

THERMAL STRATIFICATION IMPACTS MICROBIAL NITROGEN REMOVAL AND
NITROUS OXIDE PRODUCTION IN A SMALL EUTROPHIC RESERVOIR: AN
IN-SITU APPROACH TO QUANTIFYING HYPOLIMNETIC PROCESS RATES

By

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To the faculty of Washington State University:

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Abstract

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Identifying patterns and controls on microbial N removal and N₂O production is a critical step in understanding the role that freshwater systems play as both N filters and greenhouse gas sources. Efforts to discern these relationships have been limited by methodological difficulties associated with the measurement of these processes. In this study, N dynamics within the hypolimnion of a thermally stratified reservoir were examined in order to test a novel approach to measuring in situ N₂ and N₂O production rates wherein sub-thermocline gas accumulation rates serve as a proxy for sediment production rates. Water column stratification can significantly change bottom water chemistry and was thus expected to impact system N₂ and N₂O production rates. Potential denitrification rates determined by acetylene block were three orders of magnitude greater in the sediment than in the overlying water column. These assays, sediment incubation experiments, and hypolimnion NO₃⁻ data show that, over the course of the summer, progressive nitrate depletion at the sediment-water interface limited N₂ production and associated N removal. As rates of N₂ production dropped off, rates of N₂O production increased, resulting in significant increases in N₂O:N₂ ratios as the summer progressed ($p < 0.05$). These findings have important implications for the incorporation of seasonal patterns of aquatic N removal in N transport models. Our findings also suggest that physical disturbances, such as dam spills, may sufficiently destabilize the water column to alleviate sediment NO₃⁻ limitation.

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Introduction

The anthropogenic mobilization of biologically available nitrogen (N) across the landscape has resulted in a number of serious aquatic pollution issues (Vitousek 1997, Townsend et al. 2003, Diaz 2008, Heisler et al. 2008). Denitrification, defined here as the microbially mediated conversion of biologically available N to gaseous non-reactive N_2 (via either dissimilatory nitrate reduction or anaerobic ammonium oxidation), is an important N-removal pathway in aquatic systems (Saunders and Kalff 2001a). Lakes and rivers are particularly active sites for denitrification, accounting for an estimated 20% of total global N removal (Seitzinger et al. 2006). In some watersheds, aquatic ecosystems are estimated to remove upwards of 60% of terrestrial N inputs (e.g. Alexander et al. 2000), constituting a significant buffer to coastal N loading. While the relative importance of reservoirs as N-trapping systems is quite variable and system-dependent, recent modeling efforts indicate that reservoirs play a disproportionately large role with respect to N removal in comparison to lakes (Harrison et al. 2009). Model estimates show that while reservoirs account for only 6% of lentic system surface area, they contribute roughly 33% of lentic N removal (Harrison et al. 2009).

While denitrification provides an important ecosystem service, it is also associated with the production of nitrous oxide (N_2O). N_2O is a powerful greenhouse gas (IPCC 2007) that contributes significantly to the destruction of stratospheric ozone (Ravishankara et al. 2009). Previous work has quantified $N_2O:N_2$ ratios in freshwater systems as a metric of N removal efficiency (Silvennoinen et al. 2008, Woodward et al. 2009). While a number of environmental variables have been related to the $N_2O:N_2$ ratio, the controls on this ratio are also far from being understood. For instance, while some work suggests that high oxygen concentrations increase $N_2O:N_2$ ratios (Firestone et al. 1980), lab-incubated nitrifying bacteria have been found to increase N_2O production by nearly 10% under low oxygen conditions (Goreau et al. 1980) and nitrifying bacteria were implicated in producing the majority of the N_2O in a poorly ventilated Antarctic lake ($<2\text{mg L}^{-1} O_2$, Priscu et al. 2008). Similarly, high NO_3^- concentrations have been linked to high N_2O emissions at some sites (Harrison and Matson 2003), while another study of river systems found no predictable relationship between NO_3^- and N_2O production (Cole and Caraco 2001). A review of published greenhouse gas emissions from reservoirs suggests their potential importance in global budgets (St. Louis et al. 2000). Finally, the few studies that have quantified reservoir N_2O fluxes have been carried out in tropical or boreal systems

(Huttunen et al. 2002, Hendzel et al. 2005, Guérin et al. 2008), with a notable absence of measurements from temperate systems. Hence this study addresses an important gap in the existing literature.

Much of the uncertainty surrounding the patterns and controls of aquatic N_2 and N_2O production can be attributed to the assumptions and difficulties associated with measuring in-situ gas fluxes. Previous work estimating process rates has relied on approaches that are each associated with their own unique setbacks: (1) sediment incubations only represent discrete points of activity and can miss (or over-represent) processes happening during brief periods at select locations (e.g. hot spots and hot moments, Wang et al. 2006, Groffman et al. 2009); (2) ^{15}N tracers have been broadly successful in lotic systems (Mulholland et al. 2008), but remain prohibitively expensive for estimating lentic process rates; (3) black box estimates of whole-system behavior (mass balance approaches, typically for estimating denitrification) do not differentiate short-term N sinks (i.e. biotic uptake) from longer term sinks (i.e. denitrification or sediment burial); (4) thin boundary methods (Liss and Slater 1974) rely on in-situ determinations of gas transfer velocities which have been successfully employed in lotic systems (Laursen and Seitzinger 2002) and shallow lentic systems (Clark et al. 1995, Cole and Caraco 1998) but become cumbersome in deeper lake and reservoir systems. Methodological limitations have also historically resulted from the difficulty associated with quantifying the end product of denitrification (N_2) given its high background concentrations (Groffman 2006). Relatively new technology allows for the precise measurement of excess dissolved N_2 in water samples using a membrane inlet mass spectrometer (MIMS) to compare expected and actual $N_2:Ar$ ratios (Kana 1994). Great potential exists to use MIMS technology to quantify in-situ denitrification process rates, as has already been done in an estuarine setting (Kana et al. 2006). Kana et al. document how the halocline in Chesapeake Bay acts as a barrier to N_2 exchange with the atmosphere (2006). Using this natural cap, the authors were able to measure the rate of N_2 accumulation as a proxy for whole-system denitrification rate. Estimates using this in-situ approach did not differ significantly from concordant sediment incubation flux estimates and thus constitute a substantial contribution to denitrification methodology. Theoretically the thermocline of a stratified lake or reservoir could also serve as an effective cap on gas exchange.

In addition to allowing for in-situ measurements of process rates, stratification also results in important biogeochemical changes in reservoir bottom waters, with the potential to impact sediment N_2 and N_2O

production. Stratification supports the occurrence of seasonally hypoxic bottom waters and would thus boost anaerobic processes such as denitrification if these processes are indeed limited by high oxygen levels. Alternatively, if denitrification is occurring primarily in the sediment, stratification may decrease sediment NO_3^- availability by shifting oxic sites of nitrification distal to sediment denitrifier communities. Because highly impacted systems typically support amplified rates of primary production and decomposition, it could be argued that, all else being equal, these systems experience more extreme chemical stratification than their less impacted counterparts. This makes an understanding of the impacts of stratification on N removal and N_2O production relevant to understanding how humans have impacted aquatic ecosystem function. Locally, knowledge of seasonal flux patterns is also necessary to design efficient sampling regimes that will best characterize overall system N processing. At the global scale, incorporating seasonality of N biogeochemistry into models allows scientists to consider how the timing of N transport is related to seasonal or continuous events such as harmful algal blooms and coastal hypoxia. Finally, an understanding of the physical controls on N transformations in a reservoir system is particularly relevant to N management because the hydrology of these systems is controlled.

We measured accumulation of N_2 and N_2O in the hypolimnion of a small, eutrophic, seasonally stratifying reservoir during the summer of 2008 as a proxy for denitrification and N_2O production rates as well as variables likely to co-vary with or control N_2 and N_2O production. The main objectives of this study are to (1) assess the effectiveness of this method in providing reasonable flux estimates, and (2) identify controls on microbial N removal and N_2O production during stratification in this system.

Study Site

Lacamas Lake is a small (1.3km^2), monomictic reservoir in southwest Washington (45.37N , 122.25W , Figure 1). Lacamas has an average depth of 7.8m, a maximum depth of 19.8m and a capacity of $7.694,630\text{ m}^3$ (Washington State Department of Ecology, <http://www.ecy.wa.gov/services/GIS/data/elevat/lakebath.htm>). The reservoir is long and narrow, a shape characteristic of many impounded systems, with a deep basin in its center. The impoundment was built in 1938, deepening a pre-existing lake, and is currently operated primarily for recreational purposes. The system is an EPA 303[d] listed hypereutrophic system, with dominant

nutrient inputs attributed to upstream dairy farms and residential storm-water runoff. Lacamas Lake also experiences annual onset of hypolimnetic hypoxia following spring stratification (Schnabel and Hutton 2004) as well as a fall dam spill event during which time approximately 20% of the water is released downstream.

Methods

Field Sampling

Water samples were collected with a Van-Dorn sampler from the deepest part of the reservoir (deep water site, Figure 1) on 8 dates between 23 Jun 2007 and 11 Sep 2008 at 0.1, 2, 7, 13, and 15 meters as well as 0.2 meters off the lakebed. Bottom water samples for N_2O on 8 Jul 2008 and for N_2 on 15 Aug 2008 were discarded due to air bubble entrainment, which interferes with sample analysis. Samples from additional depths were taken on several days to better characterize epilimnion and hypolimnion chem-

ical patterns. A Hach DS5X Sonde was used to measure temperature, dissolved oxygen, pH, and conductivity at each sampling depth. The pH probe was always calibrated within 12 hours of sampling. The Clark Cell DO sensor was calibrated in the field directly before sampling using water-saturated air. Maximum daily wind speed data was gathered from the Fort Vancouver weather station (Weather Underground, wunderground.com), less than 5 km away from Lacamas Lake.

Laboratory Analysis

For dissolved nutrient analysis (NO_3^- , NO_2^- , NH_4^+ , and PO_4^{3-}), one filtered (Whatman GFF 0.45 μ m) water sample was collected in an acid washed 30mL nalgene and frozen until analysis on a Westco discrete nutrient analyzer using standard EPA-approved colorimetric methods (NEMI, <http://infotrek.er.usgs.gov/>

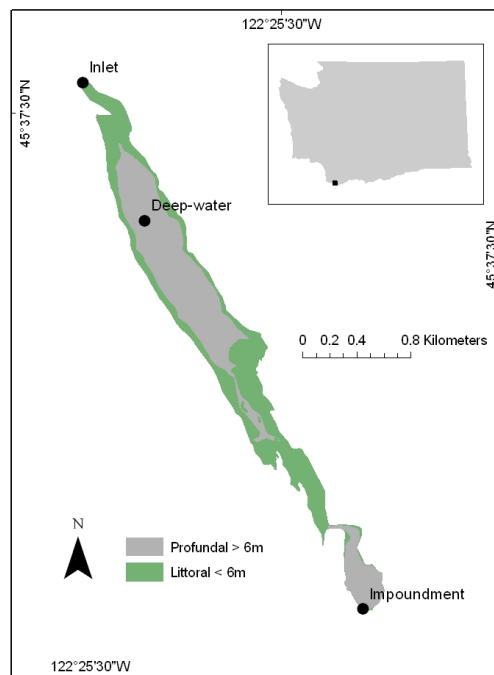


Figure 1: Profundal and littoral zones of Lacamas Lake. The deep-water site as well as the inlet and outlet (impoundment) are indicated by black dots. Black portion of insert map shows the location of Lacamas Lake in Washington State.

apex/f?p=nemi:browse_methods). Mean % error was 4.8% for NO₃-N, 2.36% for PO₄-P, 6.1% for NH₄-N, and -1.43% for NO₂-N.

N₂O samples were collected in 72mL crimp top Wheaton vials, treated with ZnCl₂, and analyzed using a gas equilibration technique as in Harrison and Matson (2003). Briefly, lab samples were brought to 25°C and 20 mL of ultra high purity helium headspace were introduced to Wheaton vials. Vials were shaken before analyzing headspace gas on a Hewlett Packard 5890 Series II Plus Gas Chromatograph equipped with an electron capture detector (ECD). The ECD contained 63 Ni as the isotope source and an argon/methane mixture was used as the carrier gas (Harrison and Matson 2003). 0.1 and 1.0 ppmv N₂O standards were run every 6-9 samples. R² values for calibration curves were never lower than 0.96 and mean coefficients of variation for standards were 11% (±1.5, n=8). Headspace N₂O concentrations were converted to original dissolved gas concentrations using the appropriate solubility tables (Weiss and Price 1980).

N₂:Ar samples were collected in duplicate 5 ml hollow penny-head ground-glass stoppered vials as in Harrison et al. (2005), treated with ZnCl₂ and refrigerated under water until analysis for N₂:Ar ratios on a MIMS as in Kana et al. (1994). Five pseudoreplicated readings were taken from the same sample vial for atomic mass 28 and 40 signals with a mean precision (coefficient of variation) of <0.025%. Precision of replicate samples from the same depth averaged <0.2%. Air-equilibrated deionized water was run as a standard every six samples and signal readings were used to correct for instrument drift. For each individual MIMS run, an equation for change in standard N₂:Ar over time was used to predict the amount of drift associated with each sample signal. The calculated drift value was then added to or subtracted from the raw signal value. Drift-corrected N₂:Ar ratios were compared to the expected N₂:Ar ratio at the measured in-situ temperature.

In-Situ Physical and Chemical Characteristics

In order to assess the degree of thermal stratification present for each sampling date, a stability index developed by Idso (1973) was used to quantify the amount of energy needed to completely mix the reservoir

on each sampling date. Stability (S) was calculated as:

$$S = \frac{g}{A_0} \int_{z_0}^{z_m} (\rho_z - \bar{\rho}) A_z (z - z_{\bar{\rho}}) dz \quad (1)$$

where A_o is the surface area in m^2 , A_z is the area in m^2 at depth z , $\bar{\rho}$ is the density after mixing to isothermy, ρ_z is the density at depth z , z is the depth interval (m), $z_{\bar{\rho}}$ is the depth at which the density $\bar{\rho}$ exists prior to mixing, z_m is the maximum depth (m), z_0 is the surface, and g is the acceleration due to gravity (9.8 m s^{-2}). Chemical stratification was also assessed by comparing epilimnion and hypolimnion DO, NO_3^- , NO_2^- , NH_4^+ , DIN, and N_2 concentrations to look for significant differences in the chemical composition of the two hydrologically distinct zones.

Summertime Accumulation and Depletion Rates in the Hypolimnion

Thermal stratification during summer months creates a strong density boundary at the reservoir thermocline that prohibits significant exchange of dissolved solutes and gasses between the hypolimnion and the epilimnion. For each sampling date, excess N_2 and N_2O as well as NO_3^- and NH_4^+ concentrations were integrated by depth in order to calculate the total moles of each solute within the hypolimnion. This method assumes that solute concentrations measured at the deep-water site are representative of all hypolimnion water at that depth. N_2O transect data collected from the hypolimnion support this notion; concentrations were uniform in the horizontal direction despite measurable differences in concentration by depth. Bathymetry data from WA Dept of Ecology (<http://www.ecy.wa.gov/services/GIS/data/elevat/lakebath.htm>) was used to calculate the volume of the reservoir represented by each sample depth. Total moles of solutes were then regressed against time to estimate summertime fluxes. Flux rates were calculated using the slope of the regression line. In order to quantify the degree of exchange across the thermocline boundary, solute fluxes across the thermocline boundary were quantified based on a heat exchange coefficient (vt). A heat exchange coefficient was used in place of a gas transfer velocity because density differences across the thermocline complicate the use of Schmidt numbers. This coefficient was calculated based on Chapra (1980):

$$V_h \frac{dT_h}{dt} = v_t A_t (T_e - T_h) \quad (2)$$

where the subscripts e and h represent epilimnion and hypolimnion respectively, V is volume (cm^3), T is temperature ($^{\circ}\text{C}$), and A_t is the thermocline area (cm^2). Thermocline area and hypolimnion volume were determined using bathymetric data from the Washington State Department of Ecology (<http://www.ecy.wa.gov/services/GIS/data/elevat/lakebath.htm>).

This equation simplifies to solve for v_t :

$$v_t = \frac{V_h(T_{h,f} - T_{h,i})}{A_t \int_{t_i}^t (T_e - T_h) dt} \quad (3)$$

where $T_{h,f}$ is the hypolimnion temperature on the final sampling date and $T_{h,i}$ is the hypolimnion temperature on the initial sampling date. Four independent values for v_t were calculated based on temperature changes experienced over 13 to 26 day increments. The mean v_t was used to calculate chemical fluxes across the thermocline using the following equation:

$$F = v_t(C_H - C_E) \quad (4)$$

where C_H and C_E are the mean concentrations of a given solute in the hypolimnion and epilimnion respectively. Flux rates calculated using this method are subject to the assumption that both the epilimnion and hypolimnion are well mixed, and thus represent an upper bound on thermocline flux rates.

Surface N_2 and N_2O Fluxes Based on Expected Gas Transfer Velocities

Excess N_2O concentrations at the surface of the reservoir were used to compare average summertime hypolimnion N_2O fluxes to those calculated across the air-water interface. N_2O fluxes at the reservoir surface are governed both by the observed concentration gradients at these interfaces and by a transfer coefficient (k). In the case of well-mixed surface waters, the gas transfer coefficient k can be derived for N_2O at ambient temperature if the gas transfer for any other gas at any other temperature is known (Jahne et al. 1987). Normalization to a Schmidt number of 600, the Schmidt number for CO_2 at 20°C , has been done in a number of studies (Clark et al. 1995, Cole and Caraco 1998, Zappa et al. 2007) and established relationships between Sc_{600} , wind speed, and k_{600} allow us to solve for k_x (Crusius and Wanninkhof 2003):

$$\frac{k_{600}}{k_x} = \left(\frac{600}{Sc_x} \right)^n \quad (5)$$

where k_{600} , or the gas transfer velocity of CO_2 at 20°C , has a constant value of 1 cm h^{-1} because maximum daily wind speeds never exceeded 3.7 m s^{-1} on any sampling date (Cole and Caraco 1998, Crusius and Wanninkhof 2003) and the exponent n has a value of $-2/3$ when maximum daily wind speeds were below 3 m s^{-1} and $-1/2$ when maximum daily wind speeds were above 3 m s^{-1} (Crusius and Wanninkhof 2003). Sc_x was calculated using sonde temperature data for each sampling date based on Wanninkhof (1992). Once k is determined, N_2O flux is calculated based on:

$$F = k(C_{measured} - C_{equilibrium}). \quad (6)$$

Where $C_{measured}$ is the actual surface gas concentrations and $C_{equilibrium}$ is the gas concentration expected if the water were in equilibrium with the atmosphere. Surface fluxes of N_2 at the air water interface were also considered, keeping in mind that N fixation and denitrification processes may both significantly influence N_2 concentrations in this zone.

Sediment Incubations

While denitrification is known to occur in low-oxygen hypolimnetic waters (Seitzinger 1988), the relative importance of sediment versus water column denitrification in lentic systems is not well understood (Seitzinger et al. 2006). Acetylene block assays were conducted along a vertical transect at the deep-water site in order to ascertain the relative importance of water column versus sediment denitrification. During August of 2007, nine replicate wheaton vials were filled with reservoir water from the surface, 5m, and 17m using a Van Dorn sampler; an additional set were filled with 3mL of sediment and overlying water collected using an Eckman Dredge. Three replicates from each depth were treated as controls, three were fertilized with KNO_3 , and three were sampled before incubating to ascertain initial N_2O concentrations. Helium headspace was introduced and evacuated, and 10mL of C_2H_2 were added to each sample. Samples were incubated and then run on a Hewlett Packard 5890 Series II-Plus Gas Chromatograph for N_2O concentrations as described

above.

Sediment cores were also collected and incubated under different treatment conditions in order to better assess factors limiting sediment NO_3^- uptake. Intact cores were collected from the deepest part of the reservoir (Figure 1) on 12 Aug 2008. Dredge contents, typically between 5 and 10 cm of sediment deep, were sub-sampled using polycarbonate cylinders. Overlying water from 0.2 meters off the bed was used to overfill core cylinders in the field. Cores were transported to the lab on ice and triplicate cores were set up for carbon (1000 μM CH_3COONa), nitrate (1000 μM KNO_3), carbon + nitrate (1000 μM CH_3COONa and KNO_3), sediment, and blank treatments. Cores were incubated in the dark at 8°C and were sampled for nitrate from an air-tight port at seven and twelve hours after set-up. Nitrate removal fluxes were calculated by quantifying change in NO_3^- concentration over the course of the incubation, taking sample volume replacement into account.

Correlations with Environmental Variables

The rate of change in hypolimnion $\text{N}_2\text{-N}$ and $\text{N}_2\text{O-N}$ mass and $\text{N}_2\text{O-N:N}_2\text{-N}$ ratios between sampling dates was tested for significant correlations with mean hypolimnion $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, and dissolved oxygen concentrations as well as temperature, conductivity, pH, and stability. This was done using linear regression analysis. The change in $\text{N}_2\text{O-N:N}_2\text{-N}$ ratio between 23 Jun 2008 and 8 Jul 2008 was omitted from the analysis because N_2 was undersaturated in the hypolimnion on 23 Jun 2008 complicating the use of $\text{N}_2\text{O-N:N}_2\text{-N}$.

Results

The Lacamas water column was highly stable during the summer of 2008 (Table 1), making the hypolimnion an excellent site to look for excess gas accumulation. Strong thermal stratification during this time (Figure 2) was accompanied by general supersaturation of hypolimnion N_2 and N_2O (Figure 3) and pronounced N_2 and N_2O accumulation in the reservoir hypolimnion (Figure 4). Patterns in hypolimnion chemistry are punctuated by two disturbance events (Figures 2 and 4): a shift in wind direction on 14 Aug 2008 (Weather Underground, wunderground.com), and a large dam spill between 9 Sep 2008 and 20 Sep 2008 that was

preceded by a smaller mud flap spill on 7 Sep 2008.

Chemical stratification was evident in the depleted dissolved oxygen readings (Figure 2) and higher NH_4^+ , N_2 , and N_2O concentrations detected below the thermocline. Patterns in relative epilimnion and hypolimnion $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations were more variable; however DIN concentrations were always higher below the thermocline than above, with the exception of 11 Sep 2008. Stability index values over this period ranged from 1807 kg s^{-2} on 23 Jun 2008 to 3243 kg s^{-2} on 18 Aug 2008, indicating high water-column stability. If dam spill sampling points are removed, stability values increase significantly with time ($p < 0.01$).

Vertical profiles of N_2 and N_2O over the summer sampling period indicate that the thermocline acts as an effective transport barrier for these solutes (Figure 3). Hypolimnion concentrations of N_2 and N_2O were, on average, much higher than epilimnion concentrations. Hypolimnion concentrations of N_2O averaged $0.17 \mu\text{mol} (\pm 0.02, n=31)$ with 0.15

$\mu\text{mol} (\pm 0.02, n=31)$ of this being gas in excess of expected equilibrated values. Hypolimnion concentrations of $\text{N}_2\text{-N}$ averaged $1392 \mu\text{mol L}^{-1} (\pm 14.2, n=31)$ with $48 \mu\text{mol L}^{-1} (\pm 7, n=31)$ of this being gas in excess of expected equilibrated values. N_2 concentrations in the upper hypolimnion were generally supersaturated (with one exception on 12 Aug 2008). In contrast, aside from two sampling dates (23 Jun 2008 and 11 Sep 2008), epilimnion N_2 concentrations were not strongly supersaturated. In fact, mean epilimnion N_2 concentrations were undersaturated on four of the eight sampling dates. A general trend of increasing N_2

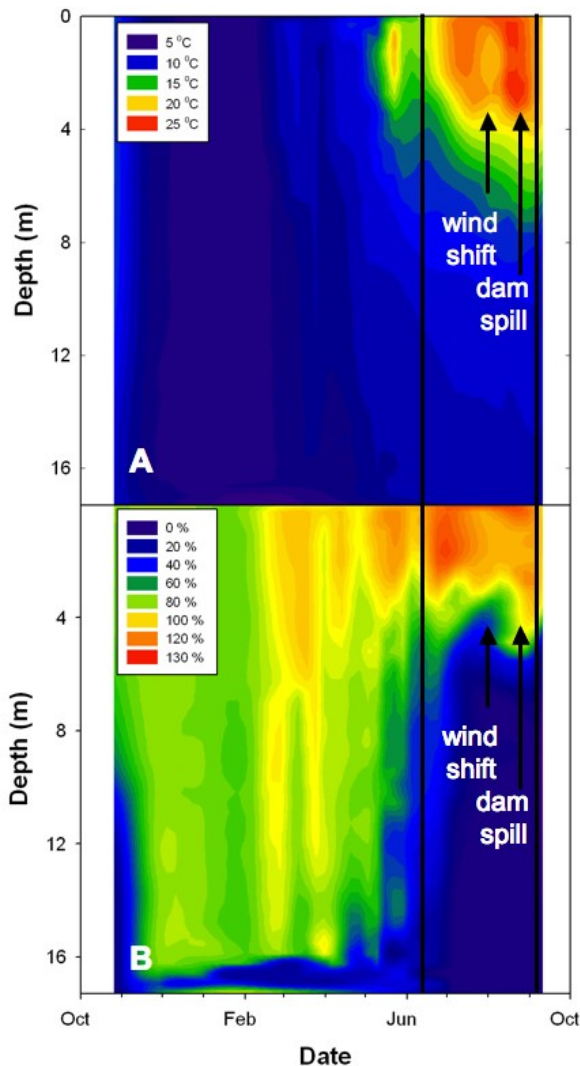


Figure 2: Water column temperature (A) and dissolved oxygen saturation (B) during water year 2008. The summer 2008 sampling period is bracketed by black lines and disturbance events are indicated by arrows.

and N_2O concentrations over the summer sampling period was observed at each sampling depth below the thermocline (Figure 3).

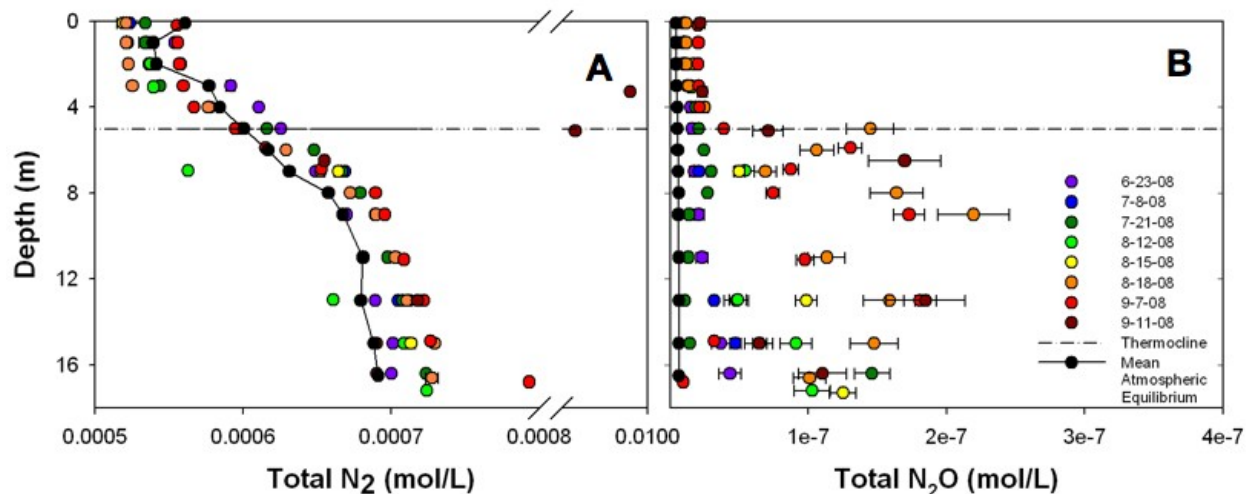


Figure 3: Expected and measured concentrations of water column N_2 (A) and N_2O (B) across the sampling period. Solid black dotted lines mark the mean concentration of solute that is expected due to atmospheric equilibration; error bars on these points represent the standard error associated with temperature changes across the sampling period. Colored data points represent measured values; error bars on these points represent coefficients of variation associated with measurement error. Dashed lines indicate the approximate location of the reservoir thermocline.

Summertime Accumulation and Depletion Rates in the Hypolimnion

The mass of both N_2 and N_2O in the reservoir hypolimnion increased significantly with time ($p < 0.05$, Figure 4), supporting the use of solute accumulation rates as a proxy for gas fluxes. Average hypolimnion accumulation flux rates were $155 \mu\text{mol } N_2\text{-N } m^{-2} h^{-1}$ and $47 \mu\text{mol } N_2O\text{-N } m^{-2} d^{-1}$ which fall within the range of values reported in other reservoir systems (Figure 5). Calculated fluxes of solutes across the thermocline were relatively minimal ($59.5 \mu\text{mol } N_2\text{-N } m^{-2} h^{-1}$, ± 3.3 , $n=8$ and $0.57 \mu\text{mol } N_2O\text{-N } m^{-2} d^{-1}$, ± 0.05 , $n=8$) compared to the hypolimnion accumulation rates observed. While thermocline transfer rates are calculated assuming a fully mixed hypolimnion, observations during a 14m dye injection suggest minimal hypolimnion mixing in the vertical direction.

While increases in N_2 mass were generally linear, there was a notable steep increase in hypolimnion N_2 observed between 23 Jun 2008 and 8 Jul 2008 followed by a slower rate of N_2 accumulation between 8 Jul 2008 and 7 Sep 2008, and, finally, a drop off in N_2 on 11 Sep 2008 (Figure 4a). In fact, removing the 11 Sep 2008 sampling point improves the linear relationship between N_2 mass and time markedly ($R^2=0.81$, $p < 0.01$).

Excluding 11 Sep 2008, mean summertime N_2 flux was $155 \mu\text{mol } N_2\text{-N m}^{-2} \text{ h}^{-1}$, but denitrification rates as high as $538 \mu\text{mol } N_2\text{-N m}^{-2} \text{ h}^{-1}$ are possible early in the stratification event, while later in the stratification event rates may be as low as $96.5 \mu\text{mol } N_2\text{-N m}^{-2} \text{ h}^{-1}$. A 31% drop in hypolimnion N_2 mass between 7 Sep 2008 and 11 Sep 2008 makes quantifying denitrification rates over this time period more complex. A large dam spill was occurring during this time, and significant changes in hypolimnion NH_4^+ (82% drop) and NO_3^- (50% drop) were also observed.

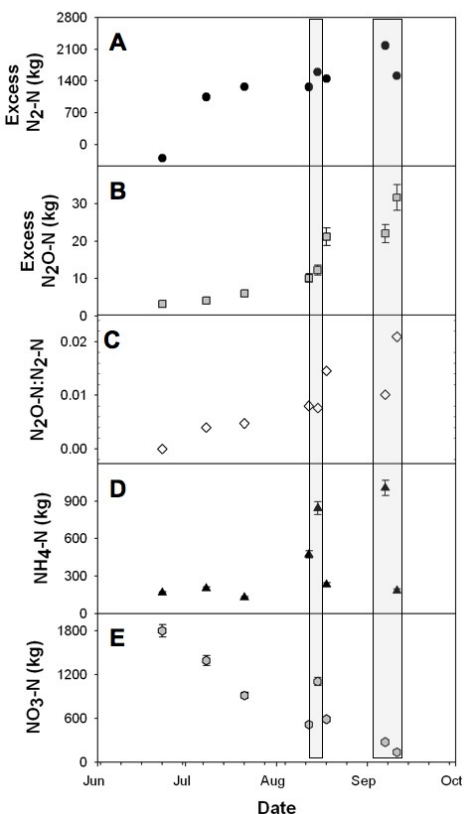


Figure 4: Excess hypolimnion mass of $N_2\text{-N}$ (A), and $N_2O\text{-N}$ (B), $N_2O\text{-N:N}_2\text{-N}$ mass ratios (C), $NH_4\text{-N}$ (D), and $NO_3\text{-N}$ (E) during the summer of 2008. Error bars represent coefficients of variation associated with measurement error. Shaded area corresponds to disturbance events.

Hypolimnion $N_2O\text{-N}$ mass also increased significantly during the sampling period ($p < 0.05$). Unlike patterns in hypolimnion $N_2\text{-N}$, $N_2O\text{-N}$ mass increased the most later in the season (Figure 4b). This resulted in $N_2O\text{-N:N}_2\text{-N}$ mass ratios that increased significantly over the summer stratified period ($p < 0.05$, Figure 4c) and averaged 0.01 (± 0.002 , $n=8$).

Measurable depletion of NO_3^- in the hypolimnion was also observed during the sampling period. $NO_3\text{-N}$ decreased significantly with time over the stratification event ($p < 0.01$, Figure 4e). A mean summertime $NO_3\text{-N}$ depletion flux of $112 \mu\text{mol } N_2\text{-N m}^{-2} \text{ h}^{-1}$ was calculated and $2.0 \mu\text{mol } NO_3\text{-N m}^{-2} \text{ h}^{-1}$ (± 0.97 , $n=8$) was calculated to pass across the thermocline. Sediment driven NO_3^- depletion could thus be overestimated by as much as 1.8%. After considering the degree to which sediment NO_3^- loss is potentially overestimated and N_2 flux underestimated (see discussion for description of potential N_2 overestimation), hypolimnion $NO_3\text{-N}$ depletion may explain as

little as 53% of the observed N_2 flux. Alternatively, if the hypolimnion is poorly mixed and limited thermocline transfer is actually occurring, as much as 69% of hypolimnion $NO_3\text{-N}$ depletion could be explained by N_2 production. Hypolimnion $NH_4\text{-N}$ generally increased over the sampling period (Figure 4d) and hypolimnion DIN concentrations generally decreased though neither of these relationships is statistically significant. If

the 11 Sep 2008 sampling point is added to these regression equations, summertime DIN decreases more steeply and less ammonium appears to accumulate, suggesting a role for larger dam spill in hypolimnion N dynamics.

Alternative Flux Estimates

Surface N₂O fluxes calculated based on estimated gas transfer velocities were approximately 6% of the value of sediment fluxes derived from hypolimnion N₂O accumulation (Figure 6), averaging 2.87 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{ d}^{-1}$ (± 0.62 , $n=8$). Surface N₂ flux was extremely variable suggesting that the dominant process, denitrification or N fixation, was shifting dramatically over time. On average, surface N₂ fluxes were -330 $\mu\text{mol N}_2 \text{ m}^{-2} \text{ d}^{-1}$ though numbers were extremely variable ranging from -2784 to 648 $\mu\text{mol N}_2 \text{ m}^{-2} \text{ d}^{-1}$.

Core incubations and potential denitrification assays produced denitrification flux estimates drastically lower than estimates derived from hypolimnion N₂ accumulation estimates (Figure 6). Nitrate uptake rates in cores with no nutrient addition averaged 0.04 $\mu\text{mol NO}_3\text{-N m}^{-2} \text{ h}^{-1}$, representing only 0.0003% of the N₂-N flux and 0.0004% of the NO₃-N flux calculated using the hypolimnion accumulation method. Acetylene block assays revealed net consumption of N₂O in vials with no nutrient addition.

Location of N₂ and N₂O Production

Potential denitrification experiments indicate that potential denitrification in sediments is much higher than in the water column. Potential denitrification rates in vials containing sediments and amendments of KNO₃ were nearly three orders of magnitude greater than in vials with KNO₃, hypolimnion water, but no sediments (0.777 $\mu\text{mol N}_2\text{O d}^{-1}$ vs. 0.00194 $\mu\text{mol N}_2\text{O d}^{-1}$). Also, field samples showed higher N₂ concentrations near the sediment-water interface than further up in the water column, indicating the predominance of sediment-based production (Figure 3a). While N₂O exhibits this pattern early in the summer, the pattern disappears in August (Figure 3b), suggesting that N₂O production may be occurring higher in the water column as the summer progresses.

Sediment denitrification was nitrate limited during the summer stratified period. While untreated acetylene block assays of reservoir sediment show net uptake of N₂O, indicating insignificant rates of denitrification, KNO₃ amended vials show positive fluxes of N₂O equivalent to denitrification rates of 0.777 $\mu\text{mol N}_2 \text{ d}^{-1}$

Table 1: Inputs to the linear regression analysis, all values are hypolimnion means.

	23 Jun- 8 Jul	8 Jul- 21 Jul	21 Jul- 12 Aug	12 Aug-15 Aug	15 Aug- 18 Aug	18 Aug- 7 Sep	7 Sep- 11 Sep
NO ₃ -N (mg L ⁻¹)	0.64	0.56	0.35	0.36	0.4	0.21	0.1
NO ₂ -N (mg L ⁻¹)	0.005	0.004	0.005	0.015	0.019	0.01	0.003
NH ₄ -N (mg L ⁻¹)	0.095	0.085	0.141	0.274	0.242	0.295	0.307
PO ₄ -P (mg L ⁻¹)	0.005	0.001	0.001	0.002	0.002	0.001	0
Stability (kg s ⁻²)	0.0018	0.0022	0.0023	0.0023	0.0025	0.0021	0.0016
Temp °C	9.7	9.9	9.98	9.96	10.4	10.8	10.42
Conductivity (mS cm ⁻¹)	75.65	88.7	101.38	307.37	193.81	83.76	92.33
pH	5.95	5.91	6.73	7.81	8.4	7.68	6.4
DO (mg/L)	2.52	0.81	0.38	0.25	0.25	0.37	0.32
kg N ₂ -N d-1	89.70	17.68	-0.45	110.55	-48.57	36.37	-167.25
kg N ₂ O-N d-1	0.06	0.14	0.19	0.69	2.98	0.043	2.40
Δ N ₂ O-N:N ₂ -N	—	6 x 10 ⁻⁵	0.0001	-0.0001	0.0023	-0.0002	0.0027

Table 2: Linear regression statistics; direction of correlation is indicated by the sign of the R^2 value.

	ΔN_2-N		ΔN_2O-N		$\Delta N_2O-N:N_2-N$	
	R^2	p	R^2	p	R^2	p
NO ₃ -N (mg L ⁻¹)	0.38	0.13	-0.17	0.36	-0.17	0.42
NO ₂ -N (mg L ⁻¹)	0.05	0.64	0.19	0.32	0.01	0.85
NH ₄ -N (mg L ⁻¹)	-0.14	0.41	0.27	0.24	0.15	0.45
PO ₄ -P (mg L ⁻¹)	0.25	0.25	-0.02	0.77	0.01	0.83
Stability (kg s ⁻²)	0.15	0.40	0.00	0.99	-0.17	0.41
Temp °C	-0.22	0.29	0.15	0.33	0.09	0.56
Conductivity (mS cm ⁻¹)	0.10	0.48	0.08	0.55	-0.004	0.90
pH	0.004	0.89	0.18	0.33	0.01	0.87
DO (mg L ⁻¹)	0.18	0.36	-0.17	0.36	-0.13	0.49

(Figure 7). Intact core experiments also showed a positive relationship between NO₃-N concentration and NO₃-N removal rate. Significantly higher NO₃-N uptake rates were observed in sediment cores amended with nitrate and carbon + nitrate than in other treatments (one-way ANOVA and Tukeys multiple range test, $p < 0.05$, Figure 5). Field data also points toward sediment nitrate limitation. NO₃-N concentrations 0.2m off the reservoir bottom were low ($< 90 \mu\text{g L}^{-1}$ with the exception of 23 Jun 2008) and significantly lower than NO₃-N concentrations in the rest of the hypolimnion (one-tailed t-test assuming unequal variance, $p < 0.05$).

Inputs to the linear regression analysis are presented in Table 1. Change in hypolimnion N₂-N and N₂O-N mass and N₂O-N:N₂-N ratios were not significantly correlated to any environmental variables. No relationship between NO₃-N or DO and N₂-N was apparent from linear regression analysis, even when bottom-water NO₃-N and DO were regressed against hypolimnion N₂-N change.

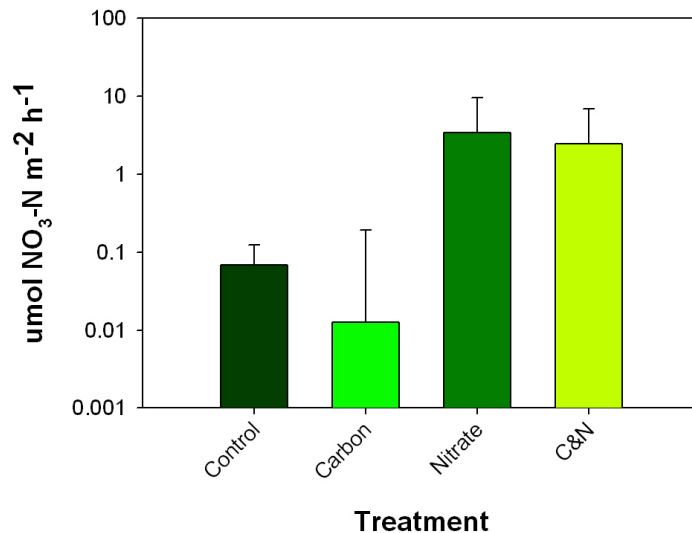


Figure 5: Mean NO₃-N removal rates by treatment for Aug 2008 sediment incubation, standard error bars shown.

Discussion

The Hypolimnion as an In-Situ Incubation Chamber

In Lacamas Lake, strong thermal stratification and measurable hypolimnion accumulation of both N_2 and N_2O make it possible to employ the novel, in-situ approach outlined here to quantify hypolimnion wide denitrification and N_2O production. Calculated rates of denitrification and N_2O fluxes based on this approach are similar to other published rates of denitrification and N_2O production in reservoirs (Figure 6). Denitrification flux estimates also compare reasonably with a preliminary mass balance N removal rate of $61 \mu\text{mol } N_2-N \text{ m}^{-2} \text{ h}^{-1}$ calculated using summer 2008 inlet and outlet NO_3-N samples (see Figure 1 for sampling locations) and 15min discharge data from the Clark County Lacamas Creek at Goodwin Road flow gauge (Clark County Water Resources, <http://www.co.clark.wa.us/water-resources/monitoring/flow.html>). Estimates of N removal rate from sediment incubations and denitrification potential assays were drastically lower than estimates produced by the hypolimnion accumulation method, suggesting significant spatial and/or temporal heterogeneity in sediment process rates.

Although hypolimnion accumulation represents a promising new way to estimate N transformations

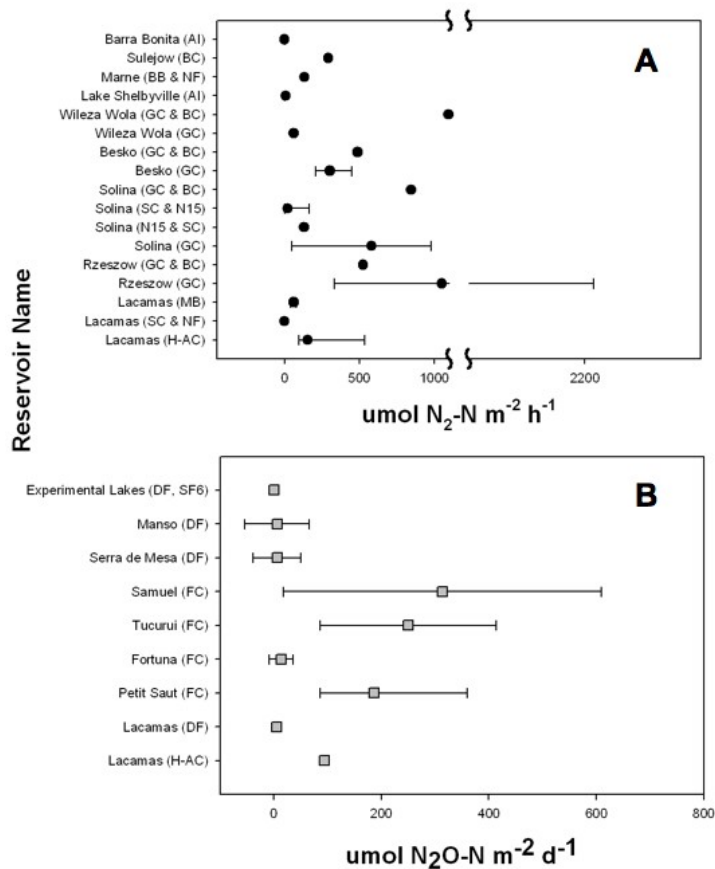


Figure 6: Denitrification rates (A) and N_2O production rates (B) published in other reservoir systems as well as rates quantified in this study. Method approaches are abbreviated in parentheses: Acetylene Inhibition (AI), Sediment Cores (SC), Nitrate Flux (NF), Hypolimnion Accumulation (H-AC), Benthic Chambers (BC), Gas Chromatography (GC), ^{15}N Isotope Pairing (N15), Diffusive Flux (DF), Floating Chambers (FC), and Diffusive Flux based on in-situ quantification of gas transfer velocity (SF6,DF). Published denitrification rates were compiled from Tomaszek and Czerwieniec 2000, Tomaszek and Czerwieniec 2003, Gruca-Rokosz & Tomaszek 2007, David et al. 2008, Josette et al. 1999, Bednarek and Zalewski 2007 and Abe et al. 2003. Published N_2O fluxes were taken from Guérin et al. 2008. Note the difference in units on the x axes.

within lakes and reservoirs, it can not be applied indiscriminately. For example, it is important to consider both thermocline transfer and hypolimnion temperature fluctuations. Temperature changes within the hypolimnion can influence the amount of excess N_2 and N_2O calculated. For instance, a general trend of increasing hypolimnion temperature during summer sampling will cause increasing amounts of atmospheric N_2 previously dissolved in the hypolimnion to be counted as excess N_2 creating an artificial accumulation of excess N_2 due only to the changing solubility-temperature relationships. In Lacamas, mean hypolimnion temperatures fell in a narrow range ($8.9^{\circ}C$ - $10.2^{\circ}C$) over the stratification event. Such a change in temperature would explain a total increase of 67 kg excess N_2 -N within the hypolimnion over the study period, constituting only 3.7% of the observed increase of 1810 kg N. Such a potential overestimation of N_2 flux is overshadowed by estimated N_2 fluxes across the thermocline, which averaged $59.5 \mu\text{mol } N_2\text{-N m}^{-2} \text{ h}^{-1}$ over the summer stratification event or 38% of summertime N_2 accumulation. Thus hypolimnion flux rates calculated by this method may underestimate denitrification rates by as much as 34.3%. It should be noted that the absolute degree of underestimation is difficult to ascertain because the extent to which the hypolimnion is well mixed is unknown. A mean flux of $208 \mu\text{mol } N_2\text{-N m}^{-2} \text{ h}^{-1}$ calculated based on a 34.3% underestimation of N_2 production thus represents an upper bound on hypolimnion denitrification rates. However, limited vertical mixing in the Lacamas hypolimnion suggests that actual mean fluxes are likely closer to the $155 \mu\text{mol } N_2\text{-N m}^{-2} \text{ h}^{-1}$ calculated based on hypolimnion accumulation. Transfer across the thermocline constitutes most of the error associated with hypolimnion N_2O flux estimates, averaging $0.57 \mu\text{mol } N_2O\text{-N m}^{-2} \text{ d}^{-1}$ or 1.2% of summertime N_2O accumulation. Hypolimnion temperature changes explain much less of the observed change in excess N_2O -N over the sampling period. A $1.3^{\circ}C$ increase in hypolimnion temperatures accounts for only 4g excess N_2O -N over the sampling period, constituting only 0.01% of the observed increase of 1.97 kg N_2O -N. Thus, hypolimnion flux rates calculated by this method likely underestimate N_2O flux by about 1.2%. The larger degree to which N_2 -N flux is underestimated relative to N_2O flux suggests that observed hypolimnion N_2O -N: N_2 -N ratios are higher than actual production ratios. The disproportionate underestimation of N_2 flux does not, however, affect the pattern of increasing N_2O -N: N_2 -N over the stratification event.

While the hypolimnion accumulation approach successfully integrates spatio-temporal variability in pro-

fundal process rates, it should not be used as a proxy for whole-system N-removal rate. Littoral and profundal sediments likely behave quite differently within a given system. The vegetated littoral zone could contribute disproportionately to whole-system denitrification (Saunders and Kalff 2001b) due to the prevalence of oxic micro-sites on plant roots which support nitrification and associated denitrifier communities (Christensen and Sorensen 1986). While some studies find no relationship between denitrification rates and sediment depth (David et al. 2006), a number of studies find higher N removal at littoral sites (Saunders and Kalff 2001b, Koszelnik et al. 2007, Gruca-Rokosz 2007). Littoral zones have also been identified as hot spots for N_2O production (Wang et al. 2006). Future studies should focus on developing similar in-situ integrative approaches to quantifying littoral process rates so that spatio-temporally integrated whole-system estimates of lentic N fluxes can better be achieved.

Stratification as a Limit to N Removal

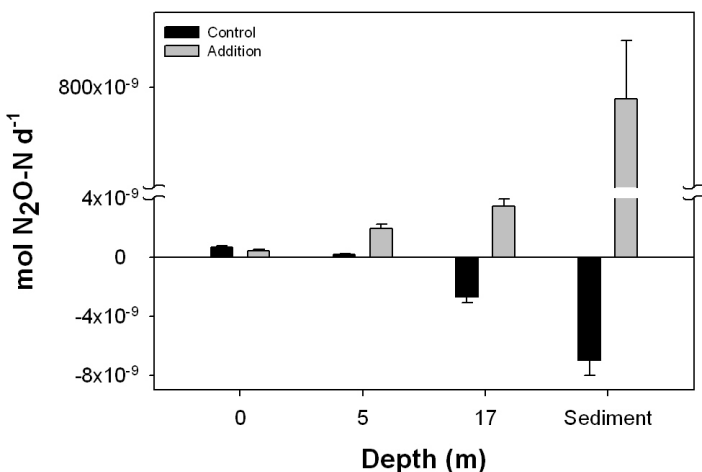


Figure 7: Potential denitrification rates from acetylene block, error bars indicate coefficient of variation associated with measurement error.

Rapid accumulation of N_2 within the hypolimnion of Lacamas Lake during the early summer accounts for the removal of at least 1345 kg N over 16 days. This is equivalent to 0.44 mg L^{-1} of N removed from the hypolimnion, 0.17 mg L^{-1} of N removed from the entire reservoir, or about 20% of the nitrate present prior to stratification (mean winter concentration is $0.84 \text{ mg L}^{-1} \pm 0.03$, unpubl. data).

Similar spring increases in denitrification rate have been observed in several Polish reservoirs (Tomaszek and Czerwieniec 2000, Tomaszek and Czerwieniec 2003). In these systems, spring algal production was suspected to relieve organic substrate limitation and enhance denitrification (Tomaszek and Czerwieniec 2000). In Lacamas, however, high profundal sediment carbon content across seasons (mean 7.8% C) and no obvious boost in N or C sedimentation rates during spring or early summer (Z. Budiselic, unpubl.) suggests that

denitrification is not carbon limited and that other factors are controlling denitrification during this time period. Thermal stratification and the resultant hypoxic hypolimnion is likely the primary driver of limited nitrate supply across the sediment-water interface, both through chemical (no oxygen for nitrification) and physical (thermocline as a transport barrier) means.

Results from acetylene block assays indicate that conditions at the sediment-water interface are most likely to control denitrification rates. Oxygen profile data from earlier during the stratification event show that boosted N removal rates between 23 Jun 2008 and 8 Jul 2008 corresponds to the time-period when bottom water is becoming hypoxic (Figure 2). It is during this time period that near-sediment oxygen conditions are sufficiently low to support high N removal, but still high enough to support nitrification proximal to denitrifying communities (ie: coupled nitrification-denitrification processes). As the summer progresses, bottom water oxygen concentrations drop off to 0.8 mg L^{-1} (± 0.02 , $n=6$). Severe nitrate limitation and a mean bottom-water $\text{NO}_3\text{-N}$ concentration of 0.05 mg L^{-1} (± 0.01 , $n=6$) between 21 Jul and 11 Sep 2008 suggest that as the summer progresses, low oxygen limits hypolimnetic nitrification and nitrate production becomes distal to denitrifying communities. Beutel and others also observed severe NO_3^- limitation of sediment NO_3^- uptake in the deoxygenated profundal sediments of a eutrophic reservoir (2008). In this case, uptake rates increased significantly under mixed conditions (Beutel et al. 2008).

Although several lines of evidence suggest that sediment denitrification is nitrate limited, there was no significant relationship between N_2 and NO_3^- . In fact, the analysis failed to identify any significant predictors of N_2 production. This is likely due to the relatively small fluctuations in bottom water NO_3^- (and O_2) observed after 23 Jun 2008. The largest fluctuations in hypolimnion chemistry are observed during the first sampling dates, which fall during the final stages of stratification, and between 7 Sep and 11 Sep 2008 when approximately 20% of the water in Lacamas was being spilled over the dam. Excluding these dates from the regression analysis does not result in any significant relationships, likely because smaller scale fluctuations in NO_3^- and O_2 availability are not captured by our sampling regime.

While some reservoir systems can act to remove a highly significant mass of N from the water column, dissolved inorganic nitrogen concentrations within Lacamas do not decrease significantly during the stratified period. Thermal stratification appears to isolate denitrifiers from a nitrate source, thus limiting the capacity

of the system to remove N efficiently.

Though for most of the summer the hypolimnion behaved as a closed system, patterns in hypolimnion chemistry during this period reveal the potential importance of disturbance events in mixing epilimnion waters downward to relieve sediment nitrate limitation. While lentic boundary mixing during stratified conditions is traditionally thought of as a process that mixes nutrients upwards into the epilimnion to stimulate primary production (Ostrovsky et al 1996), these events also appear to exert a strong effect on the composition of the hypolimnion. The fall dam spill, in particular, appears to exert the strongest effect. Water is spilled directly from the epilimnion at this time, but significant drops in hypolimnion NH_4^+ , NO_3^- , and N_2 indicate that surface disturbance impacts hypolimnion chemistry (Figure 5). N transformation processes are likely also directly impacted by dam spill events. While hypolimnion N_2 drops off during dam spill, N_2O concentrations remain relatively high. Future work should investigate how dam spill impacts these flux rates given the important management implications of such knowledge.

Controls on N_2O Production

N_2O fluxes reach their maximum during late summer despite the relatively low rates of denitrification observed during this time. Because N_2O production is typically associated with both nitrification and denitrification, the observed N_2O patterns in this system are somewhat counterintuitive. Extremely low oxygen conditions make any significant rates of nitrification unlikely, however small amounts of nitrification may produce a high proportion of N_2O , contributing significantly to total system N_2O flux. High N_2O concentrations corresponding to low oxygen conditions have been observed as far back as 1978 by Elkin and others. One study of ammonium oxidizing bacteria (*Nitrosomonas* sp.) incubated under different oxygen condition and showed a shift from 0.3% to 10% N_2O production under low oxygen conditions (Goreau et al. 1980). Some of the highest concentrations of N_2O ever reported in an aquatic system were measured in the hypoxic hypolimnion of an Antarctic lake (Priscu et al. 1996) and isotopic analysis implied that most of this N_2O was produced via incomplete nitrification (Priscu et al. 2008). Patterns in the location of summertime N_2O production suggest that early summer N_2O production is primarily due to sediment denitrification, whereas late summer N_2O production is mostly due to partial nitrification higher up in the water column

under extremely low oxygen conditions.

While N_2O production increases over the stratification period, $\text{N}_2\text{O-N:N}_2\text{-N}$ ratios remain low. Mean $\text{N}_2\text{O-N:N}_2\text{-N}$ ratios are relatively low compared to those reported for other aquatic systems (Silvennoinen et al. 2009, Woodward et al. 2009) despite N_2O fluxes that fall within the mid-range of reported values for other reservoirs (Figure 6). Using Lacamas N_2O flux rates as a conservative estimate of the average flux rate for all reservoir systems, and assuming a global reservoir surface area of $2.5 \times 10^5 \text{ km}^2$ (as in Harrison et al. 2009), annual N_2O fluxes are $6.0 \times 10^{10} \text{ g yr}^{-1}$ or about 0.003% of the global annual N_2O emissions to the atmosphere (IPCC 2001).

Alternate N Removal Pathways

In Lacamas' hypoxic hypolimnion, it is notable that a large portion of the observed N_2 accumulation is left unexplained by nitrate depletion. NO_3^- production within the hypolimnion could account for discrepancies between NO_3^- depletion and N_2 accumulation rates, but given the extremely low oxygen conditions nitrification is unlikely. While the heterotrophic reduction of NO_3^- to N_2 gas is traditionally considered the most common microbial N removal pathway, there is an emerging body of research suggesting that alternate pathways may play important roles in the removal of aquatic N (Francis et al. 2007, Jetten 2008, Arrigo 2005, Burgin and Hamilton 2007). In particular, anaerobic ammonium oxidation (anammox) is emerging as an important contributor to aquatic N removal (Francis et al. 2007). This autotrophic pathway oxidizes biologically available NH_4^+ to non-reactive N_2 gas in the absence of oxygen with NO_2^- typically serving as the electron donor. A number of marine studies have shown anammox to be a primary contributor to N removal (Thamdrup and Dalsgaard 2002, Dalsgaard and Thamdrup 2002, Kuypers et al. 2005, and Hamersley et al. 2007), but less is known about the relative role of anammox in freshwater systems. Some studies suggest high anammox potential in freshwaters (Schubert et al. 2006, Hamersley et al. 2009); one study of a temperate lake found that anammox accounted for all the wintertime microbial N removal (Hamersley et al. 2009). Other studies find relatively low anammox rates in freshwater systems (Koop-Jakoben and Giblin 2009). Anammox has been documented in two lentic systems that we are aware of, both of which are permanently stratified, and in both cases activity was documented near the thermocline (Schubert et al.

2006, Hamersley et al. 2009). Abundant hypolimnion NH_4^+ in Lacamas, combined with a nitrite maximum near the thermocline (unpubl. data) suggests that water column anammox may be a partial source of N removal and N_2 production during summer stratification. While anomalously high excess N_2 concentrations near the thermocline on 11 Sep 2008 (Figure 3) could be the result of bottom-water N_2 mixing upward, they more likely result from anammox within the thermocline. NH_4^+ and NO_2^- concentrations peak at 6m on 7 Sep 2008 and drop off by 11 Sep 2008 further supporting a hot moment for thermocline anammox during dam spill.

Conclusions

While NO_3^- availability (Gruca-Rokosz and Tomaszek 2007, Beutel et al. 2008), organic carbon availability (Bednarek and Zalewski 2007), and temperature (Gruca-Rokosz and Tomaszek 2007) have been implicated as important controllers of denitrification rate in reservoir systems, results from our study emphasize the role of stratification and physical disturbances as drivers of N transformations. In a reservoir system, this finding has particularly compelling management implications because the hydrology can be manipulated by controlling spill practices.

We emphasize the use of robust in-situ techniques in developing an understanding of the patterns and controls on aquatic N transformations. This study represents the first application of in-situ hypolimnion accumulation techniques in a freshwater system. There is strong potential for this method to be broadly applied across systems, including but not limited to groundwater, lakes, and ice-capped waterbodies. Measuring changes in hypolimnion chemistry during stratification uncovered a number of interesting relationships in the Lacamas reservoir system. High rates of N_2 accumulation early in the stratification event emphasize the potential importance of springtime as a hot moment for N removal. The causes of higher N_2O accumulation later in the summer deserve further investigation. Differences in the relative rates of NO_3^- depletion and N_2 accumulation suggest the importance of alternate N removal pathways in Lacamas Lake. Future efforts should aim to develop similar integrative in-situ estimates under de-stratified conditions and in littoral zones such that whole-system responses to changes in hydrology can be better quantified.

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