

# Nitrogen transformations at the sediment–water interface across redox gradients in the Laurentian Great Lakes

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**Abstract** The capacity of a lake to remove reactive nitrogen (N) through denitrification has important implications both for the lake and for downstream ecosystems. In large oligotrophic lakes such as Lake Superior, where nitrate ( $\text{NO}_3^-$ ) concentrations have increased steadily over the past century, deep oxygen penetration into sediments may limit the denitrification rates. We tested the hypothesis that the position of the redox gradient in lake sediments affects denitrification by measuring net N-fluxes across the sediment–water interface for intact sediment cores collected across a range of sediment oxycline values from nearshore and offshore sites in Lake Superior, as well as sites in Lake Huron and Lake Erie. Across this redox gradient, as the thickness of the oxygenated sediment layer increased from Lake Erie to Lake Superior, fluxes of  $\text{NH}_4^+$  and  $\text{N}_2$  out of the sediment decreased, and sediments shifted from a net sink to a net source of  $\text{NO}_3^-$ .

Denitrification of  $\text{NO}_3^-$  from overlying water decreased with thickness of the oxygenated sediment layer. Our results indicate that, unlike sediments from Lake Erie and Lake Huron, Lake Superior sediments do not remove significant amounts of water column  $\text{NO}_3^-$  through denitrification, likely as a result of the thick oxygenated sediment layer.

**Keywords** Denitrification · Laurentian Great Lakes · Nitrogen · Sediment

## Introduction

As a result of human activity altering the global nitrogen (N) cycle (Galloway, 1998), inputs of reactive N into aquatic ecosystems have increased N levels in many aquatic ecosystems (Galloway et al., 2004), altering species composition and ecosystem structure (Vitousek et al., 1997). In large lakes, as in other aquatic ecosystems, N concentrations are affected by external loading rates, but internal processes can also play an important role in controlling N concentrations and composition. Transformations of N at the sediment–water interface (SWI) are especially important, as sediments may contain a large pool of organic carbon and nitrogen, and often have steep redox gradients required for denitrification and other anaerobic processes.

Reactive N enters sediments as organic N (through sedimentation) and also through diffusion of overlying

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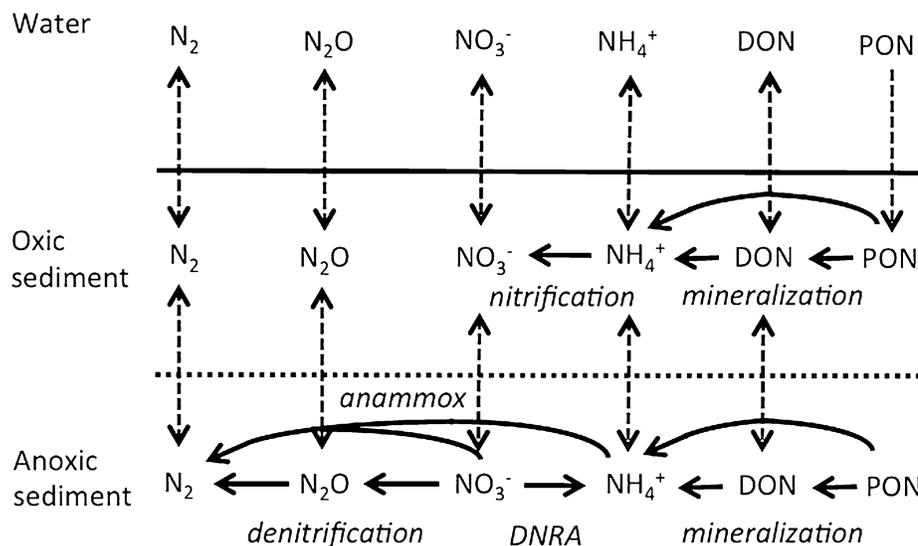
water (containing dissolved N) (Fig. 1). Within sediments, a variety of biogeochemical transformations may occur: mineralization of organic N produces ammonium ( $\text{NH}_4^+$ ), and nitrification converts  $\text{NH}_4^+$  into nitrate ( $\text{NO}_3^-$ ). Below the oxycline, denitrification, and anaerobic ammonium oxidation (anammox) convert  $\text{NO}_3^-$  (and also  $\text{NH}_4^+$  in the case of anammox) into unreactive dinitrogen ( $\text{N}_2$ ). Another potential fate of sediment  $\text{NO}_3^-$  under reducing conditions is dissimilatory nitrate reduction to ammonium (DNRA). These redox reactions within the sediment control the net fluxes of reactive N across the SWI, which in turn may profoundly alter availability of reactive N in the lake's water column and in downstream ecosystems.

The rates of these transformations depend on a variety of physical and chemical characteristics (e.g., temperature, sedimentation rates, sediment porosity, carbon (C) and N content of sediment, concentrations of dissolved N in overlying water). Two general factors can be distinguished as potential controls on rates of N transformations in lake sediments. The first is linked to substrate concentration and quality, irrespective of physical factors. For example, because organic carbon (C) is a substrate for denitrification, organic C availability in sediment may be an important control on denitrification rates (e.g., Pfenning & McMahon, 1996). The other general factor is the physical structure of the sedimentary zone such as the depth of oxygen penetration (Seitzinger et al., 2006). In sediments with deep oxygenated layers, the rate of

diffusion may limit the supply of water column  $\text{NO}_3^-$  to the zone of denitrification. These two general factors are related as, for example, deep, oligotrophic lakes may have both low organic C in the sediment and a deep redox boundary (Li et al., 2012) due to low organic carbon deposition rates.

In oligotrophic lakes where redox boundaries occur deep within the sediment, direct removal of water column  $\text{NO}_3^-$  is likely to be limited by rates of diffusion (Fig. 1), potentially contributing to very low levels of reduction of water column  $\text{NO}_3^-$  through denitrification or anammox. The thicker layer of oxygenated sediment may also result in higher areal rates of nitrification in the sediments, representing the oxidation of  $\text{NH}_4^+$  derived from mineralization within the sediments and also potentially the diffusion of  $\text{NH}_4^+$  from the water column into the upper sediment layers. Therefore, nitrification within the sediments may be the dominant source of  $\text{NO}_3^-$  fueling denitrification (i.e., coupled nitrification–denitrification), especially as the depth of the sediment redox boundary increases. As a result, increasing sediment redox boundary depths may shift sediments from a net sink of water column  $\text{NO}_3^-$  (i.e., where a significant amount of water column  $\text{NO}_3^-$  diffuses through the sediment and is denitrified) to a net source (i.e., where diffusion of water column  $\text{NO}_3^-$  to the reducing zone is limited, and  $\text{NO}_3^-$  produced from sediment nitrification diffuses out of the sediment). Sediment redox conditions may therefore play an important role in a lake's N budget: large oligotrophic lakes with deep

**Fig. 1** Nitrogen transformations occurring at sediment–water interface. Nitrification requires oxic conditions, whereas denitrification, anammox, and dissimilatory nitrate reduction to ammonium (DNRA) require anoxic conditions. Vertical, dashed arrows represent diffusion through the sediment, in the case of solutes, or sedimentation, in the case of PON. We note that N reduction may also occur within anoxic micro-sites in the overall oxic sediment layer



sediment oxygen penetration may retain external inputs of reactive N (i.e., contributing to long term increases in  $\text{NO}_3^-$  levels) if denitrification is unable to act as a negative feedback to balance N relative to C and phosphorus (P) (Redfield, 1958).

In Lake Superior,  $\text{NO}_3^-$  concentrations have increased >5-fold over the last century to current levels of  $\sim 27 \mu\text{M}$  (Sterner et al., 2007), with annual increases continuing through recent years (Sterner, 2011). Recent evidence has shown that cycling of N within the water column is high relative to both external inputs and the rate of annual increase (Finlay et al., 2007; Kumar et al., 2008; Small et al., 2013), but important questions remain about the role that sediments play in transforming nitrogen. Rates of N sedimentation reported in previous studies (Klump et al., 1989; Heinen & McManus, 2004) are comparable to loading rates of allochthonous N (Sterner et al., 2007). High  $\text{NO}_3^-$  fluxes out of the sediment were reported for the Duluth basin (Heinen & McManus, 2004), which is characterized by higher productivity and organic C sedimentation rates compared to the rest of the lake (Munawar & Munawar, 1978; Robertson, 1997). Heterogeneity in sediment redox conditions and fluxes are likely to be significant in large lakes, but previous studies have only examined a small number of sites (e.g., Gardner et al., 1987; Heinen & McManus, 2004), limiting efforts to estimate the lake-wide importance of these fluxes. An improved understanding of the environmental controls on sediment N-fluxes is needed to better understand how these biogeochemical transformations across the SWI contribute to lake-wide N budget.

We hypothesized that the depth of the oxycline controls the form of N released from the sediments, because anoxic conditions favor denitrification whereas oxic conditions favor nitrification. We predict that, in sediments with a shallow oxycline, fluxes of inorganic N out of the sediment would be dominated by the products of anoxic N transformations ( $\text{N}_2$ ,  $\text{NH}_4^+$ ) and the net flux of  $\text{NO}_3^-$  would be negative (into the sediments), whereas in sediments with deeper oxyclines, fluxes of  $\text{N}_2$ ,  $\text{NH}_4^+$  would be lower and sediment would be a net source of  $\text{NO}_3^-$ . To test this hypothesis, we analyzed N-fluxes in sediment core incubations from 16 stations across Lakes Superior representing both open-lake and nearshore locations. We then compared sediment N-flux values, sediment oxygen profiles, and sediment chemistry in Lake

Superior with measurements from Lake Huron (three stations) and Lake Erie (five stations) to evaluate how sediment N transformations change as water moves downstream through the Laurentian Great Lakes system. In order to better interpret sediment N transformations in Lake Superior, we also measured denitrification of water column  $\text{NO}_3^-$  using the isotope pairing technique, and measured profiles of porewater  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and potential nitrification rates versus sediment depth for nearshore and offshore stations.

## Methods

### Site description

Lake Superior, at the headwaters of the Laurentian Great Lakes system, is the world's largest lake by area ( $82,100 \text{ km}^2$ ) and contains 10% of Earth's surficial liquid freshwater (Matheson & Munawar, 1978). The lake's watershed is relatively small ( $128,000 \text{ km}^2$ ), mostly (95%) forested, and sparsely populated, with about half of the population concentrated in the metropolitan areas of Thunder Bay and Duluth-Superior (Matheson & Munawar, 1978). Sedimentation rates in the lake are low ( $0.1\text{--}2.0 \text{ mm y}^{-1}$ ), and recently deposited sediments are generally mixed with silty clay and organic material, overlying glaciolacustrine clay and glacial till (Thomas & Dell, 1978). Allochthonous sediment loads consists of riverine inputs and is greatly influenced by erosional inputs from red clay bluffs on the lake's southwest shore (Matheson & Munawar, 1978; Thomas & Dell, 1978). The lake is deep (mean depth 147 m, maximum depth 407 m) and cold (average temperature  $3.64^\circ\text{C}$ ; Bennett, 1978). Primary productivity is limited by bioavailable P and possibly Fe (Sterner et al., 2004), as well as light. Nitrate concentrations within the water column average  $\sim 27 \mu\text{M}$  and exhibit little spatial variability, although epilimnetic decreases in  $\text{NO}_3^-$  of  $2\text{--}4 \mu\text{M}$  occur during stratification (Sterner et al., 2007).

Sites in Lake Superior used in the study span a variety of water depths and depositional environments (Table 1), including offshore sites in all of the major basins (Thomas & Dell, 1978), and also including shallow-water inshore sites in the Saint Louis River Estuary, Thunder Bay, Black Bay, and Nipigon Bay.

**Table 1** List of stations used in core incubation study

Lake	Station	Latitude	Longitude	Collection date	Bottom depth (m)	Bottom temp. (°C)	Incubation temp. (°C)	Incubation duration (d)
Erie	879 (23)	42.506	-79.894	7/20/11	60	5	4	5
Erie	880 (84)	41.917	-81.633	7/19/11	25	9	10	5
Erie	91M	41.841	-82.917	7/18/11	10	25	10	5
Erie	CCB (1326)	41.733	-81.698	7/19/11	25	10	10	5
Erie	CCB + 3	42.100	-81.278	7/19/11	25	9	10	5
Huron	EC Gbay 17	45.245	-80.864	7/23/11	80	4	4	17
Huron	EC54	45.517	-83.417	7/16/11	125	4	4	12
Huron	EC9	43.633	-82.378	7/17/11	50	4	4	12
Superior (nearshore)	BB-2	48.692	-88.398	6/29/12	10	18	4	28
Superior (nearshore)	NB-2	48.997	-88.039	6/28/12	15	11	4	28
Superior (nearshore)	NB-5	48.874	-88.197	6/28/12	28	12	4	28
Superior (nearshore)	SLRE 32	46.749	-92.130	6/25/12	10	18	4	28
Superior (nearshore)	SLRE 34	46.715	-92.068	6/25/12	10	18	4	28
Superior (nearshore)	SLRE 35	46.763	-92.091	6/25/12	10	18	4	28
Superior (nearshore)	Sterner-C	46.802	-91.853	10/7/10, 8/15/12	40	8	4	28
Superior (nearshore)	WFB	46.667	-84.833	7/16/11, 8/15/12	65	5	4	28
Superior (nearshore)	TB-2	48.483	-88.892	6/29/12	65	4	4	28
Superior (offshore)	CD-1	47.063	-91.432	10/5/10, 7/12/11, 6/26/12, 8/16/12	250	4	4	28
Superior (offshore)	CM	48.018	-87.774	6/28/12	235	4	4	28
Superior (offshore)	EL-0	47.750	-87.500	7/15/11	165	4	4	28
Superior (offshore)	EL-2	47.000	-85.500	7/15/11	200	4	4	28
Superior (offshore)	IR	47.974	-89.468	10/3/10, 6/28/12	237	4	4	28
Superior (offshore)	Michipicotin	47.900	-85.000	7/25/11	80	4	4	28
Superior (offshore)	WM	47.297	-89.849	10/6/10, 6/27/12	160	4	4	28

### Sediment core collection

Sediments were collected between June–November 2007 and from October 2010–August 2012 from the R/V Blue Heron. Sediment cores were obtained from four different sites in Lake Superior in 2007, and from 16 different sites in Lake Superior, three sites in Lake Huron, and five sites in Lake Erie in 2010–2012 (Fig. 2).

Sediment cores were collected using an Ocean Instruments multi-corer, which allowed recovery of

sediment cores with an intact SWI. Cores were capped within 20 min of collection, and immediately placed in refrigerators at 4 or 10°C (Table 1). Niskin bottles were used to collect water from depths near the lake bottom. Water temperatures at the SWI ranged from 4 to 5.5°C for all offshore sites in Lake Superior and Lake Huron, and for the eastern basin of Lake Erie. Summer water temperatures were warmer in the shallow central and western basins of Lake Erie, and for some of the nearshore stations in Lake Superior (Table 1).

## Sediment core incubations

Incubations (two replicate cores per station) were performed to measure dissolved nutrient and gas fluxes at the SWI. During incubations, cores were stored in the dark and incubated at near-ambient temperatures (4°C for cores from Lake Superior, Lake Huron, and the eastern basin of Lake Erie; 10°C for cores from western and central basins of Lake Erie). Sediment cores were sealed with airtight caps containing sampling ports.

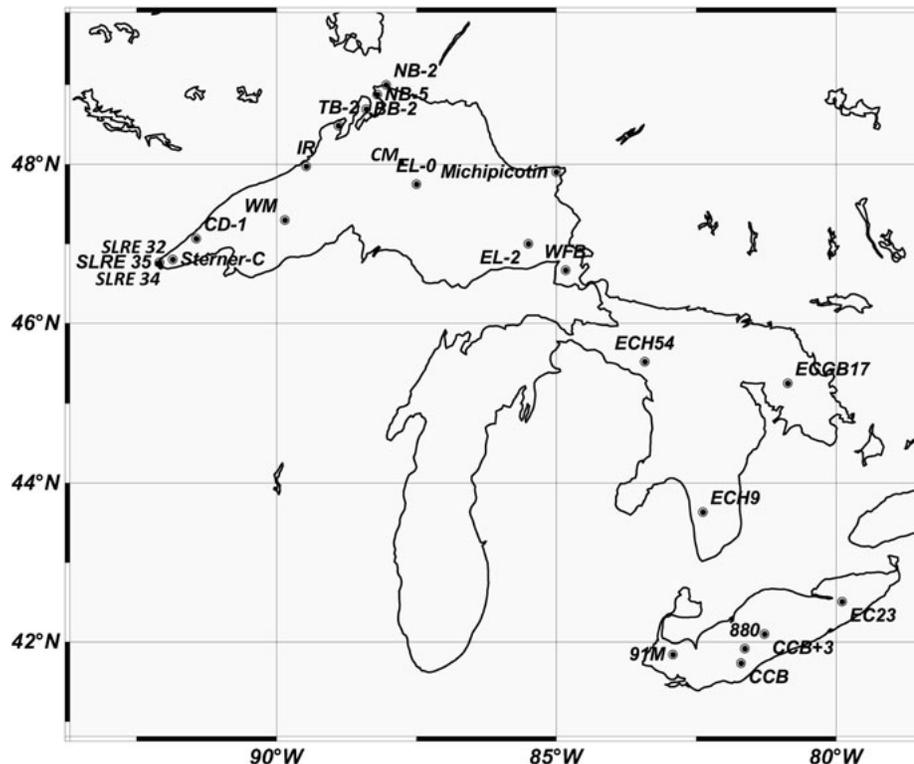
We varied incubation times based on anticipated differences in rates among lakes. Initial samples were taken 1–3 days after cores were collected. Overlying water was sampled at 7-day intervals for Lake Superior (both offshore and inshore sites), at 4-day intervals for Lake Huron, and at 1-day intervals for Lake Erie. Total incubation time ranged from 5 days for Lake Erie cores to 28 days for Lake Superior. To collect water samples during the core incubations, lake water was gravity-fed into a port at the top of the core tube, while water from the middle of the core's water column was simultaneously collected using polyvinyl chloride tubing extending from a second port in the

top of the core tube. Cores contained a mean of 1.5 l water, and at least 15 cm of intact sediment. Over the course of the incubations, we replaced a maximum of 0.25 l of water with bottom water collected from that station.

Water samples were collected for N<sub>2</sub> and total dissolved nitrogen (TDN) for all core incubations. For incubations in 2011, we also analyzed samples for NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, and N<sub>2</sub>O.

Fluxes were calculated as the rate of change in concentration of dissolved nutrients or gases in water overlying sediment cores, after correction for the effects of dilution caused by sampling procedures. Fluxes were also normalized to the standard surface area of the sediment cores. In waters overlying sediment cores, fluxes of nutrients or gas concentrations occasionally became nonlinear as incubation time increased, most likely due to equilibration with sediment concentrations. Thus, the range of linearity was determined by eye, and the rate of change within this range determined by linear regression. Positive fluxes indicate efflux of solutes from the sediment to the water column, and negative fluxes indicate uptake by the sediment from the water column.

**Fig. 2** Location of stations from which cores were collected for this study



## Nutrient and dissolved gas analysis

Water samples for TDN were collected in 20 ml glass scintillation vials, acidified and refrigerated until analysis (typically <1 week). Samples for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were collected in 20 ml and 60 ml plastic bottles, respectively, and were frozen until analysis. Samples were analyzed for  $[\text{NH}_4^+]$  using a modified version of the fluorometric method (Taylor et al., 2007), developed specifically for the measurement of low concentrations of  $\text{NH}_4^+$  in small sample volumes. Samples were analyzed for  $\text{NO}_3^-$  plus  $\text{NO}_2^-$  (hereafter reported as  $[\text{NO}_3^-]$ ) using a Lachat auto-analyzer. Samples were analyzed for TDN using a Shimadzu Total Carbon Analyzer with a TN module. These samples were acidified to  $\text{pH} < 2$  with 2 N HCl before analysis. The precision of replicate standards was regularly maintained within  $\pm 3\%$  (DOC) and  $\pm 5\%$  (TDN). [DON] was calculated as the difference between [TDN] and  $[\text{NO}_3^-] + [\text{NH}_4^+]$ .

Samples for  $\text{N}_2\text{O}$  were analyzed using the headspace equilibration method (Kling et al., 1992). Twenty ml of sample was collected using a plastic syringe. After expelling bubbles from the sample, 20 ml of ambient air was drawn into each syringe. Samples of ambient air were also taken at this time. Syringes were agitated for 5 min at  $4^\circ\text{C}$ , after which the headspace gas was collected into an evacuated serum bottle. Gas samples were analyzed (usually within 3 days) on a HP5890 Series II gas chromatograph with headspace autosampler and ECD, FID, and TCD detectors. Samples were injected into Agilent 10 ml helium-flushed headspace vials. Dissolved  $\text{N}_2\text{O}$  concentrations in liquid samples were back-calculated from headspace gas concentration as described in Kling et al. (1992).

Samples to be analyzed for dissolved  $\text{N}_2$  were collected by overfilling 12 ml Vacutainers<sup>TM</sup>, to which  $\text{HgCl}_2$  was added to a final concentration of 0.01% w/v. While filling vials for  $\text{N}_2$  analyses, care was taken to minimize opportunity for gas exchange with the atmosphere (i.e., by filling with the water outflow tube inside the vial, and by keeping the vial open for not more than 5 s). Samples were stored at  $4^\circ\text{C}$  until analysis to avoid changes in oxygen solubility, and were generally analyzed within 1 month after collection.  $\text{N}_2$  concentrations were measured using a membrane inlet mass spectrometer (Bay Instruments).

## Sediment chemistry and oxygen penetration

Sediment samples to be analyzed for C and N contents were dried at  $60^\circ\text{C}$  for 3–5 days and then homogenized by grinding. Samples were acidified in a desiccator for at least 1 week, and shaken several times throughout the process to achieve uniform acidification. Approximately, 20 mg of sediment was then weighed into  $9 \times 5$  mm pre-weighed tins. Samples were analyzed using a Perkin Elmer CHN elemental analyzer.

In order to characterize sediment redox conditions, we measured sediment oxygen profiles on additional cores collected at each of the stations described above, following methods described in Li et al. (2012). Oxygen concentrations were measured using a Unisense microelectrode (Revsbech, 1989) positioned using a micromanipulator. The electrode was calibrated in air-saturated and anoxic water. Measurements were conducted while cores were at ambient temperature ( $4^\circ\text{C}$  for most stations). Two profiles were measured on one core from each station. We calculated sediment  $\text{O}_2$  extinction rates ( $\text{mm}^{-1}$ ) as the slope of natural logarithm of sediment  $\text{O}_2$  versus depth.

## Isotope pairing technique

We quantified the amount of water column  $\text{NO}_3^-$  removed through denitrification for three sites in the Western Basin of Lake Superior, using a modification of the isotope pairing technique (Nielson, 1992). Duplicate sediment cores were collected from these three stations in August 2012. Overlying water was siphoned off, and replaced with a solution of  $27 \mu\text{M}$   $^{15}\text{NO}_3^-$ . Initial (following a 2-day equilibration period) and final (after 28 days) water samples were collected. Masses of  $^{28}\text{N}_2$ ,  $^{29}\text{N}_2$ , and  $^{30}\text{N}_2$  were measured using the membrane inlet mass spectrometer. This allowed us to partition denitrification into denitrification of overlying water ( $^{15}\text{N}$ -enriched  $\text{N}_2$ ) and coupled nitrification–denitrification within sediments. We did not attempt to correct for anammox (Risgaard-Petersen et al., 2003) because oxygen levels in the overlying water remained high ( $>6 \text{ mg l}^{-1}$ ) throughout the incubations, and previous anammox potential assays produced no evidence of anammox from sites in western Lake Superior (G.E. Small, unpublished data).

## Depth-specific measurements of sediment porewater N and potential nitrification

In order to characterize how the form of N changes with sediment depth, we measured sediment porewater  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations from four stations in western Lake Superior. Eight sediment cores collected from June to November 2007, from three nearshore stations (six cores total) and one offshore station (two cores) in western Lake Superior, were sectioned into 1 cm slices either immediately after collection or upon return to the lab. Sediment sections from depths between 0 and 20 cm below the SWI were retained for porewater extraction. Sediment samples were centrifuged in a clinical centrifuge for 15 min to separate sediment solids from water. The supernatant was removed, filtered through a 0.45  $\mu\text{m}$  GF/F, and frozen for analysis. Nitrate and ammonium concentrations were measured as described above.

We measured how potential nitrification rates varied by sediment depth for three stations in western Lake Superior in 2007. Sediment incubations were used to determine potential sediment nitrification rates as in Joye & Hollibaugh (1995), with the following modifications. Sediment cores were sectioned into 1 cm slices for 2–4 different sediment depths. These sediment samples were combined with filtered lake water in 60-ml glass serum vials to form slurries, and amended with  $\text{NH}_4\text{Cl}$  to a final concentration of 100  $\mu\text{M}$   $\text{NH}_4^+$ . Blanks consisted of serum vials containing only filtered lake water. Slurries were sampled and filtered at 0, 10, 30, and 78 h, and samples were frozen for analysis. We assume that  $\text{O}_2$  was not limiting during the incubations, because of the ample headspace (30 ml of ambient air) and the fact the vials were agitated at each sampling point. Rates of  $\text{NO}_3^-$  production were calculated as the linear rate of change in  $\text{NO}_3^-$  concentration over the incubation period, and production rates at various depths were integrated over sediment volume to estimate total potential  $\text{NO}_3^-$  production per unit area of sediment.

### Data analysis

Stations were classified by lake, and, for Lake Superior, as offshore and nearshore according to depth (>70 m was considered offshore; Table 1). The depth-dependent rate of sediment  $\text{O}_2$  depletion, %C, %N, and C:N, and flux rates were compared across

categories using ANOVAs, followed by Tukey's HSD to assess pair-wise comparisons when a significant difference was indicated.

We used Spearman's rank order correlations to investigate relationships between sediment characteristics (rate of  $\text{O}_2$  loss with depth in sediment, %C, %N, and C:N), incubation temperature, and N-fluxes. To eliminate the effect of incubation temperature, we separately conducted Spearman's rank order correlations on the subset of cores that were incubated at 4°C. Because understanding controls on sediment  $\text{NO}_3^-$  flux is of particular interest given the long-term  $\text{NO}_3^-$  increase in Lake Superior, we additionally conducted a multiple regression analysis using the predictor variables listed above. These statistical tests were all conducted using Statistica v10 (Statsoft, Inc.).

## Results

### Sediment characteristics

Sediment from offshore stations in Lake Superior had a mean organic C content of  $1.21 \pm 0.48\%$  (mean  $\pm$  SD), a mean organic N content of  $0.11 \pm 0.08\%$ , and a mean molar C:N ratio of  $17.28 \pm 10.35$ . The mean  $\text{O}_2$  extinction rate measured in sediment in offshore sediments was  $0.10 \pm 0.15 \text{ mm}^{-1}$ . Nearshore stations in Lake Superior had a mean  $\text{O}_2$  extinction rate of  $0.32 \pm 0.11 \text{ mm}^{-1}$ . Lake Huron sediment had a mean organic C content of  $1.81 \pm 0.48\%$ , a mean organic N content of  $0.21 \pm 0.12\%$ , and a mean C:N ratio of  $11.64 \pm 4.11$ . The mean  $\text{O}_2$  extinction rate measured in Lake Huron sediment was  $0.28 \pm 0.22 \text{ mm}^{-1}$ . Lake Erie sediment had a mean C content of  $2.50 \pm 0.22\%$ , a mean N content of  $0.28 \pm 0.09\%$ , and a mean molar C:N ratio of  $11.62 \pm 5.42$ . The mean  $\text{O}_2$  extinction rate in Lake Erie sediment was  $0.73 \pm 0.15 \text{ mm}^{-1}$ . Sediment  $\text{O}_2$  extinction, %C, and %N was significantly lower for offshore Lake Superior sites compared to Lake Erie, and sediment  $\text{O}_2$  extinction and %C were also significantly lower for offshore Lake Superior sites compared to all other categories ( $P > 0.05$ ). Sediment C:N ratios were not statistically different across these categories.

### Sediment core incubations

Nitrogen fluxes were highly variable within lakes.  $\text{N}_2$  flux was significantly higher for Lake Erie compared

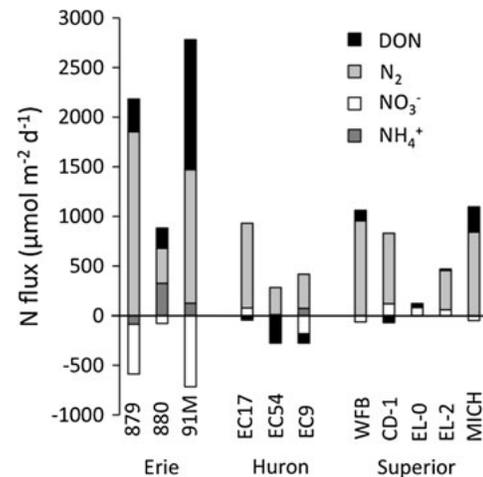
to Lake Superior (both nearshore and offshore) and Lake Huron ( $P > 0.0005$ ). All other N-fluxes were not significantly different across these categories (Table 2, Fig. 3).

Mean sediment nitrification (estimated as the sum of  $\text{N}_2$  flux and  $\text{NO}_3^-$  flux from core incubations) was  $1,057 \pm 952 \mu\text{mol N m}^{-2} \text{d}^{-1}$  (mean  $\pm$  SD) for Lake Erie,  $448 \pm 364 \mu\text{mol N m}^{-2} \text{d}^{-1}$  for Lake Huron, and  $541 \pm 339 \mu\text{mol N m}^{-2} \text{d}^{-1}$  for Lake Superior. The average fraction of estimated sediment nitrification that was subsequently denitrified (i.e., mean sediment nitrification that was not accounted for by  $\text{NO}_3^-$  flux) is 96% for Lake Erie, 93% for Lake Huron, and 67% for Lake Superior.

Several of the potential sediment predictor variables were correlated. Sediment  $\text{O}_2$  extinction rate was positively correlated with incubation temperature, sediment %C, and sediment %N, and negatively correlated with sediment C:N (Table 3). Sediment %C was positively correlated with %N, and %N was negatively correlated with C:N. Core incubation temperature was positively correlated with %C.

Of all potential predictors our focus was mainly on the position of the redox gradient, which we measured by the oxygen extinction coefficient. Sediment  $\text{O}_2$  extinction rate was positively correlated with  $\text{N}_2$  (Fig. 4a) and  $\text{NH}_4^+$  (Fig. 4c) fluxes, and significantly negatively correlated with  $\text{NO}_3^-$  flux (Fig. 4b). Total dissolved N, DON, and  $\text{N}_2\text{O}$  showed no significant correlation with sediment  $\text{O}_2$  extinction (Fig. 4d, e). No N-fluxes were significantly correlated with other N-fluxes (Table 2).

In the subset of core incubations conducted at  $4^\circ\text{C}$  (which excludes most Lake Erie cores), sediment  $\text{O}_2$  extinction rate was negatively correlated with  $\text{NO}_3^-$  and TDN fluxes, and TDN flux was positively correlated with sediment C:N.  $\text{NO}_3^-$  flux was negatively correlated with DON flux (Table 4).



**Fig. 3** Summary of N-fluxes from core incubations from Lakes Erie, Huron, and Superior, conducted in July 2011. Values here are means of duplicate cores

Results of multiple regression modeling showed that  $\text{NO}_3^-$  fluxes were positively related to incubation temperature and negatively related to sediment  $\text{O}_2$  extinction (Table 5). Sediment chemistry was not a significant predictor of  $\text{NO}_3^-$  fluxes. When the analysis was restricted to incubations conducted at  $4^\circ\text{C}$ , sediment  $\text{O}_2$  extinction explained 39% of the variance in  $\text{NO}_3^-$  flux.

#### Isotope pairing technique

For three stations in the western arm of Lake Superior, denitrification of  $\text{NO}_3^-$  from overlying water increased linearly with sediment  $\text{O}_2$  extinction rate ( $P < 0.05$ ,  $R^2 = 0.87$ ; Fig. 5). The highest measured rate,  $25 \mu\text{mol N m}^{-2} \text{d}^{-1}$  at the nearshore Ste-C station, was 14% of the total  $\text{N}_2$  flux for this station and is approximately 5% of the mean  $\text{N}_2$  flux from offshore Lake Superior sediments.

**Table 2** Summary of N-fluxes (mean  $\pm$  SD) measured in this study

N-flux ( $\mu\text{mol N m}^{-2} \text{d}^{-1}$ )	Lake Superior (offshore)	Lake Superior (nearshore)	Lake Huron	Lake Erie
$\text{NO}_3^-$	$31 \pm 78$		$-38 \pm 138$	$-175 \pm 307$
$\text{NH}_4^+$	$-1 \pm 4$		$22 \pm 54$	$121 \pm 210$
DON	$59 \pm 173$		$-150 \pm 279$	$372 \pm 510$
TDN	$76 \pm 168$	$383 \pm 1129$	$194 \pm 136$	$291 \pm 279$
$\text{N}_2$	$444 \pm 250$	$561 \pm 248$	$485 \pm 276$	$1232 \pm 989$
$\text{N}_2\text{O}$	$0.03 \pm 0.07$		$0.07 \pm 0.04$	$-0.07 \pm 0.31$

**Table 3** Spearman rank order correlations between sediment chemistry variables and N-fluxes, for all incubations

	Sed O <sub>2</sub>	Incubation temp	Sed C	Sed N	Sed C:N	NH <sub>4</sub> <sup>+</sup> flux	NO <sub>3</sub> <sup>-</sup> flux	N <sub>2</sub> O flux	N <sub>2</sub> flux	TDN flux	DON flux
Sed O <sub>2</sub>	1.00										
Incubation temp	<b>0.63</b>	1.00									
Sed C	<b>0.60</b>	<b>0.67</b>	1.00								
Sed N	<b>0.59</b>	0.29	<b>0.68</b>	1.00							
Sed C:N	<b>-0.44</b>	-0.02	-0.20	<b>-0.82</b>	1.00						
NH <sub>4</sub> <sup>+</sup> flux	<b>0.49</b>	<b>0.63</b>	0.06	-0.05	0.05	1.00					
NO <sub>3</sub> <sup>-</sup> flux	<b>-0.36</b>	-0.16	-0.16	-0.28	0.16	-0.03	1.00				
N <sub>2</sub> O	-0.21	-0.24	-0.11	-0.24	0.25	0.10	0.23	1.00			
N <sub>2</sub> flux	<b>0.32</b>	<b>0.30</b>	0.31	0.18	0.04	-0.18	-0.15	0.19	1.00		
TDN flux	-0.15	<b>0.34</b>	0.35	-0.06	<b>0.46</b>	0.43	-0.17	-0.27	0.10	1.00	
DON flux	0.21	0.20	0.27	0.24	-0.07	-0.18	-0.17	-0.25	0.26	0.09	1.00

Sed O<sub>2</sub> rate of sediment O<sub>2</sub> loss (mm<sup>-1</sup>), incubation temp incubation temperature (°C), sed C sediment carbon (%), sed N sediment nitrogen (%), sediment C:N sediment carbon:nitrogen (molar ratio)

Correlations in bold are significant at  $P < 0.05$

#### Depth-specific measurements of sediment porewater N and potential nitrification

In sediment porewater from station Ste-C, [NO<sub>3</sub><sup>-</sup>] decreased from 11 μM at the surface to 2 μM at a depth of 3 cm (Fig. 6). At the surface, [NH<sub>4</sub><sup>+</sup>] was 7 μM, and increased linearly with depth to ~50 μM (Fig. 6). Other nearshore stations in western Lake Superior showed similar patterns, with a shift in porewater N from NO<sub>3</sub><sup>-</sup> dominance to NH<sub>4</sub><sup>+</sup> dominance at a depth of between 1 and 4 cm. By contrast, the deep and offshore station, WM, shifted from NO<sub>3</sub><sup>-</sup> dominance to NH<sub>4</sub><sup>+</sup> dominance at a much greater depth (~11 cm). [NO<sub>3</sub><sup>-</sup>] was 36 μM at the surface, and decreased linearly through 12 cm to approximately 2 μM, while [NH<sub>4</sub><sup>+</sup>] increased from <1 μM at 10 cm to 10 μM at 20 cm (Fig. 6).

Potential nitrification rates for the offshore station WM were approximately 1.3 μmol N cm<sup>-3</sup> d<sup>-1</sup> in the top 3 cm, before declining to 0 by 6 cm. Nearshore stations B and G had lower potential nitrification rates at the surface (0.4 and 0.2 μmol N cm<sup>-3</sup> d<sup>-1</sup>, respectively), and declined to 0 by approximately 5 cm (Fig. 7).

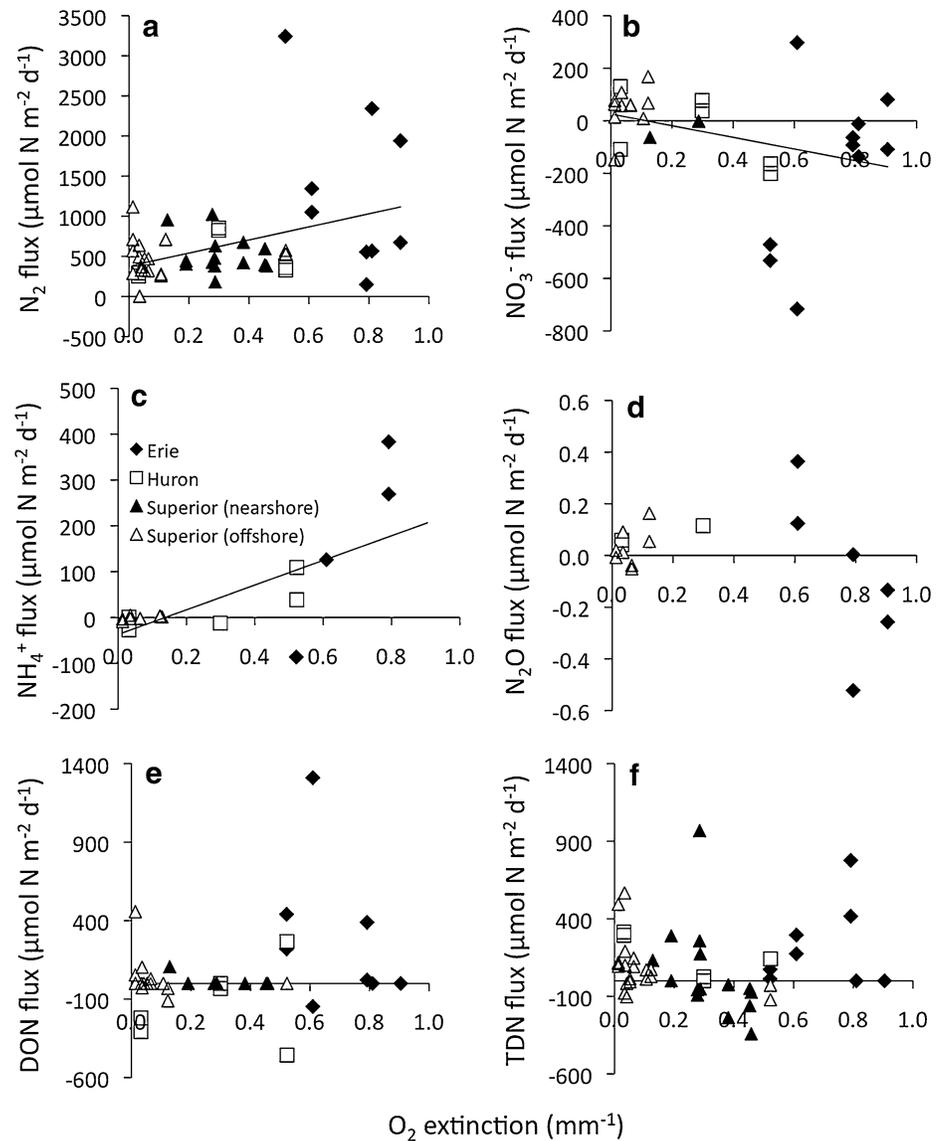
#### Discussion

Our results support the hypothesis that the thickness of the oxygenated layer in sediment is an important

predictor of N-fluxes across the SWI. Across a broad gradient of sedimentary redox conditions observed for Lakes Superior, Huron, and Erie, sediments with deeper oxygenated layers (i.e., lower O<sub>2</sub> extinction rates) were characterized by decreased fluxes of N<sub>2</sub> and NH<sub>4</sub><sup>+</sup> out of the sediment, and increased fluxes of NO<sub>3</sub><sup>-</sup>. However, because multiple predictor variables such as sediment chemistry and water temperature were correlated with the thickness of the oxygenated layer in our observational data set, we cannot definitively attribute causality to sediment O<sub>2</sub> penetration. Sediment chemistry and water temperatures can affect N-fluxes directly, and ultimately influence the position of the sediment oxycline. At least for NO<sub>3</sub><sup>-</sup> fluxes, we found that sediment O<sub>2</sub> levels were an important predictor after accounting for these other variables (Fig. 4).

Offshore sites in Lake Superior are characterized by deep O<sub>2</sub> penetration and high nitrification potential. For one of these offshore stations (WM), NO<sub>3</sub><sup>-</sup> increases from near 0 at the redox boundary (at approximately 10 cm deep) to a concentration of 36 μM at the surface, exceeding water column NO<sub>3</sub><sup>-</sup> concentration by nearly 10 μM (Fig. 6). In contrast, nearshore sites have thinner oxygenated sediment layers, lower potential nitrification rates, and porewater NO<sub>3</sub><sup>-</sup> at the sediment surface that was lower than that of the water column. These data are consistent with our observations from core flux

**Fig. 4** Nitrogen fluxes versus  $O_2$  extinction (i.e., rate of  $O_2$  decline with depth) for cores from nearshore and offshore stations in Lake Superior, as well as Lake Huron and Lake Erie. Positive values denote net fluxes out of the sediment; negative values denote net removal from the water column



measurements, in which offshore stations in Lake Superior generally had positive net fluxes of  $NO_3^-$  where nearshore fluxes were slightly negative (Fig. 4b). The results of the  $^{15}NO_3^-$  enriched core incubations indicate that denitrification of  $NO_3^-$  from overlying water increases as the depth of the oxygenated sediment layer decreases, pointing to diffusion limitation (Fig. 5). Moreover, these data indicate that  $NO_3^-$  from the water column represents a small fraction ( $\sim 5\%$ ) of total denitrification, which is predominantly driven by nitrification within the oxygenated sediment. The oxygenated sediment layer effectively separates water column  $NO_3^-$  from the reducing environment in the deeper sediment, so that

the  $NO_3^-$  that reaches the anoxic environment is primarily generated within the sediments.

Across a wide range of aquatic systems, the source of  $NO_3^-$  fueling denitrification typically shifts from nitrification within sediments (i.e., coupled nitrification–denitrification) to the water column  $NO_3^-$  levels above approximately  $10 \mu M$  (Seitzinger et al., 2006). However, in Lake Superior, unlike other high  $NO_3^-$  aquatic systems, water column  $NO_3^-$  supports a small fraction of total denitrification, presumably due to the thick oxygenated layer of sediments. Our results suggest that, even in nearshore environments, denitrification rates in Lake Superior sediments are not directly acting as a large sink for

**Table 4** Spearman rank order correlations between sediment chemistry variables and N-fluxes, for all incubations run at 4°C

	Sed O <sub>2</sub>	Sed C	Sed N	Sed C:N	NH <sub>4</sub> <sup>+</sup> flux	NO <sub>3</sub> <sup>-</sup> flux	N <sub>2</sub> O flux	N <sub>2</sub> flux	TDN flux	DON flux
Sed O <sub>2</sub>	1.00									
Sed C	0.19	1.00								
Sed N	<b>0.55</b>	<b>0.84</b>	1.00							
Sed C:N	<b>-0.75</b>	-0.32	<b>-0.72</b>	1.00						
NH <sub>4</sub> <sup>+</sup> flux	0.14	<b>-0.52</b>	-0.29	0.06	1.00					
NO <sub>3</sub> <sup>-</sup> flux	<b>-0.46</b>	-0.27	-0.37	0.26	0.22	1.00				
N <sub>2</sub> O flux	0.37	0.24	0.13	-0.46	0.45	0.02	1.00			
N <sub>2</sub> flux	0.18	0.23	0.10	-0.06	-0.29	-0.14	0.12	1.00		
TDN flux	<b>-0.43</b>	-0.03	-0.23	<b>0.66</b>	0.16	0.01	-0.15	0.08	1.00	
DON flux	0.11	0.21	0.21	-0.13	-0.29	<b>-0.59</b>	-0.36	0.40	0.03	1.00

Sed O<sub>2</sub> rate of sediment O<sub>2</sub> loss (mm<sup>-1</sup>), sed C sediment carbon (%), sed N sediment nitrogen (%), sediment C:N sediment carbon:nitrogen (molar ratio)

Correlations in bold are significant at  $P < 0.05$

**Table 5** Results of multiple regression modeling for NO<sub>3</sub><sup>-</sup> fluxes

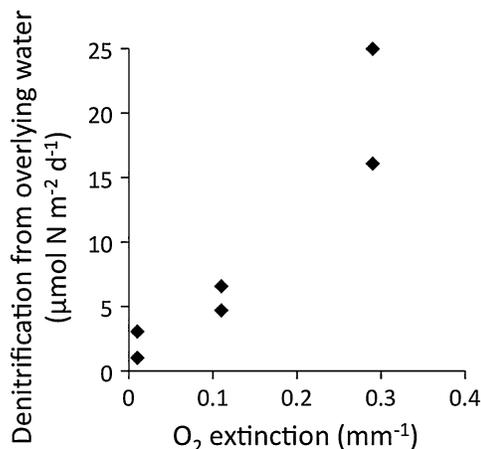
Parameter	<i>b</i>	SE	<i>t</i> (19)	<i>P</i> value
Intercept	0.02	222.86	0.00	1.000
Sed O <sub>2</sub>	-819.94	332.23	-2.47	0.023
Incubation temp	80.05	35.83	2.23	0.038
Sed C	-105.44	127.96	-0.82	0.420
Sed N	410.53	1024.30	0.40	0.693
Sed C:N	-7.63	11.04	-0.69	0.497

Sed O<sub>2</sub> rate of sediment O<sub>2</sub> loss (mm<sup>-1</sup>), incubation temp incubation temperature (°C), sed C sediment carbon (%), sed N sediment nitrogen (%), sediment C:N sediment carbon:nitrogen (molar ratio)

$N = 25$ . Adjusted  $R^2 = 0.119$

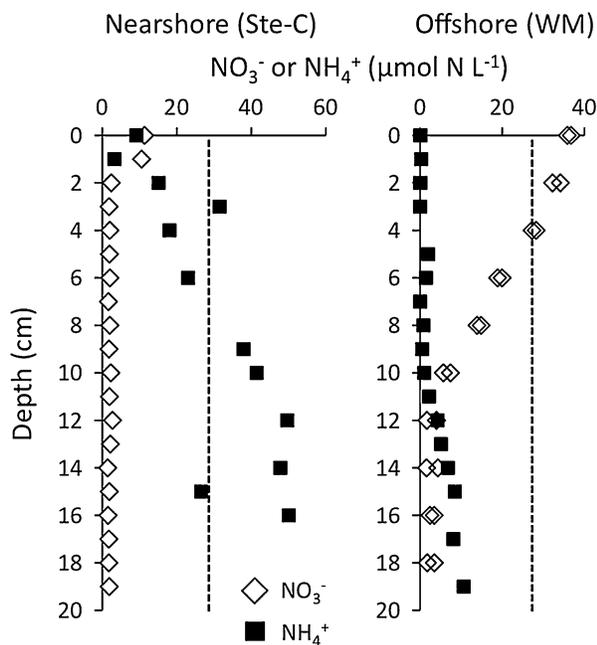
water column NO<sub>3</sub><sup>-</sup>. Even the highest rate of direct NO<sub>3</sub><sup>-</sup> removal observed in Lake Superior (150 μmol N m<sup>-2</sup> d<sup>-1</sup>) represents only 4% of mean water column nitrification rates in the lake (3.6 mmol N m<sup>-2</sup> d<sup>-1</sup>; Small et al., 2013).

Instead, nitrification within the sediments supplies nearly all of the NO<sub>3</sub><sup>-</sup> for denitrification in Lake Superior. Most water column NO<sub>3</sub><sup>-</sup> that is ultimately denitrified therefore takes a circuitous path that may take decades or centuries: it first must be taken up by phytoplankton, deposited through organic matter sedimentation, buried, mineralized, and nitrified. The mean of N<sub>2</sub> fluxes that we measured from offshore Lake Superior cores (440 μM N m<sup>-2</sup> d<sup>-1</sup>) is reasonably similar to estimates of average rates of NO<sub>3</sub><sup>-</sup> uptake by phytoplankton (230 μM N m<sup>-2</sup> d<sup>-1</sup>;

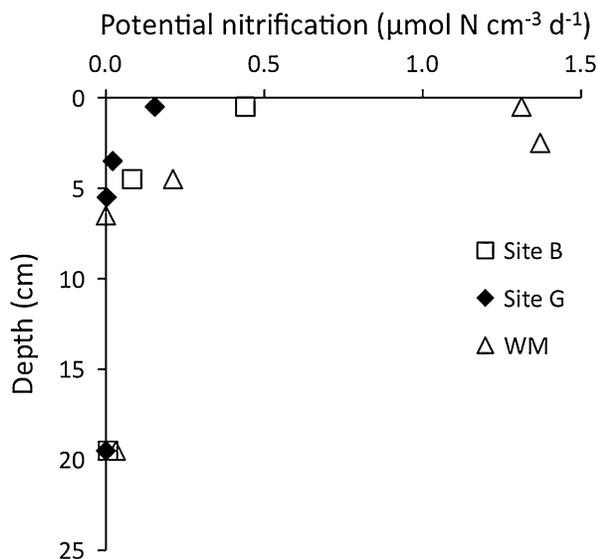


**Fig. 5** Denitrification from overlying water, as measured by buildup of <sup>15</sup>N-N<sub>2</sub> for three stations in western Lake Superior (Ste-C, CD-1, and WM) that vary in depth of O<sub>2</sub> penetration. O<sub>2</sub> extinction rates used here were measured in October 2010; isotope-enriched core incubations were conducted in August 2012

Kumar et al., 2008) and of organic N sedimentation rates (170–410 μmol N m<sup>-2</sup> d<sup>-1</sup>; Heinen & McManus, 2004) in Lake Superior, suggesting that denitrification is the ultimate fate of much of the lake's organic N. However, even the highest N<sub>2</sub> flux value that we measured in Lake Superior (1,120 μM N m<sup>-2</sup> d<sup>-1</sup>) is less than one-third of mean areal water column nitrification rates (Small et al., 2013), indicating that cycling of N within the lake predominates over removal. Likewise, the flux of NO<sub>3</sub><sup>-</sup> out of sediments in offshore Lake Superior sites seems to be a minor contributor to water column



**Fig. 6** Representative sediment  $\text{NO}_3^-$  and  $\text{NH}_4^+$  profiles from a nearshore station (Ste-C) and an offshore station (WM) in Lake Superior



**Fig. 7** Potential nitrification versus sediment depth for nearshore stations (B and G) and an offshore station (WM) in Lake Superior

nitrate. For offshore sites, even the highest observed rates of  $\text{NO}_3^-$  fluxes out of the sediment (approximately  $200 \mu\text{mol N m}^{-2} \text{d}^{-1}$ ) are only 6% of the mean areal water column nitrification rate.

In contrast to Lake Superior, Lake Erie is characterized by oxygenated sediment layers of not more than several mm (sediment  $\text{O}_2$  extinction rates of  $0.5\text{--}0.9 \text{ mm}^{-1}$ ). Observed net fluxes of  $\text{NO}_3^-$  were negative in 8 of 10 cores from Lake Erie (mean value of  $-175 \mu\text{mol N m}^{-2} \text{d}^{-1}$ ), indicating that sediment denitrification is a sink for water column  $\text{NO}_3^-$ . Mean total  $\text{N}_2$  efflux was seven times higher than the mean net flux of  $\text{NO}_3^-$ , however, indicating that nitrification within the very thin (1–3 mm) oxygenated layer was still the primary driver of denitrification.

While it is not surprising that total denitrification rates in Lake Erie were highest among the three large lakes considered in this study, it is somewhat surprising that there was not a greater difference in total denitrification (i.e.,  $\text{N}_2$  flux) among these lakes, given the substantial difference in redox conditions. We considered the possibility that  $\text{N}_2$  flux could have been overestimated from the deep Lake Superior sites, if gasses trapped in the sediment take several days or more to completely equilibrate with atmospheric pressure. However, we found no evidence for this in our data set; after controlling for temperature, and accounting for sediment  $\text{O}_2$  extinction rate, there was no relationship between  $\text{N}_2$  flux and water column depth at each station. Instead, our results suggest that rates of coupled nitrification–denitrification may not depend on the depth of the oxycline per se; regardless of the depth of the oxycline,  $\text{NO}_3^-$  generated within oxygenated sediments just above the oxycline is still available to fuel denitrification in the adjacent anaerobic zone (Seitzinger et al., 2006). In Lake Erie, higher sediment %N, and warmer temperatures during the summer in the western and central basins (and warmer incubation temperature during our study) are likely explanations for slightly elevated rates of coupled nitrification–denitrification. The more important direct consequences of relatively deep sediment  $\text{O}_2$  penetration for N-cycling relate to the fraction of water column  $\text{NO}_3^-$  that is denitrified (low in Lake Superior), and the fraction of sediment nitrification that escapes denitrification and diffuses into the water column (relatively high in Lake Superior).

The range of denitrification values that we measured from these core incubations were similar to values reported from Lake Michigan (Gardner et al., 1987), and other lakes (Seitzinger, 1988). The highest denitrification value that we measured in Lake Erie sediments ( $3 \text{ mmol N m}^{-2} \text{d}^{-1}$ ; Fig. 4) was lower

than denitrification rates measured in many coastal marine systems, however (Seitzinger, 1988).

Temperature is an important control on N-fluxes from sediments, as has been shown in other lakes (Anthony & Lewis, 2012). Because we incubated cores from the western and central basins of Lake Erie at warmer, near-ambient temperatures to simulate realistic conditions (Table 1), it is difficult to separate the effects of temperature from the very shallow oxycline in these sediments. Similarly, some cores from nearshore environments in Lake Superior collected in June 2012 were incubated at temperatures that were cooler than ambient conditions, leading to potential underestimates of these rates (although temperatures are around 4°C for much of the year at these sites). Despite this limitation, the range of conditions and sites used in this experiment are representative of the range of physical and chemical conditions in the Laurentian Great Lakes and the data allow us to begin to untangle cause and effect in this complex suite of biogeochemical relationships.

## Conclusions

Bodies of water with long residence times are typically highly efficient at removing N through denitrification (Seitzinger et al., 2006). However, in Lake Superior, which has a residence time of nearly 200 years,  $\text{NO}_3^-$  levels have increased steadily over the past century. Our results indicate that very little water column  $\text{NO}_3^-$  is removed through direct denitrification in Lake Superior; indeed there is a net flux of  $\text{NO}_3^-$  out of the sediments. Most denitrification in Lake Superior occurs via a very slow indirect pathway: a small fraction of water column  $\text{NO}_3^-$  that is taken up by phytoplankton ultimately enters the sediment PON pool through sinking and subsequent burial, and may eventually be mineralized, nitrified, and denitrified. Because of the low rates of primary production and sedimentation in Lake Superior, denitrification occurring today may represent  $\text{NO}_3^-$  that was removed from the water column decades or centuries ago, possibly before the long-term  $\text{NO}_3^-$  rise began. Furthermore, unlike direct denitrification, which is generally not limited by phosphorus, this indirect pathway involving algal uptake and burial is limited by the low phosphorus levels in Lake Superior. The combination of this time lag and phosphorus

dependence suggests that this indirect denitrification pathway would not be able to play an important role in regulating the lake's  $\text{NO}_3^-$  levels. Thus, while the century-long  $\text{NO}_3^-$  increase in Lake Superior may have been caused by relatively small changes in external loading, the lack of direct denitrification in this large lake has certainly played an important role in allowing for this buildup.

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