that significant surface-atmosphere CH_4 fluxes are supported. The median CH_4 flux from Mono Lake surface waters during 1985–1987 was $312 \mu\text{mol m}^{-2} \text{ d}^{-1}$ ($12.8 \mu\text{mol m}^{-2} \text{ h}^{-1}$; Miller and Oremland 1988), and the CH_4 concentration in surface water was low ($1.3 \mu\text{M}$; Miller et al. 1993), similar to that measured in July 1994 ($1.5 \mu\text{M}$; Table 3). Because of thermal mixing and diffusion across the thermocline, Mono Lake surface waters are always supersaturated with respect to CH_4 (Miller and Oremland 1988). Since the median CH_4 surface flux was well correlated with CH_4 surface concentration (Miller and Oremland 1988), we applied the average fluxes given in Miller and Oremland (1988) to estimate surface-atmosphere exchange during our study period and then compared those fluxes to the integrated CH_4 oxidation rate. The basic assumption required for this calculation is that the surface-atmosphere flux during our study was similarly proportional to the surface-atmosphere concentration gradient.

The integrated CH_4 oxidation rates and fluxes across the chemocline were calculated using the data presented in Table 3 and Fig. 2. Fluxes across the chemocline were 620 , 440 , and $800 \mu\text{mol m}^{-2} \text{ d}^{-1}$ in July 1994, April 1995, and July 1995, respectively. The highest integrated oxidation rate was observed in July 1994 ($702 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ d}^{-1}$); rates in April and July 1995 were much lower, being 76.5 and $120 \mu\text{mol CH}_4 \text{ oxidized m}^{-2} \text{ d}^{-1}$, respectively. Thus, the integrated CH_4 oxidation rates were lower than the integrated CH_4 fluxes across the chemocline on two of the three sampling dates.

In July 1994, the surface CH_4 concentration was similar to that reported by Miller and Oremland (1988), so we have assumed the average surface-atmosphere flux was similar (ca. $312 \mu\text{mol m}^{-2} \text{ d}^{-1}$). Given this, the integrated CH_4 oxidation rate was approximately twice the surface CH_4 flux. During 1995, surface CH_4 concentrations decreased ($<0.5 \mu\text{M}$), and the surface flux was likely reduced by a factor comparable to ca. $100 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ d}^{-1}$. This value is

comparable to the integrated oxidation rates of 76.5 and 120 $\mu\text{mol CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ in April and July, respectively. Based on these data and considerations, in situ CH₄ oxidation could reduce the surface CH₄ flux by a factor of two to three.

When considering oxidation and (potential) lake-atmosphere exchange, it becomes obvious that microbial oxidation plays an important role in the lake CH₄ cycle. Another interesting observation with respect to Mono Lake CH₄ biogeochemistry is that nearly all of the CH₄ isotope fractionation occurs within or above the oxycline (Oremland et al. 1987). Oremland et al. (1987) documented a 70‰ difference between sediment pore water (−90‰) and surface water (−20‰) $\delta^{13}\text{C-CH}_4$. The CH₄ in anoxic bottom waters exhibited a C isotope ratio of ca. −75‰; thus, most of the fractionation occurred within or above the oxycline. Isotope fractionation could be a function of the oxidation rate, with slow (aerobic) oxidation being an equilibrium process that results in isotope fractionation while fast (anaerobic) oxidation is a nonequilibrium process that results in less isotope fractionation. A similar observation was made in Lake Fryxell (Smith et al. 1993), namely, that aerobic CH₄ oxidation was a minor component in the CH₄ cycle but resulted in a larger $\delta^{13}\text{C}$ shift when compared to anaerobic oxidation. Alternatively, anaerobic oxidation may yield a smaller fractionation than aerobic oxidation (Alperin et al. 1988).

Conclusions

Biologically mediated oxidative processes are responsible for efficient and rapid recycling of NH₃ and CH₄ in Mono Lake. NH₃-oxidizing bacteria transform a volatile gas, NH₃, into an ionic form NO₂[−], which may be retained within the system to serve as a nutrient source for phytoplankton. Considering the data presented in this paper and in previous papers (Jellison et al. 1993; Miller et al. 1993), NH₃ oxidation could play a central role in the nitrogen biogeochemistry of alkaline lake ecosystems by reducing nitrogen losses associated with NH₃ volatilization. The occurrence of NH₃ oxidation within aerobic (surface) and oxycline waters suggests dynamic interactions between primary producers, zooplankton grazers, bacterial NH₃ remineralizers, and NH₃ oxidizers. Whether the NO₂[−] produced by NH₃ oxidizers is taken up by phytoplankton or consumed by denitrifying bacteria is unknown. It is unlikely that O₂-sensitive denitrifiers are active in surface waters; however, they may be active within the oxycline. The fate of NO₂[−] produced in situ by NH₃ oxidizers should be studied in detail in the future. Methane oxidation rates were strongly inhibited by the addition of NH₃, indicating that co-metabolism of these substrates may occur in Mono Lake aerobic and microaerophilic waters and that NH₃ oxidizers could contribute to aerobic CH₄ oxidation. However, the primary biological sink for CH₄ is anaerobic oxidation. Methane oxidation reduces the (potential) surface flux by a factor of two to three. However, unlike NH₃ oxidation, anaerobic CH₄ oxidation does not appear to be closely linked to plankton dynamics.

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