1-1-2013

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Gaston E. Small
University of Minnesota Twin Cities

George S. Bullerjahn
Bowling Green State University

Robert W. Sterner
University of Minnesota Twin Cities

Benjamin F.N. Beall
Bowling Green State University

Sandra Brovold
University of Minnesota Twin Cities

See next page for additional authors

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Authors

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Rates and controls of nitrification in a large oligotrophic lake

Gaston E. Small,[a,1,]* George S. Bullerjahn,[b] Robert W. Sterner,[a] Benjamin F. N. Beall,[b]
Sandra Brovold,[a] Jacques C. Finlay,[a] Robert M. L. McKay,[b] and Maitreyee Mukherjee [b]

[a] Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, Minnesota
[b] Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio

Abstract

Recent discoveries have altered prevailing paradigms concerning the conditions under which nitrification takes place and the organisms responsible for nitrification in aquatic ecosystems. In Lake Superior, nitrate (NO_3^-) concentrations have increased fivefold in the past century. Although previous evidence indicated that most NO_3^- is generated by nitrification within the lake, important questions remain concerning the magnitude and controls of nitrification, and which microbial groups are primarily responsible for this process. We measured water-column nitrification rates in the western basin of Lake Superior during five research cruises from November 2009 to March 2011. Using in situ bottle incubations at 10 depths, we quantified nitrification rates using both the oxidation of ^{15}N-labeled ammonium (NH_4^+) and the uptake of ^{14}C associated with nitrification. Average rates of NH_4^+ oxidation ranged from 18–34 nmol N L^{-1} d^{-1} across the five cruises, similar to values reported for the coastal ocean, and two orders of magnitude lower than values reported from other lakes. Low nitrification rates observed in the epilimnion corresponded to the absence of ammonium-oxidizing archaea and nitrite-oxidizing bacteria. The measured rates of nitrification are > 50-fold greater than the long-term NO_3^- rise in the lake, indicating that N is actively cycling and that long-term change in this ecosystem is mediated by internal dynamics.

Nitrification is a key transformation in the nitrogen (N) cycle, important in determining the distribution of N among pools of dissolved inorganic nitrogen (Ward 2011), which in turn can affect rates of primary production (Howarth and Marino 2006) and greenhouse gas emissions (Stadmark and Leonardson 2007). Recent findings have challenged prevailing assumptions about the conditions under which nitrification occurs, and the organisms responsible for nitrification. Recent studies in marine environments have shown that nitrification can occur in the euphotic zone (Fernandez and Raimbault 2007; Clark et al. 2008), challenging the fundamental assumption of the classic "new production" paradigm (Dugdale and Goering 1967). The recent discovery that Archaea are the most abundant ammonia oxidizers in the ocean (Könneke et al. 2005) highlights how little is understood about the ecological controls underpinning nitrification.

Nitrification rates are potentially subject to control by physical, biological, and chemical factors. Growth of ammonium-oxidizing archaea (AOA) in cultures is directly inhibited by light (French et al. 2012; Merbt et al. 2012). Heavy grazing pressure can maintain low populations of slow-growing nitrifiers (Lavrentyev et al. 1997). The availability of ammonium (NH_4^+) is a potentially important control on nitrification rates, given the demand for NH_4^+ by heterotrophic microbes and photoautotrophs. Photoautotrophs may outcompete nitrifiers for NH_4^+ in the presence of light, and heterotrophic microbes may outcompete nitrifiers when organic carbon (OC) availability is

high (Strauss and Lamberti 2000; Taylor and Townsend 2010).

Despite recent advances, measurements of rates and controls of nitrification are relatively rare in lake ecosystems, and there is a specific need for temporally replicated measurements of nitrification throughout the water column, linking rates with abundances of nitrifiers. Lake Superior is a propitious environment for testing these hypotheses regarding N cycling. In this lake, which contains ~ 10% of the liquid freshwater on Earth's surface, nitrate (NO_3^-) levels have increased fivefold over the past century, from historic levels of ~ 5 μmol L^{-1} to present-day concentrations of ~ 26 μmol L^{-1} (Sterner et al. 2007). This increase continues albeit at a somewhat lower rate (Sterner 2011). Although this rise has previously been attributed to external loading and low rates of NO_3^- assimilation in the oligotrophic lake, recent evidence has shown that N cycles actively within the lake (Kumar et al. 2007, 2008), and that nearly all of the lake's NO_3^- (93–100%) has been produced directly from in situ nitrification rather than by external loading (Finlay et al. 2007). The mass-balance of N in the lake indicates a net NO_3^- production of 21.7 mmol m^{-2} yr^{-1} (Sterner et al. 2007) and a net removal of 14–27 mmol NH_4^+ m^{-2} yr^{-1} (Kumar et al. 2007), indicating that nitrification plays an important role in altering the chemistry of water leaving the lake. Because of the likely importance of internal dynamics driving long-term changes in the lake ecosystem (McDonald et al. 2010), it is necessary to understand the rates and controls on nitrification within the water column.

Lake Superior has relatively low rates of primary production (Sterner 2010) and is low in both OC and NH_4^+, characteristics shared by other high-latitude oligotrophic lakes (Sterner 2011). We hypothesize that during stratification, nitrification rates in the epilimnion should be

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* Corresponding author: gesmall@umn.edu

[1] Present address: Department of Biology, University of St. Thomas, St. Paul, Minnesota

low due to a combination of direct light inhibition, grazing pressure, and competition for NH_4^+ with photoautotrophs and heterotrophic microbes. In the hypolimnion, these pressures should be relived, resulting in higher nitrification rates. In order to characterize controls on nitrification in Lake Superior, we analyzed how nitrification rates measured throughout the water column and in different seasons varied with physical (depth, temperature), chemical (NH_4^+, OC), and biological (chlorophyll *a* [Chl *a*], primary production) variables.

Results are based on a series of five research cruises from November 2009 to March 2011 during which we measured in situ nitrification rates throughout the water column by measuring both the oxidation of ^{15}N-NH_4^+ and the uptake of ^{14}C associated with nitrification. We also measured primary production rates and other water chemistry parameters as potential explanatory variables. For a subset of these cruises, nitrifier abundance was measured throughout the water column. We performed additional experiments measuring the effect of light and NH_4^+ concentrations on nitrification rates.

Methods

In situ nitrification from $^{15}NH_4^+$ oxidation—We measured in situ nitrification rates during five research cruises from November 2009 to March 2011 (Table 1) at Sta. Western Midlake (WM) in the Western Basin of Lake Superior (47°20′0″N, 89°48′0″W). This station is > 45 km from land, with a depth of 160 m, and is site of a weather buoy operated by the National Oceanic and Atmospheric Administration (NOAA), a mooring with instrumentation, and a previous study on primary production in the lake (Sterner 2010). Cages containing incubation bottles were secured to a ''drifter'' (cable attached to a float, as described in Kumar et al. 2008) at 10 depths (2, 5, 10, 20, 30, 40, 60, 90, 120, and 150 m). Incubations ran for ∼ 18 h on each sampling date. Upon arrival at the station, we collected water from these 10 depths. Clean 250 mL polycarbonate Nalgene incubation bottles were rinsed 3× with lake water and filled to overflowing. Bottles were secured inside of canvas bags to keep incubations in the dark, and bags were secured inside of cages corresponding to the appropriate depth.

We measured rates of NH_4^+ oxidation following the addition of $^{15}NH_4Cl$, after published methods (Beman et al. 2008; Carini and Joye 2008). Bottles were enriched with $^{15}NH_4^+$ to achieve a concentration of 0.03 μmol L^{-1} $^{15}NH_4^+$. This concentration was designed to be < 10% of ambient lake NH_4^+, but NH_4^+ concentrations are temporally and spatially variable (Kumar et al. 2007), and low levels at certain depths during some cruises occasionally resulted in $^{15}NH_4^+$ spikes as high as 25% of ambient concentrations. After an incubation period of 18–20 h, the floating array was recovered. Samples were syringe-filtered (0.45 μm) and frozen for analysis of final NO_3^- and ^{15}N-NO_3^-. NO_3^- was measured at the University of Minnesota using a Lachat QuickChem© 8500 Flow Injection Analysis System and a cadmium reduction column. ^{15}N-NO_3^- was measured at the University of California, Davis Stable

Isotope Facility using the denitrifier method (Sigman et al. 2001). Nitrification rates were calculated using the equation:

$$(^{15}NO_3^- \text{ excess}) \times [NO_3^-]$$
$$/[(^{15}NH_4^+)/[NH_4^+] \times \text{incubation time}] \tag{1}$$

where $^{15}NO_3^-$ excess is the difference between final and initial concentrations of atom % $^{15}NO_3$, $[NO_3^-]$ is the final concentration of nitrate in the sample, $^{15}NH_4^+$ is the concentration of $^{15}NH_4^+$ added to the incubation bottle, and $[NH_4^+]$ is the total initial concentration of NH_4 in the incubation bottle (ambient NH_4^+ + $^{15}NH_4^+$). Some measurements resulted in negative estimates of nitrification where $^{15}NO_3^-$ was slightly depleted relative to background.

In situ nitrification from ^{14}C uptake—We independently quantified nitrification rates by measuring carbon uptake associated with nitrification. For this approach, we measured ^{14}C uptake in dark bottle incubations with a pair of nitrification inhibitors: nitrapyrin (which blocks the transformation of NH_4^+ to nitrite $[NO_2^-]$) and sodium chlorate (which blocks the oxidation of NO_2^- to NO_3^-), and reference dark bottles with no inhibitors. We added 250 μL of nitrapyrin (final concentration 5 mg L^{-1}) to a pair of 250 mL polycarbonate incubation bottles for each depth, 4 h prior to filling with lake water to allow ethanol to evaporate. Upon collecting water using a conductivity–temperature–depth rosette with Niskin bottles, four incubation bottles, including the two bottles containing the inhibitor nitrapyrin, were filled to overflowing from each depth. Sodium chlorate was then added to the inhibitor bottles (final concentration 10 mg L^{-1}). Bottles were spiked with 250 μL of H$^{14}CO_3$ (80 μCi, MP Biomedicals No. 17441H25, specific activity 30–60 mCi mmol^{-1}, not chelex rinsed), placed in opaque canvas bags, and secured inside the cages of the floating array. Following the ∼ 18 h deployment, samples were filtered through 0.2 μm Millipore polycarbonate filters. Vials containing filters were acidified with 0.25 mL 0.5 mol L^{-1} HCl, evaporated to dryness, filled with fluor, and counted on a Beckman-Coulter LS 6500 liquid scintillation counter within 10 d of collection.

Production in each bottle was calculated as:

$$\text{Particulate production } (\text{mg C L}^{-1} \text{ d}^{-1})$$
$$= (F/V) \times (0.00025C/T) \times (1.05/D) \tag{2}$$

where F is disintegrations per minute (DPMs) in filtered sample, V is volume filtered (L), C is dissolved inorganic carbon concentration (mg C L^{-1}), T is total ^{14}C DPMs (in 0.25 mL), and D is incubation duration (fraction of 1 d). The value 0.00025 converts total DPMs to 1 liter volume, and 1.05 represents the correction for the lower uptake of ^{14}C compared to ^{12}C. Production associated with nitrification for each depth was calculated as the difference between the mean production of the two control bottles and the mean production of the two inhibited bottles. To convert C uptake to NH_4^+ oxidation rates, we used a conversion factor of 25 mol of N required to fix 1 mol of C for

Table 1.   Physical and chemical parameters, and rates of primary production and nitrification (with standard deviation). The two NH_4^+ values and associated nitrification rates in italics were excluded from the statistical model because of likely overestimation. Temp, temperature; ND, no data.

Cruise date	Depth (m)	Temp (°C)	Chl a (μg L^{-1})	POC (μg L^{-1})	DOC (mg L^{-1})	NH_4^+ (μmol L^{-1})	Primary production (μmol C L^{-1} d^{-1})	Nitrification (SD) (nmol N L^{-1} d^{-1})
11 Nov 09	2	7.1	0.62	104.0	1.3	0.17	0.588	22.8(3.6)
11 Nov 09	5	7.1	0.78	111.6	1.4	*0.63*	0.492	*83.0*(1.1)
11 Nov 09	10	7.1	0.91	111.2	1.5	0.18	0.260	20.3(5.3)
11 Nov 09	20	7.0	0.66	89.5	1.2	0.25	0.053	34.5(1.2)
11 Nov 09	30	7.0	0.98	106.5	1.3	0.28	0.005	23.5(8.8)
11 Nov 09	40	7.0	0.78	100.1	1.3	0.18	−0.001	30.0(1.8)
11 Nov 09	60	6.9	0.50	97.3	1.3	0.21	0.007	40.8(1.3)
11 Nov 09	80	4.3	0.19	48.8	1.2	0.13	−0.001	30.2(5.8)
27 Jun 10	2	7.8	0.32	103.4	1.2	0.12	0.470	7.4(0.2)
27 Jun 10	5	7.6	0.38	117.5	1.2	0.25	0.487	13.7(0.6)
27 Jun 10	10	6.3	0.55	114.2	1.4	0.23	0.617	13.2(0.0)
27 Jun 10	20	4.9	0.18	101.1	1.2	0.10	0.342	6.3(0.5)
27 Jun 10	30	4.5	0.16	98.4	1.2	0.16	0.273	10.4(0.6)
27 Jun 10	40	4.3	0.10	83.1	1.2	*1.11*	0.088	*71.9*(0.5)
27 Jun 10	60	4.0	0.07	73.1	1.2	0.33	0.009	21.8(0.4)
27 Jun 10	90	4.0	0.08	62.9	1.3	0.50	0.000	32.5(1.3)
27 Jun 10	120	3.9	0.07	67.8	1.3	0.35	0.000	24.8(0.9)
27 Jun 10	150	3.9	0.07	57.3	1.2	0.35	0.000	23.4(0.5)
19 Aug 10	2	11.2	0.27	153.4	1.3	0.29	0.352	−1.8(0.0)
19 Aug 10	5	11.1	0.26	146.1	1.3	0.25	0.642	−2.9(2.0)
19 Aug 10	10	10.6	0.37	162.9	1.6	0.28	0.742	−0.1(0.7)
19 Aug 10	20	5.5	0.38	167.9	1.3	0.25	0.488	1.8(0.4)
19 Aug 10	30	4.4	0.64	168.5	1.3	0.31	0.166	6.3(1.8)
19 Aug 10	40	4.3	0.40	114.6		0.30	0.030	11.0(2.6)
19 Aug 10	60	4.0	0.16	58.9	1.3	0.15	0.000	22.7(4.8)
19 Aug 10	90	4.0	0.10	45.8		0.09	0.000	20.6(0.4)
19 Aug 10	120	3.9	0.07	31.7	1.2	0.31	0.000	36.3(2.3)
19 Aug 10	150	3.9	0.09	45.1	1.3	0.14	0.000	12.3(0.0)
12 Oct 10	2	9.6	1.05	165.5	1.0	0.17	0.820	−1.0(0.1)
12 Oct 10	5	9.6	0.97	161.4	1.5	0.17	0.571	−1.1(0.1)
12 Oct 10	10	9.5	1.23	169.3	1.4	0.18	0.673	−0.7(0.1)
12 Oct 10	20	9.3	1.18	148.6	1.3	0.19	0.353	−0.2(0.1)
12 Oct 10	30	8.3	0.95	128.0	1.3	0.17	0.084	14.4(1.6)
12 Oct 10	40	6.5	0.45	105.0	1.3	0.18	0.022	35.9(1.7)
12 Oct 10	60	4.9	0.22	62.9	1.6	0.11	0.001	30.2(0.5)
12 Oct 10	90	4.3	0.20	55.3	1.4	0.09	0.000	28.6(2.9)
12 Oct 10	120	4.0	0.23	53.3	1.3	0.13	0.000	40.4(1.9)
12 Oct 10	150	4.0	0.23	58.9	1.0	0.15	0.000	36.0(3.9)
30 Mar 11	2	1.1	0.43	117.8	1.1	0.15	0.138	1.8(0.1)
30 Mar 11	5	1.3	0.43	106.5	1.3	0.32	0.238	4.5(1.9)
30 Mar 11	10	1.4	0.48	129.8	1.1	0.19	0.343	1.8(ND)
30 Mar 11	20	1.4	0.54	125.9	1.2	0.15	0.302	2.6(0.3)
30 Mar 11	30	1.4	0.53	116.0	1.2	0.21	0.231	2.6(0.9)
30 Mar 11	40	1.4	0.55	114.4	1.2	0.20	0.083	3.5(0.8)
30 Mar 11	60	1.4	0.53	106.9	1.2	0.24	0.006	5.5(0.1)
30 Mar 11	90	1.6	0.39	96.7	1.2	0.21	0.000	7.4(0.3)
30 Mar 11	120	3.1	0.31	95.1	1.2	0.17	0.000	31.3(3.3)
30 Mar 11	150	3.3	0.14	115.0	1.1	0.25	0.000	50.5(ND)

bacterial nitrifiers (Ward 2011). We note, however, that for AOA, the efficiency may be lower (Hallam et al. 2006) so these C uptake measurements may correspond to somewhat higher NH_4^+ oxidation rates.

To test the effectiveness of the inhibitors, in March 2011 we added the two inhibitors as described above to an additional pair of bottles at 5 m and 150 m that were spiked with $^{15}NH_4Cl$. We then measured NH_4^+ oxidation rates as described above, and compared these values to the NH_4^+ oxidation rates of the bottles at those depths without inhibitors.

Primary production and water chemistry—During these five cruises, primary production was measured along with nitrification in the in situ bottle array, following the

protocols described in Sterner (2010). Briefly, light and dark incubation bottles were filled from each depth and spiked with $NaH^{14}CO_3$. For each depth, one bottle was filtered immediately onto pre-combusted 25 mm GF/F filters to estimate non-biological ^{14}C uptake associated with the filter. The remaining bottles were filtered following the ~ 18 h incubation.

Water chemistry parameters, including NH_4^+, dissolved organic C (DOC), particulate organic C (POC), and Chl a, were measured throughout the water column on each cruise. For NH_4^+ samples, ~ 300 mL of lake water was filtered using pre-combusted Whatman GF/F filters. Filters were first flushed with 500 mL of sample water to saturate them with NH_4^+. Samples were stored in high-density polyethylene bottles, and frozen until analysis. Ammonium samples were analyzed using the fluorometric method (described in Kumar et al. 2007), and we corrected for matrix effects and background fluorescence following Taylor et al. (2007).

POC samples were collected by filtering ~ 1 liter of sample water onto a 0.7 μm GF/F filter (pre-ashed at 450°C). Samples were transported frozen, then dried at 60°C for 24 to 48 h, and prepared for analysis. Samples were analyzed on a Perkin Elmer 2400 CHN analyzer calibrated with an acetanilide standard. DOC samples were collected in pre-ashed brown vials, rinsed with sample water, then filled and sealed with a Teflon-faced septum cap. Samples were analyzed within 1 week of collection on a Shimadzu Total Organic Carbon analyzer using a platinum catalyst. For Chl a analysis, 200 mL of lake water was filtered through 25 mm 0.2 μm cellulose nitrate filters and immediately frozen until analysis. Chl a was determined using the standard fluorometric method involving 90% acetone extraction (Welschmeyer 1994).

Controls on nitrification rates—The effects of physical, chemical, and biological parameters on nitrification rates measured in the in situ incubations were analyzed using multiple regression. We considered seven candidate variables (NH_4^+, temperature, Chl a, primary productivity, POC, DOC, and log(depth)). Depth was log transformed so that it would act as a surrogate for light. Cruise identity was also included as a fixed effect in all models. Two observations were removed from the model data set because of high NH_4^+ values that were likely erroneous (based on observations at neighboring depths) led to high apparent rates of nitrification. We used backwards step-wise regression to select the most parsimonious model according to Akaike's Information Criterion with correction for finite sample sizes (AIC_c; Burnham and Anderson 2002).

To measure the potential for light to directly inhibit nitrification in Lake Superior, during two of the bottle array incubations, in August and October 2010, we added two light bottles spiked with $^{15}N\text{-}NH_4^+$ in cages in the epilimnion (2, 5, 10, 20, 30, 40, and 60 m), in addition to the two dark bottles described above. Differences in ammonium oxidation rates over the ~ 20 h incubation period were attributed to light inhibition.

In August 2010, we measured the potential for substrate limitation of nitrification. Duplicate incubation bottles containing water from 5 m and 150 m were enriched at eight different concentrations of $^{14}N\text{-}NH_4^+$ ranging from 0.13–5.30 μmol L^{-1}. Spikes of $^{15}N\text{-}NH_4^+$ corresponded to 10% of the added $^{14}N\text{-}NH_4^+$. These bottles were incubated in the dark for 22 h at ambient temperature, either in an on-deck flow-through incubator (for 5 m) or in a refrigerator (for 150 m).

Enumeration of nitrifiers—The nitrifier population in Lake Superior was enumerated using the Catalyzed Reporter Deposition Fluorescence in situ Hybridization method (CARD-FISH; Pernthaler et al. 2002). The following modifications were made to the established methods: the hybridization temperature was 46°C, and the first washing step was performed at 48°C. The CARD amplification step was performed at 46°C. We used probe Cren554 for total AOA counts (Massana et al. 1997), NIT3 (Wagner et al. 1996) and Ntspa712 (Daims et al. 2001) for nitrite-oxidizing bacteria (NOB) counts, and Nso1225 and Nsm156 (Mobarry et al. 1996) for ammonium-oxidizing bacteria (AOB) counts. In addition, bacterial probes Eub338I, Eub338II, and Eub338III, and non-hybridizing probe NON338 were used in concert with each CARD-FISH run as positive and negative controls, respectively. All probes were horseradish peroxidase (HRP)-labeled at the 5′ end, and fluorescein isothiocyanate was used for the CARD step. Each probe was used at a final concentration of 50 ng μL^{-1}. Filters were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) for total cell counts on each filter. Direct microscopic counts by fluorescence microscopy (Zeiss Axiophot HB0100) were performed at 1000× magnification.

Results

Over the five in situ water-column incubation experiments and across all depths, water temperature ranged from 1.1–11.2°C, Chl a ranged from 0.07–1.23 μg L^{-1}, NH_4^+ ranged from 0.09–1.11 μmol L^{-1}, POC ranged from 31.7–169.3 μg L^{-1}, and DOC ranged from 1.0–1.6 mg L^{-1}. Measured rates of primary production ranged from 0–1.03 μmol C L^{-1} d^{-1} (Table 1).

Individual observations of NH_4^+ oxidation rates ranged from 0–83 nmol N L^{-1} d^{-1} (Table 1). Mean NH_4^+ oxidation rates (using trapezoidal integration to calculate an areal value, then dividing by depth) ranged from 14.7 nmol N L^{-1} d^{-1} (March 2011) to 33.9 nmol N L^{-1} d^{-1} (November 2009) (Fig. 1). During the November 2009 cruise, measured rates were > 20 nmol L^{-1} d^{-1} throughout the water column. By contrast, in the other four experiments, epilimnetic NH_4^+ oxidation rates were low, < 15 nmol N L^{-1} d^{-1}. The overall, mean NH_4^+ oxidation rate was 24.1 nmol N L^{-1} d^{-1}.

Mean nitrification rates measured by ^{14}C uptake across the five cruises ranged from 0.8 nmol C L^{-1} d^{-1} (March 2011) to 1.5 nmol C L^{-1} d^{-1} (November 2009). Depth profiles for these nitrification measurements showed no clear trends (Fig. 2), although variability among replicate control and treatment incubation bottles was high in shallow depths, contributing to several high and low (negative) estimates. The mean C uptake rates measured
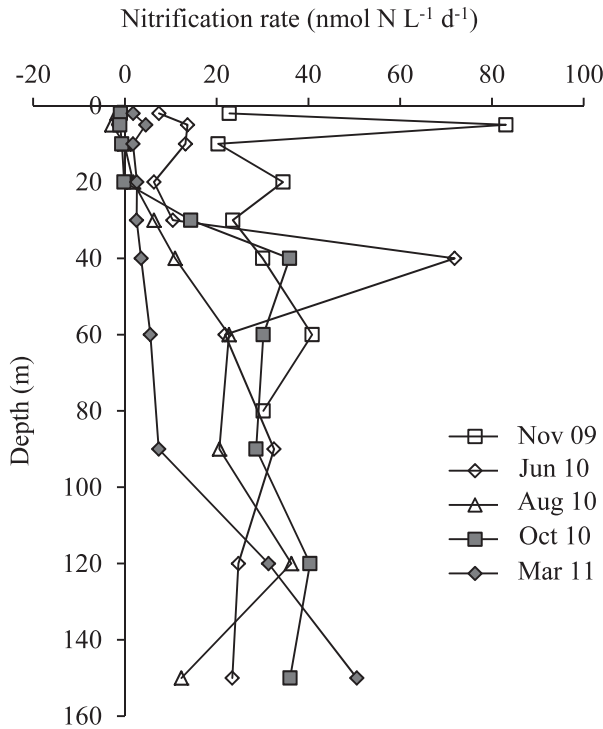
Table 2. Model selection criteria for multiple regression. Seven candidate variables were considered, in addition to cruise. Variables in bold denote $p < 0.05$, and (+) or (−) denotes direction of relationship. Adj, adjusted.

			Variables					ΔAIC_c	Adj r^2
cruise	NH_4^+ **(+)**		**Chl*a*(−)**				**logDepth(+)**		0.721
cruise	NH_4^+ **(+)**		**Chl*a*(−)**		POC(−)		**logDepth(+)**	0.23	0.727
cruise	NH_4^+ **(+)**		**Chl*a*(−)**	PrimProd(−)	POC(−)		logDepth(+)	1.20	0.726
cruise	NH_4^+ **(+)**		**Chl*a*(−)**	**PrimProd(−)**	POC(−)			1.97	0.716
cruise	NH_4^+ **(+)**	Temp(+)	**Chl*a*(−)**	PrimProd(−)	POC(−)		logDepth(+)	2.62	0.731
cruise	NH_4^+ **(+)**	Temp(+)	**Chl*a*(−)**	PrimProd(−)	POC(−)	DOC(−)	logDepth(+)	4.67	0.728

AOA had a maximum abundance of 1.9×10^4 cells mL^{-1} at a depth of 40 m, *Nitrobacter* had a maximum abundance of 1.4×10^4 cells mL^{-1} at a depth of 100 m, and *Nitrospira* had a maximum abundance of 1.5×10^4 cells mL^{-1} at a depth of 80 m. AOB were also enumerated but never exceeded 5 cells mL^{-1}.

Discussion

Controls on nitrification in Lake Superior—We found that AOA are the dominant ammonium oxidizers in Lake Superior, as in marine environments (Könneke et al. 2005). Our results indicate that during stratification, nitrification rates in the epilimnion are low because of a scarcity of these organisms, which may be due to light-inhibited growth, predation by grazers, or competition for NH_4^+ with photoautotrophs and heterotrophic microbes. Although corresponding measurements of nitrifier abundance and nitrification rates are limited to one cruise, additional observations in Lake Superior have shown that nitrifiers are detected at the surface only during seasonal isothermal mixing (M. Mukherjee, A. Ray, and G. Bullerjahn unpubl. data).

The higher nitrification rates observed in the hypolimnion ($\sim$ 80% of the water column) correspond with higher abundance of nitrifiers. These rates appear to be limited by NH_4^+ availability, given the elevated nitrification rates observed when additional NH_4^+ was added (Fig. 6). The strong negative relationship between nitrification rates and Chl *a* and log(depth) likely reflects the low abundance of nitrifiers in the epilimnion (Fig. 7). Nitrification rates were not significantly related to POC and DOC, as would be expected if competition for NH_4^+ with heterotrophic microbes was a primary control of nitrification (Taylor and Townsend 2010). In more heterotrophic aquatic environments (e.g., stream sediments; Strauss and Lamberti 2000), this effect may be more pronounced. Although DOC in Lake Superior is considered to be "semi-labile" with an age $\leq$ 60 yr (Zigah et al. 2012), it is likely that the bulk of the DOC pool does not stimulate microbial growth in oligotrophic lakes (del Giorgio and Cole 1998), and that heterotrophic activity is more tightly linked to algal exudates.

Implications for lake-wide N budget—Across five different months, we measured a mean nitrification rate of 24.1 nmol N L^{-1} d^{-1} in Lake Superior. This value is 1–2 orders of magnitude higher than values reported for the Pacific (Raimbault et al. 1999) and Atlantic (Clark et al. 2008) Oceans, and similar to rates measured in Monterey Bay (Ward 2005) and the Gulf of California (Bemen et al. 2008). However, nitrification rates for smaller freshwater
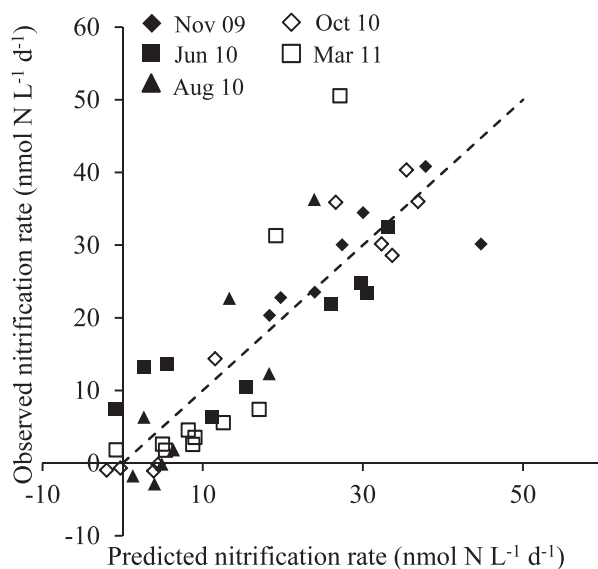

Fig. 4. Observed nitrification rates (based on ^{15}N-NH$_4^+$ oxidation) compared to corresponding values from multiple regression model (*see* Table 3 for model parameters). A 1 : 1 line is shown for reference.

Table 3. Results of best multiple regression model (based on lowest AIC$_c$) to predict nitrification rates. Adjusted r^2 value is 0.73. Cruise identity is considered a fixed effect, and the cruise number term gives the modeled difference in nitrification rates between each consecutive cruise compared to the November 2009 cruise (e.g., the Cruise 2 term describes the difference between the November 2009 and June 2010 cruises). NH_4^+ is in μmol N L^{-1}, Chl *a* is in μg L^{-1}, and log(depth) is in meters.

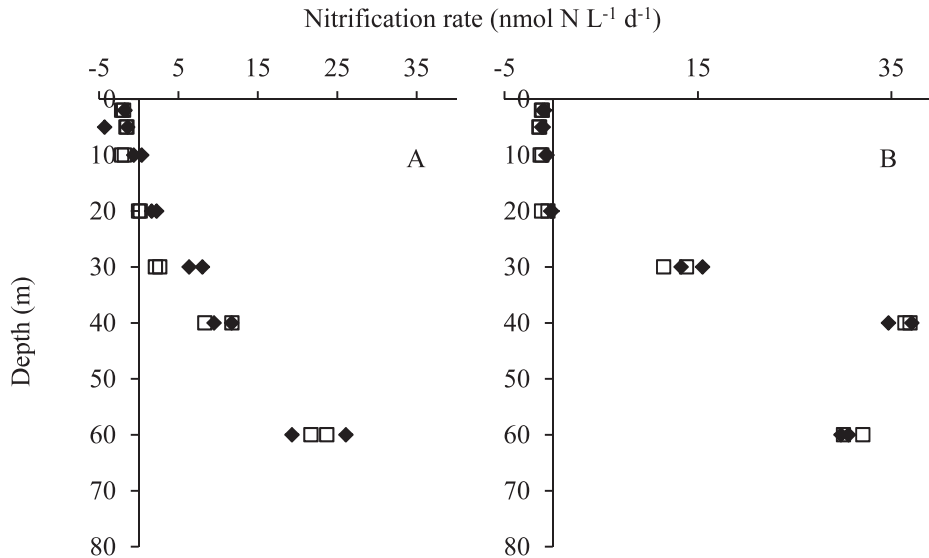| Parameter	Estimate	SE	df	t	p
Intercept	28.44	7	1	4.06	0.0003
Cruise 2	−27.25	4.96	1	−5.49	<0.0001
Cruise 3	−31.87	4.78	1	−6.66	<0.0001
Cruise 4	−9.71	3.95	1	−2.46	0.019
Cruise 5	−25.26	4.04	1	−6.52	<0.0001
NH_4^+	33.09	17.49	1	1.89	0.0665
Chl *a*	−27.62	5.83	1	−4.74	<0.0001
log(depth)	9.06	2.5	1	3.62	<0.0001

Fig. 5. Comparison of nitrification rates (based on ^{15}N-NH_4^+ oxidation) measured in dark incubation bottles (black diamonds) and transparent incubation bottles (open squares) at seven depths during (A) August 2010 and (B) October 2010 research cruises.

lakes are 1–3 orders of magnitude greater than this mean value for Lake Superior (Table 4).

Several independent lines of evidence indicate that this mean nitrification rate of 24.1 nmol N L^{-1} d^{-1}, derived from the $^{15}NH_4^+$ oxidation measurements, is a realistic estimate of nitrification rates in the lake. Nitrification measurements using the ^{14}C uptake method indicate similar or slightly higher mean nitrification rates (Fig. 3). The average nitrification rate per cell in our study (5.2 fmol cell^{-1} d^{-1}) is similar to values reported in the North Sea (4.6 fmol cell^{-1} d^{-1}; Wuchter et al. 2006) and in cultures of *Nitrosopumilus maritimus* (4.0 fmol cell^{-1} d^{-1}; Könneke

et al. 2005). Additionally, the nitrification rate that we measured is consistent with the observation, based on isotopic signatures, that nearly all of the lake's NO_3^- (93–100%) was derived from nitrification (Finlay et al. 2007). Applying the external NO_3^- loading rate (24 mmol m^{-2} yr^{-1}; Sterner et al. 2007) to our mean measured nitrification rate, on an areal basis (1300 mmol m^{-2} yr^{-1}, based on 150 m mean depth) indicates that 98% of lake NO_3^- is derived from nitrification. Furthermore, the observed enrichment of $\delta^{18}O$-NO_3^- and $\delta^{15}N$-NO_3^- in the epilimnion from June to October (Finlay et al. 2007) indicates an increase in NO_3^- uptake relative to NO_3^- inputs, consistent with the
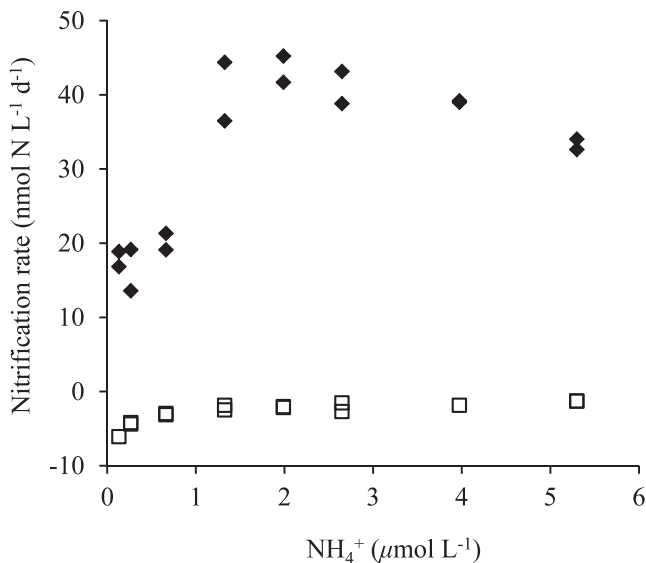

Fig. 6. Nitrification rates (based on ^{15}N-NH_4^+ oxidation) measured at eight different levels of NH_4^+ from lake water collected from 5 m (open squares) and 155 m (black diamonds).
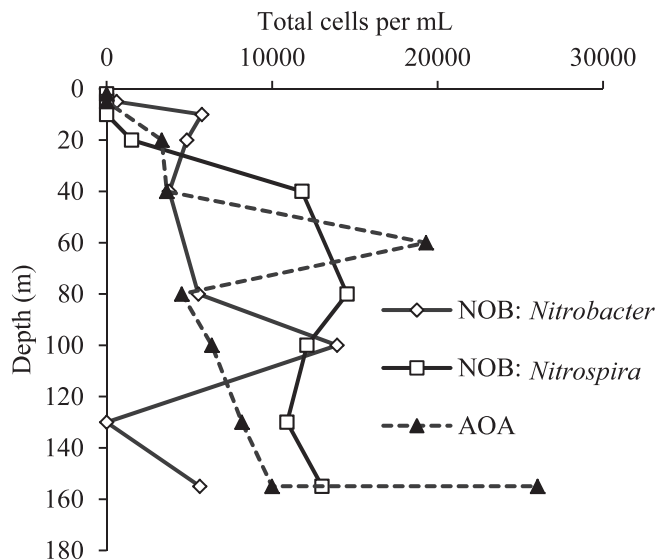

Fig. 7. Abundance data for AOA (Crenarchaeota) and NOB (*Nitrobacter* and *Nitrospira*) from August 2010 cruise. AOB were also enumerated, but were found in concentrations < 5 cells mL^{-1}.

Table 4. Comparison of nitrification rates reported for pelagic environments. The range of nitrification rates observed across the five cruises is reported for Lake Superior's epilimnion ($\leq$ 30 m) and hypolimnion (> 30 m).

Location	Nitrification rate (nmol L^{-1} d^{-1})	Reference
Pacific Ocean (oligotrophic, shallow)	0.4	Raimbault et al. 1999
Pacific Ocean (oligotrophic, deep)	0.7	Raimbault et al. 1999
Pacific Ocean (upwelling)	2.9	Raimbault et al. 1999
Atlantic Ocean	1–10	Clark et al. 2008
Lake Superior (epilimnion)	0–35	This study
Lake Superior (hypolimnion)	4–51	This study
Monterey Bay (shallow)	18–37	Ward 2005
Monterey Bay (deep)	19–99	Ward 2005
Gulf of California	40–80	Bemen et al. 2008
Mono Lake	60–335	Carini and Joye 2008
Lake Mendota (epilimnion)	1700–5000	Hall 1986
Lake Mendota (hypolimnion)	4000–26,000	Hall 1986

observation of diminished nitrification rates that we documented during these months.

Putting these observations of nitrification rate into the context of the long-term rise of NO_3^- in the lake is a desired outcome of this work. However, scaling our estimate of nitrification to the entire lake on an annual basis has considerable uncertainty. We cannot account for possible spatial heterogeneity, as these measurements occurred at a single offshore station. Compared to offshore rates, we might expect nearshore rates to be lower due to a larger fraction of the water column that is epilimnetic, or higher due to the influence of river plumes, which are often associated with higher nitrification in the coastal zone (Bianchi et al. 1999). And, although these five cruises were spread fairly evenly across a 16 month period, our ability to assess temporal heterogeneity is limited. Given its extensive volume of water and relatively low biogeochemical rates, Lake Superior is chemically relatively homogeneous, but our data suggest that populations of nitrifying organisms are variable and could contribute to spatial and temporal heterogeneity in nitrification rates.

With this caveat, our quantification of nitrification rates highlights its importance in Lake Superior's N budget. Although nitrification rates on a per-volume basis were low compared to other freshwater lakes, nitrification constitutes a large flux relative to other measured components of Lake Superior's N cycle (Fig. 8), confirming previous evidence of rapid NH_4^+ turnover rates in the lake. At the mean rate of 24.1 nmol L^{-1} N d^{-1}, nitrification alone would deplete the NH_4^+ pool (mean concentration
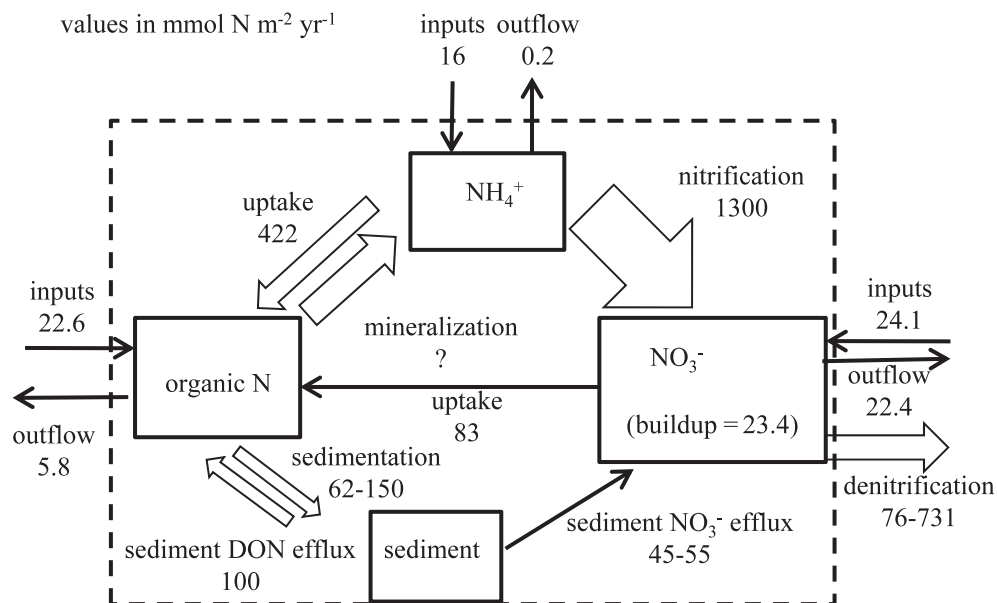
Fig. 8. Updated nitrogen budget for Lake Superior. Arrow width is proportionate to magnitude of flux. The dashed line represents the boundaries of the lake ecosystem. Nitrification value is from this study; inputs, outflow, and NO_3^- buildup values are from Sterner et al. (2007); NO_3^- and NH_4^+ uptake values are from Kumar et al. (2008); sedimentation values are from McManus et al. (2003); sediment NO_3^- efflux values are from Heinen and McManus (2004); and sediment dissolved organic nitrogen (DON) efflux and denitrification values are from Stark (2009).

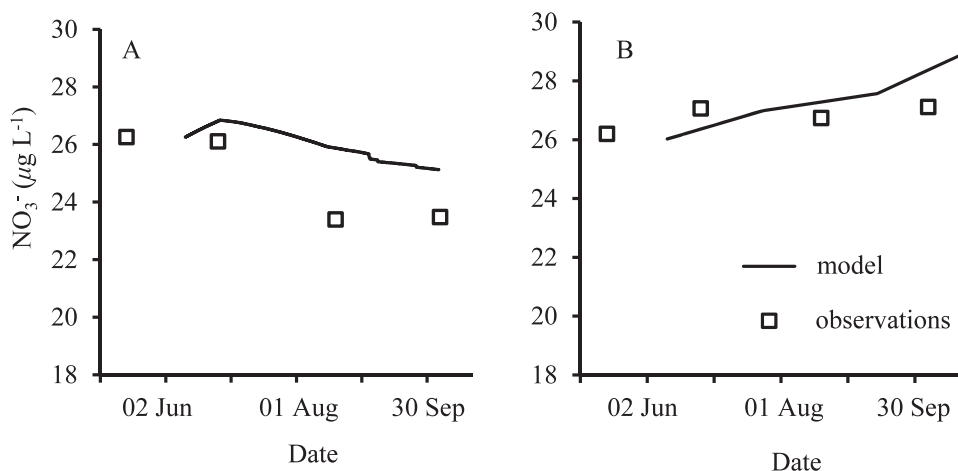Fig. 9. Observed and modeled seasonal NO_3^- trends for (A) epilimnion (0–30 m) and (B) deep hypolimnion (140–160 m). Model for epilimnion assumes that NO_3^- drawdown begins with onset of stratification, that there is no nitrification following our measurement on 26 June, and that NO_3^- uptake is a function of temperature as described by Kumar et al. (2008). Model for deep hypolimnion similarly estimates NO_3^- uptake, and interpolates nitrification values between observations by extending each measurement to the midpoint between consecutive observations.

0.21 μmol L^{-1}) in 8.7 d, and would replace the NO_3^- pool (mean concentration 26 μmol L^{-1}) in $\sim$ 1000 d, or $\sim$ 69 times during the 190 yr residence time of water. This mean nitrification rate is $>$ 50-fold greater than the long-term rate of NO_3^- increase in the lake, further emphasizing the importance of internal processes in mediating this long-term trend (McDonald et al. 2010). This finding suggests that the long-term change in this ecosystem represents a small imbalance in the N cycle that has been maintained over time, as opposed to N loading that overwhelms the processing capacity of the ecosystem.

Our measured nitrification rates yield much more NO_3^- than can be currently accounted for based on previous measurements of denitrification and NO_3^- uptake (Fig. 8). The magnitude of the long-term NO_3^- increase is well constrained, as is the flux of NO_3^- out of the lake via the St. Mary's River. One possibility is that denitrification rates are substantially higher than suggested by current estimates. Sediment denitrification is poorly constrained, but estimates are very low (0–27 mmol N m^{-2} yr^{-1}; Carlton et al. 1989). Denitrification in the water column is unlikely due to high oxygen levels, and oxygen penetration extends $>$ 2 cm into sediments in offshore areas throughout much of lake (Li et al. 2012). However, the spatial extent of previous characterizations of denitrification is limited and spatial heterogeneity is likely. Nearshore areas such as bays and harbors could have substantially higher denitrification rates due to higher inputs of organic carbon. It remains to be seen whether these areas are sufficient to remove substantial amounts of NO_3^-.

Even if new data lead to higher estimates of lake-wide denitrification, this presents a tension between lake-wide NO_3^- and total N budgets. The difference between total N inputs to the lake plus NO_3^- buildup, and total N output from the lake, has been estimated to be 11.1 mmol N m^{-2} yr^{-1} (Sterner et al. 2007), which reflects denitrification plus long-term burial of N. Denitrification rates approaching

1000 mmol N m^{-2} yr^{-1} would be required to balance the NO_3^- budget, but this would require additional, as yet undiscovered, inputs of reduced N to balance the lake's total N budget. Although we may have underestimated denitrification, rates of that magnitude are not realistic given what we know, suggesting that the dominant fate of this additional NO_3^- may be assimilation rather than denitrification.

Given the relatively low external inputs of reduced N to Lake Superior (Sterner et al. 2007), NO_3^- assimilation should be similar in magnitude to nitrification to support N cycling within the lake. Measurements of NO_3^- assimilation reported by Kumar et al. (2008) focused on the epilimnion and did not measure assimilatory NO_3^- uptake by plankton smaller than 0.7 μm, which may be important in Lake Superior. Uptake deep in the water column, or by small size fractions could lead to underestimation of NO_3^- assimilation.

An analysis of seasonal NO_3^- trends during 2010 suggests that NO_3^- uptake is higher than previously reported. In the epilimnion (0–30 m), NO_3^- concentration decreased from 26.2 μmol L^{-1} NO_3^- on 15 May to 23.4 μmol L^{-1} NO_3^- on 19 August (Fig. 9A). Based on surface-water temperature recorded by the NOAA weather buoy at Sta. WM, stratification occurred on 11 June 2010. Assuming that the NO_3^- drawdown began with the onset of stratification, this corresponds to a NO_3^- loss rate of 41 nmol L^{-1} N d^{-1}. Conservatively assuming that nitrification stops for the remainder of the stratified period, immediately after our measurement on 26 June, then the decline would be entirely attributed to NO_3^- uptake (in the well-oxygenated water column, other NO_3^- losses such as denitrification or dissimilatory nitrate reduction to ammonium [DNRA] are thermodynamically unfavorable; Burgin and Hamilton 2007). However, daily NO_3^- uptake, calculated as a function of water temperature based on the model of Kumar et al. (2008), only accounts for a cumulative NO_3^- loss of 1.13 μmol L^{-1} during the 117 d period between stratification and our final observation on

06 October. A possible explanation for this discrepancy is that the remainder of the observed drawdown during this period, 1.65 μmol L^{-1}, may be explained in part by NO$_3^-$ uptake by plankton smaller than 0.7 μm that were not accounted for in the study by Kumar et al. (2008).

In addition to highlighting the apparent underestimate of NO$_3^-$ uptake in Lake Superior, this simple model also illustrates the importance of temporal dynamics of nitrification in contributing to the seasonal NO$_3^-$ drawdown. If we assume that the observed NO$_3^-$ drawdown is completely due to assimilatory uptake, then the average rate of NO$_3^-$ uptake over the 117 d period between stratification and our final observation on 06 October is 22.5 nmol L^{-1} d^{-1}. If nitrification rates in the epilimnion maintained the overall average of 24.1 nmol L^{-1} N d^{-1} instead of falling to zero during the stratified period, then there would be a slight NO$_3^-$ increase, rather than a drawdown, during the summer months. This calculation suggests that the seasonal cessation of nitrification in the upper water column is a primary factor in the seasonal NO$_3^-$ drawdown.

In the deep hypolimnion (140–160 m), we observed a slight rise in NO$_3^-$ from 26.2 μmol L^{-1} on 15 May to 27.1 μmol L^{-1} on 06 October (Fig. 9B). Combining observed nitrification rates (interpolating between observations by extending each measurement to the midpoint between observations) with modeled NO$_3^-$ uptake (based on Kumar et al. 2008, using a constant temperature of 4.0°C) produces a rise in NO$_3^-$ that exceeds the observed levels. Assuming nitrification rates are correct, NO$_3^-$ uptake would have to be > 10-fold higher (18 nmol L^{-1} NO$_3^-$ d^{-1} instead of 1 nmol L^{-1} NO$_3^-$ d^{-1}) to fit the observations over the 117 d period. As before, this calculation is conservative, as it does not account for the contribution of NO$_3^-$ flux from sediments ($\sim$ 135 μmol NO$_3^-$ m^{-2} d^{-1}; Heinen and McManus 2004). Loss of water-column NO$_3^-$ due to denitrification or DNRA are likely to be very low, as oxic conditions extend > 2 cm into the sediment (Li et al. 2012). Thus, calculations from both the upper and lower water column, suggests that NO$_3^-$ uptake rates by plankton smaller than 0.7 μm, and subsequent rapid mineralization of organic N, may help account for this apparent imbalance in the lake NO$_3^-$ budget. Higher algal production in nearshore environments is another potentially important NO$_3^-$ sink that is poorly quantified (Bennington et al. 2012). It is clear from simple models of lake nitrification that further measurements are required to achieve a more complete understanding of nitrogen cycling in one of Earth's largest lakes.

In conclusion, our results suggest that physical and ecological factors constraining AOA abundance in the water column are important controls on the nitrogen cycle in Lake Superior. These measurements confirm previous evidence that nearly all NO$_3^-$ in Lake Superior is derived from in situ nitrification. The fact that nitrification rates are > 50-fold greater than the long-term NO$_3^-$ rise in the lake indicates that N is actively cycling, and that long-term change in this ecosystem is not due to N loading overwhelming the processing capacity of the ecosystem, but instead represents a small imbalance in the N cycle that has been maintained over time.

References

BEMAN, J. M., B. N. POPP, AND C. A. FRANCIS. 2008. Molecular and biogeochemical evidence for ammonia oxidation by marine *Chrenarchaeota* in the Gulf of California. ISME J. **2:** 429–441, doi:10.1038/ismej.2007.118

BENNINGTON, V., G. A. MCKINLEY, N. R. URBAN, AND C. P. MCDONALD. 2012. Can spatial heterogeneity explain the perceived imbalance in Lake Superior's carbon budget? A model study. J. Geophys. Res. **117:** G03020, doi:10.1029/2011JG001895

BIANCHI, M., FELIATRA, AND D. LEFEVRE. 1999. Regulation of nitrification in the land-ocean contact area of the Rhône River plume (NW Mediterranean). Aquat. Microb. Ecol. **18:** 301–312, doi:10.3354/ame018301

BURGIN, A. J., AND S. K. HAMILTON. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Front. Ecol. Environ. **5:** 89–96, doi:10.1890/1540-9295(2007)5[89:HWOTRO]2.0.CO;2

BURNHAM, K. P., AND D. R. ANDERSON. 2002. Model selection and multimodel inference: A practical information-theoretic approach, 2nd ed. Springer-Verlag.

CARINI, S. A., AND S. B. JOYE. 2008. Nitrification in Mono Lake, California: Activity and community composition during contrasting hydrological regimes. Limnol. Oceanogr. **53:** 2546–2557, doi:10.4319/lo.2008.53.6.2546

CARLTON, R., G. WALKER, M. KLUG, AND R. WETZEL. 1989. Relative values of oxygen, nitrate, and sulfate to terminal microbial processes in the sediments of Lake Superior. J. Great Lakes Res. **15:** 133–140, doi:10.1016/S0380-1330(89)71467-2

CLARK, D. R., A. P. REES, AND I. JOINT. 2008. Ammonium regeneration and nitrification rates in the oligotrophic Atlantic Ocean: Implications for new production estimates. Limnol. Oceanogr. **53:** 52–62, doi:10.4319/lo.2008.53.1.0052

DAIMS, H., J. L. NIELSEN, P. H. NIELSEN, K. H. SCHLEIFER, AND M. WAGNER. 2001. In situ characterization of *Nitrospira*-like nitrite-oxidizing bacteria active in wastewater treatment plants. Appl. Environ. Microbiol. **67:** 5273–5284, doi:10.1128/AEM.67.11.5273-5284.2001

DEL GIORGIO, P. A., AND J. J. COLE. 1998. Bacterial growth efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst. **29:** 503–541, doi:10.1146/annurev.ecolsys.29.1.503

DUGDALE, R. C., AND J. J. GOERING. 1967. Uptake of new and regenerated forms of nitrogen in marine production. Limnol. Oceanogr. **12:** 196–206, doi:10.4319/lo.1967.12.2.0196

FERNANDEZ, C., AND P. RAIMBAULT. 2007. Nitrogen regeneration in the NE Atlantic Ocean and its impact on seasonal new, regenerated and export production. Mar. Ecol. Prog. Ser. **337:** 79–92, doi:10.3354/meps337079

FINLAY, J. C., R. W. STERNER, AND S. KUMAR. 2007. Isotopic evidence for in-lake production of accumulating nitrate in Lake Superior. Ecol. Appl. **17:** 2323–2332, doi:10.1890/07-0245.1

FRENCH, E. J., J. KOZLOWSKI, M. MUKHERJEE, G. BULLERJAHN, AND A. BOLLMAN. 2012. Niche differentiation between ammonium-oxidizing archaea and bacteria based on differences in their growth responses to environmental factors. Appl. Environ. Microb. **78:** 5773–5780, doi:10.1128/AEM.00432-12

HALL, G. H. 1986. Nitrification in lakes, p. 127–156. *In* J. I. Prosser [ed.], Nitrification. IRL Press.

HALLAM, S. J., T. J. MINCER, C. SCHLEPER, C. M. PRESTON, K. ROBERTS, P. M. RICHARDSON, AND E. F. DELONG. 2006. Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine *Chrenarchaeota*. PLoS Biol. **4:** e95, doi:10.1371/journal.pbio.0040095

HEINEN, E. A., AND J. MCMANUS. 2004. Carbon and nutrient cycling at the sediment-water boundary in western Lake Superior. J. Great Lakes Res. **30:** 113–132, doi:10.1016/S0380-1330(04)70381-0

HOWARTH, R. W., AND R. MARINO. 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. Limnol. Oceanogr. **51:** 364–376, doi:10.4319/lo.2006.51.1_part_2.0364

KÖNNEKE, M., A. E. BERNHARD, J. R. DE LA TORRE, C. B. WALKER, J. B. WATERBURY, AND D. A. STAHL. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature **437:** 543–546, doi:10.1038/nature03911

KUMAR, S., R. S. STERNER, AND J. C. FINLAY. 2008. Nitrogen uptake dynamics in Lake Superior. J. Geophys. Res. -Biogeosci. **113:** G04003, doi:10.1029/2008JG000720

———, ———, J. C. FINLAY, AND S. BROVOLD. 2007. Spatial and temporal variation of ammonium in Lake Superior. J. Great Lakes Res. **33:** 581–591, doi:10.3394/0380-1330(2007)33[581:SATVOA]2.0.CO;2

LAVRENTYEV, P. J., W. S. GARDNER, AND J. R. JOHNSON. 1997. Cascading trophic effects on aquatic nitrification: Experimental evidence and potential implications. Aquat. Microb. Ecol. **13:** 161–175, doi:10.3354/ame013161

LI, J., S. A. CROWE, D. MIKLESH, M. KISTNER, D. E. CANFIELD, AND S. KATSEV. 2012. Carbon mineralization and oxygen dynamics in sediments with deep oxygen penetration, Lake Superior. Limnol. Oceanogr **57:** 1634–1650, doi:10.4319/lo.2012.57.6.1634

MASSANA, R., A. E. MURRAY, C. M. PRESTON, AND E. F. DELONG. 1997. Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. Appl. Environ. Microbiol. **63:** 50–56.

MCDONALD, C. P., N. R. URBAN, AND C. M. CASEY. 2010. Modeling historical trends in Lake Superior total nitrogen concentrations. J. Great Lakes Res. **36:** 715–721, doi:10.1016/j.jglr.2010.07.008

MCMANUS, J., E. A. HEINEN, AND M. M. BAEHR. 2003. Hypolimnetic oxidation rates in Lake Superior: Role of dissolved organic material on the lake's carbon budget. Limnol. Oceanogr. **84:** 1624–1632, doi:10.4319/lo.2003.48.4.1624

MERBT, S. N., D. A. STAHL, E. O. CASAMAYOR, E. MARTÍ, G. W. NICOL, AND J. A. PROSSER. 2012. Differential photoinhibition of bacterial and archaeal ammonia oxidation. FEMS Microbiol. Lett. **327:** 41–46, doi:10.1111/j.1574-6968.2011.02457.x

MOBARRY, B. K., M. WAGNER, V. URBAIN, B. E. RITTMANN, AND D. A. STAHL. 1996. Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. Appl. Environ. Microbiol. **62:** 2156–2162.

PERNTHALER, A., J. PERNTHALER, AND R. AMANN. 2002. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol. **68:** 3094–3101, doi:10.1128/AEM.68.6.3094-3101.2002

RAIMBAULT, P., AND OTHERS. 1999. Carbon and nitrogen uptake and export in the equatorial Pacific at 150 degrees W: Evidence of an efficient regenerated production cycle. J. Geophys. Res. -Oceans **104:** 3341–3356, doi:10.1029/1998JC900004

SIGMAN, D. M., K. L. CASCIOTTI, M. ANDREANI, C. BARFORD, M. GALANTER, AND J. K. BÖHLKE. 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal. Chem. **73:** 4145–4153, doi:10.1021/ac010088e

STADMARK, J., AND L. LEONARDSON. 2007. Greenhouse gas production in a pond sediment: Effects of temperature, nitrate, acetate, and season. Sci. Total Environ. **387:** 194–205, doi:10.1016/j.scitotenv.2007.07.039

STARK, R. 2009. Nitrate production and nitrogen and carbon cycling in Lake Superior sediments. M.S. thesis. Univ. of Minnesota.

STERNER, R. W. 2010. In situ measured primary production in Lake Superior. J. Great Lakes Res. **36:** 139–149, doi:10.1016/j.jglr.2009.12.007

———. 2011. C : N : P stoichiometry in Lake Superior: Freshwater sea as end member. Inland Waters **1:** 29–46.

———, AND OTHERS. 2007. Increasing stoichiometric imbalance in North America's largest lake. Geophys. Res. Lett. **34:** L10406, doi:10.1029/2006GL028861

STRAUSS, E. A., AND G. A. LAMBERTI. 2000. Regulation of nitrification in aquatic sediments by organic carbon. Limnol. Oceanogr. **45:** 1854–1859, doi:10.4319/lo.2000.45.8.1854

TAYLOR, B. W., C. A. KEEP, A. S. FLECKER, R. O. HALL, B. J. KOCH, L. M. TRONSTAD, AND A. J. ULSETH. 2007. Improving the fluorometric ammonium method: Matrix effects, background fluorescence, and standard additions. J. N. Am. Benthol. Soc. **26:** 167–177, doi:10.1899/0887-3593(2007)26[167:ITFAMM]2.0.CO;2

TAYLOR, P. G., AND A. R. TOWNSEND. 2010. Stoichiometric control of organic carbon–nitrate relationships from soils to the sea. Nature **464:** 1178–1181, doi:10.1038/nature08985

WAGNER, M., G. RATH, H. P. KOOPS, J. FLOOD, AND R. AMANN. 1996. In situ analysis of nitrifying bacteria in sewage treatment plants. Water Sci. Technol. **34:** 237–244.

WARD, B. B. 2005. Temporal variability in nitrification rates and related biogeochemical factors in Monterey Bay, California. Mar. Ecol. Prog. Ser. **292:** 97–109, doi:10.3354/meps292097

———. 2011. Nitrification in the ocean, p. 325–345. *In* B. B. Ward, D. J. Arp, and M. G. Klotz [eds.], Nitrification. ASM Press.

WELSCHMEYER, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol. Oceanogr. **39:** 1985–1992, doi:10.4319/lo.1994.39.8.1985

WUCHTER, C., AND OTHERS. 2006. Archael nitrification in the ocean. Proc. Natl. Acad. Sci. USA **103:** 12317–12322, doi:10.1073/pnas.0600756103

ZIGAH, P. K., E. C. MINOR, AND J. P. WERNE. 2012. Radiocarbon and stable-isotope geochemistry of organic and inorganic carbon in Lake Superior. Global Biogeochem. Cycles **26:** GB1023, doi:10.1029/2011GB004132