HOW DOES NO_3^- REDUCTION IN ONONDAGA LAKE SEDIMENTS RESPOND TO NO_3^- AMENDMENT?

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Abstract. Methylmercury in Onondaga Lake (Syracuse, NY) causes health problems for the Lake's fish and fish consumers. In 2004, the town of Syracuse's wastewater treatment plant, which discharges effluent into the lake, added a terminal nitrification step to reduce the amount of ammonium (NH_4^+) added to the lake by converting it to nitrate (NO_3^-). Since that time, mercury methylation has declined. Additional NO_3^- supplementation during summer stratification may further decrease methylation by favoring nitrate reducing bacteria over sulfate reducing bacteria, which are responsible for methylmercury production. Before beginning lake-wide NO_3^- supplementation, we seek to understand what happens to added NO_3^-. Added NO_3^- may either remain as NO_3^- or be reduced to one of three possible products: N_2 through complete denitrification, the greenhouse gas N_2O through incomplete denitrification, or the mildly toxic ammonium (NH_4^+) through dissimilatory nitrate reduction to ammonium (DNRA). To determine how NO_3^- amendments affect NO_3^- removal to N_2, N_2O, and NH_4^+, we incubated anaerobic sediments from three lake sites at low (sediment+DI water), ambient (sediment+3mg NO_3^--N/L lakewater), and high (sediment+32.7mg NO_3^--N/L amended lakewater) NO_3^- levels. Because high NO3- concentrations are thought to favor denitrification over DNRA, we expected increased rates of nitrate removal at higher nitrate concentrations. Conversely, under NO_3^--limited conditions, we expected DNRA to be favored over denitrification. Lake sediments increase denitrification rates several fold in response to NO_3^- supplementation, but produce minimal amounts of NH_4^+ and N_2O even when NO_3^- levels are elevated by an order of magnitude. Our findings suggest that NO_3^- addition to maintain lakebottom levels at 2mg NO_3^--N/L will not cause NO_3^- accumulation, NH_4^+ accumulation, or excessive N_2O production.

INTRODUCTION

Onondaga Lake is a culturally eutrophic, dimictic freshwater lake in northern New York State. Prior to 1988, mercury cell chlor-alkali plants released large amounts of mercury into the lake. Over the past 100 years, the lake has also received wastewater discharge from Syracuse, NY. Currently, 20% of the lake's inflow comes from the Metro wastewater treatment plant in the lake's southeast corner. Concern over levels of ammonium (NH_4^+) and methylmercury (MeHg) led the US Environmental Protection Agency to place the Lake on its National Priorities List in 1994 (Upstate Freshwater Institute, 2007).

Sulfate-reducing bacteria (SRB) are believed to be the primary source of methylmercury in the water column (Upstate Freshwater Institute, 2007). SRB do not become active until supplies of more energetically favorable electron acceptors, including NO_3^-, are locally depleted. Hence, the Upstate Freshwater Institute proposed NO_3^- addition to reduce MeHg production.

Water column measurements have responded favorably to NO_3^- addition. In 2004, to reduce levels of NH_4^+ in the lake, Metro began nitrification of wastewater prior to discharge. Wastewater nitrification increased hypolimnetic NO_3^- concentrations, postponed the time of NO_3^- depletion during summer stratification, postponed the onset of hydrogen sulfide accumulation, and decreased MeHg production (Upstate Freshwater Institute 2007). Although NO_3^- addition sounds both promising and economical, the dynamics of NO_3^- in the sediment and the ecological consequences of NO_3^- metabolism warrant exploration before further nitrate amendments begin.
NO_3^- added to lakes may be assimilated to organic N by plants and microbes, reduced to nitrous oxide (N_20) or dinitrogen gas (N_2) by denitrification, or reduced to ammonium (NH_4^+) by Dissimilatory Nitrate Reduction to

Ammonium (DNRA) (Burgin and Hamilton 2007). Of these three processes, only denitrification removes nitrogen from the lake to the atmosphere. NO_3^- that remains in the lake is safe even to sensitive freshwater species at 2mg NO_3^--N/L (Camargo et al 2005). N_2O production is concerning because of its greenhouse gas potential. Although less mobile than NO_3^-, NH_4^+ is highly bioavailable to algae and toxic to fish at lower levels than is NO_3^- (USEPA, 1985).

To evaluate the safety of additional NO_3^- enrichment, we examined the products of NO_3^- reduction different levels of addition. We considered four possible fates of NO_3^- in the sediment: (1) Added NO_3^- could remain in the lake as NO_3^- if NO_3^- reducers are already operating at capacity. (2) Added NO_3^- could be partially denitrified to N_20. (3) NO_3^- could be fully denitrified to N_2, and (4) NO_3^- could undergo DNRA to generate NH_4^+.

We expected that low levels of NO_3^- would favor DNRA over denitrification because DNRA uses the electron acceptor, NO_3^-, more efficiently (Kelso et al 1997). DNRA transfers eight electrons per NO_3^- molecule, whereas reduction to N_2 transfers only five. Conversely, high levels of NO_3^- were expected to favor denitrification over DNRA because denitrification uses the electron donor, organic matter, more efficiently (Kelso et al 1997).

We predicted that sediment incubated with DI water would have lower ratios of N_2:NH_4^+ production than samples incubated with lakewater (3 mg NO_3^- -N/L), and that samples amended with 30 mg NO_3^--N/L would have the highest ratios of N_2:NH_4^+ production.

METHODS

Sample collection and storage

We collected sediment in late June from the 18m South Deep site, an 8m site west of South Deep, and an 8m site east of South Deep (Figure 1) using a dredge box. Samples were separated by depth (0-2cm and 2-4cm) with PVC pipe core, ruler, and spatula. We combined individual cores into a 0-2cm bag and a 2-4cm bag from each site. Sediment was stored at 4C in freezer bags until sampling. At sampling time, the shallow and deep bags from each site were pooled due to shortage of sample.

Denitrification and DNRA assays

To determine rates of denitrification in the three sites under 3 levels of nitrate, we incubated sediments in 140mL serum bottles at room temperature for 6h. Each 5-replicate group of vials received 10g sediment and 40mL water as DI water, lake water (3mg NO_3^--N/L), or NO_3^--amended water (33mg NO3- N/L). Vials were crimped with rubber septa and aluminum rings to form an airtight seal and flushed with helium for a minimum of 1h to eliminate ambient N_2 and O_2. We sampled N_2O, CO_2, and N_2 on a gas chromatograph (Shimadzu) immediately after flushing (t=0h), after three hours' incubation (t=3h), and after 6h incubation (t=6h). Between samplings, the transparent vials were covered with Al foil to prevent photosynthesis and incubated upside-down underwater in plastic extraction cups on a shaker table. Vials containing DI water only controlled for N2 leakage through the septa during the incubation.

To determine rates of DNRA, we measured NO_3^- and NH_4^+ in the sediment-water slurry at the beginning and end of incubation. For the initial measurement, we mixed 5g site-matched sediment with 20g treatment-matched water in a plastic extraction cup. (Because vials were already sealed and flushed, we could not filter their contents for this initial measurement) After 30 min incubation on a shaker table, sediment was allowed to settle and overlying water was filtered through a 1µm glass filter. For the final measurement, vials were decrimped, allowed to settle, and filtered through 1µm filter paper.

Subsamples from each site were dried at 65C for 48h for moisture content.

Analysis

Rates of N_2O, CO_2, and N_2 production were calculated as µmol/dry gram/h using a best-fit slope of amount vs. time for each vial's t=0, t=3h, and t=6h timepoints. Rates of NO_3^- removal and NH_4^+ production were derived from t=0 and t=6h timepoints. ANOVA analyzed overall and site-specific effects of treatment on changes in NO_3^-, N_2, N_2O, and NH_4^+.

RESULTS

We incubated sediments from three lake locations with DI water, lakewater, or NO_3^--amended lakewater. Both overall (F=11.042, P=0.000) and within each site (East site: F=43.294, P=0.000; West site: F=147.134, P=0.000; South Deep site: F=940.536, P=0.000), sediments increased rates of NO_3^- removal in response to NO_3^- addition (Figure 2).

In all treatments, most of the NO_3^- reduction occurred as denitrification to N_2 (Figure 3). Both across the 3 sites and within each site, there was significant increase in N_2 production with NO_3^- addition (pooled sites: F=11.06, P=0.000, East site: F=8.095, P=0.010; West site: F=12.423, P=0.005; South Deep site: F=157.585, P=0.000).

Little NO_3^- was reduced to N_2O (Figure 4), or NH_4^+ (Figure 5), even in the high NO_3^- treatment. There were no statistical trends toward increased N_2O or NH_4^+ production with additional NO_3^-.

DISCUSSION

NO_3^- reduction in Onondaga Lake appears dominated by denitrification over DNRA. Sediments are below NO_3^--reducing capacity at 3mg NO_3^--N/L; rates of NO_3^- reduction increase with NO_3^- addition. Most NO_3^- reduction is in the form of complete denitrification to N_2, with no trend towards increased N_2O or NH_4^+ production even at 10x ambient NO_3^- concentrations. If our results accurately portray sediment NO_3^- reduction, then the UFI suggestion to maintain NO_3^- levels at less than 2mg NO_3^--N/L during the summer should not cause worrisome increases in NH_4^+ or N_2O.

Although our observations showed a minimal role for DNRA, we caution that our assay was relatively insensitive to NH_4^+ production. We measured NH_4^+ in water filtered from sediment-water slurries through 1µm glass filters. This filtering did not collect NH_4^+ sorbed to the negatively charged clay particles in the sediment.

The possibility of increased DNRA with NO_3^- addition is concerning and warrants thorough investigation. Fishkills from toxic NH_4^+ concentrations were one reason for beginning nitrification of wastewater discharges (Upstate Freshwater Institute, 2007). If NO_3^--N is converted to NH_4^+ by DNRA, then the lake community would be reversing the nitrification process, which suggests that further NO_3^- addition would be harmful. NH_4^+ may also harm fish indirectly by depleting lake oxygen levels. Nitrification of NH_4^+-rich upwelling water during the fall mixing period exerts increases oxygen demand in surface waters (Gelda et al 2000). Fish normally leave the lake during this time of oxygen depletion (Tango and Ringler 1996), but they may still be vulnerable to rapid late-summer changes in oxygen levels.

Our study could not account for either the effects of cold storage or seasonal variables that might influence denitrification patterns. We gathered our sediments before the onset of stratification and at a time when O_2 was still present 2cm deep in the sediment (Todorova, personal comm.). Increasing HS^- levels, particularly after DO depletion in lower sediments, could hinder denitrification (Brunet and Garcia-Gil 1996), perhaps favoring NO_2^-, N_2O or NH_4^+ production.

The accumulation NO_2^- , a product of incomplete denitrification, needs addressing (Wetzl 2001). NO_2^- oxidizes hemoglobin to methemoglobin, interfering with its ability to bind O_2 in humans and fish (Russo et al 1981). Onondaga Lake water had some of the highest NO_2^- values ever measured in lakes over the 1989-1998 interval

(Gelda et al 1999). Characterization of NO_2^- production and its relationship to NO_3^- amendment would help to weigh the risks of NO_3^- supplementation against the benefits of MeHg decreases.

Future studies should use stable isotope techniques to more closely evaluate the importance of DNRA, measure responses to NO_3^- addition within the lake itself, and consider the activity of the sediment community at different times of the year in response to changing DO and HS^- levels.

Nitrification of wastewater appears to have positive effects on both NH_4^+ and MeHg levels. Our results tentatively support the safety of maintaining 2mg NO_3^--N/L with respect to NO_3^- removal, NH_4^+ production (DNRA) and N_2O production.

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APPENDIX

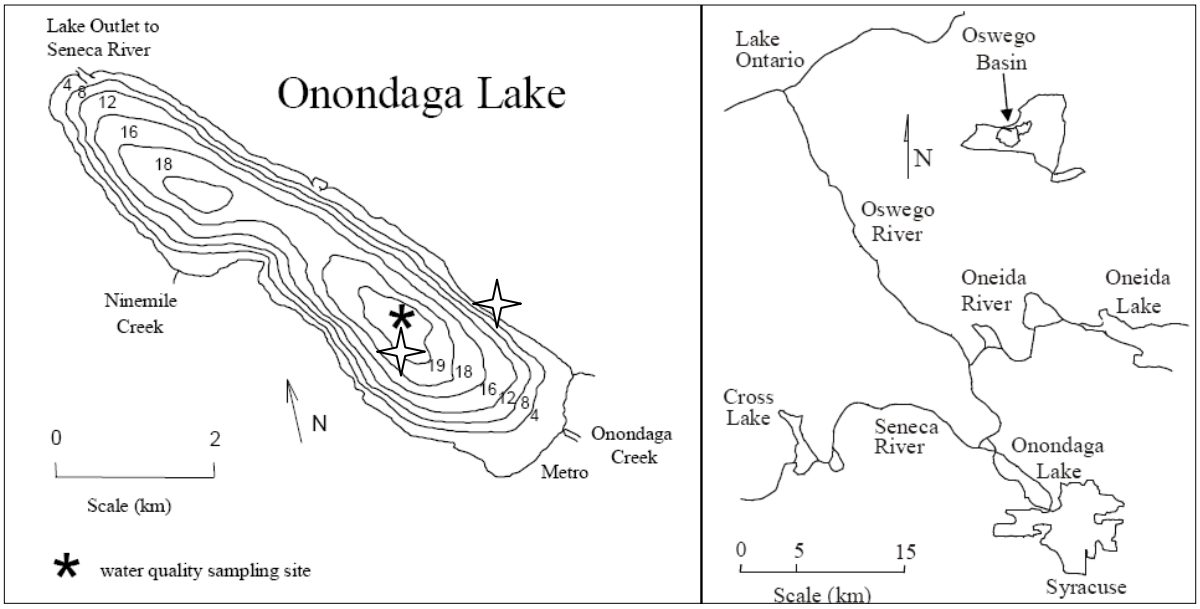

FIGURE. 1. Onondaga Lake and sampling sites South Deep (18m, asterisk), East site (8m, 4-point star northeast of South Deep), and West site (8m, 4-point star southwest of South Deep).

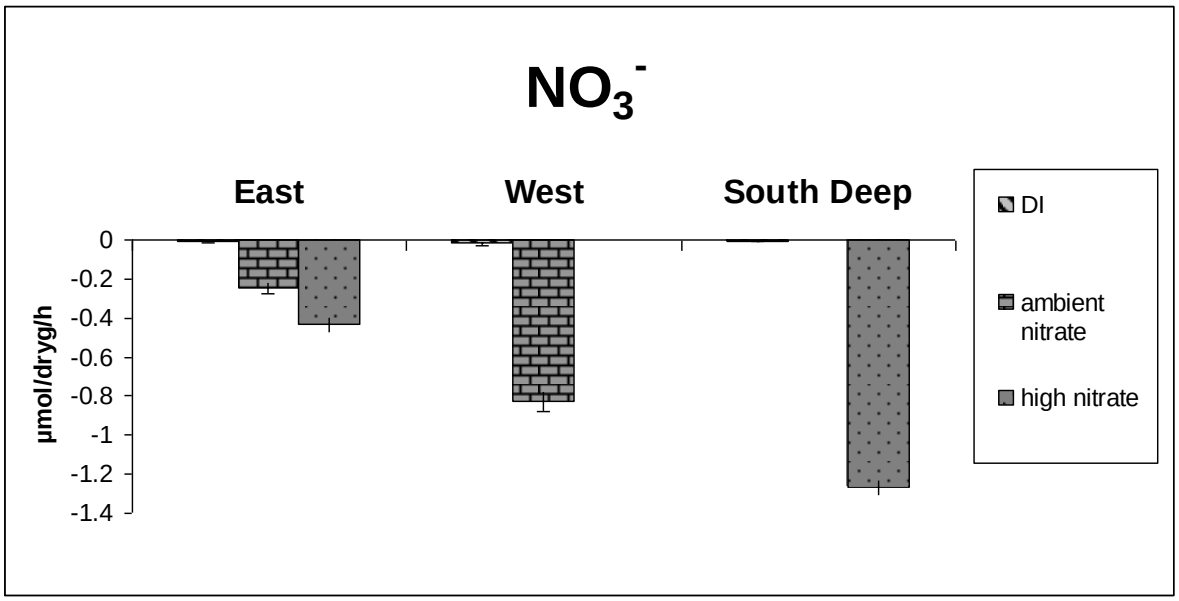

FIGURE 2. Rates of NO_3^- reduction in sediments from 3 Onondaga Lake sites. Error bars represent 1 S.E. from mean.

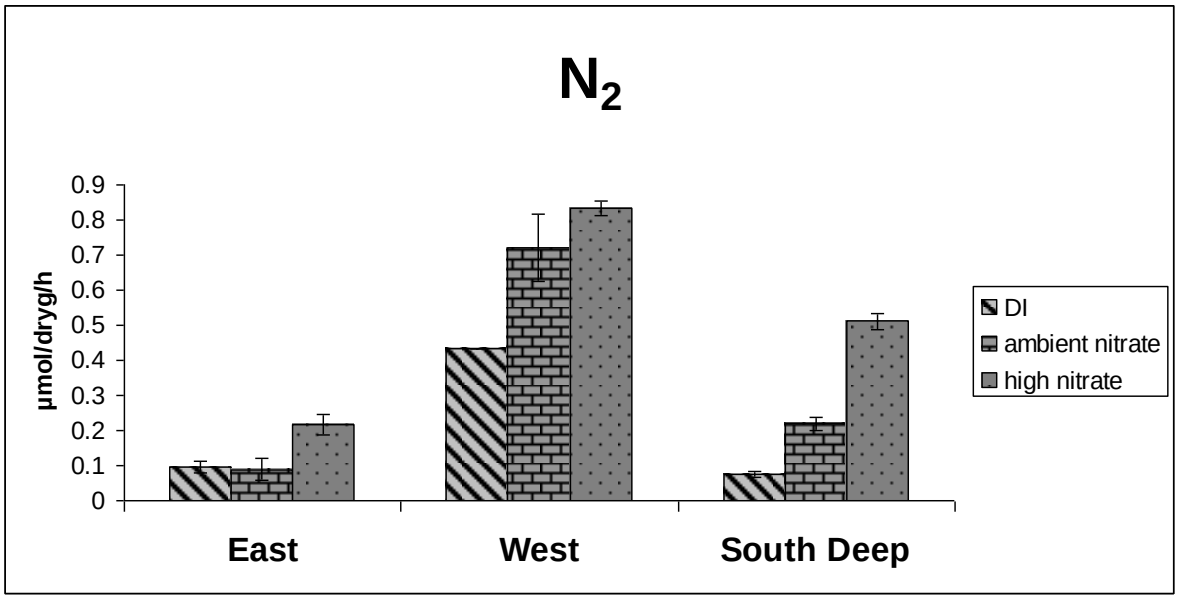

FIGURE 3. Rates of N_2 production in sediments from 3 Onondaga Lake sites. Error bars represent 1 S.E. from mean.

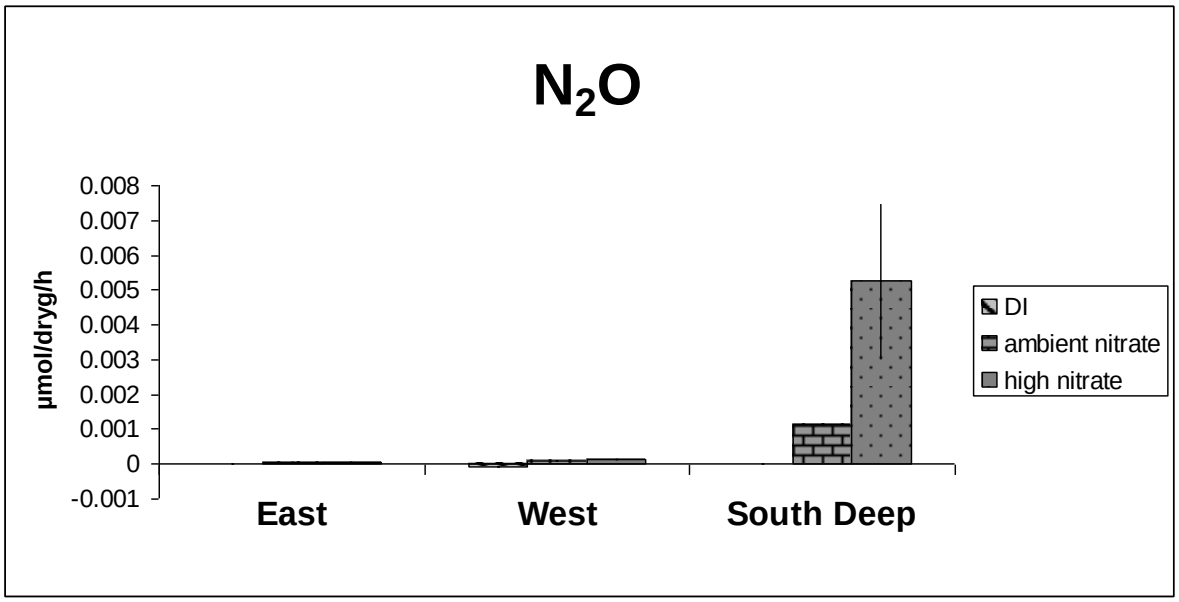

FIGURE 4. Rates of N_2O production in sediments from 3 Onondaga Lake sites. Error bars represent 1 S.E. from mean.

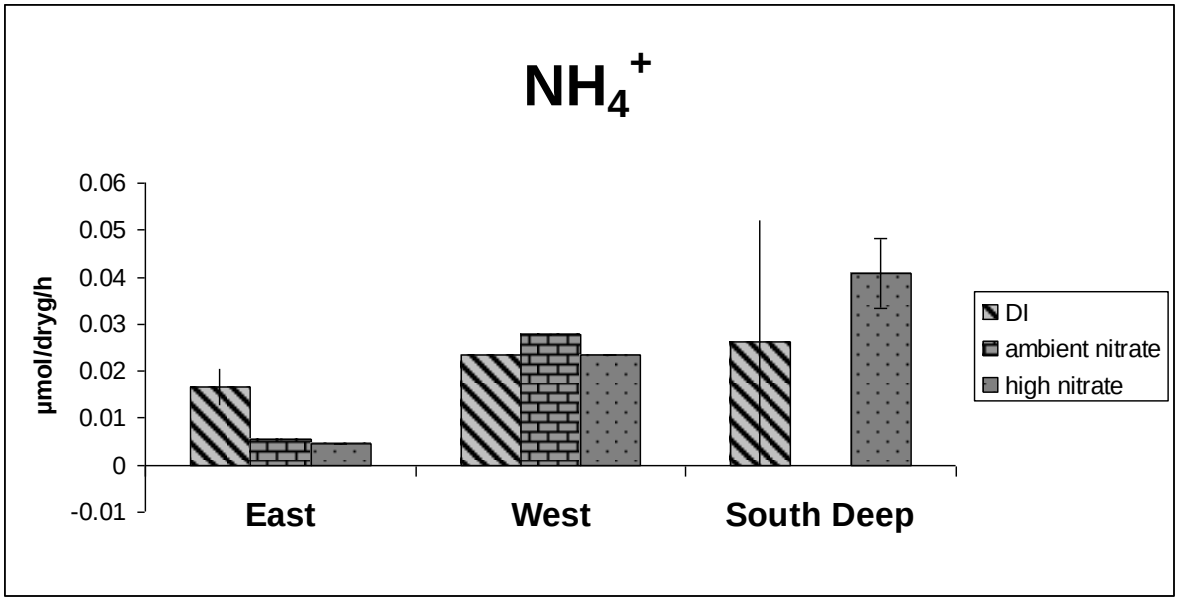

FIGURE 5. Rates of NH_4^+ production in sediments from 3 Onondaga Lake sites. Error bars represent 1 S.E. from mean.