12-2011

Nitrogen Retention and Denitrification in Reservoirs

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NITROGEN RETENTION AND DENITRIFICATION IN RESERVOIRS

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Crop, Soil and Environmental Science

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ABSTRACT

Studies coupling direct measurements of micro-scale nitrogen (N) cycle processes with ecosystem-scale flux estimates are needed to determine N retention hotspots within river networks, where up to 50% of terrestrial loading to aquatic systems is retained. This study examined the role of denitrification, a microbially-mediated reactive N removal pathway, in ecosystem-scale N retention in 3 small (< 1 km^2), shallow flood-control reservoirs. Annual reservoir N retention was estimated through mass balance modeling of system inputs and outputs. Annual denitrification rates were estimated by combining multiple measurements of seasonal, habitat-specific dinitrogen gas (N_2) fluxes. Annual reservoir N retention ranged from 14 - 19 g m^{-2} in the reservoirs, while reactive N removal through denitrification was 13 - 25 g m^{-2}. Denitrification efficiency, or the portion of the retained N load that was denitrified, was high relative to other lentic systems and was > 100% at 2 sites. Previous lentic denitrification studies may have underestimated denitrification efficiency by not considering water column denitrification, which was 50% of total denitrification at one study reservoir. However, not all potential inputs, most importantly biological N_2 fixation, were included in this study's mass balance model, which likely led to underestimation of N retention. This study's findings indicate that reservoirs are N sinks in the landscape, and that denitrification plays a major role in regulating long-term storage of both watershed and biologically-fixed N loads in lentic systems.

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ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. J. Thad Scott for his support and guidance during my graduate studies. Thank you for the many opportunities to grow as a scientist during our work together. I would also like to thank Brad Austin, Bryant Baker, Wade Cash, Stephanie Cepeda, Bodie Drake, Stephanie Kulesza, Alden Smartt, Ben Thompson, Erin Scott, Lucy Stowe and Joe Wyatt for assistance with field and lab work related to this project. Funding for the project was provided by the Arkansas Water Resource Center through the USGS 104b National Institute of Water Research Program.

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LIST OF PAPERS

Grantz, Erin M., A. Kogo and J. Thad Scott. In Review. Partitioning ecosystem-scale denitrification in three shallow reservoirs using in situ dinitrogen gas accumulation and intact sediment core experiments. Limnology and Oceanography.

Grantz, Erin M., J. Thad Scott and Brian E. Haggard. Nitrogen retention and denitrification efficiency in reservoirs.

1. INTRODUCTION

Anthropogenic activities including the production and widespread application of synthetic nitrogen (N) fertilizers have dramatically increased the modern biologically-active terrestrial N pool and subsequent fluxes to adjacent aquatic systems (Vitousek et al. 1997, Galloway et al. 2008). The negative environmental impacts of increased N availability in aquatic ecosystems, particularly in sensitive coastal waters, are now well-recognized and include eutrophication, hypoxia, fish kills and biodiversity loss (Howarth et al. 1996, Vitousek et al. 1997). Hypoxia in the Gulf of Mexico not only affects the ecosystem's health and biodiversity, but also negatively impacts local economies dependent on fishing and tourism.

Despite the clearly identified impacts of increased N loading to the Gulf of Mexico, as much as 50% of terrestrial N loading to aquatic systems does not reach coastal waters and is intercepted within river networks (Wollheim 2008). These river networks are comprised of wetlands, headwater streams, higher order rivers, and natural and man-made lakes, all of which have variable hydrologic regimes and nutrient cycling rates (Sanders and Kalff 2001, Seitzinger et al. 2002, 2006). Within these varied ecosystems N is retained and transformed through N cycle processes including biological uptake, sedimentation, or denitrification, which is the microbially-mediated conversion of reactive N to inert dinitrogen gas (N_2) and the only permanent removal pathway for bioavailable N from the biosphere. Quantifying the rates of N cycle processes such as N retention and denitrification in the landscape is vital in developing management strategies for reducing N loading to fragile coastal ecosystems.

The relative magnitude of N retention and denitrification is not well understood across aquatic systems. Within river networks of the northeastern United States, Seitzinger et al. (2002) estimated that individual stream/river reaches retained approximately 20% of loading, though

retention increased to 37 – 76% when summed across all reaches within a watershed. Mullholand et al. (2008) found that denitrification accounted for more than 43% of N removal in a quarter of 72 stream reaches. On average, wetlands and natural lakes retain approximately 64 and 34% of N inputs, respectively (Saunders and Kalff 2001), though some natural lakes have exhibited retention efficiency as high as 70% (Menghis et al. 1997). Within wetlands and natural lakes, denitrification is believed to contribute significantly to N retention due to increased hydraulic residences times relative to lotic systems (Sanders and Kalff 2001).

Fewer studies have specifically investigated N retention and denitrification in reservoir ecosystems (David et al. 2006, Kozelnik et al. 2007). However, high area-specific nutrient loading (Kozelnik et al. 2007) and high rates of carbon burial (Downing et al. 2008) make man-made impoundments potential landscape-scale denitrification hotspots. The currently available data suggest that reservoirs may play a disproportionate role in landscape-scale N retention relative to natural lakes, which comprise a larger proportion of the global lentic system surface area (Harrison et al. 2009). As much as 33% of global N retention in river networks may occur in reservoirs, despite the fact that reservoirs account for only 6% of the total surface area of lentic systems. When reservoir N retention rates are normalized to reservoir surface area, estimated retention is estimated to be 7-8 x greater than by natural lakes.

Estimates of ecosystem N retention in lentic systems are also rarely paired with direct measurements of denitrification. Lake and reservoir N budgets have often simply equated N removal through denitrification with “missing” N in mass balance models (Jensen et al. 1992, Molot and Dillon 1993, Garnier et al. 1999). Two recent studies paired N retention estimates with direct laboratory measurements of denitrification, but reached divergent conclusions. David et al. (2006) measured denitrification using acetylene inhibition in sediment slurry assays,

concluding that over 50% of N retention in Shelbyville Reservoir (Illinois, USA) could be attributed to denitrification, while Kozelnik et al. (2007) used isotope pairing techniques during intact sediment core incubations and found low denitrification rates that accounted for only 16.4% of N retention at Solina Reservoir in Poland. These findings may represent the naturally occurring variability in lentic denitrification efficiency, but may also reflect limitations of the methods employed to measure denitrification, particularly in regards to describing ecosystem-scale rates (Groffman et al. 2006, 2009).

Studies of lentic denitrification are largely limited by the cost, feasibility, and range of currently available methods. Methods like ^{15}N tracer studies that have been employed successfully in relatively small-volume lotic systems are difficult and costly to apply in larger-volume lakes and reservoirs (Mulholland et al. 2008). Other techniques that measure denitrification indirectly, such as acetylene block in sediment slurries (David et al. 2006), are unable to provide information encompassing micro-scale intricacies, and are also difficult to make relevant at the ecosystem scale (Groffman et al. 2006, 2009).

Intact sediment cores used in conjunction with membrane inlet mass spectrometry (MIMS; Kana et al. 1994) can provide direct and accurate measurements of denitrification (Scott et al. 2008), but these studies are also limited by the spatial scale represented by the cores and the relatively small number of cores that can practically be accommodated. Other recent studies have used MIMS to measure naturally-occurring temporal and spatial N_2 gradients in aquatic systems (Deemer et al. 2011). This technique exploits water density gradients which temporarily isolate the lower water column of many estuaries, lakes, and reservoirs. Transformation into virtually closed systems results in a predictable depletion of oxidized compounds like oxygen (O_2) and

nitrate, accompanied by the accumulation of reduced compounds like N_2. No studies have yet combined these approaches to generate whole-ecosystem denitrification estimates.

The objectives of my study were 1) to estimate annual N retention in 3 reservoirs representing a relatively understudied, but potentially important, size class (< 1 km^2) of reservoirs, 2) to estimate annual whole-ecosystem denitrification rates in the study reservoirs by combining multiple direct measurements of denitrification suited to specific habitats in reservoir environments, and 3) to compare N retention and denitrification estimates to determine denitrification efficiency, or the proportion of retained N permanently removed from the study reservoirs as inert N_2. The whole-reservoir total nitrogen (TN) flux was estimated as the difference between system inputs and outputs in a mass balance model. Reactive N removal through denitrification was estimated using intact sediment core incubations of epilimnetic sediments and from N_2 accumulation rates in the reservoir hypolimnia and anoxic metalimnia.

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2. PARTITIONING ECOSYSTEM-SCALE DENITRIFICATION IN THREE SHALLOW RESERVOIRS USING IN SITU DINITROGEN GAS ACCUMULATION AND INTACT SEDIMENT CORE EXPERIMENTS

2.1 Introduction

Anthropogenic activities have increased the modern reactive nitrogen (N) pool (Vitousek et al. 1997), and the resulting terrestrial loading of bioavailable N to adjacent aquatic ecosystems has been tied to adverse environmental impacts and human economic losses (Galloway et al. 2008). Inland freshwater networks transport terrestrially-applied N to sensitive downstream ecosystems, but freshwater networks also retain up to 50% of watershed N inputs (Wollheim et al. 2008). The magnitude of this ecosystem service differs between aquatic systems, with greater N retention efficiency in systems with long water residence times, such as natural lakes and manmade reservoirs (Sanders and Kalff 2001, Kozelnik et al. 2007).

Watershed-scale N retention occurs through the sum of micro-scale processes that retain, transform, and remove reactive N, but the relative contribution of these processes is not well understood across freshwater ecosystems. Denitrification, defined here as microbial dinitrogen gas (N_2) production through dissimilatory nitrate reduction or anaerobic ammonium oxidation (annamox), occurs readily in aquatic sediments (Seitzinger et al. 1988) and permanently removes reactive N from the system. An estimated 16% and 63% of N retained in lotic systems and lakes, respectively, has been attributed to denitrification (Mulholland et al. 2008, Saunders and Kalff 2001). Fewer estimates of the contribution of denitrification to N retention in reservoirs exist. However, high area-specific nutrient loading (Kozelnik et al. 2007) and high rates of carbon burial (Downing et al. 2008) make man-made impoundments potential landscape-scale

denitrification hotspots. Reservoirs that experience seasonal thermal stratification also represent predictable hot moments for denitrification (Groffman et al. 2009).

Methods for estimating denitrification in lentic systems are often limited by their feasibility and cost-effectiveness in large water bodies. The ^{15}N tracer studies employed successfully in relatively small-volume lotic systems are difficult to apply in larger-volume lakes and reservoirs (Mulholland et al. 2008). Other techniques that measure denitrification indirectly, such as acetylene block in sediment slurries (David et al. 2006), are unable to provide information encompassing micro-scale intricacies, and are also difficult to make relevant at the ecosystem scale (Groffman et al. 2006, 2009).

Intact sediment cores used in conjunction with membrane inlet mass spectrometry (MIMS; Kana et al. 1994) can provide direct and accurate measurements of denitrification (McCarthy et al. 2007, Scott et al. 2008), but these studies are also limited by the spatial scale represented by the cores and the relatively small number of cores that can practically be accommodated. Other recent studies have used MIMS to measure naturally-occurring temporal and spatial N_2 gradients in aquatic systems (Deemer et al. 2011). This technique exploits water density gradients which temporarily isolate the lower water column of many estuaries, lakes, and reservoirs. Transformation into virtually closed systems results in a predictable depletion of oxidized compounds like oxygen (O_2) and nitrate, accompanied by the accumulation of reduced compounds like N_2. Combining these different approaches, particularly those that measure N_2 directly, may allow for highly accurate and detailed measurements of ecosystem-scale denitrification. But, no studies to our knowledge have combined these approaches into system-level studies.

The objective of this study was to combine multiple direct measurements of denitrification suited to specific habitats in reservoir environments to derive whole-ecosystem denitrification estimates. We monitored hypolimnetic and metalimnetic N_2 concentrations through time in 3 shallow reservoirs with inherently low stratification stability and combined these estimates with denitrification measurements from intact cores collected from sediments in contact with reservoir epilimnia. This approach allowed us not only to derive accurate estimates of denitrification at the ecosystem scale, but to also quantify the relative contribution of distinct ecosystem compartments to the overall denitrification rate. We also used laboratory experimentation to confirm in situ patterns of N_2 accumulation in temporarily isolated and anoxic environments.

2.2 Methods

2.2.1 *Study Sites*

Lakes Elmdale (36º11'45.5"N, 94º12'50.8"W), Fayetteville (36º08'11.5"N, 94º07'46.7"W) and Wedington (36º05'27.05"N, 94º22'02.9"W) are small (surface area < 1 km^2), shallow (average depth 3 m, maximum depth 9-10 m), and eutrophic flood control impoundments located in and around Fayetteville, Arkansas, United States of America. Watersheds for each of the reservoirs are approximately 30 – 40x greater than the surface area of the reservoirs, indicating that the reservoirs have comparable hydrologic residence times, a factor known to impact rates of N cycling and retention (Seitzinger 2002). The reservoirs differ in their primary watershed land use/land cover, introducing potential variability in external N loading. The reservoirs all experience thermal stratification during summer and anoxia within the

hypolimnion and metalimnion. However, the timing and severity of these patterns differs between reservoirs.

2.2.2 *Reservoir chemical stratification and seasonal N_2 accumulation*

Vertical profile data and water samples were collected at the location of maximum depth in each reservoir weekly to biweekly from April – August 2010. Temperature, dissolved oxygen (DO) and oxidation-reduction potential (ORP) measurements were taken at 0.5-1 m intervals using a multiparameter datasonde (Yellow Springs, Inc.). Water samples were collected with a Van Dorn horizontal sampler at 2-3 depths which were assigned based on the depth of the oxycline. Sample depths were distributed evenly across the hypoxic/anoxic water column and varied among weeks, but were approximately 4, 6 and 8 m at Lakes Elmdale and Fayetteville and 6 and 8 m at Lake Wedington. Dissolved gas samples were transferred into 300 mL Wheaton bottles by slowly filling from the bottom up. Samples were immediately preserved by adding 3.9 mL of 50% w/v zinc chloride ($ZnCl_2$) solution, and bottles were capped with a ground-glass stopper and wrapped with parafilm to prevent atmospheric exchange. Water chemistry samples were collected concurrently at each depth and transferred into acid-washed bottles. All samples were stored on ice and returned to the laboratory.

Dissolved gas samples were analyzed for oxygen gas to argon ratios (O_2:Ar) and N_2:Ar using MIMS (Kana et al. 1994). Samples were refrigerated in the dark prior to analysis, which was conducted within 1 week of collection. Prior to MIMS analysis, sample temperature was brought back to in situ temperature, and the temperature of the standard solution for the MIMS was adjusted to match each sample. The MIMS method assumes 100% Ar saturation that varies with temperature and salinity. Biological effects on the O_2 and N_2 pool of samples were

separated from physical effects using the Ar signal. Sample N_2 concentration ($[N_2]_{sample}$) was defined as:

$$[N_2]_{sample} = \left(N_2{:}\,Ar_{sample} \times [Ar]_{exp}\right)\left(\frac{[N_2]{:}\,[Ar]_{exp}}{N_2{:}\,Ar_{standard}}\right) \quad (1)$$

where N_2:Ar_{sample} is the measured sample signal, $[Ar]_{exp}$ and $[N_2]$:$[Ar]_{exp}$ are saturated concentrations or ratios at a given temperature, and N_2:$Ar_{standard}$ is the measured signal for well-mixed deionized water open to the atmosphere at the same temperature as the samples. A similar equation was used to calculate sample O_2 concentrations. Excess N_2 ($[N_2]_{excess}$) was the N_2 concentration exceeding saturation for a given temperature and was defined as:

$$[N_2]_{excess} = [N_2]_{sample} - [N_2]_{exp} - \min[N_2]_{excess} \quad (2)$$

where $[N_2]_{exp}$ is the saturated N_2 concentration at a given temperature, and $\min[N_2]_{excess}$ is the lowest value $[N_2]_{excess}$ in the dataset for each reservoir. Due to matrix differences between the standard and samples, some $[N_2]_{sample}$ measurements were less than $[N_2]_{exp}$, yielding $[N_2]_{excess}$ slightly less than zero. The $\min[N_2]_{excess}$ was used to correct for these matrix effects and to redistribute the data so that saturation values ($[N_2]_{excess} = 0$) were site-specific.

Water chemistry samples were filtered through acid-washed Whatman GFF filters within 24 hrs of collection and were frozen for later nitrate-N (NO_3^--N) and ammonia-N (NH_3-N) analysis. Nitrate-N was analyzed colorimetrically using the cadmium reduction method, and NH_3-N was determined fluorometrically according to Holmes et al. (1999). Both analyses were carried out on a Turner Designs Trilogy Lab Fluorometer, with a spectrophotometer adaptor containing a 600 nm filter cell for NO_3^--N analysis.

Gas and water chemistry data were grouped as either hypolimnion (Elmdale and Fayetteville, ~6 and 8 m; Wedington, ~8 m) or metalimnion (Elmdale and Fayetteville, ~4 m; Wedington ~6 m) for each reservoir. Metalimnion $[N_2]_{excess}$ data were divided into early and late

summer subsets and compared with reservoir stratification stability. Changes in the thermocline depth and temperature were assumed to be indicative of partial mixing events and were described through the stratification stability index (SSI; Yu et al. 2010), which was calculated for each reservoir on each date as:

$$SSI = \frac{\Delta T}{\Delta z} \quad (3)$$

where ΔT and Δz are the change in temperature and depth, respectively, between the bottom of the epilimnion and the top of the hypolimnion. When SSI > 1 ºC m^{-1}, the thermocline was assumed to exist, and increasing SSI indicated increasing thermal stability (Horne and Goldman 1994).

Volumetric denitrification rates (k_{N2}) for the hypolimnion and metalimnion were estimated as the slope of a linear regression analysis on $[N_2]_{excess}$ versus time conducted in SAS 9.1. Trends in NO_3^--N and NH_3-N concentrations through time were also quantified using linear regression in SAS 9.1, with negative and positive slopes indicating solute consumption and production, respectively. Boundaries between thermally stratified layers were determined for each sampling date with depth profiles and thermocline boundary estimates from SSI analyses, which were then averaged across the study period. Epilimnion thickness was defined as the vertical distance between the surface and the upper thermocline boundary. The anoxic metalimnion thickness was the distance between the average anoxic depth in the metalimnion and the average hypolimnion depth. The hypolimnion was considered to be the remainder of the underlying water column. The volume and surface area of the thermal strata were estimated using bathymetry data from Lake Wedington. No bathymetry data were available for Lakes Elmdale and Fayetteville. But, because the reservoirs are located within close proximity to each other, it was assumed that their underlying topography was similar, and bathymetry models were

developed by interpolation using data from Lake Wedington. The water column volume corresponding to each depth and the associated surface area at these depths was determined by calculating the volumes of sequential conical frustra. Volumetric hypolimnion and anoxic metalimnion denitrification rates (k_{N2}) were converted to areal denitrification rates (K_{dnf}) for these locations using the estimates of water volume and surface area associated with each stratum:

$$K_{dnf} = \frac{k_{N2} \times V_s}{A_s} \quad (4)$$

where V_s and A_s are the volume and surface area, respectively, of a thermal stratum.

2.2.3 *Metalimnetic water column denitrification experiment*

Preliminary data suggested that the metalimnia of these reservoirs could experience hypoxia or anoxia. A laboratory experiment was conducted to estimate the time needed to induce water column denitrification following a partial mixing event in the metalimnion, and to quantify what resource limitations may have existed on metalimnetic denitrification. Twenty-five liters of water were collected from Lake Fayetteville in June 2011 at a depth where DO was < 1 mg L^{-1} and ORP was ~ -100 mV. Two subsamples were immediately preserved in 300 mL Wheaton bottles as previously described. A concentrated seston slurry was also collected by repeated vertical tows of the upper 4 m depth with an 80μm mesh size Wisconsin net. In the laboratory, the 25 L water sample was aerated for 24 hours, and the seston slurry was further concentrated by centrifugation and refrigerated overnight. The following day the aerated water was divided into 24- 300 mL Wheaton bottles which were grouped into the following four treatments: 1) a control receiving no additions, 2) a 71.4 μmol L^{-1} NO_3^--N addition, 3) a 0.5 mmol L^{-1} particulate carbon (PC) addition, and 4) a combined NO_3^--N and PC addition. The selected NO_3^--N addition

rate reflected typical pre-stratification concentrations at Lake Fayetteville. The PC addition was derived from the concentrated seston slurry and was intended to mimic a typical rate of organic C sedimentation in a eutrophic reservoir over a two week period (Molongoski and Klug 1980). The experimental rate of PC loading to bottles was determined post-hoc by filtering 0.5 mL of slurry onto a Whatman GFF filter and analyzing the filter for organic C on a Thermo Flash 2000 Organic Elemental Analyzer. The PC loading rate to the bottles was approximately equivalent to a 0.13 mol C m^{-2} day^{-1} sedimentation rate. Bottles were incubated at the average metalimnion in situ temperature (15 ºC) and analyzed for O_2:Ar and N_2:Ar on days 1, 2, 5, 8, 13, and 16 as described previously.

2.2.4 *Intact core experiments*

Intact cores were collected from sediments in contact with reservoir epilimnia in February, May, June, August and December 2010. Three to four cores with overlying water were collected from each reservoir on each date in clear plastic tubes (surface area = 40.6 cm^2, height = 70 cm) using a manual gravity corer. Vertical temperature and DO profiles were collected on each date to determine in situ temperature and sampling depth (May, June and August), which ranged from 5 to 7 m during seasonal mixing (February and December) and from 2 to 3 m during seasonal stratification (May, June and August). Epilimnetic water was collected in acid-washed carboys from each reservoir on each date to supply inflow water for continuous-flow cores.

Continuous-flow sediment core incubations were conducted similarly to those described by Scott et al. (2008). Cores were sealed airtight with rubber stoppers and fitted with Teflon tubing through the stopper to provide inflow and outflow paths for the incubation water. The experiment was conducted inside an incubator set to in situ temperature. The inflow water supply

was constantly aerated to simulate reservoir mixing and was pumped into cores at a rate of 0.50-0.75 mL min^{-1}. A control chamber (shorter core without sediment) for each water supply was set up similarly using source water from each reservoir. After a 12-18 hour pre-incubation period, effluent from each core chamber and influent from each reservoir were collected in 20 mL glass vials. Samples were immediately preserved by adding 0.26 mL 50% w/v $ZnCl_2$, and vials were capped with ground glass stoppers and wrapped with parafilm. Inflow and outflow N_2:Ar was analyzed within 24 hours using MIMS and were converted to N_2 concentrations as described in Eq. 1. Areal sediment denitrification (K_{dnf}, μmol m^{-2} h^{-1}) for each core was calculated as:

$$K_{dnf} = \frac{([N_2]_{out} - [N_2]_{in} - [N_2]_{control}) \times Q}{A} \quad (5)$$

where $[N_2]_{out}$, $[N_2]_{in}$ and $[N_2]_{control}$ were the outflow, inflow and control N_2 concentrations, respectively, Q was the measured flow rate for each core in L h^{-1}, and A was the core surface area in square meters. In February and May, control chambers were not used; therefore, N_2 fluxes measured on these dates were not corrected for potential activity in the overlying water ($[N_2]_{control} = 0$). Mean K_{dnf} and associated error rates were calculated from replicate cores from each reservoir on each date. Positive values indicated sediment N_2 production (i.e, net denitrification), while negative fluxes indicated sediment N_2 consumption (i.e. net N_2 fixation). In addition to intact core experiments, water samples were collected from the upper water column of each reservoir approximately monthly throughout 2010 and analyzed for NO_3^--N.

2.2.5 *Annual whole-ecosystem denitrification estimates*

Annual areal whole-ecosystem denitrification rates (DNF_{annual}) were estimated by summing the habitat-specific and seasonally-specific areal rates over the appropriate areas and durations, respectively. Denitrification rates were assumed to represent average seasonal rates;

therefore, assigning time intervals to each K_{dnf} for all habitats was required. Mean K_{dnf} rates from sediment cores collected in February, May, June, August and December 2010 sediment incubations were divided over a one-year period as follows: January 1-April 14, April 15-May 31, June 1-July 31, August 1-October 15, and October 16-December 31 2010, respectively. Negative N_2-N fluxes indicative of net N_2 fixation were considered as K_{dnf}=0 in calculating DNF_{annual}. Denitrification rates from the hypolimnion and metalimnion were scaled up only during the stratified period (April 15-October 15). Metalimnetic K_{dnf} rates were not extrapolated past August and K_{dnf} was set to zero on days when DO was greater than 1 mg L^{-1} throughout the metalimnion.

An annual, whole lake estimate of N loss to denitrification was calculated as follows:

$$DNF_{annual} = \frac{\sum(K_{dnf} \times t \times A_s)}{A_0} \quad (6)$$

where *t* is number of days corresponding to a specific K_{dnf} for a habitat, A_s is the surface area of the habitat corresponding to a specific measured K_{dnf}, and A_0 is the reservoir surface area. Habitat-specific denitrification rates for each reservoir were divided by the estimated total denitrification rate to obtain the percent denitrification that occurred in each habitat. Error rates from habitat-specific and seasonal-specific K_{dnf} were propagated appropriately to provide error rates for the annual whole-ecosystem denitrification rates for each reservoir.

2.3 Results

2.3.1 *Reservoir chemical stratification and seasonal N_2 accumulation*

Hypolimnion DO was rapidly depleted from all reservoirs following seasonal stratification and DO was already below 31 µmol L^{-1} at Lake Fayetteville when sampling commenced in April (Figure 1 A-C). Hypolimnion ORP also declined rapidly at all sites,

fluctuating between -150 and -200 mV in late summer (Figure 1 D-F). Hypoxic and anoxic conditions also occurred in the reservoirs' metalimnia. The average anoxic metalimnion depth was 2.6 ± 0.744, 2.8 ± 0.799 and 5.4 ± 1.03 at Elmdale, Fayetteville and Wedington, respectively. The reservoirs all maintained seasonal thermal stratification, but some variation in thermal stability did exist, which also influenced redox conditions in the metalimnia. At Lake Elmdale, metalimnion ORP declined from 100 to -112 mV from early June to mid-July, but increased rapidly to ~ 100 mV in late-July when the reservoir experienced a major decline in thermal stability (Figure 1D). Metalimnetic ORP in Lake Elmdale had decreased again to ~ -100 mV by the end of August. At Lake Fayetteville, ORP at 4 m was similar to hypolimnion ORP throughout the summer (Figure 1E). At Lake Wedington, metalimnetic ORP remained high relative to the other reservoirs, not declining below zero until late-July (Figure 1F).

Hypolimnetic NO_3^--N declined over the study period from 25.5, 19.8, and 12.4 µmol L^{-1} in April at Elmdale, Fayetteville and Wedington, respectively, to below detection by mid-June at all reservoirs (Figure 2). Hypolimnetic NH_3-N concentrations increased over this time, from relatively low concentrations in all reservoirs in April to 158, 359, and 117 µmol L^{-1} in August at Elmdale, Fayetteville and Wedington, respectively (Figure 2). Nitrate-N and NH_3-N patterns in the metalimnion (data not shown) were similar to those observed in the hypolimnion, but were less pronounced and more variable through time.

Hypolimnion N_2-N concentration increased by 66.6 ± 10.1, 63.0 ± 10.2, and 50.3 ± 10.4 nmol L^{-1} h^{-1} at Elmdale, Fayetteville and Wedington, respectively (Figure 3). Metalimnetic N_2-N concentrations experienced two periods of approximate linear change in all three reservoirs and these periods were separated by a brief period of drastic thermal instability (Figure 4). The two distinct periods of metalimnetic thermal stability in each reservoir resulted in metalimnetic

denitrification rates ranging from 95 ± 37.5 to 152 ± 84.3 nmol L^{-1} h^{-1}, which were 2 – 3x greater than the rates of volumetric denitrification observed in the reservoir hypolimnia.

2.3.2 *Metalimnetic water column denitrification experiment*

Following aeration of water to simulate a partial metalimnetic mixing event, complete water column anoxia was only observed in the treatments that received organic matter (PC) inputs (Figure 5A). Samples receiving PC were hypoxic by day 8 and completely anoxic by day 13. Samples which did not receive PC remained at 70% DO saturation by the end of the experiment on day 16. Samples which did not experience hypoxia or anoxia exhibited no denitrification, but denitrification occurred in both the PC-containing treatments which experienced anoxia (Figure 5B). Denitrification in these samples was proportional to NO_3^--N availability. Approximately 80% of NO_3^--N added to the PC plus NO_3^--N treatment was denitrified in the 16 day experimental period. The rates of denitrification observed in the PC and PC plus NO_3^--N treatments were ~ 100 and 200 nmol L^{-1} h^{-1} and were very similar to in situ denitrification rates estimated for the reservoir metalimnia (Figure 4).

2.3.3 *Intact core experiments*

Net denitrification was observed in all intact cores collected from sediments exposed to the epilimnion during winter conditions (Figure 6). In contrast, net N_2 fixation was observed in all intact cores collected during spring and summer, with the exception of very slight net denitrification in Lake Fayetteville in July. Nitrate concentrations in the epilimnion of all three reservoirs were at or below detection levels during summer, with the exact duration of NO_3^--N depletion varying by reservoir (Figure 6). However, annual NO_3^--N concentrations were always

at their maximum in winter and the annual maximum NO_3^--N concentrations always coincided with net denitrification in sediments exposed to the epilimnion.

2.3.4 *Annual whole-lake denitrification rates*

Table 1 provides a summary of annual whole-lake denitrification estimates in the study reservoirs, the error associated with these rates, and the relative contribution of the three major habitats to total denitrification in each reservoir. Whole-ecosystem denitrification ranged from 13 – 25 g N m^{-2} $year^{-1}$. Twenty percent of annual denitrification in all reservoirs occurred consistently in the reservoir hypolimnion during summer stratification. Between 50% and 70% of annual denitrification in Lakes Fayetteville and Wedington occurred in sediments exposed to the epilimnion. The metalimnetic contribution to annual denitrification in these lakes was 10% to 30%. In contrast, 50% of annual denitrification occurred in the metalimnion and only 30% occurred in epilimnetic sediments in Lake Elmdale.

2.4 Discussion

Measuring and modeling denitrification in diverse environments is challenging and until recently has primarily been accomplished with indirect measurements from limited samples (Groffman et al. 2006, Groffman et al. 2009). Monitoring N_2 accumulation in thermally stratified waters may represent the most accurate method for estimating denitrification in lakes and reservoirs at very large scales. To our knowledge the current study represents only the second attempt to quantify denitrification via hypolimnetic N_2 accumulation in freshwater lakes and reservoirs. Interestingly, hypolimnetic N_2 accumulation rates observed in this study were strikingly similar to the rate observed by Deemer et al. (2011) for Lacamas Lake in Washington, U.S.A. (Table 2). Our study went one step further in that we combined hypolimnetic and

metalimnetic N_2 accumulation rates with denitrification estimates from intact cores to derive ecosystem-scale denitrification measurements that included error estimates.

Denitrification estimates from the hypolimnion were the least variable among habitat-types (13 – 22% error). Denitrification rates obtained from replicate intact core experiments were somewhat more variable (20-25% error). But, denitrification rates estimated through intact core experiments were in range with other studies that have employed similar methods in freshwater ecosystems (McCarthy et al. 2007, Scott et al. 2008). In all the reservoirs, hypolimnion denitrification accounted for approximately 20% of total annual denitrification. The relatively small contribution of hypolimnetic denitrification to whole-ecosystem denitrification was probably constrained by NO_3^--N availability in the hypolimnion. Nitrate was not measureable in the hypolimnion throughout most of summer stratification. The contribution of hypolimnetic denitrification to whole-ecosystem denitrification was also likely constrained by the relatively small proportion of hypolimnetic volume to total volume in these shallow reservoirs. The proportion of denitrification occurring in the hypolimnion may be much greater in much deeper lakes and reservoirs like Lacamas Lake (Deemer et al. 2011).

The magnitude of denitrification rates observed in the metalimnia of our study reservoirs was unexpected and substantial compared to the other denitrification habitats. In Lake Elmdale, denitrification in the metalimnion during short-term anoxic conditions accounted for more than 50% of annual ecosystem denitrification (Table 1). The temporal separation of two distinct hypoxic/anoxic periods in the metalimnia of all three reservoirs appeared to be controlled by a substantial decrease in thermal stability (Figure 4). This pattern was most obvious at Lake Elmdale, where thermal stability decreased by more than 4 °C m^{-1} during July, which corresponded with an approximate 150 µmol L^{-1} decrease in $[N_2]_{excess}$. We assume that this

supersaturated N_2 was lost to the atmosphere during metalimnion mixing. Although the regression fits for excess N_2 through time in the metalimnia of each reservoir were admittedly weak (Figure 4), experimental data confirmed that a relatively deep mixing event followed by a long period of stable chemical stratification could result in denitrification rates similar to estimates for reservoir metalimnia (Figure 5). These results gave us confidence that the observed metalimnetic denitrification was a valid component of ecosystem-scale denitrification, but more work is needed to understand the physical-chemical controls on denitrification in hydrologically-dynamic locations.

The annual whole-ecosystem denitrification rates for the study reservoirs are in the lower to middle range of annual denitrification rates reported for other reservoirs, and were also similar to annual denitrification rates reported for natural lakes and riverine ecosystems (Table 2). Kozelnik et al. (2007) used an isotope-pairing technique on sediment cores from Solina Reservoir, Poland to show that denitrification in sediments resulted in the removal of approximately 6 g N m^{-2} $year^{-1}$, but denitrification was positively correlated with total N concentrations in the reservoir. In contrast, David et al. (2006) reported denitrification in Shelbyville Reservoir, Illinois, USA was as high as 230 g N m^{-2} $year^{-1}$, but NO_3^--N concentrations in this reservoir were commonly > 5 mg N L^{-1} and presumably never limited denitrification. Clearly the potential for more denitrification existed in our study reservoirs, but constraints on NO_3^--N availability most likely kept rates low relative to the range reported at Shelbyville Reservoir. Estimated annual reactive N removal through denitrification was of similar magnitude at Lakes Elmdale and Fayetteville, but was approximately 50% less at Lake Wedington. Seasonal DIN fluxes and concentrations were consistently lowest at Lake Wedington, indicative of a smaller reactive N supply to fuel denitrification.

Whole-ecosystem denitrification estimates from this study were also similar to recent global model estimates of denitrification in lakes and rivers. Seitzinger et al. (2006) estimated a global average lentic denitrification rate of 11.5 g N m^{-2} yr^{-1} based primarily on data from large lakes (surface area > 55 km^2). The estimate of global denitrification in rivers was slightly higher, at 13 g N m^{-2} annually. Denitrification rates at Lakes Elmdale and Fayetteville were approximately 2x greater than model estimates for natural lakes and rivers. These findings support the idea that manmade impoundments act as hybrid ecosystems, sharing characteristics of both lakes and rivers that could enhance denitrification (Thornton et al. 1990). Denitrification rates from Lake Wedington were more similar to global averages for both lakes and rivers.

In modeling N retention globally across lakes and reservoirs, Harrison et al. (2009) found not only that reservoirs, but particularly small reservoirs retain disproportionately large N loads relative to natural lakes, estimating annual N retention in small reservoirs at 31 g N m^{-2} yr^{-1}. If these estimates were accurate for our study reservoirs then approximately 40-80% of N retained would have been denitrified in the reservoirs in 2010, which would be among the highest reported rates of denitrification efficiency for aquatic ecosystems (Kozelnik et al. 2007).

Some issues associated with our methods for measuring and scaling denitrification were worthy of explanation. A plateau in N_2 production did not accompany the exhaustion of NO_3^--N in the hypolimnion of the study reservoirs. If these hypolimnia were indeed functioning as pseudo-closed systems, a saturation effect should have occured in $[N_2]_{excess}$. Although hypolimnetic NO_3^--N concentrations rapidly fell below detection limits following thermal stratification, N_2 accumulation in lower strata continued. In some cases, N_2 accumulation was more than 5x greater than NO_3^--N consumption over the summer. This indicates that either unmeasured inputs of NO_3^--N occurred in the hypolimnion or that another microbial process

such as anaerobic ammonium oxidation (annamox) were contributing to N_2 accumulation in the hypolimnion. Although sufficient ammonium certainly existed, the contribution from annamox seems unlikely because this process also requires available nitrite (NO_2^--N; Burgin and Hamilton 2007). Our method for measuring NO_3^--N did not distinguish NO_2^--N, therefore hypolimnetic NO_2^--N concentrations were also below detection levels throughout most of the summer stratified period in all three reservoirs.

Groundwater percolating into the reservoirs through sediment may have provided a constant NO_3^--N source to the hypolimnion and stimulated denitrification throughout the summer. High surface to groundwater connectivity is a defining characteristic of the Ozark Plateau's topography, resulting in similar water chemistry between surface and groundwater in both pristine and non-point source impacted zones (Petersen et al. 1999, Haggard et al. 2005). In urban and agricultural watersheds like those of the study reservoirs, stream and groundwater NO_3^--N concentration can reach levels of up to 4 mg L^{-1}. Springs that formerly fed the reservoirs' parent streams would now discharge into the reservoirs directly, creating secondary denitrification zones deep within sediments (Sanders et al. 2007). Potential groundwater NO_3^--N inputs were not detected in the late summer hypolimnion NO_3^--N pool, as they were likely denitrified within the sediment before entering the water column, leaving only the constant increase in $[N_2]_{excess}$ as a signal of this activity.

None of the methods employed to estimate denitrification in this study captured secondary denitrification products, the most important of which is nitrous oxide (N_2O). Elevated N_2O production relative to N_2 in reservoirs has been observed in the littoral zone (Wang et al. year) and within the water column in conjunction with disturbance (Deemer et al. 2011). The maximum N_2O-N: N_2-N ratio observed in Lacamas Lake was approximately 0.02, suggesting

that failure to quantify N_2O would not lead to gross underestimation of total denitrification. However, understanding production hotspots and hot moments of N_2O production is important due to the role of N_2O as a potent greenhouse gas.

It is also worth noting that estimating denitrification from hypolimnetic N_2 accumulation as done in this study has inherent limitations. Deemer et al. (2011) reported that hypolimnion N_2 fluxes could have been underestimated by up to 36% at Lacamas Lake due to gas transfer across thermal boundaries. Loss rates were not quantified in this study, but are likely similar to those in the hypolimnion of Lacamas Lake and may be even more important in anoxic metalimnetic environments, where we found substantial denitrification.

Results of this study indicate that denitrification in shallow impoundments may be accurately and rapidly estimated by combining habitat-specific techniques. The rates of hypolimnetic denitrification reported here were strikingly similar to those reported in the only other study (Deemer et al. 2011) known to us to have applied the in situ N_2 accumulation technique for estimating denitrification in stratified freshwater ecosystems. Our study builds on this previous study by integrating hypolimnetic denitrification estimates with estimates from the anoxic metalimnion and epilimnetic sediments. Experimental data supported the idea that the anoxic metalimnion can be a location of significant denitrification. Hypolimnetic denitrification was consistent across the three study reservoirs, accounting for approximately 20% of whole-ecosystem denitrification. However, the percent contribution from the anoxic metalimnion and epilimnetic sediments varied substantially between reservoirs and was strongly dependent on stratification stability and NO_3^--N availability.

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Table 2.1 Habitat-specific and whole-ecosystem denitrification rates (K_{dnf}) with associated error estimates and the percent contribution from each habitat type. Epilimnion error rates were derived by summing the standard error of the mean K_{dnf} from February and December incubations. Metalimnion and hypolimnion error rates were derived from the standard error of the slopes from linear regression equation(s) used to estimate K_{dnf}. All stratum estimates are normalized to the total reservoir surface area.

	K_{dnf} in g N m^{-2} $year^{-1}$ ± S.E. (% of total)		
Habitat	Lake Elmdale	Lake Fayetteville	Lake Wedington
Epilimnion Sediments	6.9 ± 1.4 (28%)	12.5 ± 2.6 (52%)	8.5 ± 2.1 (69%)
Metalimnion	13 ± 5.7 (52%)	4.8 ± 2.8 (28%)	1.3 ± 0.52 (11%)
Hypolimnion	5.0 ± 0.70 (20%)	6.7 ± 0.84 (20%)	2.5 ± 0.53 (21%)
Whole Ecosystem	25 ± 7.8 (100%)	24 ± 6.2 (100%)	13 ± 3.2 (100%)

Table 2.2 Freshwater denitrification rates compiled from a variety of ecosystems derived from multiple habitats using multiple methods. Methods are abbreviated DA for dinitrogen accumulation, CMS for sediment cores with MIMS, SAB for sediment slurry with acetylene block, CPI for cores with paired isotope techniques, MB for mass balance studies, RSI for reach-scale tracer studies, and M for global modeling estimates.

			Denitrification Rate	
Site	Ecosystem	Method	μmol m^{-2} hr^{-1}	g m^{-2} $year^{-1}$
Elmdale[1]	Reservoir	DA, CMS	193	25
Fayetteville[1]	Reservoir	DA, CMS	179	24
Wedington[1]	Reservoir	DA, CMS	137	13
Lacamas[2]	Reservoir	DA	183	__
Shelbyville[3]	Reservoir	SAB	__	62 - 230
Solina[4]	Reservoir	CPI	__	5.5 – 6.1
Sobygard[5]	Lake	MB	__	55
Harp[6]	Lake	MB	__	1.2
East Fork Walker Branch[7]	Stream, For.	RSI	12	__
Sugar Creek[7]	Stream, Ag.	RSI	120	__
Global Lakes[8]	Large Lakes	M	__	11.5
Global Rivers[8]	Rivers	M	__	13

[1]Present study; [2]Deemer et al. (2011); [3]David et al. (2006); [4]Kozelnik et al. (2007); [5]Jensen et al. (1992); [6]Molot and Dillon (1993); [7]Mulholland et al. (2004); [8]Seitzinger et al. (2006)

Figure Legends

Figure 2.1. Hypolimnion and metalimnion DO concentration (A-C) and ORP (D-F) during stratification at Elmdale, Fayetteville and Wedington, respectively.

Figure 2.2. Hypolimnion NO_3^--N and NH_3-N concentration during stratification, with regression r^2, *p* values and rates of concentration loss and accumulation, respectively, at (A) Elmdale, (B) Fayetteville and (C) Wedington.

Figure 2.3. Hypolimnion excess N_2-N concentration during stratification, with regression r^2, *p* values and concentration accumulation rate at (A) Elmdale, (B) Fayetteville and (C) Wedington.

Figure 2.4. Metalimnion excess N_2-N concentration and SSI during stratification at (A) Elmdale, (B) Fayetteville and (C) Wedington. Early and late summer regression r^2, *p* values and rates of accumulation used in whole-lake denitrification calculations are shown.

Figure 2.5. Dissolved oxygen (A) and excess N_2-N (B) concentration over time in the 4 metalimnetic water column incubation treatments. Regression r^2, *p* values and rates of excess N_2-N accumulation in PC and NO_3^--N + PC treatments are shown.

Figure 2.6. Epilimnion denitrification rates estimated from 5 intact sediment core incubations in contact with the mixed water column at Elmdale (A), Fayetteville (B) and Wedington. Mean monthly surface NO_3^-concentration at each reservoir from January-December 2010 are shown.

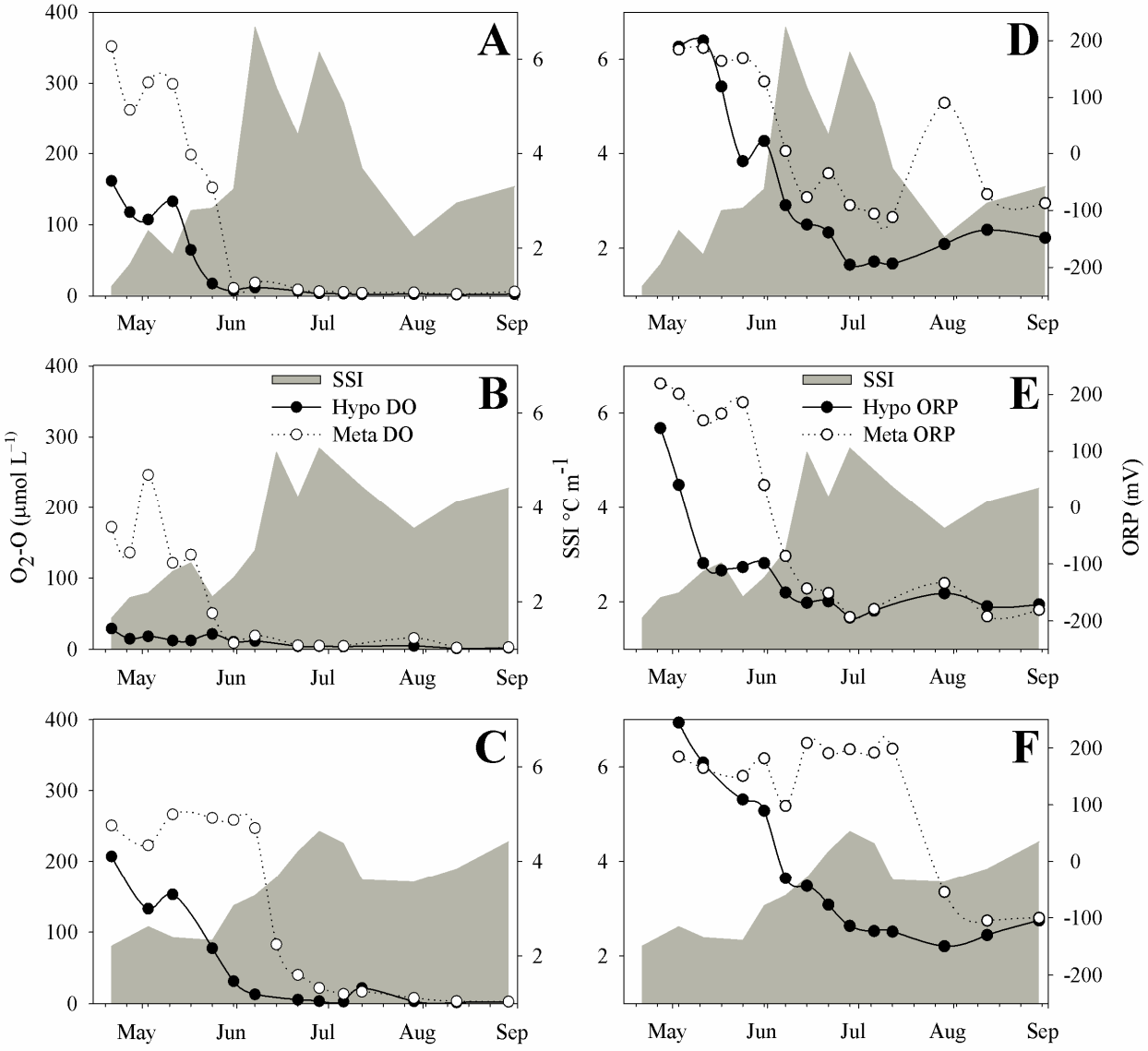
A
B
C
D
E
F
SSI
Hypo DO
Meta DO
Hypo ORP
Meta ORP
O2-O (µmol L-1)
SSI °C m-1
ORP (mV)
May
Jun
Jul
Aug
Sep

Figure 1.1

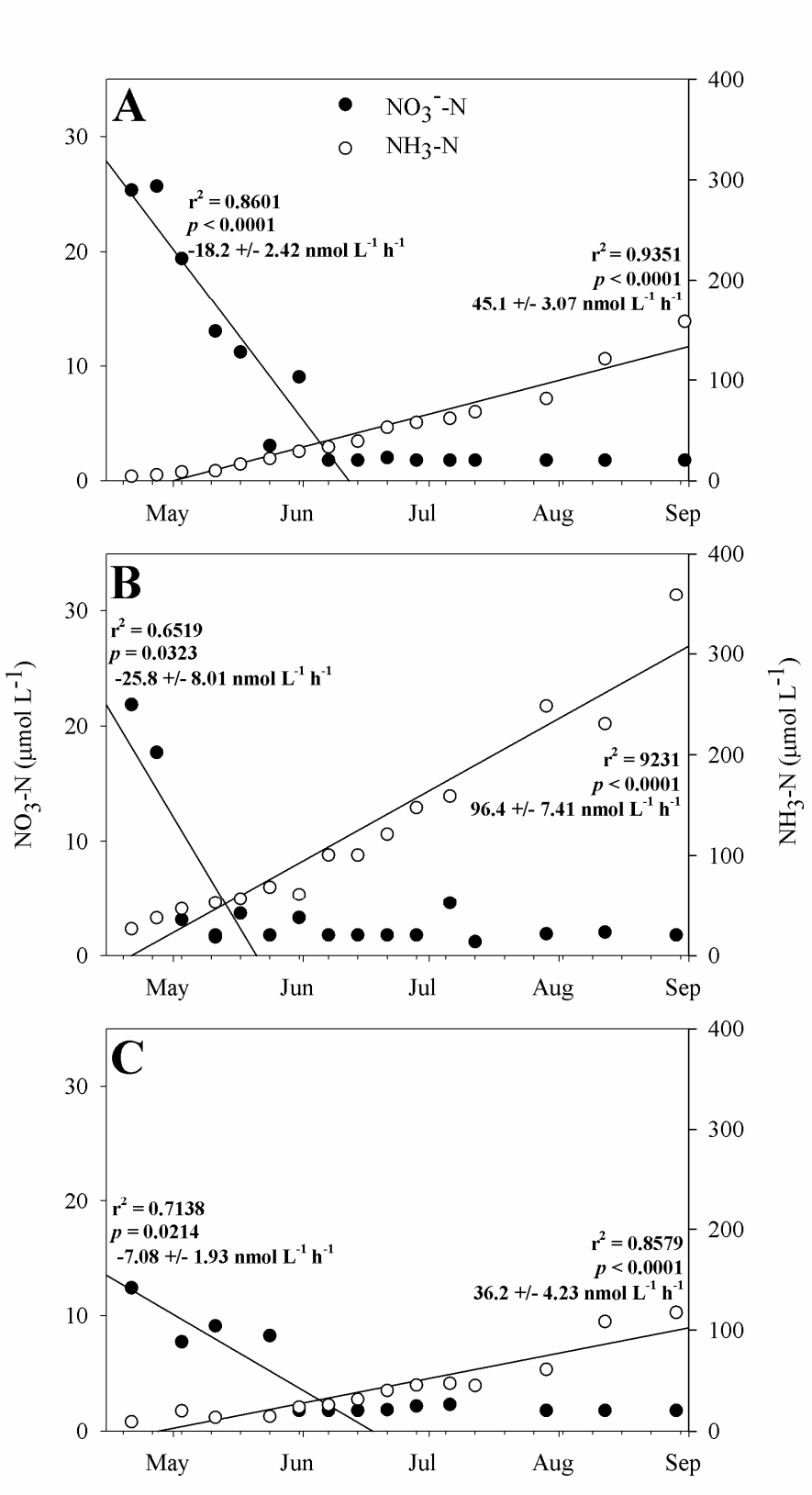
A
NO_3^--N
NH_3-N
r^2 = 0.8601
p < 0.0001
-18.2 +/- 2.42 nmol L^{-1} h^{-1}
r^2 = 0.9351
p < 0.0001
45.1 +/- 3.07 nmol L^{-1} h^{-1}
B
r^2 = 0.6519
p = 0.0323
-25.8 +/- 8.01 nmol L^{-1} h^{-1}
r^2 = 9231
p < 0.0001
96.4 +/- 7.41 nmol L^{-1} h^{-1}
C
r^2 = 0.7138
p = 0.0214
-7.08 +/- 1.93 nmol L^{-1} h^{-1}
r^2 = 0.8579
p < 0.0001
36.2 +/- 4.23 nmol L^{-1} h^{-1}
NO_3-N (µmol L^{-1})
NH_3-N (µmol L^{-1})
0
10
20
30
100
200
300
400
May
Jun
Jul
Aug
Sep

Figure 2.1

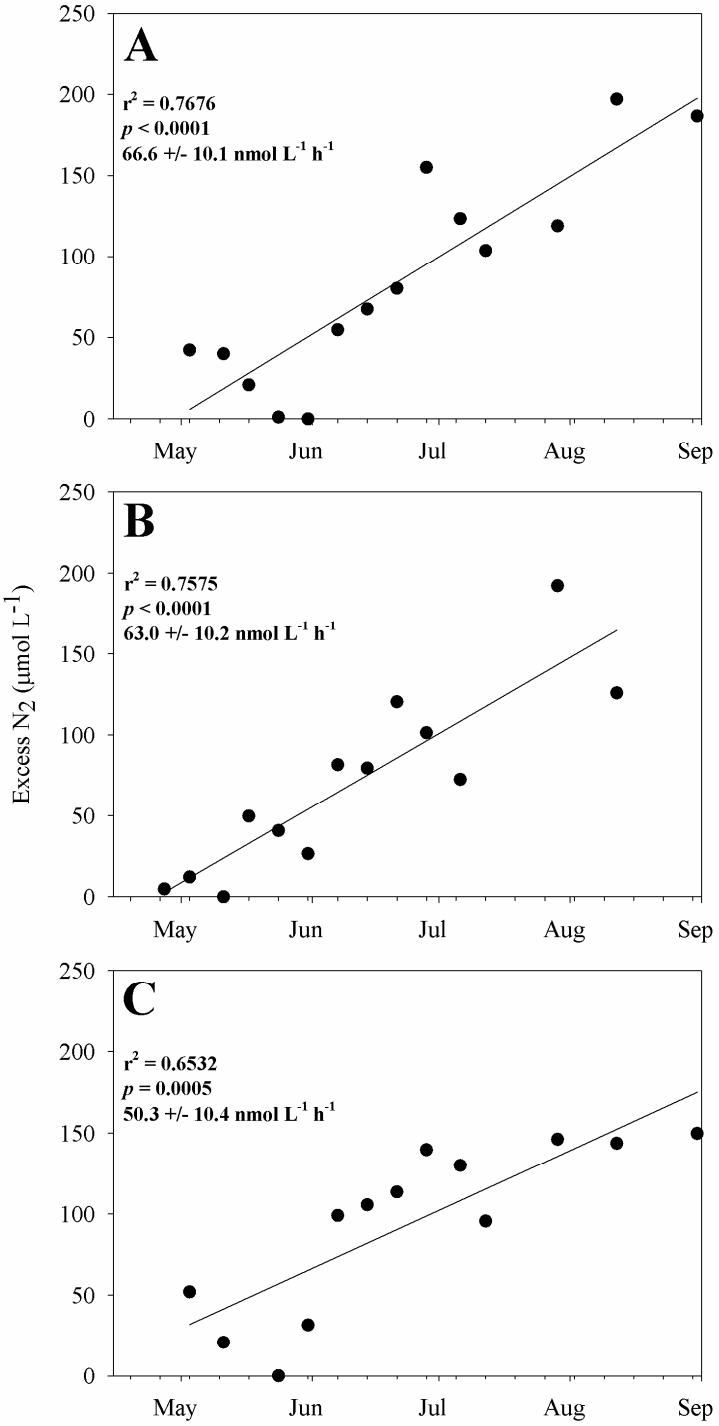
A
r² = 0.7676
p < 0.0001
66.6 +/- 10.1 nmol L⁻¹ h⁻¹
B
r² = 0.7575
p < 0.0001
63.0 +/- 10.2 nmol L⁻¹ h⁻¹
C
r² = 0.6532
p = 0.0005
50.3 +/- 10.4 nmol L⁻¹ h⁻¹
Excess N2 (µmol L-1)
0
50
100
150
200
250
May
Jun
Jul
Aug
Sep

Figure 3.1

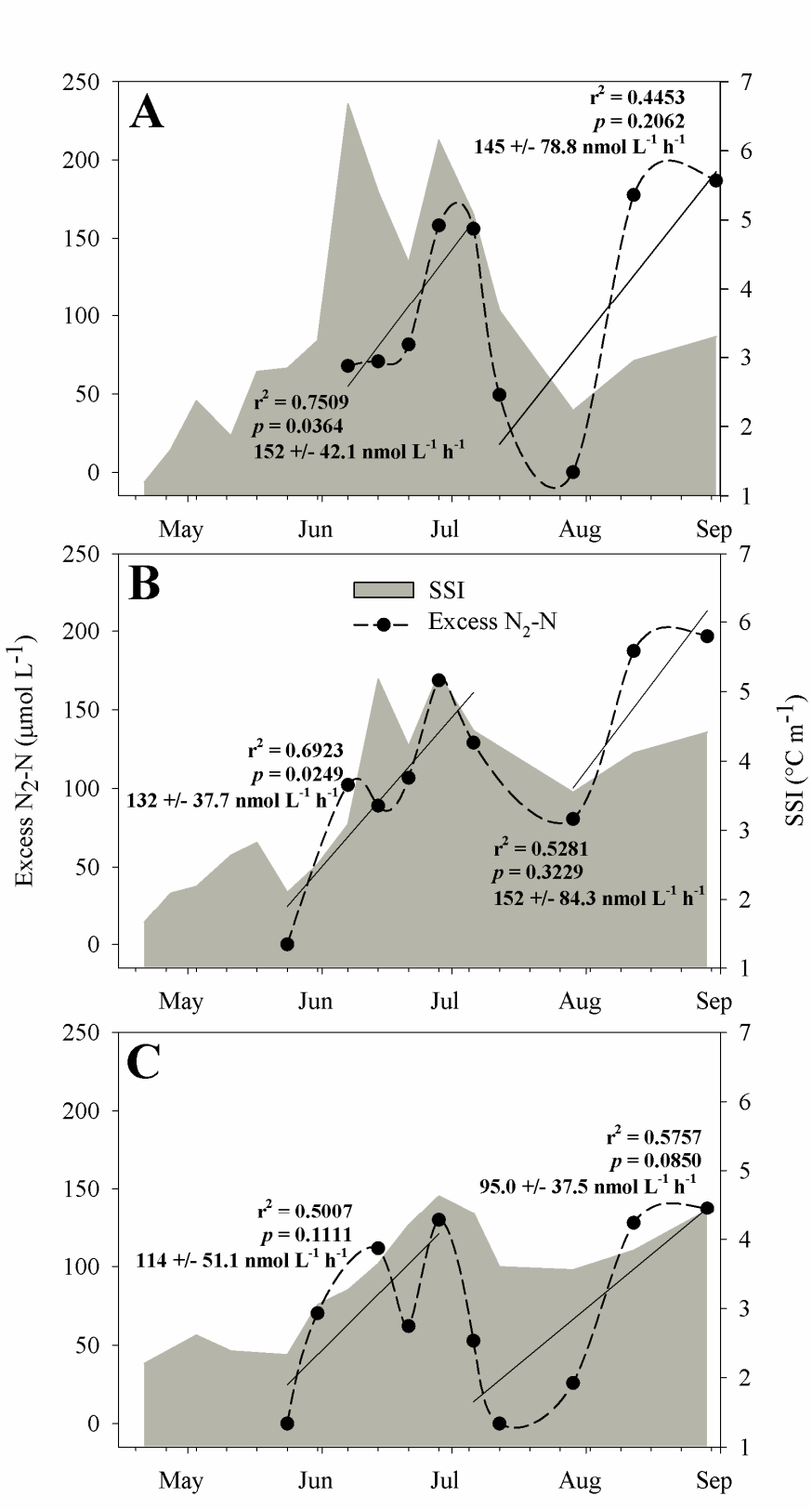
A
r² = 0.4453
p = 0.2062
145 +/- 78.8 nmol L⁻¹ h⁻¹
r² = 0.7509
p = 0.0364
152 +/- 42.1 nmol L⁻¹ h⁻¹
B
SSI
Excess N2-N
r² = 0.6923
p = 0.0249
132 +/- 37.7 nmol L⁻¹ h⁻¹
r² = 0.5281
p = 0.3229
152 +/- 84.3 nmol L⁻¹ h⁻¹
C
r² = 0.5757
p = 0.0850
95.0 +/- 37.5 nmol L⁻¹ h⁻¹
r² = 0.5007
p = 0.1111
114 +/- 51.1 nmol L⁻¹ h⁻¹
May
Jun
Jul
Aug
Sep
Excess N2-N (µmol L⁻¹)
SSI (°C m⁻¹)

Figure 4.1

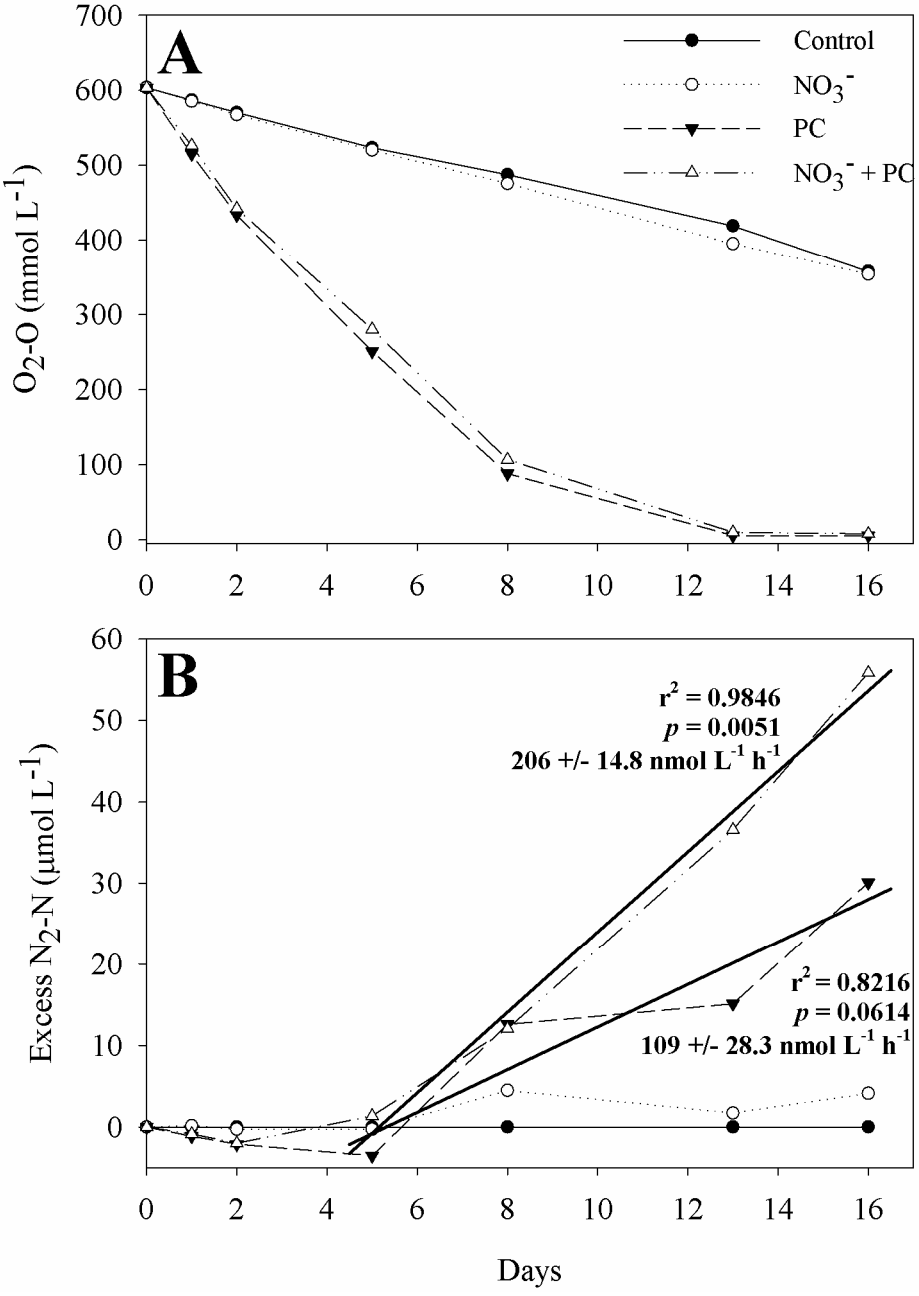
A
Control
NO3-
PC
NO3- + PC
O2-O (mmol L-1)
B
r2 = 0.9846
p = 0.0051
206 +/- 14.8 nmol L-1 h-1
r2 = 0.8216
p = 0.0614
109 +/- 28.3 nmol L-1 h-1
Excess N2-N (µmol L-1)
Days

Figure 5.1

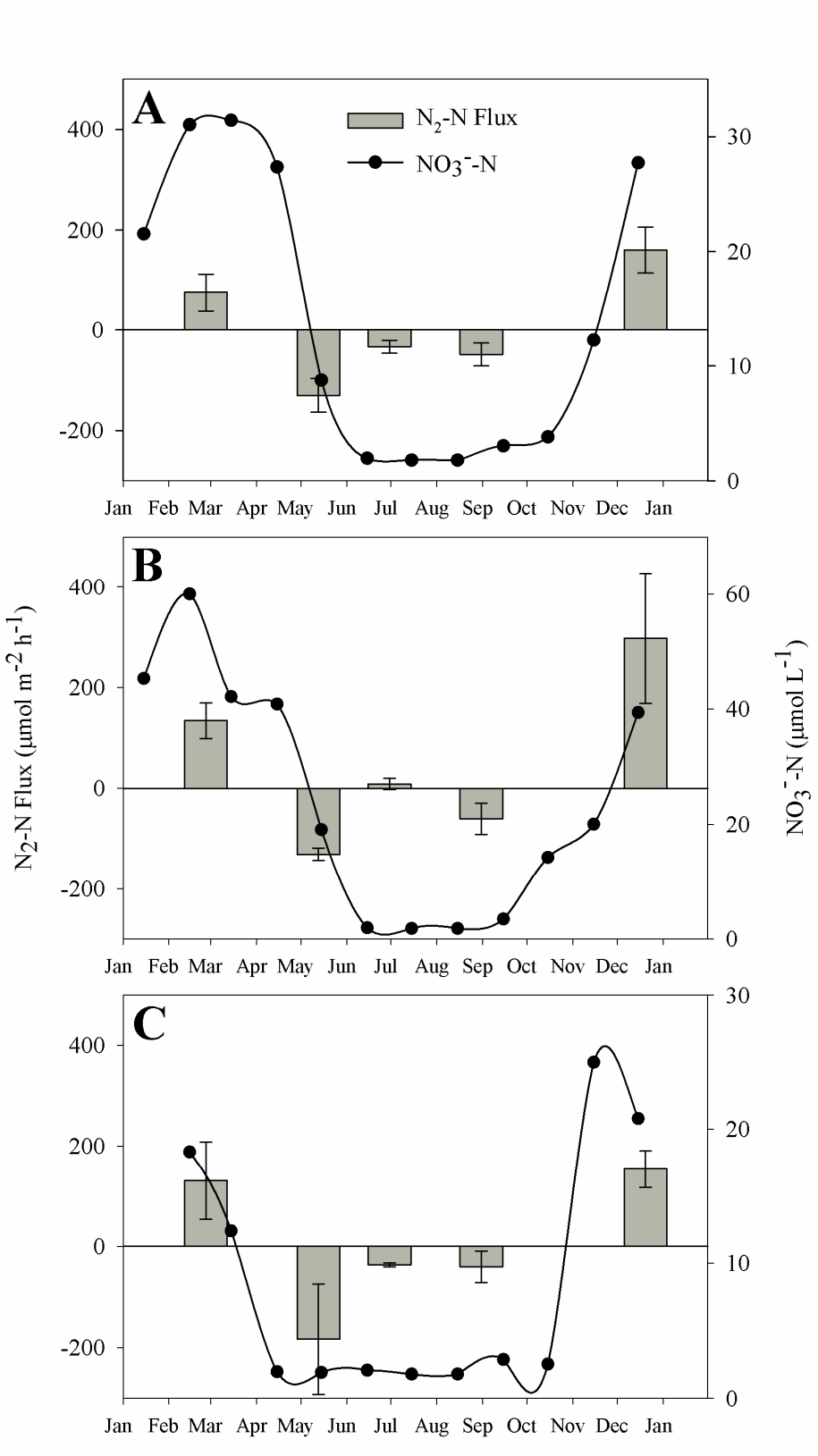
A
B
C
N_2-N Flux
NO_3^--N
N_2-N Flux (µmol m^{-2} h^{-1})
NO_3^--N (µmol L^{-1})
400
200
0
-200
30
20
10
60
40
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan

Figure 6.1

3. NITROGEN RETENTION AND DENITRIFICATION EFFICIENCY IN SMALL RESERVOIRS

3.1 Introduction

Anthropogenic activities have greatly increased the terrestrial reactive nitrogen (N) pool and subsequent N fluxes to adjacent aquatic ecosystems (Vitousek et al. 1997, Galloway et al. 2008). Nitrogen export to sensitive downstream water bodies, such as marine coastal waters, has been tied to adverse environmental impacts and human economic loss (Howarth et al. 1996, Vitousek et al. 1997). However, the river networks that transport N loads to marine systems may also retain up to 50% of watershed inputs (Wollheim et al. 2008). These river networks are comprised of wetlands, headwater streams, higher order rivers, and natural and man-made lakes, all of which have variable hydrologic regimes and nutrient cycling rates (Sanders and Kalff 2001, Seitzinger et al. 2002, Seitzinger et al. 2006). These ecosystems all retain and transform reactive N to inert dinitrogen gas (N_2), but the relative magnitude of N retention and transformations remain poorly understood.

Nitrogen retention, or the portion of the influent N load not accounted for in the effluent load, varies across aquatic ecosystems and is positively correlated with hydraulic residence time and N loading rates (Kelly et al. 1987, Sanders and Kalff 2001). Lentic water bodies, such as natural lakes and man-made impoundment reservoirs, have long water residence times relative to lotic systems and therefore exhibit greater N retention. Nitrogen retention in natural lakes has been widely studied (Jensen et al. 1992, Molot and Dillon 1993, Menghis et al. 1997), but few observations are available from manmade impoundment reservoirs (but see David et al. 2006, Kozelnik et al. 2007). Nevertheless, the currently available data suggest that reservoirs receive high area-specific nutrient loads relative to natural lakes and may play a disproportionate role in landscape-scale N retention (Harrison et al. 2009). These data indicate that as much as 33% of

global N retention in river networks occurs in reservoirs, despite the fact that reservoirs account for only 6% of the total surface area of these networks (Harrison et al. 2009). When N retention rates of reservoirs are normalized to reservoir surface area, estimated retention by is estimated to be 7-8 x greater than by natural lakes.

Additionally, Harrison et al. (2009) explored potential N retention variability related to lake and reservoir size, dividing data between large (surface area > 50 km^2) and small (0.001 km^2 < surface area < 50 km^2) systems. Estimated small reservoir areal retention exceeded that of large reservoirs by more than 25% and was almost 10x greater than areal retention by large lakes. Variability in N retention may also occur within the small reservoir class. Downing et al. (2008) showed that organic carbon burial rates in 40 small impoundments (approximately 0.1-50 km^2) increased by orders of magnitude as impoundment surface area decreased. Because N is generally transported to sediments as organic matter, a similar pattern for N storage should be expected.

Estimates of ecosystem-scale lentic N retention are rarely paired with direct measurements of denitrification, the only permanent removal pathway for reactive N from the biosphere. The inherent difficulties associated with measuring denitrification products and scaling rates to the whole ecosystem have presented major challenges in studies of lentic denitrification (Groffman et al. 2006, Groffman et al. 2009). Lake and reservoir N budgets bypass direct measurements of denitrification, equating N removal through denitrification with "missing" N in mass balance models (Jensen et al. 1992, Molot and Dillon 1993, Garnier et al. 1999). Two recent studies paired N retention estimates with direct laboratory measurements of denitrification, but reached divergent conclusions. David et al. (2006) measured denitrification using acetylene inhibition in sediment slurry assays, concluding that over 50% of N retention in

Shelbyville Reservoir could be attributed to denitrification, while Kozelnik et al. (2007) used isotope pairing techniques during intact sediment core incubations, estimating low denitrification rates that accounted for only 16.4% of N retention at Solina Reservoir. These findings may represent the naturally occurring variability in lentic denitrification efficiency, but may also reflect limitations of the methods employed to measure denitrification, particularly in regards to describing ecosystem-scale rates (Groffman et al. 2006, Groffman et al. 2009). More work is still needed to understand the contribution of denitrification to the ecosystem-scale N flux in lakes and reservoirs.

The objectives of this study were 1) to estimate annual areal N retention in 3 reservoirs representing a relatively understudied, but potentially important, size class (< 1 km^2) of reservoirs, and 2) to combine retention estimates with previously estimated annual denitrification rates from the study reservoirs that combined multiple seasonal and habitat-specific denitrification measurements (Chapter 2). We estimated whole-reservoir total nitrogen (TN) fluxes as the difference between system inputs and outputs. Annual reactive N removal through denitrification was estimated in reservoir epilimnion sediments during incubations of intact sediment cores repeated over a one-year period and from N_2 accumulation rates within reservoir hypolimnia and anoxic metalimnia (Chapter 2). Reservoir N retention and denitrification efficiency, or the proportion of N retention representing permanent reactive N removal through denitrification, were determined by comparing model inputs, retention, and denitrification rates.

3.2 Methods

3.2.1 *Study Sites*

Lakes Elmdale (36º11'45.5"N, 94º12'50.8"W), Fayetteville (36º08'11.5"N, 94º07'46.7"W) and Wedington (36º05'27.05"N, 94º22'02.9"W) are small (surface area < 1 km^2), shallow (average depth 3 m, maximum depth 9-10 m), and eutrophic flood control impoundments located in and around Fayetteville, Arkansas, USA. The watershed areas of each of the reservoirs are approximately 30 – 40x greater than the reservoir surface area, indicating comparable hydrologic residence times, a factor known to impact rates of N cycling and retention (Seitzinger et al. 2002). The reservoirs differ in their primary watershed land use/land cover, introducing potential variability in external N loading that could impact reservoir N retention efficiency (Haggard et al. 2003). Urban and agricultural land use/land cover dominates the Lake Elmdale and Lake Fayetteville watersheds, while 80% of Lake Wedington's catchment is forested. Brush Creek, Clear Creek and an unnamed stream within the Lake Wedington State Park are the primary impounded streams that supply flow to Lakes Elmdale, Fayetteville and Wedington, respectively.

3.2.2 *Nitrogen mass balance model*

A whole-reservoir mass balance model was created to quantify ecosystem areal N flux ($Flux_{2010}$) at each site for the year 2010. Positive fluxes represented net retention, while negative fluxes indicated net release. Model inputs were the sum of all estimated 2010 daily watershed TN (TDN + PN) influent loads, annual NH_3-N release through mineralization of buried or settling organic N at oxic-anoxic boundaries or in reduced in-lake environments, and wet and dry

atmospheric deposition. Estimated 2010 daily TN outflow loads were considered the only output. The mass balance is therefore summarized:

$$Flux_{2010} = \frac{\left(\sum_{2010} L_{flux} + M_{annual} + D_{wd}\right)}{A_0} \quad (1)$$

Where M_{annual} is the 2010 organic N mineralization rate for the whole lake, D_{wd} is the sum of wet and dry atmospheric deposition to the reservoir surface, A_0 is the reservoir surface area, and Lflux is described as:

$$L_{flux} = \frac{L_{inflow}}{A_g} - L_{outflow} \quad (2)$$

Where L_{inflow} is the influent load estimated for the gauged portion of the watershed, $L_{outflow}$ is the estimated effluent load, and A_g is the proportion of the total watershed gauged in this study. The N yield of ungauged sub-watersheds was assumed to be equivalent to that of the gauged area. Regional wet deposition rates (1.29 g N m^{-2}) were obtained for the year 2010 from the National Atmospheric Deposition Program/ National Trends Network site AR27 (*http://nadp.sws.uiuc.edu*). Annual 2010 dry deposition was assumed to be 40% of wet deposition (0.52 g N m^{-2}) as in David et al. (2006).

3.2.3 *Watershed inflow (Linflow) and outflow nitrogen loads(Loutflow)*

At each reservoir, stream stage was measured continuously in the primary impounded streams immediately up and downstream of the reservoir during August 2009-December 2010 and April-May 2011. Gauging was conducted similarly to Schoonover et al. (2006). At each site, gauging stations were constructed within a deep pool, where the continuous presence of standing water was expected, by anchoring a T-post firmly in the stream bed and attaching a PVC conduit casing at the base of the post. Casings were perforated to allow entry of flow.

Each casing housed an Onset Hobo Water Level Data Logger, which logged the overlying absolute pressure (water + atmospheric pressure; kPa) at 5-minute intervals. Atmospheric pressure was recorded simultaneously with a pressure transducer stored in the lab.

From August 2009-August 2010, point discharge measurements were collected at base and storm flow conditions once monthly at a single stream transect at each stream site, when possible. Total discharge was estimated as the sum of at least 10 cross-sectional discharge measurements calculated as the product of cross-section depth, width and average velocity (Hauer and Lamberti 2007). A Marsh-McBirney Flo-Mate flowmeter was used to measure velocity. At the Lake Elmdale inflow site and Lake Fayetteville and Wedington outflow sites, a Teledyne RD Streampro Acoustic Doppler Current Profiler was used to collect discharge measurements during May 2011 high flow events when the streams were not wadeable.

For each gauging site, a rating curve relating discharge measurements to corresponding stream stage readings was developed using linear regression analysis in JMP 9.0. A square-root transformation was applied to discharge measurements to maximize linearity in the relationship between the variables (Hirsch et al. 1993). These models are summarized:

$$Q^{\frac{1}{2}} = a + bS \quad (3)$$

Where S is stage and *a* and *b* are linear regression equation parameters corresponding to the y-intercept and slope, respectively. For any measurement where S was less than or equal to the y-intercept, Q was estimated to be 0.

Concurrent with point discharge measurements, water chemistry samples were collected from the thalweg of the inflow streams (Hauer and Lamberti 2007). Outflow water chemistry samples were collected directly from the upper mixed water column of the reservoirs from near the outlet structure. Outflow samples were collected at least once monthly throughout the study

period, when possible, but were not collected in tandem with point discharge measurements after March 2010 because preliminary data indicated no effect of discharge on reservoir N concentration. Following collection, all samples were stored on ice until return to the laboratory.

Water chemistry samples were processed in the lab within 24 hours of collection using vacuum filtration to collect fractions for PN and TDN analysis (APHA 2005). Particulate N sub-samples were collected on 25mm Whatman glass fiber GFF filters, while filtrate passing through the filter was collected for TDN analysis. Sub-samples were preserved by freezing. Filters were dried at 55ºC for 24 hours and then analyzed for PN using a Thermo Flash 2000 Organic Elemental Analyzer. Sample TDN concentration was determined using a Shimadzu TOC analyzer equipped with a TNM-1 module (Shimadzu Scientific Instruments, Columbia MD).

At inflow sites, sample TDN and PN loads were estimated as the product of point discharge and TDN or PN concentration measurements, respectively. Rating curves relating TDN and PN loads to discharge were then developed using linear regression analysis in JMP 9.0. Natural logarithm transformations were applied to both load and discharge variables to maximize linearity, and a bias correction factor (BCF) was calculated for each model (Hirsch et al. 1993). Models for estimating inflow loads (L) are summarized:

$$\ln L = a + b \ln Q \times BCF \quad (4)$$

Season was considered the dominant control on variability in outflow PN and TDN concentration. Fifth order polynomial (Poly 5) or multiple-sine periodic (Multi-Sin) expressions relating natural log transformed TDN or PN concentration to time were selected as possible models to estimate daily concentration (Hirsch et al. 1993) and were generated using multiple linear regression analysis in JMP 9.0. Fifth order polynomial models are generalized in equation 5, while equation 6 represents Multi-Sin models:

$$\ln C = a + bT + c(T - \overline{T})^2 + d(T - \overline{T})^3 + e(T - \overline{T})^4 + f(T - \overline{T})^5 \times BCF \quad (5)$$

$$\ln C = a + b \sin 2\pi T + c \cos 2\pi T + d \sin 4\pi T + e \cos 4\pi T \times BCF \quad (6)$$

Where T is days elapsed since January 1, 2009, $\overline{T}$ is the mean value of T at each site, and *a-f* are regression coefficients. The adjusted coefficient of variation (r^2_a), root mean square error (RMSE), PRESS statistic, sum of squared error (SSE) were calculated for each possible model using JMP 9.0 and compared. The optimal model for each N constituent at each site was described as that with the highest r^2_a and lowest RMSE, PRESS, SSE and MSPE (Hirsch et al. 1993).

Discharge, influent TDN and PN loads, and effluent TDN and PN concentration corresponding to each 5-minute stage reading logged during the study period were estimated using equations 3-6. Effluent loads were then calculated for each 5-minute interval as the product of corresponding discharge and concentration estimates. Five-minute interval estimates were combined and scaled-up to daily influent and effluent loads. Daily TDN and PN riverine fluxes were calculated according to equation 2.

3.2.4 *Annual mineralization rates*

Epilimnetic sediment and hypolimnion (sediment and water column) contributions to whole-reservoir mineralization were estimated separately based upon thermal stratum-specific NH_3-N fluxes. Epilimnetic sediment NH_3-N fluxes were measured from continuous flow-through incubations of intact sediment cores. Sediment cores were collected from sediments in contact with reservoir epilimnia in February, May, June, August and December 2010. At each site, 3 - 4 cores with overlying water were collected in clear plastic tubes (surface area = 40.6 cm^2, height = 70 cm) using a manual gravity corer. Epilimnetic water was collected

simultaneously in acid-washed carboys at each site to serve as the incubation inflow water supply.

Incubations were conducted in the lab similarly to those described by Scott et al. (2008) at in situ temperature. Cores were sealed airtight with rubber stoppers fitted with Teflon tubing to provide inflow and outflow paths for the incubation water. The inflow water supply was constantly aerated to simulate reservoir mixing and was pumped through the cores at a rate of 0.50-0.75 mL min^{-1}. After a 12-18 hour pre-incubation period, effluent from each core chamber and influent from each reservoir inflow water supply was harvested on 2 consecutive days and was filtered through 47 mm 0.60 µm Whatman GFF glass fiber filters under vacuum pressure. Filtrate was transferred to acid-washed dark bottles and preserved by freezing until subsequent fluorometric NH_3-N analysis on a Turner Designs Lab Fluorometer (Holmes et al. 1999). Areal mineralization rates (K_m) were then calculated for each sediment core as:

$$K_m = \frac{([NH_3]_{out} - [NH_3]_{in}) \times Q}{A_c} \quad (7)$$

Where $[NH_3]_{out}$ and $[NH_3]_{in}$ are the NH_3-N concentration of core chamber effluent and influent, respectively, Q is the continuous flow rate and A_c is the surface area of the sediment core. The mean K_m and associated standard error rates were calculated from replicate cores from each reservoir on each date.

Hypolimnion mineralization was estimated based upon hypolimnion NH_3-N accumulation rates during stratification. From April – August 2010, hypolimnion water samples were collected weekly to biweekly at the location of maximum depth in each reservoir. Samples were collected with a Van Dorn horizontal sampler at approximately 6 and 8 m at Lakes Elmdale and Fayetteville and 8 m at Lake Wedington. Samples were transferred to acid-washed dark bottles and stored on ice until return to the laboratory, where samples were processed and later

analyzed for NH_3-N concentration as described above. Volumetric hypolimnion mineralization rates (k_{NH3}) were estimated as the slope of a linear regression analysis on NH_3-N concentration versus time conducted in JMP 9.0, with a positive or negative slope indicating solute production or consumption, respectively. Volumetric rates were converted to area-specific mineralization rates (K_m) as described below:

$$K_m = \frac{k_{NH3} \times V_h}{A_h} \quad (8)$$

Where V_h and A_h are the volume and surface area of the hypolimnion, respectively. All thermal strata dimensions were taken from Chapter 2.

Annual whole-ecosystem areal mineralization rates (M_{annual}) were estimated by summing habitat- and seasonally-specific areal rates over the appropriate areas and durations, respectively. Measured mineralization rates were assumed to represent average seasonal rates; therefore, assigning time intervals to each K_m for all habitats was required. Mean K_m rates from sediment cores collected in February, May, June, August and December 2010 were divided over a one-year period as follows: January 1-April 14, April 15-May 31, June 1-July 31, August 1-October 15, and October 16-December 31 2010, respectively. Hypolimnion mineralization rates were scaled up only during the stratified period (April 15-October 15). Whole reservoir annual mineralization rates are therefore expressed as:

$$M_{annual} = \frac{\sum(K_m \times t \times A_s)}{A_0} \quad (9)$$

Where *t* is the number of days over which a measured K_m was assumed to represent in situ seasonal rates, and A_s is the seasonal surface area of the stratum corresponding to that K_m.

3.2.5 *Whole-reservoir annual denitrification rates*

Seasonal stratum-specific denitrification rates in epilimnetic sediments, the anoxic metalimnion and the hypolimnion at each of the study reservoirs was estimated in Chapter 2. Denitrification rates were measured simultaneously with mineralization during sediment core incubation and hypolimnion accumulation experiments described above, with N_2-N fluxes representing denitrification rates. Additional field and lab procedures required for estimating denitrification rates during these experiments are described in detail in Chapter 2. In order to approximate annual, whole reservoir areal denitrification rates, stratum-specific N_2-N fluxes measured during these experiments were adjusted to the seasonal, whole-stratum scale and were normalized to the whole reservoir surface area, as outlined for calculating mineralization rates in equations 8 and 9, respectively.

3.2.6 *Reservoir nitrogen retention and denitrification efficiency*

The annual areal TN flux for the year 2010 in the study reservoirs was calculated using equation 1. Positive flux rates indicated net reservoir N retention in 2010, while negative fluxes indicated net export. Reservoir N retention efficiency ($\%N_{ret}$) of model TN inputs, as well as watershed TDN and PN loading, was estimated as:

$$\%\mathrm{N_{ret}} = \frac{\mathrm{Flux_{2010}}}{\mathrm{Input_{2010}}} \times 100\% \quad (10)$$

Where $Input_{2010}$ is the sum of all model inputs described in equation 1. Reservoir denitrification efficiency ($\%L_{DNF}$) was then estimated in this study as:

$$\%\mathrm{L_{DNF}} = \frac{\mathrm{DNFannual}}{\mathrm{Flux_{2010}}} \times 100\% \quad (11)$$

Finally, other published estimates of areal N loading and retention in lentic systems were compiled and compared to findings from the study reservoirs (David et al. 2006, Kozelnik et al. 2007).

3.3 Results

3.3.1 *Watershed nitrogen loading models*

A strong linear relationship ($p < 0.0001$) between stream stage and square root transformed discharge was found across the sampled range at all stream sites (Table 1). At all inflow sites, both TDN and PN loads were strongly related to discharge across the sampled range (Figure 1). Fifth order polynomial models were selected to predict TDN concentration at Lakes Elmdale and Wedington (Table 2). The models for predicting TDN concentration at Lake Fayetteville ranked similarly, but the multi-sine periodic equation was selected because the PRESS statistic was lower. The model selected to estimate PN concentration was a 5^{th} order polynomial equation at Lakes Elmdale and Fayetteville and a multi-sine periodic equation at Lake Wedington. Across sites, 47-78% of variability in outflow PN and TDN concentration was explained by the selected seasonal models. In 2009 and 2010, TDN concentration peaked in November following lake turnover, remained high through winter, and reached a minimum during the summer months (Figure 2A-C). Reservoir PN concentration exhibited the reverse trend, peaking in late summer and declining during winter months (Figure 2D-F).

3.3.2 *Daily nitrogen watershed fluxes*

Across sites, daily riverine TDN fluxes were most often positive, indicating net reservoir retention of the dissolved N fraction (Figure 3A-C). At Lakes Elmdale and Fayetteville, daily

riverine PN fluxes were most often negative, indicating net export (Figure 3D-F). However, high magnitude PN storage events occurred concurrently with high flow, though high magnitude PN export followed these events during summer months when reservoir PN concentrations were at their maximum. In contrast to Lakes Elmdale and Fayetteville, daily PN fluxes at Lake Wedington were most often positive, except following storm events and when the reservoir discharged during summer months. Both base and storm flow daily TDN and PN fluxes at Lake Wedington were low in magnitude relative to fluxes at Lakes Elmdale and Fayetteville.

3.3.3 *Reservoir mineralization and denitrification rates*

Table 3 provides a summary of the annual epilimnetic sediment, hypolimnion, and whole-reservoir mineralization rates estimated at Lakes Elmdale, Fayetteville and Wedington. Whole-ecosystem mineralization ranged from 2.22 - 7.38 g N m^{-2} and accounted for approximately 10 – 20 % of total TN model inputs. Whole-reservoir mineralization rates were dominated by hypolimnion rates, which were an order of magnitude greater than epilimnetic sediment rates. Whole-ecosystem denitrification rates for the year 2010 estimated by Chapter 2 are included in Table 4.

3.3.4 *Annual reservoir nitrogen retention and denitrification efficiency*

Table 4 provides a summary of the annual 2010 TN load inputs, outputs and fluxes, as well as riverine TDN and PN inflow and outflow loads and fluxes for Lakes Elmdale, Fayetteville and Wedington. The reservoirs were consistent sinks for riverine TDN loads, but Lakes Elmdale and Fayetteville were net PN sources in 2010. Particulate nitrogen export occurred at a low rate relative to TDN retention in the lakes. Despite net PN export at 2 sites, all

reservoirs acted as TN sinks in the landscape, with annual N retention ranging from 13.6 – 18.7 g m^{-2}. Areal retention in the study reservoirs and the compiled natural lakes and reservoirs was a function of N loading rates (Figure 4). Retention efficiency ranged from 49% at Lakes Elmdale and Fayetteville to 84% at Lake Wedington (Table 4). Estimated annual denitrification exceeded retention rates at Lakes Elmdale and Fayetteville and accounted for approximately 70% of TN retention at Lake Wedington.

3.4 Discussion

3.4.1 *Ecosystem-scale N retention*

The cross-system comparison of lentic loading and retention indicated that the study reservoirs received and retained areal N loads that are intermediate among lentic systems and low relative to other reservoirs (Figure 4). Though in range with global model estimates of small reservoir N retention, annual N retention in 2010 at Lakes Elmdale, Fayetteville and Wedington was 40 – 60 % of the 31 g N m^{-2} y^{-1} estimated as the global average by Harrison et al. (2009). The magnitude of N retention in the study reservoirs relative to natural lakes supported model findings indicating a significant role for small reservoirs in retention (Harrison et al. 2009). Estimated N retention in the study reservoirs was an order of magnitude greater than in most similarly-sized natural lakes (Molot and Dillon 1993, Ahlgren et al. 1994, and van Luijn et al. 1996).

Among the study reservoirs, Lake Wedington exhibited the greatest N retention efficiency. Total nitrogen loading to Lake Wedington was only 58% of Lake Fayetteville's annual TN inputs, but Lake Wedington retained a nearly identical N load. Lake Wedington was also most efficient at retaining riverine TDN and PN loading, suggesting that hydrologic

variability might explain elevated efficiency at Lake Wedington. Discharge and TN loading out of Lake Wedington frequently ceased due to the intermittent nature of the streams in this basin, but outflow was always observed at Lakes Elmdale and Fayetteville (Figure 2). Lake Wedington's watershed also had the highest proportion of forested land use/land cover, a watershed characteristic associated with lower magnitude maximum storm flow, as well as reduced high flow and pulse frequency, particularly relative to urban land use/land cover (Schoonover et al. 2006) . The low magnitude and relative infrequency of major spikes and dips in daily riverine fluxes at Lake Wedington supports this interpretation (Figure 3). Lake Wedington N retention efficiency was comparable to larger lakes with long hydraulic residence times (Menghis et al. 1997) and was among the highest documented in lentic systems (Kozelnik et al. 2007, Harrison et al. 2009).

3.4.2 *Denitrification efficiency*

Denitrification efficiency at Lake Wedington was also high relative to other lentic systems (Kozelnik et al. 2007), but estimated annual denitrification rates were almost 50% less than at Lakes Elmdale and Fayetteville (Table 4). Annual denitrification rates at Lakes Elmdale and Fayetteville exceeded annual N retention, indicating denitrification efficiency greater than 100%. These findings were unexpected, and denitrification could have been overestimated, particularly epilimnetic rates based upon sediment core incubation N_2 fluxes that were assumed to uniformly represent seasonal denitrification rates in all epilimnetic sediments (Groffman et al. 2006, Groffman et al. 2009). It is also likely that these processes are not comparable over simultaneous intervals, as N import, denitrification, and export do not occur instantaneously and are variable in space and time. For example, up to 20% of N loading to the study reservoirs in

2010 was due to a single seasonal event, namely fall lake turnover and the associated release of accumulated hypolimnion NH_3-N. A similar release can be assumed to have occurred in 2009, and subsequent elevated TDN concentrations persisting in reservoir waters through late spring in 2010 suggest that this N input likely contributed to denitrification at least 6 months after mixing.

It is also possible that denitrification exceeded N retention at 2 of the study reservoirs because we were not able to estimate all potential N inputs as part of the study's mass balance model. In Chapter 2, disparity in hypolimnion NO_3^- supply and depletion rates relative to N_2 production was noted. It was hypothesized that NO_3^- in groundwater entering the lakes through sediments was fueling hypolimnetic denitrification up to 4 months after in-lake NO_3^- supplies were exhausted. The Ozark Plateau exhibits high surface to groundwater connectivity, resulting in similar water chemistry between surface and groundwater in both pristine and non-point source impacted zones (Petersen et al. 1999, Haggard et al. 2005). Springs that formerly fed the reservoirs' parent streams would now discharge directly into the reservoirs, and stream and groundwater NO_3^--N concentrations can reach levels of up to 4 mg L^{-1} in urban and agricultural watersheds like those of the study reservoirs.

Nitrogen loading through fixation of atmospheric N_2 in algal biomass could also be a significant reservoir N input in all three reservoirs. During summer months, lentic N_2 fixation can represent virtually all of the N load to some ecosystems (Patoine et al. 2006), though rates are typically highly variable between systems and years (Scott et al. 2008). Annual water column N_2 fixation at Lake Fayetteville was approximately 9 g m^{-2} $year^{-1}$ in 2008 and 2009 (J. Thad Scott, unpublished data). If these estimates are representative of 2010 rates, Lake Fayetteville water column N_2 fixation was roughly equivalent to the 2010 riverine N flux. In Chapter 2, net sediment N fixation was measured at multiple sites, in 3 of 5 2010 core incubation

experiments, though these fluxes were not scaled up to ecosystem rates. Clearly, N_2 fixation is a major source of N loading in the study reservoirs, but developing detailed estimates of N_2 fixation rates was beyond the scope of this study.

Previous studies may have underestimated lentic denitrification based upon limitations inherent to the methods employed and associated assumptions. The mass balance approach to estimating denitrification equates N removed through denitrification to the deficit between system inputs and outputs and N burial. Previous studies defining denitrification rates as "missing" N are therefore inherently unable to estimate denitrification rates exceeding retention. Previous lentic ecosystem denitrification estimates based upon direct measurements have solely focused on sediments (David et al. 2006, Kozelnik et al. 2007). However, in Chapter 2, indications of significant N_2 production within the reduced water column at the study reservoirs was found. Up to 50% of annual denitrification occurred in the anoxic metalimnion at Lake Elmdale, also the site with the greatest difference between retention and denitrification estimates. Anoxic metalimnion denitrification was strongly tied to water column stability, with brief N_2 production bursts occurring during stable periods following disturbance events. This type of transient water column activity falls outside the scope of any previous lentic denitrification study.

Incongruities in estimated N fluxes also highlight the limitations associated with mass balance studies. The most significant of these limitations is the relatively brief temporal scale of most studies. Considerations of cost and available manpower limit studies to estimating N fluxes over periods of 1 - 2 years, or less, (David et al. 2006; Kozelnik et al. 2007, present study), but significant interannual variability in N loading and retention likely occurs (Garnier et al. 1999, Ferris and Lehman 2006). While relatively constant seasonal patterns are important in shaping N

retention (Figure 2), in our study, extreme hydrologic events that vary in their temporal distribution between years frequently reversed base flow fluxes and compounded the effects of seasonal variability on effluent loads (Figure 3). This is best illustrated through comparison of daily PN fluxes at Lake Elmdale and Fayetteville associated with major Oct. 2009 and July 2010 storm events. High magnitude PN retention occurred during both events, but accompanying high magnitude PN export was observed only following the July event, when reservoir algal biomass is at its highest. The reverse trend was seen in daily TDN fluxes at Lake Fayetteville.

It should be noted that some extrapolation of rating curves for estimating flow and influent N loads was required due to equipment limitations preventing collection of discharge measurements at high flow exceeding wadeability. At most sites, extrapolation was relatively minor, with point discharge and water chemistry samples collected at stream stages equivalent to 80-95% of the maximum average daily stage. At the Lake Elmdale outflow site, however, the maximum stream stage at which discharge was measured was equivalent to just 57% of maximum average daily stage. Despite the limited range of point discharge measurements, modeled discharge out of Lake Elmdale always remained within range of simultaneous estimates at the gauged sites upstream and at the other study reservoirs during high flow events. In general, the modeling strategies employed by this study were highly successful considering the potential problems associated with this type of gauging and sampling regime (Toor et al. 2008).

Another source of error in our study arises from the manner in which gauged area hydraulic and N yields were scaled up to represent the total reservoir watershed. Equal yield was assumed across all sub-watersheds regardless of differences size or land use/land cover. However, in small and/or impacted catchments, high nutrient and hydraulic yields relative to larger watersheds may occur (Smith et al. 1997, Schoonover et al. 2006). Adjusting influent

loading estimates based upon sub-watershed characteristics might have increased total N inputs, but we believe this would have been difficult to accomplish at the resolution of available topographic and land use/land cover data.

3.4.3 *Summary and conclusions*

The results of this study provide estimates of annual N retention rates and efficiency in 3 man-made reservoirs representing a relatively understudied lentic size class. Areal N retention in the study reservoirs was low relative to retention documented in other reservoirs (David et al. 2006, Kozelnik et al. 2007), but retention efficiency was moderate to high. Our study builds upon previous lentic N retention studies by estimating the proportion of the retained annual N load that is permanently removed from the system through denitrification. In contrast to other studies of denitrification efficiency, our whole-reservoir denitrification estimates were generated by combining multiple seasonal and habitat-specific N_2 flux measurements and represent both sediment and anoxic water column contributions. Denitrification rates exceeded N retention estimates in 2 of the 3 reservoirs, but model inputs did not include potentially significant N_2 fixation inputs. These findings suggest that denitrification may play a greater role in regulating long-term reservoir storage of watershed and biologically-fixed N loads than previously understood.

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Table 3.1. Linear regression equation coefficients *a* and *b* as described in Eq. 3 relating square root transformed point discharge measurements to stream stage measured as water pressure at in-stream inflow and outflow sites. The adjusted coefficient of determination (r^2_a) and root mean square error (RMSE), sum of square error (SSE), and ρ values are included for each model.

Reservoir	Site	a (SE)	b (SE)	r^2_a	RMSE	SSE	ρ
Elmdale	Inflow	-1.22 (0.120)	0.322 (0.0212)	0.924	0.142	0.364	<0.0001
	Outflow	-1.91 (0.151)	0.379 (0.0251)	0.9339	0.03327	0.0166	<0.0001
Fayetteville	Inflow	-3.28 (0.120)	0.529 (0.0174)	0.9809	0.04518	0.0347	<0.0001
	Outflow	- 2.27 (0.102)	0.476 (0.0167)	0.9782	0.06586	0.0737	<0.0001
Wedington	Inflow	-1.38 (0.161)	0.257 (0.0247)	0.8842	0.09081	0.1072	<0.0001
	Outflow	-1.72 (0.218)	0.266 (0.0250)	0.9332	0.1539	0.1659	<0.0001

Table 3.2. Comparative statistics from the multi-sine periodic and 5th degree polynomial regression models developed to describe seasonal variability in total dissolved nitrogen (TDN) and particulate nitrogen (PN) concentration in the study reservoirs. The optimal model for each variable at each site is in bold. The adjusted coefficient of determination (r^2_a) and root mean square error (RMSE), sum of square error (SSE) and PRESS statistic were compared to select the overall best model with the highest r^2_a and lowest RMSE, SSE and PRESS.

Site	Variable	Model	r^2_a	RMSE	SSE	PRESS
Elmdale	TDN	Multi Sin	0.557	0.199	1.19	2.60
	TDN	**Poly 5**	**0.639**	**0.180**	**0.935**	**1.55**
	PN	Multi Sin	0.672	0.424	6.82	11.4
	PN	**Poly 5**	**0.692**	**0.411**	**6.24**	**10.0**
Fayetteville	**TDN**	**Multi Sin**	**0.732**	**0.210**	**1.24**	**2.61**
	TDN	Poly 5	0.735	0.209	1.18	4.03
	PN	Multi Sin	0.697	0.382	5.56	13.3
	PN	**Poly 5**	**0.783**	**0.324**	**3.88**	**7.38**
Wedington	TDN	Multi Sin	0.524	0.178	0.602	1.23
	TDN	**Poly 5**	**0.580**	**0.167**	**0.503**	**0.774**
	PN	**Multi Sin**	**0.474**	**0.313**	**2.64**	**3.57**
	PN	Poly 5	0.449	0.320	6.05	4.12

Table 3.3. Mean (standard error) annual epilimnion and hypolimnion (thermal stratum) contributions to the annual ammonia-nitrogen (NH_3-N) flux due to mineralization of organic N at in-lake oxic-anoxic boundaries and in reduced environments.

Site	Thermal Stratum	NH_3-N Flux ($g\ m^{-2}$)
Elmdale	Epilimnion	0.322 (0.0611)
	Hypolimnion	3.42 (0.232)
	Total	3.74 (0.293)
Fayetteville	Epilimnion	0.0541 (0.0486)
	Hypolimnion	7.32 (0.563)
	Total	7.38 (0.612)
Wedington	Epilimnion	0.373 (0.0764)
	Hypolimnion	1.85 (0.216)
	Total	2.22 (0.292)

Table 3.4. Study reservoir annual total nitrogen (TN) mass balance load input, output and flux for the year 2010, including watershed total dissolved nitrogen (TDN) and particulate nitrogen (PN) loads, mineralization and wet and dry atmospheric deposition. Influent TDN and PN loads only reflect watershed N inputs. Reservoir retention efficiency for TN inputs and watershed TDN and PN loads are given as %Ret. Whole-reservoir denitrification rates estimated for the year 2010 in Chapter 2 are included, as well as denitrification efficiency (%DNF), or the proportion of the TN load retained accounted for by denitrification.

		Load in	Load out	Retention		DNF	
Site	Variable	($g\ m^{-2}$)			% Ret	($g\ m^{-2}$)	%DNF
Elmdale	TN	28	14	14	49	25	180
	TDN	19	7.8	12	60		
	PN	2.9	6.5	-3.6	-120		
Fayetteville	TN	38	19	19	49	24	130
	TDN	22	12	9.5	43		
	PN	6.7	7.7	-0.94	-14		
Wedington	TN	22	3.5	19	84	13	70
	TDN	16	2.2	14	86		
	PN	2.3	1.3	1.0	44		

Figure Legends

Figure 3.1. Natural log transformed total dissolved nitrogen (TDN) and particulate nitrogen (PN) loads versus point discharge measurements from Lake (A) Elmdale, (B) Fayetteville, and (C) Wedington inflow streams with linear regression equations, r^2_a, F statistics and ρ values.

Figure 3.2. Natural log transformed observed and predicted total dissolved nitrogen (TDN; A-C) and particulate nitrogen (PN; E-F) outflow concentration through at the study reservoir. Panel rows correspond to Lakes Elmdale, Fayetteville, and Wedington, respectively, descending from the top.

Figure 3.3. Modeled study reservoir total dissolved nitrogen (TDN; A-C) and particulate nitrogen (PN; D-E) load fluxes. Panel rows correspond to Lakes Elmdale, Fayetteville, and Wedington, respectively, descending from the top.

Figure 4.3. Areal nitrogen (N) retention rates versus areal N loading rates reported in natural lakes and reservoirs (Kozelnik et al. 2007), including the study reservoirs indicating that retention increases exponentially as loading increases. Regression equation, r^2_a, F statistic and p value are given.

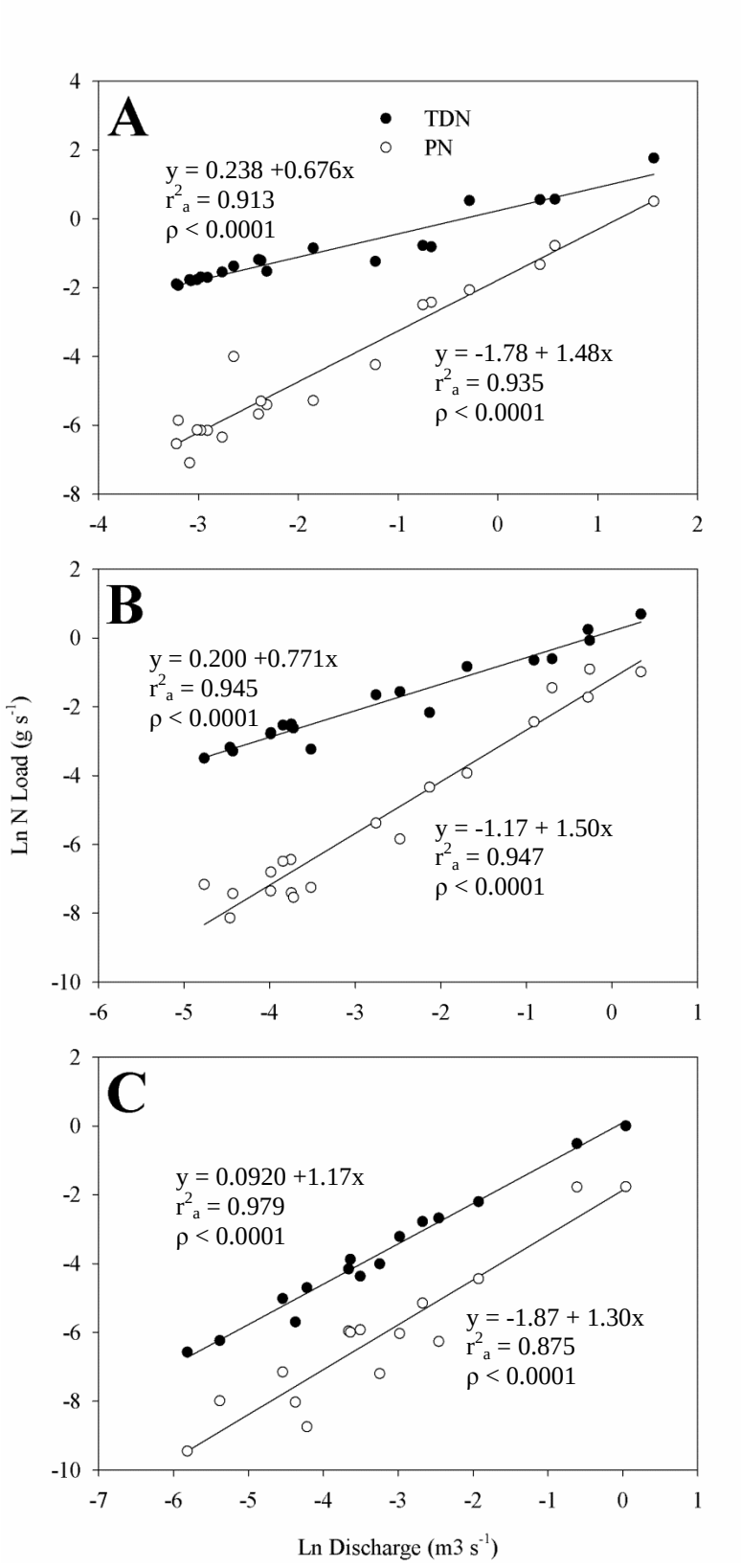
A
TDN
PN
y = 0.238 +0.676x
r2a = 0.913
ρ < 0.0001
y = -1.78 + 1.48x
r2a = 0.935
ρ < 0.0001
B
y = 0.200 +0.771x
r2a = 0.945
ρ < 0.0001
y = -1.17 + 1.50x
r2a = 0.947
ρ < 0.0001
C
y = 0.0920 +1.17x
r2a = 0.979
ρ < 0.0001
y = -1.87 + 1.30x
r2a = 0.875
ρ < 0.0001
Ln N Load (g s-1)
Ln Discharge (m3 s-1)

Figure 3.1.

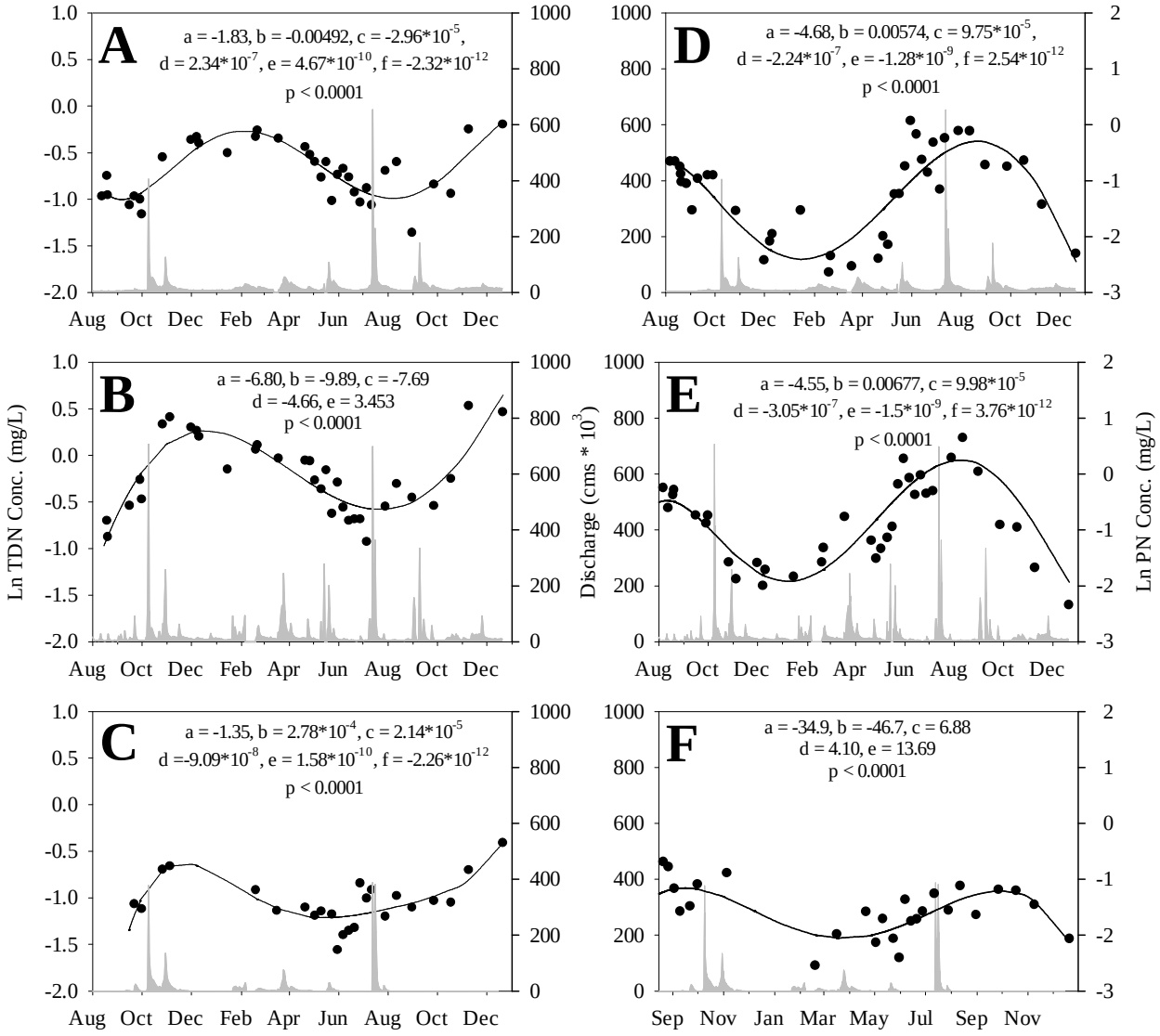
A
a = -1.83, b = -0.00492, c = -2.96*10^-5,
d = 2.34*10^-7, e = 4.67*10^-10, f = -2.32*10^-12
p < 0.0001
B
a = -6.80, b = -9.89, c = -7.69
d = -4.66, e = 3.453
p < 0.0001
C
a = -1.35, b = 2.78*10^-4, c = 2.14*10^-5
d =-9.09*10^-8, e = 1.58*10^-10, f = -2.26*10^-12
p < 0.0001
D
a = -4.68, b = 0.00574, c = 9.75*10^-5,
d = -2.24*10^-7, e = -1.28*10^-9, f = 2.54*10^-12
p < 0.0001
E
a = -4.55, b = 0.00677, c = 9.98*10^-5
d = -3.05*10^-7, e = -1.5*10^-9, f = 3.76*10^-12
p < 0.0001
F
a = -34.9, b = -46.7, c = 6.88
d = 4.10, e = 13.69
p < 0.0001
Ln TDN Conc. (mg/L)
Discharge (cms * 10^-3)
Ln PN Conc. (mg/L)
Aug Oct Dec Feb Apr Jun Aug Oct Dec
Sep Nov Jan Mar May Jul Sep Nov

Figure 3.2.

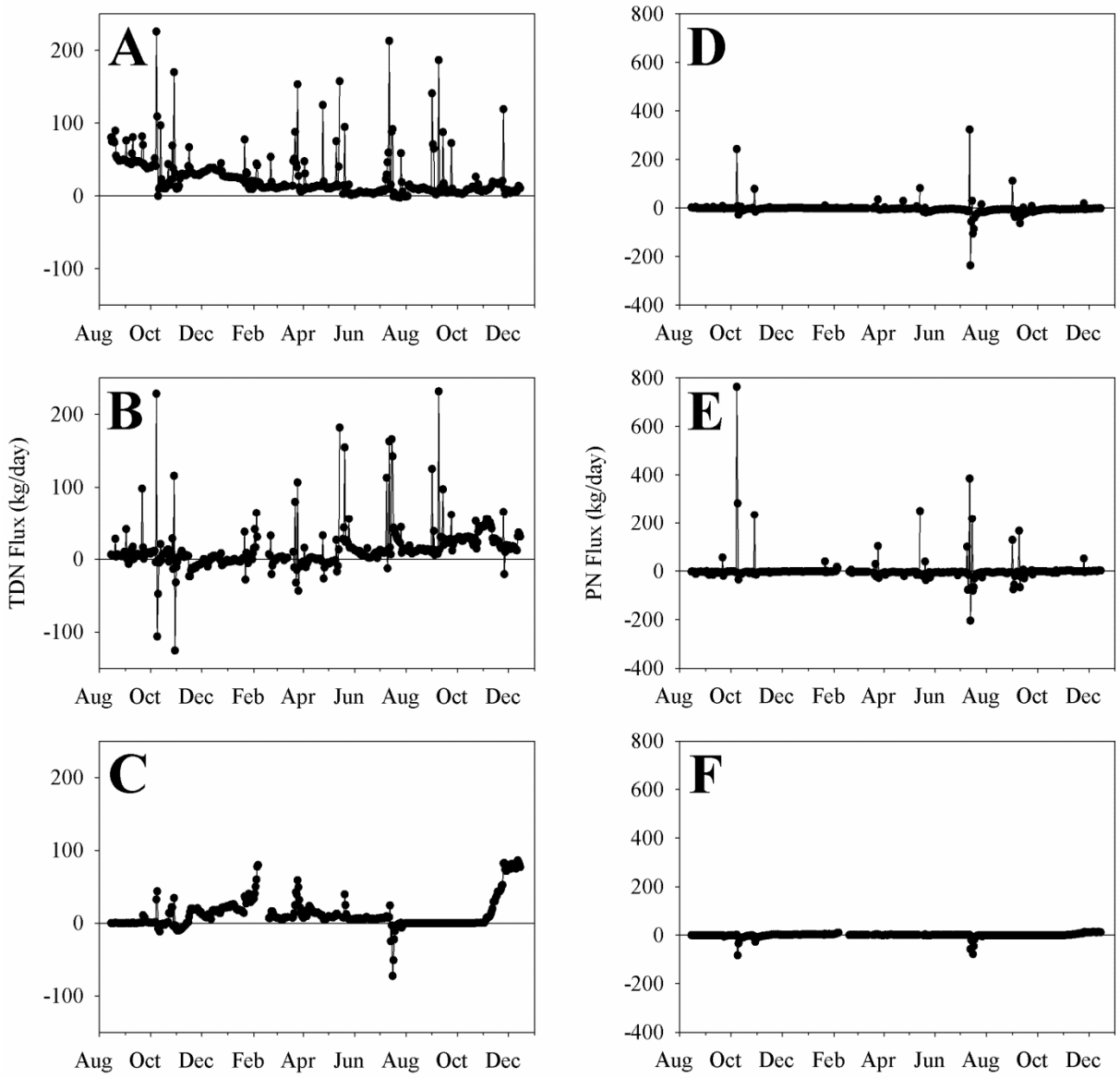
A
B
C
D
E
F
TDN Flux (kg/day)
PN Flux (kg/day)
200
100
0
-100
800
600
400
200
0
-200
-400
Aug Oct Dec Feb Apr Jun Aug Oct Dec

Figure 3.3.

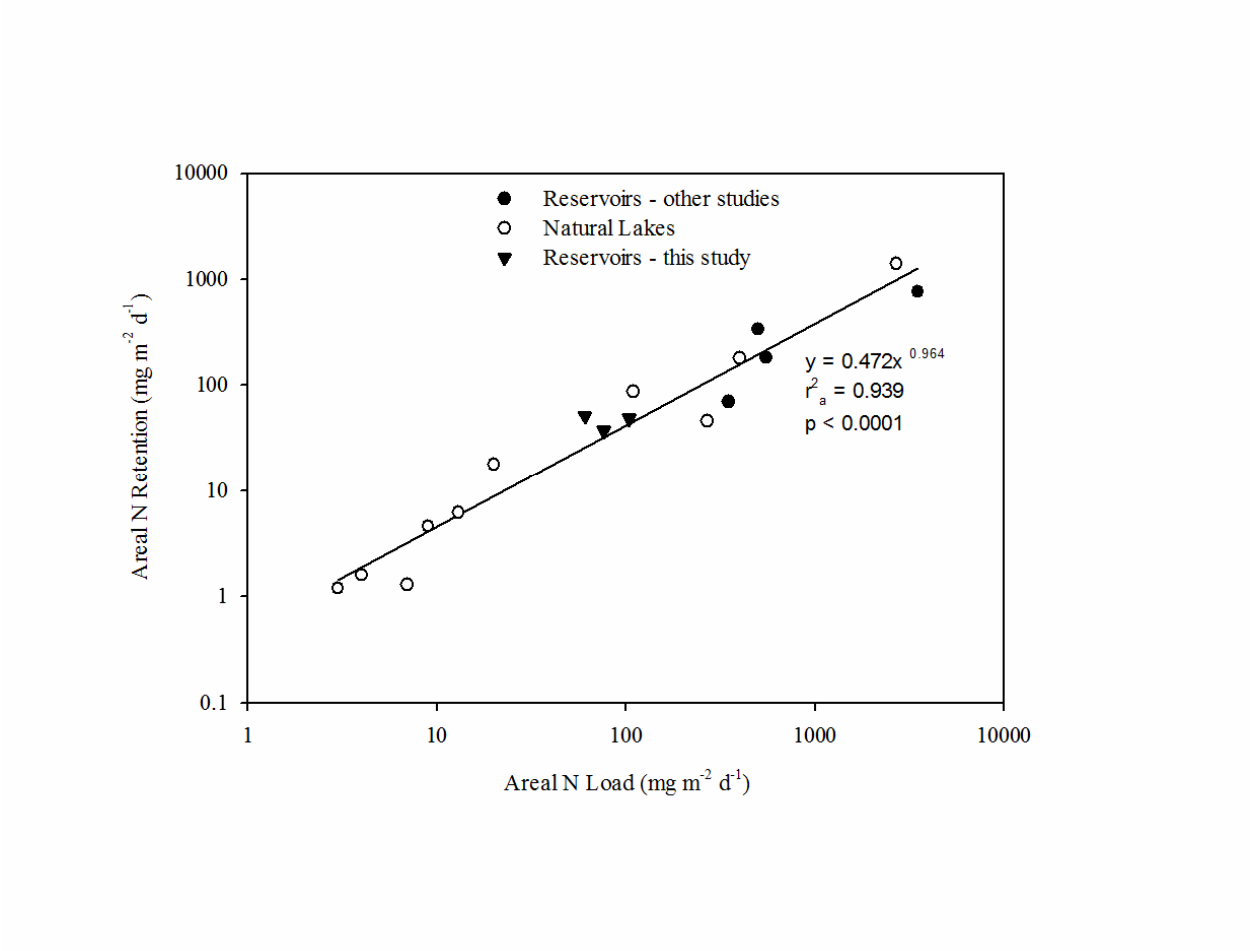
10000
1000
100
10
1
0.1
1
10
100
1000
10000
Areal N Retention (mg m-2 d-1)
Areal N Load (mg m-2 d-1)
Reservoirs - other studies
Natural Lakes
Reservoirs - this study
y = 0.472x 0.964
r2a = 0.939
p < 0.0001

Figure 3.4

4. CONCLUSION

The goals of this study were to generate estimates of annual N retention and denitrification in 3 reservoirs and compare these estimates to determine reservoir denitrification efficiency, or the proportion of N retention represented by reactive N removal through denitrification. In lentic systems, denitrification studies are largely limited by the cost, feasibility, and range of currently available methods. However, no studies had yet integrated multiple techniques for measuring denitrification into a single system-level study. This study demonstrated that denitrification in small, shallow impoundments may be accurately and rapidly estimated by combining habitat-specific techniques and advances the study of lentic denitrification by integrating hypolimnetic denitrification estimates with estimates from the anoxic metalimnion and epilimnetic sediments. Application of the N_2 accumulation technique to measure denitrification in the anoxic metalimnion is also unique to this study.

The results of this study provide estimates of annual N retention rates and efficiency in 3 man-made reservoirs representing a relatively understudied lentic size class (< 1 km^2). This study builds upon previous lentic N retention studies by using direct measurements of denitrification to estimate the contribution of denitrification to N retention. In contrast to other studies of denitrification efficiency, whole-reservoir denitrification estimates in this study were generated not only through direct measurement of N_2 fluxes, but by combining multiple seasonal and habitat-specific N_2 flux measurements that represent both sediment and anoxic water column contributions.

Denitrification efficiency in the study reservoirs was high relative to rates reported in other lakes and reservoirs, and denitrification rates exceeded N retention estimates in 2 of the 3 reservoirs. While method limitations may have resulted in underestimates of denitrification by

previous studies, this study's findings likely reflect the fact that potentially significant N inputs were not included in the mass balance model, most notable biological N_2 fixation. Microbially-mediated processes may contribute more greatly to both N loading and retention in lentic systems than previously understood, and this study indicates that denitrification plays a major role in regulating long-term storage of watershed and biologically-fixed N loads.