

Stoichiometric imbalance in rates of nitrogen and phosphorus retention, storage, and recycling can perpetuate nitrogen deficiency in highly-productive reservoirs

Erin M. Grantz,^{1,*} Brian E. Haggard,² and J. Thad Scott¹

¹Crop, Soil, and Environmental Science Department, University of Arkansas, Fayetteville, Arkansas

²Arkansas Water Resources Center, University of Arkansas, Fayetteville, Arkansas

Abstract

We measured the nutrient stoichiometry of inputs, outputs, retention, storage, and recycling in three seasonally nitrogen (N)-deficient reservoirs by incorporating watershed mass balances with measurements of internal N and phosphorus (P) transformations. Our objective was to determine if the reservoirs were accumulating N and thereby likely to develop strict P deficiency over time. For the eutrophic reservoirs, the N:P (by atoms) of annual outputs was two to five times greater than that of inputs, reflecting higher retention efficiency for P than N (~90% vs. ~50%, respectively) and resulting in retention stoichiometry indicative of N deficiency (N:P < 20). The N:P of these fluxes differed less for the mesotrophic reservoir because of similar N and P retention efficiencies, and the N:P of retained nutrients indicated strict P deficiency (N:P > 50). Denitrification (12–23 g N m⁻² yr⁻¹) removed ~50–100% of N retained by the reservoirs annually, increasing N deficiency in storage relative to retention for all the reservoirs (N:P < 1–30). The combined effects of more efficient P than N retention and efficient denitrification were also evident in the low N:P (< 10) of internal recycling. N₂ fixation (7–11 g N m⁻² yr⁻¹) was inefficient in balancing system N deficits and did not increase the low N:P of annual watershed inputs or seasonal epilimnion nutrient concentrations into the range of strict P deficiency. Low N:P storage and internal recycling strongly suggested that these reservoirs are not accumulating N relative to P and are thereby unlikely to become strictly P deficient over time.

River networks retain and export nitrogen (N) and phosphorus (P) at disparate rates (Alexander et al. 2008; Wollheim et al. 2008; Harrison et al. 2010). Differences in retention are caused by inherent dissimilarities in the N and P biogeochemical cycles (Galloway et al. 2004; Filippelli 2008; Buowman et al. 2013) and in the effects of spatially and temporally variable hydrology (Green and Finlay 2010). Impoundment reservoirs are important nutrient sinks within these networks (Harrison et al. 2009) and may be hotspots where internal processes decouple ecosystem-scale N and P fluxes (Fig. 1). Indeed, recent studies have shown that the N to P ratio (N:P) of watershed inputs to reservoirs can differ widely from that of reservoir output and sediment storage pools (Cook et al. 2010; Vanni et al. 2011).

In these studies, spatial patterns in N:P between watershed inputs and reservoir outputs were tied to reservoir nutrient limitation status. In N-deficient systems, total N:P was approximately two times higher in reservoir output than in watershed inputs (Cook et al. 2010; Vanni et al. 2011). This pattern indicated that the reservoirs more efficiently retained P than N and is supported by similar studies in other reservoirs (Gill et al. 1976; Garnier et al. 1999; Bosch et al. 2009). For the Lower Lakes in South Australia, Cook et al. (2010) attributed doubling of N:P between inflow and outflow loads to a significant but unaccounted-for internal N input from biological dinitrogen (N₂) fixation. In contrast to increased N:P in reservoir outputs, sediment N:P in reservoirs is typically much lower than the N:P of watershed inputs (Filstrup et al. 2010; Vanni et al. 2011; Burford et al. 2012). Low N:P in

sediments relative to watershed inputs and reservoir outputs suggests that many reservoirs not only retain but also store P at disproportionately high rates relative to N over potentially long time scales. Biological removal of N through denitrification may be the mechanism for low N:P sediment nutrient stoichiometry (Vanni et al. 2011), especially as no congruent internal removal process exists for P. Thus, in lakes and reservoirs, the balance between internal N addition through N₂ fixation and N loss through denitrification likely provides a substantial control on ecosystem stoichiometry and on whether a system exhibits N or P deficiency to primary production.

The annual balance between N₂ fixation and denitrification rates and the effect these processes have on ecosystem stoichiometry are relevant to current debate regarding how nutrient limitation status changes over time in lentic systems. Some have argued that fixed N accumulates in ecosystems and that, as a result, ecosystems evolve P limitation (Schindler 1977, 2012; Schindler and Hecky 2009). But a long-term experiment on Lake 227 in the Experimental Lakes area of Canada has indicated that fixed N does not accumulate proportionally with P inputs and that ecosystem stoichiometry may be more broadly influenced by denitrification or other N loss processes (Scott and McCarthy 2010). Interestingly, a recent study showed that increased ecosystem productivity due to increased P inputs associated with eutrophication enhanced denitrification (Finlay et al. 2013). Thus, denitrification may have a disproportionate effect on the stoichiometry and relative N and P deficiency of eutrophic systems in particular. New information on the ecosystem balance between rates of N₂ fixation and denitrification, as well as the role these processes have in controlling ecosystem

* Corresponding author: egrantz@uark.edu

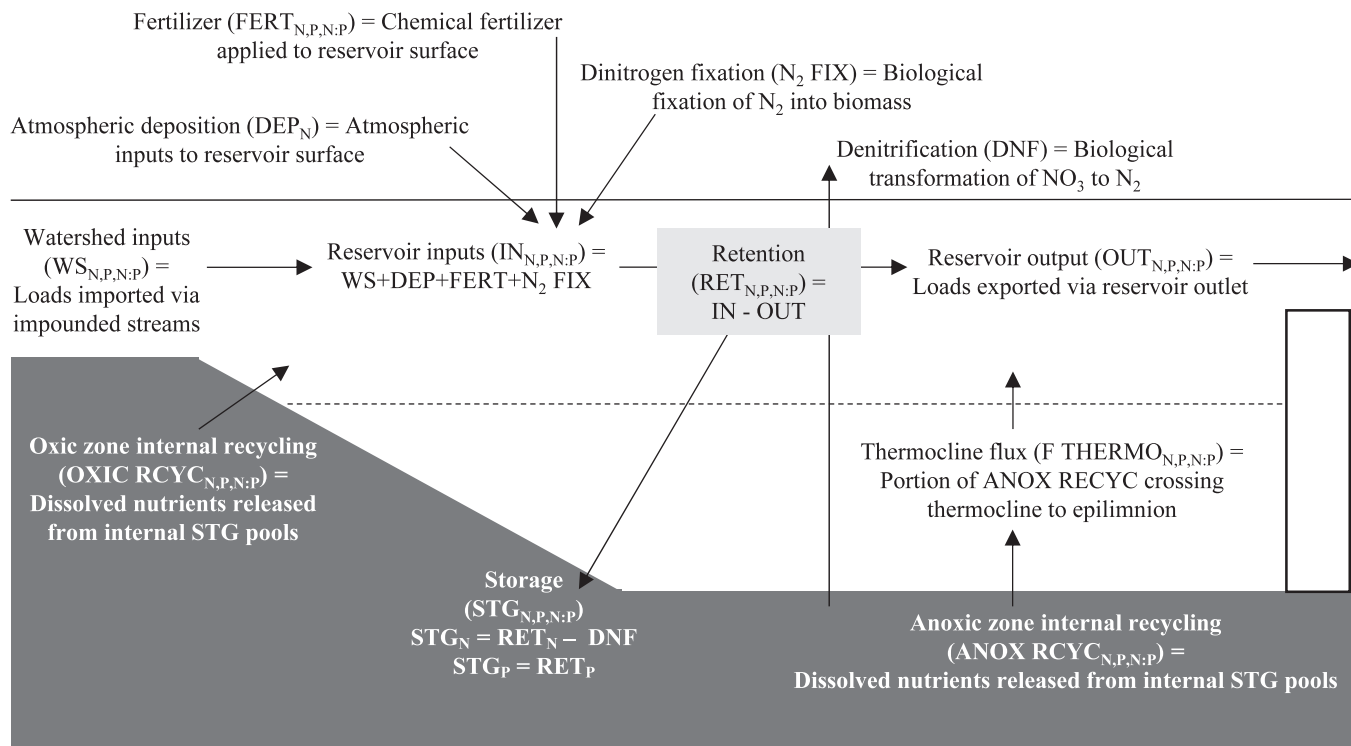


Fig. 1. Conceptual model and definitions of ecosystem fluxes in lakes and reservoirs. These fluxes were measured in the present study, and abbreviations for terms appear throughout the results and discussion sections and with annual ecosystem-scale rates for the study reservoirs in Fig. 7. For processes that directly transport or transform both N and P, subscripts on abbreviations indicate whether measurements were made or were available for N, P, or both elements in the present study.

stoichiometry, is critical to resolving the debate on the need for P only or dual N and P management to control accelerated eutrophication.

In this study, we developed N and P mass balance models (Fig. 1) for three small (< 1 km²), warm monomictic impoundment reservoirs that included estimates of internal processes including N_2 fixation (N_2 FIX), denitrification (DNF), nutrient retention (RET), storage (STG), and internal recycling (RCYC) through biological organic matter mineralization and anaerobic respiration of mineral substrates. Our objective was to determine if these annual ecosystem-scale fluxes differed stoichiometrically and how including N additions through N_2 fixation and N losses through denitrification changed the N:P stoichiometry of relevant reservoir fluxes. We defined a change in N:P as a shift relative to thresholds for strict N and P deficiency (20 and 50, respectively; from Guildford and Hecky 2000). For N_2 fixation, we explored specifically whether including internal fixed N additions altered the nutrient deficiency status implied by the N:P of watershed inputs or ambient nutrient availability in the photic zone, and hypothesized that fixed N additions would not increase the N:P of these pools and fluxes into the range of strict P deficiency (N:P > 50). For denitrification, we explored whether internal N removal altered fluxes representative of future internal nutrient supply, namely, retention, storage, and recycling. Retention was defined as a positive difference between cumulative reservoir inputs and reservoir outputs, whereas storage was defined identically for P, but as the difference

between reservoir inputs, outputs, and internal N loss to denitrification for N. We hypothesized that partitioning N retention into removal and storage components would result in the N:P of loads available for long-term storage in reservoir sediments in the range of strict N deficiency or N + P co-deficiency (N:P < 20 or 20–50, respectively). We predicted that this pattern would be enhanced by more efficient P than N retention and that this mechanism would be reflected in N-deficient internal recycling rates.

Methods

Study sites—Lakes Elmdale (36°11'45.5"N, 94°12'50.8"W), Fayetteville (36°08'11.5"N, 94°07'46.7"W), and Wedington (36°05'27.05"N, 94°22'02.9"W) are small (surface area < 1 km²), shallow (average depth 3 m, maximum depth 9–10 m), mesotrophic (Lake Wedington) to nearing hypertrophic (Lakes Elmdale and Fayetteville) warm monomictic flood-control impoundments located in and around Fayetteville, Arkansas. The reservoirs have comparable residence times (Table 1), but differ in their primary watershed land use–land cover, introducing potential variability in hydrology and external nutrient loading that could affect reservoir nutrient retention efficiency (Harrison et al. 2009; Finlay et al. 2013). Urban and animal-agricultural land use–land cover dominates the Lake Elmdale and Lake Fayetteville watersheds. Approximately 80% of Lake Wedington's relatively undisturbed catchment is forested, but nutrients were added directly to the reservoir in

Table 1. Summary characteristics of the study reservoirs (Grantz et al. 2012; Scott and Grantz 2013) and their watersheds (ADEQ 2004). Residence time was calculated based on 30 yr average mean daily flows measured at United States Geological Survey gauging stations downstream of the reservoirs, assuming equal hydraulic yield per unit of watershed area. Sestonic chlorophyll *a* (Chl *a*) concentrations represent average warm-season values that were measured approximately weekly from May to October in 2009 and 2010.

Site	Surface area (km ²)	Volume (m ³)	Watershed					Residence time (d)	Sestonic Chl <i>a</i> (μg L ⁻¹)
			% Forest	% Urban	% Agriculture	Area (km ²)	% Gauged area		
Elmdale	0.499	3.1×10 ⁶	5.2	61	27	19.3	60	167	24
Fayetteville	0.604	3.8×10 ⁶	9.8	41	37	24.0	50	164	38
Wedington	0.337	2×10 ⁶	80	1.7	13	10.3	55	202	11

each summer of the study as liquid chemical fertilizer to enhance fisheries productivity (R. Whalen, U.S. Forest Service pers. comm.). Brush Creek, Clear Creek, and an unnamed stream within Lake Wedington State Park in the Ozark National Forest are the primary impounded streams supplying flow to Lakes Elmdale, Fayetteville, and Wedington, respectively, and comprise 50–60% of the drainage area of each of the lakes. All the study reservoirs discharge downstream flow from the reservoir surface.

Nutrient mass balance models—We constructed N and P mass balance models (Fig. 1) for the study reservoirs by calculating annual watershed N and P inputs (WS) and reservoir outputs (OUT), as well as internal P recycling rates, for the calendar year 2010. We combined these estimates with previously published rates for other internal processes. Our previous work estimating annual whole-lake denitrification and internal N recycling rates (Grantz et al. 2012; Scott and Grantz 2013) corresponded temporally with the present study. We also previously estimated average annual N₂ fixation rates for the study reservoirs by summing 2010 epilimnion sediment rates with the mean of 2008–2009 lacustrine rates (Scott and Grantz 2013). Cumulative reservoir inputs (IN) in the mass balance model included watershed inputs and chemical fertilizer (FERT), where applicable, for both N and P, as well as biological N₂ fixation and atmospheric N deposition for N. In 2010, chemical fertilizer was added to Lake Wedington only (FERT = 0.19 g N m⁻² and 0.28 g P m⁻²). Internally recycled N and P were not considered new inputs and were not included in cumulative reservoir inputs. Regional wet deposition rates (0.93 g N m⁻²) for 2010 were obtained from the National Atmospheric Deposition Program National Trends Network site AR27 (<http://nadp.sws.uiuc.edu>). Dry deposition equaling 40% of wet deposition (0.37 g N m⁻²) was assumed as in David et al. (2006) and was summed with wet deposition (DEP). Estimates of atmospheric P deposition were not available. For calculating retention rates (difference between inputs and outputs), nutrients leaving the reservoirs via stream flow were the only model output. Denitrification was included as an additional N output for calculating storage rates. For the purpose of stoichiometric comparisons, the atomic N : P of all relevant fluxes was calculated.

Watershed inputs and reservoir outputs—From August 2009 to December 2010 nutrient loads input to the study

reservoirs from the watershed via impounded streams and output from the reservoir outlets were estimated using rating curves and seasonal models to relate stage, discharge, day of the year, and nutrient loads or concentrations. During this period, stage was measured continuously in the primary impounded stream immediately up and downstream of each reservoir. Gauging stations were constructed within perennial pools by anchoring a T post firmly in the stream bed and attaching a polyvinylchloride conduit casing designed to house a data logger near the base of the post. Casings were perforated for entry and exit of flow. Onset Hobo Water Level data loggers were deployed at each station, and overlying absolute pressure (water + atmosphere; kPa) was recorded at 5 min intervals. Atmospheric pressure was recorded simultaneously in the lab. Following correction for differences between lab and study site elevations, corresponding atmospheric pressure was subtracted from each absolute pressure reading to generate a continuous stage record for each stream site.

During the first 12 months of gauging, point discharge measurements were collected at base and storm flow approximately once monthly, when possible, at a stream transect near each station. Discharge was estimated as the sum discharge of at least 10 cross-section areas. Cross section areas were calculated as the product of average cross-section depth, width, and velocity measured with a Marsh-McBirney Flo-Mate flowmeter. At the Lake Elmdale inflow and Lake Fayetteville and Lake Wedington outflow sites, discharge was measured during extreme high-flow events in May 2011 using a Teledyne Streampro Acoustic Doppler Current Profiler. A linear regression analysis relating discharge to stage was conducted in John's Macintosh Program (JMP) 9.0 for each stream site. Square-root transformation of discharge maximized linearity in the relationship between discharge and stage (Hirsch et al. 1993):

$$\sqrt{Q} = (a + bS) \quad (1)$$

where *Q* is discharge, *S* is stage, and *a* and *b* are the *y*-intercept and slope of the regression equation, respectively. For all stage measurements ≤ *a*, discharge was assumed to equal 0.

At inflow sites, water samples were collected from the stream thalweg concurrently with discharge measurements. Outflow samples were collected from the reservoir surface

near the outlet structure. Preliminary analysis indicated no relationship between reservoir surface nutrient concentrations and discharge; therefore, after March 2010, outflow samples were collected separately from discharge measurements at least once monthly. Samples were stored on ice until returned to the laboratory for immediate processing (< 24 h holding time; APHA 2005). Using vacuum filtration, subsamples were collected on precombusted, acid-washed Whatman pore size F glass fiber filters for particulate N and P (PN and PP) analysis, and filtrate passing through the filter was collected in acid-washed dark bottles for total dissolved N and P (TDN and TDP) analysis. Samples were preserved by freezing. Filter samples for PN analysis were dried at 55°C for 24 h and combusted using a Thermo Flash 2000 organic elemental analyzer. TDN was measured by combusting filtrate using a Shimadzu total organic carbon analyzer with total N module-1. Both PP and TDP were analyzed using the ascorbic acid method following persulfate digestion (APHA 2005).

In base-flow stream samples and in reservoir surface samples, TDP concentration was almost always below detection. Therefore, measurements of PP and TDP were summed to estimate total P (TP) concentration for modeling purposes. The detection limit (0.014 mg P L⁻¹) was substituted for censored TDP values. Preliminary analysis indicated that TDN and PN concentrations and loads exhibited distinctly different patterns relative to discharge in the inflow streams and relative to time in reservoir surface waters. Therefore, the TDN and PN fractions were modeled separately, and modeled loads were later summed (TDN + PN = total N [TN]).

Prior to model fitting, corresponding TDN, PN, and TP concentrations were each multiplied by corresponding discharge to calculate loads for inflow streams. Linear regression analyses of the nutrient fraction loads vs. discharge were conducted in JMP 9.0. Both nutrient loads and discharge were log transformed to maximize linearity, and a bias correction factor (BCF) was calculated for each model (Hirsch et al. 1993). Models are summarized:

$$\log L = (a + b \log Q) \times \text{BCF} \quad (2)$$

where L is a nutrient fraction load, Q is discharge, and a and b are the y-intercept and slope of the regression equation, respectively.

Outflow concentrations were assumed to vary seasonally. Fifth-order polynomial (P5; Eq. 3) and multiple-sine periodic (MS; Eq. 4) models were fit to log-transformed TDN, PN, and TP concentrations vs. time for each reservoir (Hirsch et al. 1993). Models are summarized:

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

$$\log C = a + b \sin 2\pi T + c \cos 2\pi T + d \sin 4\pi T + e \cos 4\pi T \times \text{BCF} \quad (4)$$

where T is days elapsed since 01 January 2009, \bar{T} is the mean T on which samples were collected, and a–f are

regression coefficients. For model comparison, the adjusted coefficient of variation (r_a^2), predicted residual sum of squares (PRESS), and sum of squared error (SSE) were calculated. Larger r_a^2 and smaller PRESS and SSE indicated the best-fit model. We also visually assessed models for fit and compared the difference between measured and predicted nutrient concentrations for each model. Less complex third- and fourth-order polynomial models were considered in addition to P5 and MS models, but were never the best fit to the data according to these criteria.

For each 5 min interval stage measurement logged during the study, discharge and watershed input load or reservoir output concentration of TDN, PN, and TP were estimated (Eqs. 1–4). Watershed input loads were scaled to the whole watershed by dividing by the proportion of the drainage area gauged in the study. The yield of ungauged sub-watersheds was assumed to be equivalent to that of the gauged area. Reservoir output loads were calculated by multiplying corresponding interval discharge and concentration estimates. Interval load estimates were summed to approximate daily nutrient loads and scaled to the reservoir surface area.

N and P cycling at the sediment–water interface in the epilimnion—Internal N and P fluxes were estimated using habitat-specific measurements. For sediments in contact with reservoir epilimnia, fluxes of N (as nitrate + nitrite-N [NO₃-N], ammonium-N [NH₄-N], and N₂-N) and P (as soluble reactive P [SRP]) at the sediment–water interface were measured during continuous-flow incubations of intact sediment cores. The protocols and calculations involved in collecting and using these measurements to estimate denitrification, sediment N₂ fixation, and N recycling rates have been described in detail by Grantz et al. (2012) and Scott and Grantz (2013). Briefly, three to four intact sediment cores with overlying water were harvested from each reservoir in February, May, June, August, and December 2010. In the lab, continuous-flow incubations using epilimnetic water from each reservoir were conducted in the dark at in situ temperature. After a pre-incubation period, effluent from each core and influent from each reservoir were collected for N₂:argon (Ar) analysis using membrane-inlet mass spectrometry (MIMS; Kana et al. 1994) and for analysis of dissolved inorganic N (DIN) and P. Samples were processed and stored as described previously and were analyzed fluorometrically for NH₄-N (Holmes et al. 1999) and colorimetrically for NO₃-N and SRP (cadmium reduction and ascorbic acid methods, respectively; APHA 2005). All analyses were carried out on a Turner Designs Lab Fluorometer, with a spectrophotometer adaptor containing 600 and 880 nm filter cells for NO₃-N and SRP analysis, respectively.

Areal flux rates (mg m⁻² d⁻¹) were calculated for each sediment core as the difference between effluent and influent solute concentrations, multiplied by the experimental flow rate, and divided by the core surface area. Experiments were grouped temporally as representing cool-season mixed (February and December) or warm-season stratified (May, June, and August) conditions. Seasonal

N_2 -N, DIN (sum of positive NH_4 -N and NO_3 -N fluxes), and SRP flux rates were calculated as the product of mean daily rates from replicate cores, the number of days of seasonal mixing or stratification, and the ratio of epilimnion sediments to reservoir surface area. Stratum dimensions used in all calculations were determined previously using detailed bathymetry of Lake Wedington (Grantz et al. 2012). For DIN and SRP, annual recycling rates were the sum of warm- and cool-season fluxes. For N_2 -N, positive cool-season fluxes and negative warm-season fluxes were considered indicative of net sediment denitrification and N_2 fixation, respectively.

N and P cycling in anoxic zones and internal transport between thermal strata—In warm-season anoxic zones, defined here as the hypolimnion (including sediments) and the portion of the metalimnion where dissolved oxygen $< 1 \text{ mg L}^{-1}$, internal N and P fluxes were estimated by modeling increasing in situ N_2 -N, NH_4 -N, and TDP concentrations using linear regression analysis. The protocols and calculations involved in collecting and using these measurements to estimate denitrification and N recycling rates have been described in detail by Grantz et al. (2012) and Scott and Grantz (2013). Additionally, NH_4 -N fluxes from the hypolimnion and lower metalimnion origin across the thermocline to the epilimnion were estimated by Scott and Grantz (2013). Here we briefly summarize these methods, which were used in this study to estimate TDP fluxes.

Water samples were collected approximately weekly from April to August 2010 at the location of maximum depth in each reservoir at two or three depths within both the epilimnion and the oxycline. The locations of the oxycline and thermal boundaries were determined using vertical profiles collected at 0.5–1 m intervals using a multiparameter datasonde (Yellow Springs) on each sampling date. Dissolved gas samples were collected by filling 300 mL glass biological oxygen demand bottles from the bottom. These samples were preserved, sealed, and analyzed in the lab for N_2 :Ar using MIMS. Water chemistry samples were transferred to acid-washed dark bottles and stored on ice until return to the laboratory, where samples were processed, stored, and analyzed for NH_4 -N concentration as described previously. Filtrate subsamples for TDP analysis were collected and acidified to $pH \sim 2$ to avoid potential rebinding with free iron and manganese ions under oxic conditions. TDP was analyzed using an inductively coupled plasma mass spectrometer. Concentrations of N_2 -N, NH_4 -N, and TDP (which was assumed to approximately equal SRP in the anoxic water column) were expected to increase over time in seasonal anoxic zones and were modeled using linear regression analysis in JMP 9.0. Masses of reactive N and P accumulated in anoxic strata and entrained at fall turnover were estimated by multiplying the slopes of the linear regression analyses by stratum volume and dividing by the number of days of stratification and the reservoir surface area.

In order to estimate exchange rates between solutes accumulating in lower strata and the reservoir epilimnia

during summer stratification (F THERMO), Scott and Grantz (2013) calculated heat transfer coefficients specific to hypolimnia and lower metalimnia. Fluxes of hypolimnion and lower metalimnion NH_4 -N (Scott and Grantz 2013) and TDP (present study) across the thermocline (functionally defined as the top of the metalimnion) were calculated for each sampling date as the product of a stratum-specific heat transfer coefficient and the difference in concentrations of NH_4 -N or TDP in that stratum and in the epilimnion. The estimated concentrations of NH_4 -N and TDP in the lower metalimnion that were present because of hypolimnion fluxes through the metalimnion to the epilimnion were subtracted from lower-metalimnion NH_4 -N and TDP concentrations for each sample date to avoid overestimating recycling in anoxic zones. It was assumed that fluxes across the thermocline would increase over time as solute concentrations increased in lower strata. Therefore, linear regression analyses of hypolimnion and metalimnion fluxes vs. time were used to model daily fluxes across the thermocline into the epilimnion throughout stratification. Daily fluxes were summed and multiplied by the ratio of the thermocline and lake surface areas.

Estimating lacustrine N_2 fixation rates—The protocols and calculations involved in measuring and scaling lacustrine N_2 fixation rates are described in detail by Scott and Grantz (2013), and are briefly summarized here. Surface-water samples were collected monthly (May and October) to weekly (June–September) during the 2008 and 2009 warm seasons, in addition to two cool-season sampling events (December 2008 and February 2009). Concurrently, euphotic zone depth was estimated as the depth where measured light intensity was 1% of the surface irradiance. Water samples were immediately returned to the lab for incubations using the acetylene reduction method (Flett et al. 1976; Howarth et al. 1988) across an artificial photosynthetically active radiation (PAR) gradient and at in situ temperature. No N_2 fixation was detected during cool-season incubations. For warm-season months, Michaelis-Menten kinetics were used to model the relationship between PAR and volumetric N_2 fixation rates for each lake in each month of the study using the nonlinear regression tool in SigmaPlot 12.0. Estimated rates were scaled spatially using model parameters, 2008 and 2009 vertical light profiles, a continuous PAR dataset collected at a central location to the study reservoirs, and estimates of the volumes of 0.5 m thick frusta within the euphotic zone.

Results

Watershed loading models—A strong linear relationship ($r^2 \geq 0.88$, $p < 0.0001$) between square root-transformed discharge and stage was found across the sampled range at all stream sites (Table 2). For watershed inputs (WS), TDN, PN, and TP loads were all positively related to discharge (Table 2; Fig. 2A–C). P5 functions were selected to predict variability through time in concentrations of the modeled nutrient fractions for all reservoirs (Table 3; Fig. 3A–C), except TDN for Lake Fayetteville and PN

Table 2. Summary statistics and parameters for the linear regressions used to model discharge (Q) to and from the study reservoirs, as well as PN, TDN, and TP watershed inputs ($WS_{N:P}$) to the reservoirs. Raw data and model fit are shown for nutrient fraction vs. discharge relationships in Fig. 2A–C.

Reservoir	Site	Parameter	n	Model statistics			Model parameters		BCF
				r^2	p	SSE	a	b	
Elmdale	Up	Q	20	0.92	<0.0001	0.364	-1.22	0.322	—*
Elmdale	Down	Q	17	0.93	<0.0001	0.0166	-1.91	0.379	—
Elmdale	Up	PN	19	0.93	<0.0001	5.60	-1.78	1.48	1.21
Elmdale	Up	TDN	20	0.91	<0.0001	1.68	0.238	0.676	1.04
Elmdale	Up	TP	19	0.95	<0.0001	4.43	-2.22	1.78	1.10
Fayetteville	Up	Q	19	0.98	<0.0001	0.0347	-3.28	0.529	—
Fayetteville	Down	Q	19	0.98	<0.0001	0.0737	-2.27	0.476	—
Fayetteville	Up	PN	19	0.95	<0.0001	5.81	-1.17	1.50	1.17
Fayetteville	Up	TDN	19	0.94	<0.0001	1.58	0.200	0.771	1.04
Fayetteville	Up	TP	18	0.97	<0.0001	4.04	-1.43	1.74	1.08
Wedington	Up	Q	15	0.88	<0.0001	0.107	-1.38	0.257	—
Wedington	Down	Q	9	0.93	<0.0001	0.165	-1.72	0.266	—
Wedington	Up	PN	15	0.88	<0.0001	1.17	-1.87	1.30	1.25
Wedington	Up	TDN	15	0.98	<0.0001	0.127	0.0920	1.17	1.03
Wedington	Up	TP	14	0.97	<0.0001	2.00	-2.94	1.32	1.07

* BCFs were used only in load vs. discharge regressions where variables were log transformed.

for Lake Wedington, for which multi-sine periodic functions were selected as the best fit models. Across the reservoirs, 34–78% of variability in surface TDN, PN, and TP concentrations was explained by season. In 2009 and 2010, across all sites, TDN concentration peaked in November to December following fall mixing, remained high through early spring, and reached a minimum during the summer months (Fig. 3A–C). Both PN and TP concentrations exhibited the reverse trend, peaking in late summer and declining during winter months.

For Lake Elmdale and Fayetteville watershed inputs (WS), the magnitude of flow response differed between N and P, and the N:P of nutrient loading ($WS_{N:P}$) was negatively related to discharge (Fig. 4A). The magnitudes of N and P flow responses for Lake Wedington watershed inputs were more similar, with no relationship between the N:P of nutrient loading and flow ($p > 0.05$). The magnitude of seasonal variability differed between in-lake surface TN and TP concentrations, resulting in total N:P variability in reservoir outputs ($OUT_{N:P}$) through time (Fig. 4B). Generally, N:P was lowest ($OUT_{N:P} = 20$ –50) during warm-season months, when the reservoirs were stratified, and highest ($OUT_{N:P} = 60$ –200) during cool-season months, when the reservoirs were mixed. The daily record of the modeled nutrient loads showed that $WS_{N:P}$ varied by up to six orders of magnitude with flow for Lakes Elmdale and Fayetteville (Fig. 5A,B). For these reservoirs, $WS_{N:P}$ most often exceeded $OUT_{N:P}$ outside of storm events, but the bulk of N and P transfer by mass was associated with very low $WS_{N:P}$ during high flow. For Lake Wedington, $WS_{N:P}$ and $OUT_{N:P}$ varied within the same range (Fig. 5C). Lake Wedington also frequently did not discharge between large storm events.

Internal P flux estimates—For all the reservoirs, hypolimnion and lower metalimnion TDP concentrations increased over time ($r^2 = 0.57$ –0.86 and $p = 0.0183$ to <

0.0001; data not shown). Rates of TDP accumulation were 3.89 and 3.55 $\mu\text{g L}^{-1} \text{d}^{-1}$, 4.87 and 4.13 $\mu\text{g L}^{-1} \text{d}^{-1}$, and 3.39 and 1.71 $\mu\text{g L}^{-1} \text{d}^{-1}$ in the hypolimnion and lower metalimnion of Lakes Elmdale, Fayetteville, and Wedington, respectively. Fluxes across the thermocline of TDP originating in the hypolimnion and lower metalimnion also increased over time at all sites (F THERMO; Fig. 6A–C). Increasing export across the thermocline was a function of both rates of TDP accumulation and the thermal stability of lower strata. Because of inherently lower metalimnion thermal stability, fluxes from the lower metalimnion exceeded those from the hypolimnion at all sites, despite the fact that hypolimnion accumulation rates were either similar in magnitude to or greater than metalimnion rates. For sediments in contact with reservoir epilimnia, the SRP concentrations of both inflow and outflow samples from February and May core incubation experiments were always below detection. Therefore, SRP was not analyzed for subsequent incubations, and SRP fluxes from epilimnetic sediments were considered to be undetectable for all the reservoirs throughout the year (Fig. 7A–C).

Reservoir mass balances and nutrient stoichiometry—Watershed inputs to the study reservoirs were approximately 20–30 $\text{g N m}^{-2} \text{yr}^{-1}$ and < 1–7 $\text{g P m}^{-2} \text{yr}^{-1}$ in 2010 (all fluxes normalized to reservoir surface area; Fig. 7A–C). On average, the study reservoirs received significant N_2 fixation inputs ($\text{N}_2 \text{FIX} = 7$ –11 $\text{g m}^{-2} \text{yr}^{-1}$) during warm-season months, indicating that ~ 30% of total new N inputs may have been internally derived during 2010. Chemical fertilizer P additions to Lake Wedington were equivalent to ~ 40% of watershed inputs, but fertilizer N additions were equivalent to only 1% of watershed inputs. The most notable spatial differences in annual N:P loads were for Lakes Elmdale and Fayetteville, where N:P increased by four to six times from watershed inputs to reservoir outputs ($WS_{N:P} = 9$ –15; $OUT_{N:P} = 56$ –63). In

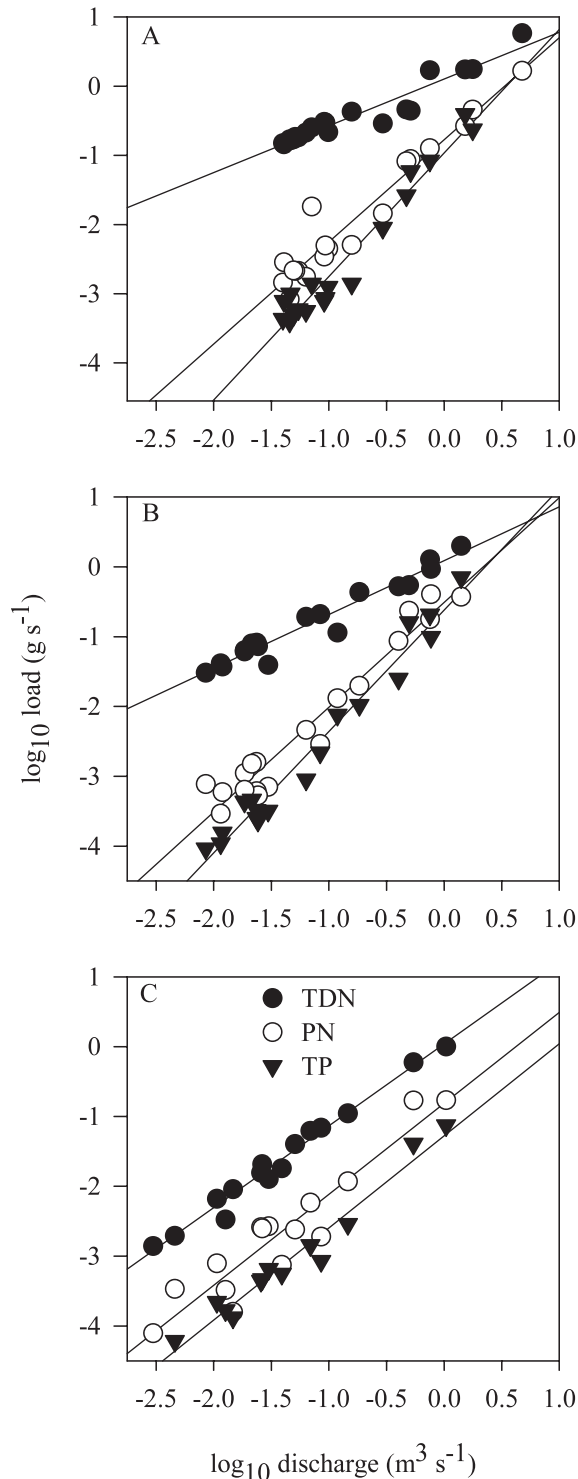


Fig. 2. Inflow stream TDN, PN, and TP load vs. discharge models for the impounded streams upstream of Lakes (A) Elmdale, (B) Fayetteville, and (C) Wedington. These models were used to estimate a continuous record of watershed inputs ($WS_{N,P}$) for the study reservoirs. Model parameters and summary statistics are provided in Table 2.

contrast, for mesotrophic Lake Wedington, N : P decreased from annual watershed inputs to reservoir outputs ($WS_{N:P} = 61$; $OUT_{N:P} = 46$). Including internal new N (N_2 fixation, atmospheric deposition, and fertilization) and P inputs (fertilization) in the mass balances increased the N : P of cumulative annual reservoir inputs slightly above that of watershed inputs for all the reservoirs ($IN_{N:P} = 13$ – 63 ; $WS_{N:P} = 9$ – 61) and decreased differences in N : P between inputs and outputs to two to five times for Lakes Elmdale and Fayetteville.

All the study reservoirs were net sinks for both N and P in 2010, and the N : P of retained loads was among the lowest in the study for Lakes Elmdale and Fayetteville ($RET_{N:P} = 7$ – 16). For Lake Wedington, the N : P of retained loads was close within range of inputs ($RET_{N:P} = 67$; $IN_{N:P} = 63$). Differences in the N : P of retained loads reflected that N and P were retained with different relative efficiencies and also differently between the eutrophic and mesotrophic sites. Lakes Elmdale and Fayetteville retained 85–90% of P inputs, but only 50–55% of N inputs. In contrast, Lake Wedington retained N and P at more similar rates (~ 80 – 85%). Each reservoir retained a similar mass of N annually ($RET_N = 19$ – 23 $g\ N\ m^{-2}$), though N inputs to Lake Fayetteville were up to 50% greater than to Lake Wedington. The study reservoirs retained different masses of P that were proportional to P watershed loading rates, which varied by more than eight times between Lakes Wedington and Fayetteville.

Annually, denitrification potentially removed 12–23 $g\ N\ m^{-2}\ yr^{-1}$ from the reservoirs. For all sites, denitrification rates were approximately two times greater than internal N additions through biological N_2 fixation. For the eutrophic reservoirs, denitrification rates were also approximately equivalent to retained N loads (or $\sim 100\%$ denitrification efficiency), whereas denitrification efficiency was lower ($\sim 50\%$) for Lake Wedington. Therefore, the estimated N : P of storage loads was unmeasurably low and was reported as < 1 for the eutrophic reservoirs. For Lake Wedington, estimated storage (STG) loads were low N : P compared to inputs, outputs, or retained loads ($STG_{N:P} = 30$; IN , OUT , and $RET_{N:P} = 50$ – 60), but were higher N : P than for the eutrophic reservoirs ($STG_{N:P} < 1$). Internal loading contributed substantial recycled N ($RCYC_N = 2$ – 14 $g\ m^{-2}\ yr^{-1}$) and P ($RCYC_P = 1$ – 6 $g\ m^{-2}\ yr^{-1}$) to the reservoirs. The N : P of internal recycling was low for all the study reservoirs ($RCYC_{N:P} = 4$ – 10). For Lakes Elmdale and Fayetteville, the N : P of internal recycling was in range with that of watershed inputs and retained loads (N : P of all fluxes < 20). For Lake Wedington, however, the N : P of internal recycling was an order of magnitude lower than any other mass balance flux.

Discussion

Our study demonstrated that stoichiometric differences between reservoir inputs, outputs, retention, storage, and internal recycling rates were largely a function of differences in N and P retention efficiencies and permanent removal of N through denitrification. Although N_2 fixation was substantial in these reservoirs and provided a necessary

Table 3. Summary statistics and regression equation parameters for the best-fit seasonal models used to estimate surface PN, TDN, and TP concentrations for the study reservoirs. Raw data and model fit are shown in Fig. 3A–C. Nutrient concentrations were \log_{10} transformed prior to model fitting. Elm, Elmdale; Fay, Fayetteville; Wed, Wedington.

Site	Fraction	r^2	p	SSE	\bar{T}	Model parameters					
						a	b	c	d	e	f
Elm	PN	0.69	<0.0001	6.24	408	-4.68	0.00573	9.74×10^{-5}	-2.24×10^{-7}	-1.28×10^{-9}	2.54×10^{-12}
Elm	TDN	0.64	<0.0001	1.55	455	1.83	-0.00492	-3.00×10^{-5}	2.34×10^{-7}	4.67×10^{-10}	-2.32×10^{-12}
Elm	TP	0.46	0.0004	3.94	466	-8.15	0.00935	3.37×10^{-5}	-4.07×10^{-7}	-5.82×10^{-10}	3.94×10^{-12}
Fay	PN	0.78	<0.0001	5.56	403	-4.55	0.00677	9.98×10^{-5}	-3.05×10^{-7}	-1.5×10^{-9}	3.76×10^{-12}
Fay	TDN	0.74	<0.0001	1.24	—*	-6.80	-9.89	-7.69	-4.66	3.45	—*
Fay	TP	0.64	<0.0001	2.26	472	-8.60	0.011	2.75×10^{-5}	-5.24×10^{-7}	-5.02×10^{-10}	5.27×10^{-12}
Wed	PN	0.47	0.0002	2.64	—*	-34.9	-46.66	6.88	4.10	13.69	—*
Wed	TDN	0.58	0.0007	0.503	508	-1.35	2.78×10^{-4}	2.14×10^{-5}	-9.01×10^{-8}	1.58×10^{-10}	2.26×10^{-12}
Wed	TP	0.38	0.022	2.03	519	-8.19	0.00842	-1.44×10^{-5}	-5.49×10^{-7}	3.75×10^{-10}	6.88×10^{-12}

* The best-fit models for these site–nutrient fraction combinations were MS functions, which included only model parameters a – e and did not require an estimate of the mean of the numbered days (where day 1 = 01 Jan 2009) on which samples were collected.

input to N budgets, internal N losses to denitrification always exceeded N_2 fixation rates. Stoichiometric differences in reservoir fluxes were consistent with previous studies (Cook et al. 2010; Vanni et al. 2011) and indicated significant shifts in N:P stoichiometry between in-lake locations relative to thresholds for strict N and P deficiency to primary production (20 and 50, respectively; Guildford and Hecky 2000). Two clear patterns emerged from our study that suggested mechanisms for potential internal controls on ecosystem stoichiometry. The stoichiometry of nutrient retention ($RET_{N:P}$), storage ($STG_{N:P}$), and recycling ($RCYC_{N:P}$) was almost always indicative of some degree of N deficiency, especially for the eutrophic reservoirs. These reservoirs not only received low N:P watershed inputs, but also retained P more efficiently than N and had the highest rates of N removal through denitrification. The N deficits, which were obvious in watershed inputs and internal recycling rates, were not balanced by N_2 fixation. This indicated that, even though N_2 fixation rates in these reservoirs are some of the highest reported, N_2 fixation was not efficient at alleviating ecosystem N deficiency as indicated by N:P stoichiometry.

Efficient P retention and permanent N removal create internal stoichiometric imbalance—The eutrophic study reservoirs (Lakes Elmdale and Fayetteville) retained nutrients with an N:P ratio indicative of N deficiency ($RET_{N:P} = 7$ –16). Thus, the difference between N:P in ecosystem inputs and outputs indicated more efficient P than N retention, or, otherwise stated, more N loss to outflow than P. These differences in retention efficiency were consistent with previous studies in reservoirs (Gill et al. 1976; Garnier et al. 1999; Bosch et al. 2009) and reflected the substantial difference in the way N and P concentrations responded to high-flow events in the impounded streams compared to within the reservoirs themselves. High flow upstream of the reservoirs reduced the N:P of watershed inputs by disproportionately increasing P loads compared to N loads. However, surface N and P concentrations at the reservoir outflows were not influenced by the magnitude of flow passing through the

reservoirs, and only varied because of comparatively small seasonal changes in N:P. Because the largest N and P transport by mass occurred during these events, disparity in daily flow response between the elements and between inflows and outflows ultimately shaped spatial stoichiometric differences in annual fluxes. In contrast, for mesotrophic Lake Wedington, the N:P of inputs, outputs, and retained loads was high (~ 50 or greater) and stoichiometric differences between fluxes likely did not exceed the error associated with these measurements. For this reservoir, N:P varied with flow in the impounded stream within the same range as seasonal variations in reservoir surface waters. A secondary factor driving differences in the stoichiometry of watershed inputs and outputs was reduced N retention efficiency in the eutrophic reservoirs relative to the mesotrophic reservoir (50–55% vs. 85%). This important difference was related to elevated cool-season TDN concentrations that were ~ 2 times higher in Lakes Elmdale and Fayetteville than in Lake Wedington. Concurrently, concentrations of all P fractions were at an annual low in the water column. Winter–spring is a period of high precipitation and low evapotranspiration in the region, which translated to enhanced reservoir flushing and, thereby, N export during seasonal mixing, especially for the eutrophic reservoirs receiving runoff from anthropogenically disturbed watersheds.

Denitrification also induced stoichiometric imbalance in ecosystem fluxes that comprise future internal nutrient sources to primary production, removing $\sim 50\%$ and ~ 95 –100% of retained N from the mesotrophic and eutrophic reservoirs, respectively. Denitrification rates were low to moderate relative to estimates from other lakes and reservoirs, but denitrification efficiencies (proportion of retained N that was denitrified) were high (David et al. 2006; Koszelnik et al. 2007). In contrast to N, all retained P was assumed to be eventually stored in sediments. Therefore, for Lakes Elmdale and Fayetteville, where retained P loads were largest by mass and denitrification activity resulted in near-zero net N storage, the N:P of stored materials was an order of magnitude lower than the N:P of retained loads ($RET_{N:P} = 7$ –16; $STG_{N:P} < 1$) and

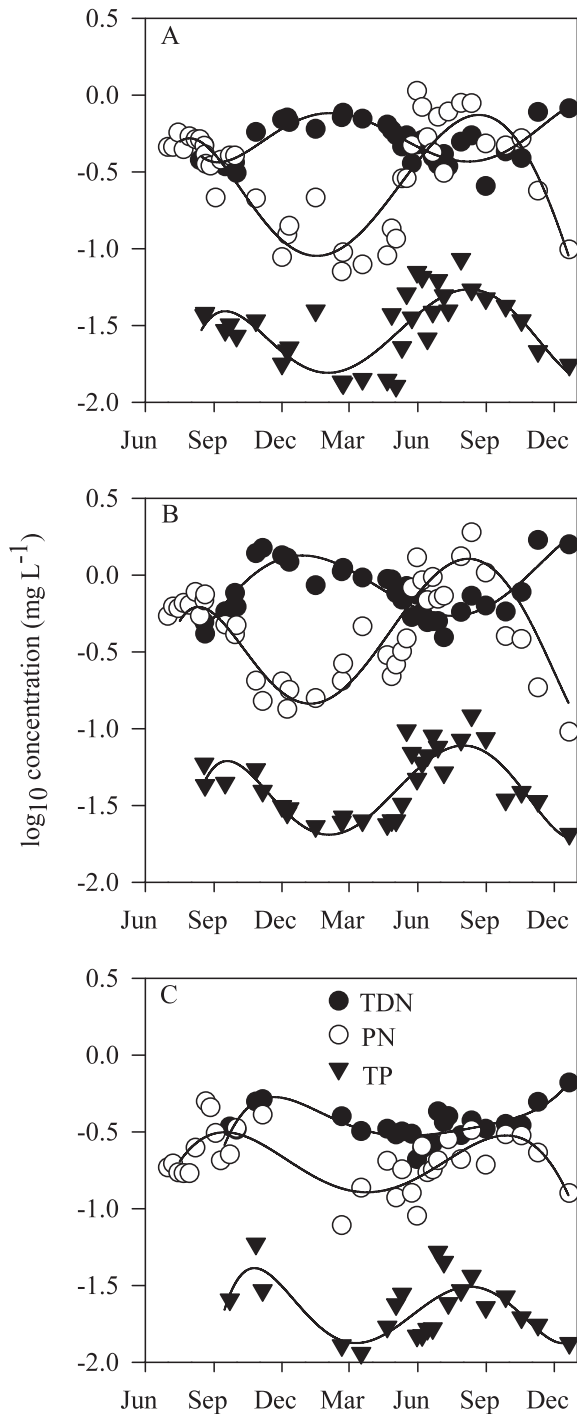


Fig. 3. Reservoir surface TDN, PN, and TP concentrations through time from July 2009 to December 2010 for Lakes (A) Elmdale, (B) Fayetteville, and (C) Wedington. These models were used estimate a continuous record of reservoir outputs ($OUT_{N:P}$). Model parameters and summary statistics are provided in Table 3.

was always reflective of N deficiency ($N:P < 20$). The difference between retained and stored $N:P$ ($RET_{N:P} = 67$; $STG_{N:P} = 30$) for mesotrophic Lake Wedington was also pronounced, but stored $N:P$ was indicative of N + P co-deficiency (20–50), rather than strict N deficiency. These findings indicate that increased denitrification in

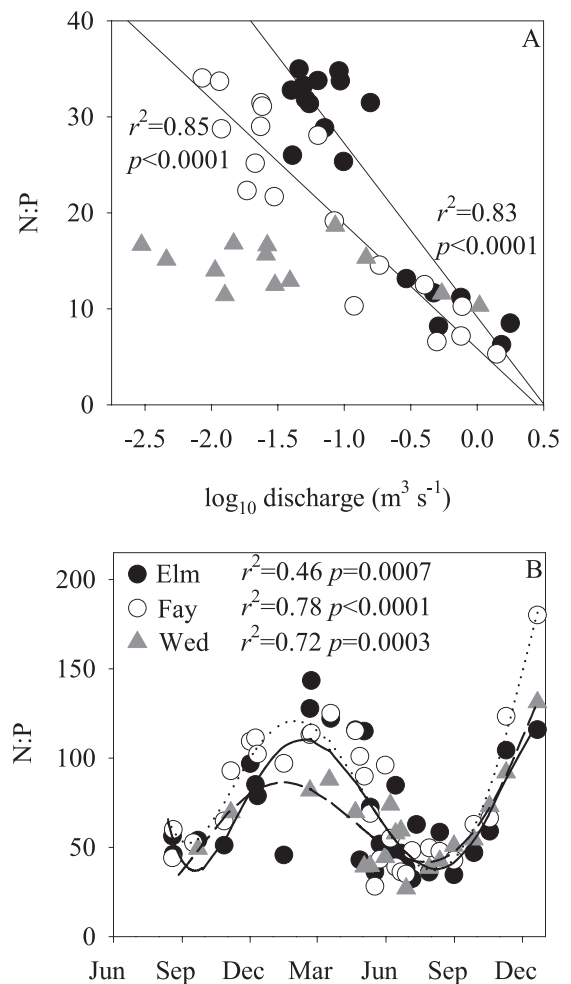


Fig. 4. Measured $N:P$ in (A) watershed inputs ($WS_{N:P}$) vs. discharge and (B) reservoir outputs ($OUT_{N:P}$) through time for Lakes Elmdale (Elm), Fayetteville (Fay), and Wedington (Wed).

P-enriched eutrophic lakes (Finlay et al. 2013) provided an internal feedback that drives these systems toward N deficiency. However, mesotrophic systems may not support enough denitrification to generate this imbalance, especially if N retention efficiencies are high.

We did not directly measure the nutrient content of sediments in the study reservoirs, but storage $N:P$ estimates were in range with consistently low $N:P$ sediment measurements from other reservoirs across a trophic gradient ($N:P < 10$; Filstrup et al. 2010; Vanni et al. 2011; Burford et al. 2012). Moreover, for all the study reservoirs, we measured low $N:P$ in internally recycled loads ($RCYC_{N:P} < 10$). Based on the assumption that internally recycled loads are largely derived from particulate matter stored in sediments in previous years, the stoichiometric similarity between these fluxes also suggested that the estimated rates may be representative of storage in the reservoirs in previous years or on average over time.

N deficiency in nutrient supply exceeded fixed N additions—As discussed above, internal feedbacks in the reservoirs appeared to drive these systems toward N deficiency because

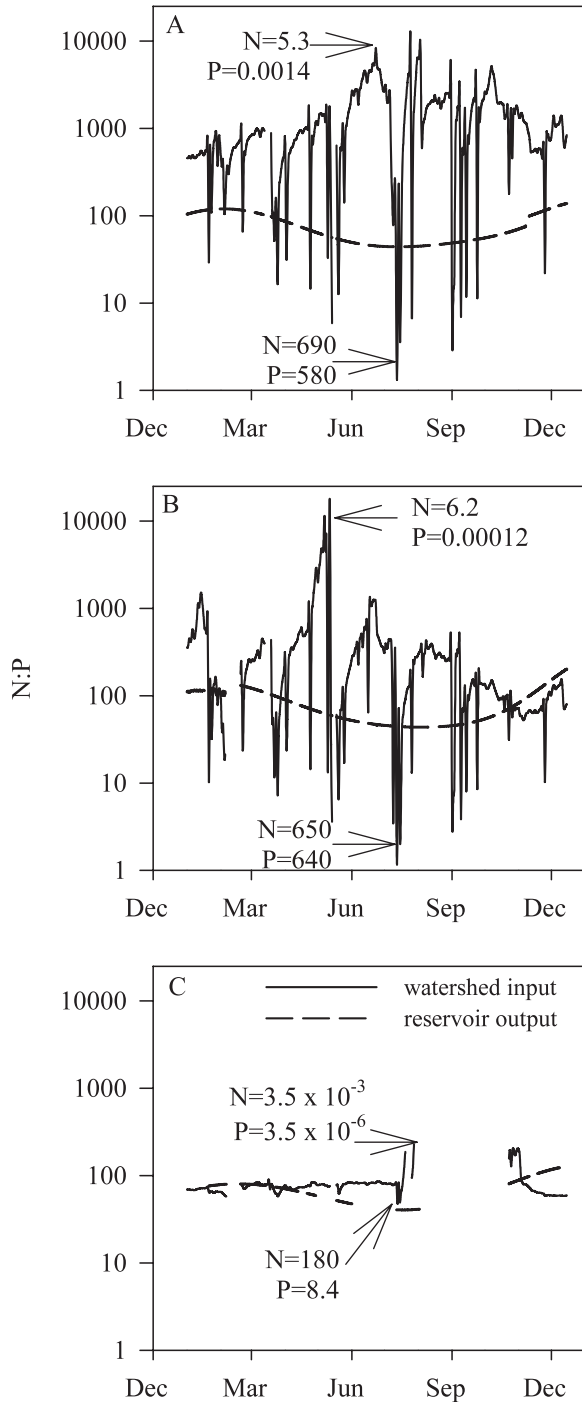


Fig. 5. Daily record of model estimates of the N:P of watershed inputs ($WS_{N:P}$) and reservoir outputs ($OUT_{N:P}$) for Lakes (A) Elmdale, (B) Fayetteville, and (C) Wedington. Breaks in the daily record indicate that discharge vs. stage models estimated no flow in the impounded stream or out of the reservoir, thereby setting nutrient loads = 0. Periods of no flow were primarily observed for Lake Wedington and occurred both upstream and downstream of the reservoir. Example daily N and P loads ($kg\ d^{-1}$) associated with extreme values of $WS_{N:P}$ are included to illustrate differences in mass transfer between high and low $WS_{N:P}$ at base and storm flow, respectively.

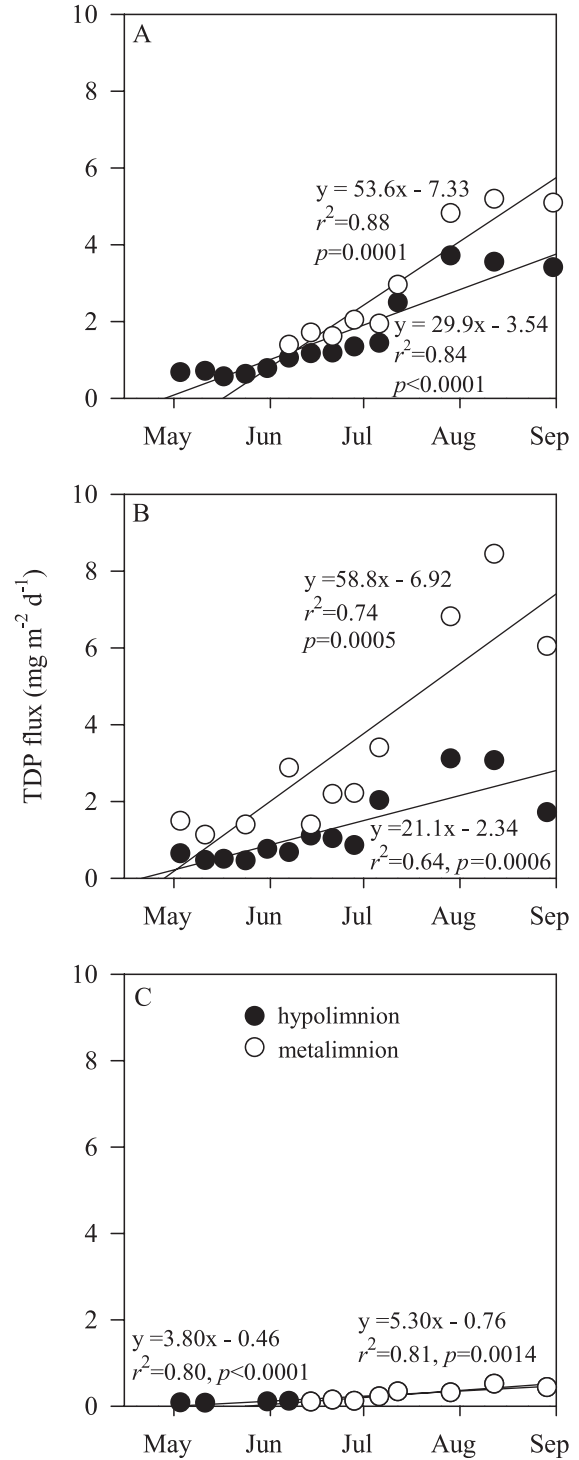


Fig. 6. Estimated TDP flux (F_{THERMO_P}) out of the hypolimnion and anoxic lower metalimnion into the epilimnion through time during reservoir stratification for Lakes (A) Elmdale, (B) Fayetteville, and (C) Wedington. These relationships were derived from heat transfer coefficients and measurements of P accumulating because of organic matter mineralization and anaerobic respiration of mineral substrates in anoxic zones over time ($ANOX\ RCYC_P$).

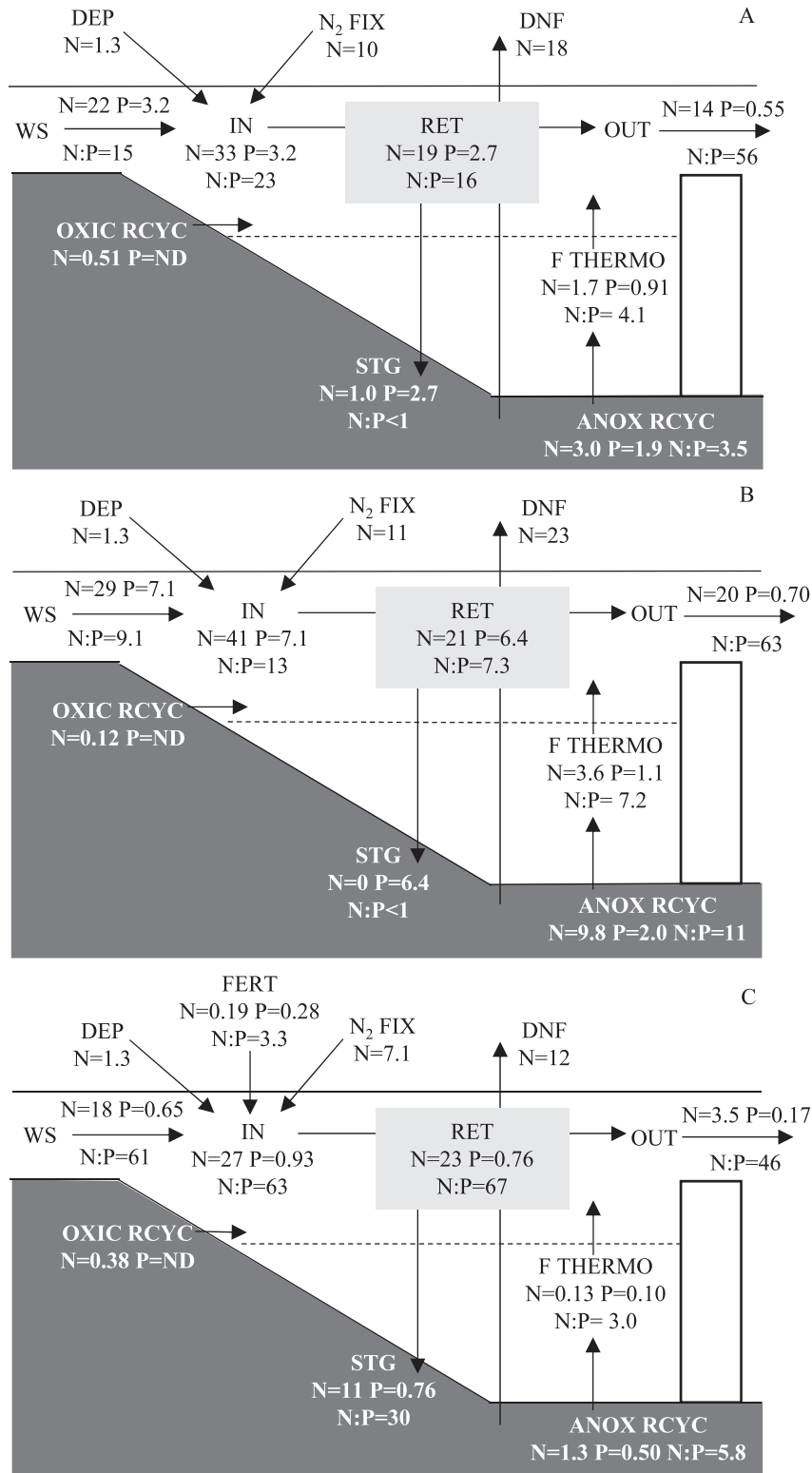


Fig. 7. Model illustrations of annual mass balance fluxes for Lakes (A) Elmdale, (B) Fayetteville, and (C) Wedington. See Fig. 1 for definitions and terms corresponding to flux abbreviations. For all fluxes, N = TN and P = TP, except internal recycling, where N was measured as NH_4 -N and P was measured as TDP in anoxic zones (ANOX RCYC_{N,P}) and N was measured as NH_4 -N and NO_3 -N and P was measured as SRP at the sediment-water interface of epilimnion sediments (OXIC RCYC_{N,P}).

of efficient denitrification. Furthermore, all the study reservoirs received N-deficient nutrient supply from their watersheds or from internal sources. The eutrophic study reservoirs received low N:P annual watershed inputs ($WS_{N:P} = 9\text{--}15$) that reflected these reservoirs' anthropogenically disturbed watersheds (Downing and McCauley 1992). Mesotrophic Lake Wedington received high N:P annual watershed inputs indicating strict P deficiency ($WS_{N:P} = 61$), but one third of annual P inputs were due to addition of low N:P liquid chemical fertilizer ($FERT_{N:P} = 3.3$) applied to the reservoir surface early in the growing season (May and June). During periods of rapid N_2 fixation, flow in the impounded streams was low or ceased completely outside of storm events, and internal loading from nutrients recycled in the anoxic metalimnion and hypolimnion likely became an important nutrient source to the photic zone for the reservoirs (Scott and Grantz 2013). Across reservoirs, the N:P of the flux of internally recycled nutrients crossing the thermocline ($F_{THERMO_{N:P}} = 3\text{--}7$) was among the lowest in the study.

The N_2 fixation rates measured in the study reservoirs ($N_2\text{ FIX} \sim 7\text{--}11\text{ g m}^{-2}\text{ yr}^{-1}$) were among the highest ever reported (Howarth et al. 1988) and resulted from the low N:P watershed and internal nutrient supply ratios, which have been shown to control N_2 fixation rates (Levine and Schindler 1999). Nevertheless, including fixed N inputs did not increase the N:P of cumulative reservoir inputs above that of watershed inputs or out of the range of ratios indicative of N (co-)deficiency for the eutrophic reservoirs ($WS_{N:P} = 9\text{--}15$; $IN_{N:P} = 13\text{--}23$). In fact, assuming that all other new inputs remained constant, achieving N:P of cumulative inputs > 50 would have required average annual N_2 fixation rates to be approximately 4–10 times greater for Lakes Elmdale and Fayetteville, respectively. In contrast, N_2 fixation rates appeared to balance excess P loading to Lake Wedington on an annual scale, maintaining the N:P of cumulative inputs > 50 . Potential differences in N_2 fixation efficiency between the reservoirs likely occurred because the N deficit induced by higher denitrification efficiency and lower N:P watershed inputs in the eutrophic reservoirs was far greater by mass than the deficit induced by fertilization in Lake Wedington.

Within the growing season, N_2 fixation efficiency was limited in all the reservoirs. As a result, none of the reservoirs were able to overcome N deficiency in seasonally important nutrient sources to the photic zone, such as thermocline nutrient transfer to the epilimnion, cyanobacterial recruitment from sediments (Xie et al. 2003), or sediment resuspension (Niemistö et al. 2008). Internal N addition through N_2 fixation has been proposed as the mechanism for increased N:P in reservoir outputs from N-limited systems relative to inputs from the watershed (Cook et al. 2010). Although this was possible for the study reservoirs, the current study has demonstrated that N_2 fixation and reservoir exports were decoupled by predictable patterns in flow, and that the N_2 fixation–reservoir export N:P dynamic involves a more complicated set of interactions between N and P retention efficiency and permanent N removal. On the seasonal scale, N_2 fixation had no sustained effect on the N:P of mixed layer nutrient

concentrations or fluxes. The N:P of mixed layer nutrient concentrations for all the reservoirs declined from > 100 early in the growing season, remaining in the range of N + P co-deficiency (20–50) throughout summer and early fall. Mixed layer N:P remained low until fall turnover, despite very high N_2 fixation rates during this period. This suggests that short-term controls on N_2 fixation efficiency, such as export of fixed N out of the photic zone via particle settling, may also limit the effects of N_2 fixation on ecosystem stoichiometry.

Study limitations and implications—Modeling studies have numerous limitations, including scaling assumptions, unexplained natural variability, data availability, extrapolation, and bias. However, we believe that predictive relationships were strongly evident in the data and were well represented in our models. Where a large degree of extrapolation was required because of equipment and access limitations (discharge for Lake Elmdale outflow), or where limited data points were available (discharge for Lake Wedington outflow), model estimates were compared to estimates from more robust models for other sites. In these cases, estimates were always close in range, which we expected because of the geomorphologic similarity between the impounded streams. Output from models based on log-transformed data was subject to bias correction (Hirsch et al. 1993), with calculated BCFs indicating model estimates within 25% of measured values, on average.

The annual fluxes estimated in this study were not all measured simultaneously. Specifically, lacustrine N_2 fixation rates were not measured in 2010. Nevertheless, the conditions indicative of warm-season N deficiency in 2008–2009 (low N:P and depleted DIN in the mixed layer; Scott et al. 2008; Scott and Grantz 2013) recur annually in the study reservoirs and were present in 2010. Most fluxes in the mass balances were also measured for only 1 yr, and constraining estimates within a calendar year ignores interannual variability. In 2008–2009, lacustrine N_2 fixation rates indeed varied considerably, especially for Lake Fayetteville ($3.4\text{--}16\text{ g N m}^{-2}\text{ yr}^{-1}$; Scott and Grantz 2013). Our conclusion that N_2 fixation did not meet stoichiometric N deficits would be refuted only, however, if average annual N_2 fixation rates were much larger (> 10 times for Lake Fayetteville), which seems unlikely because these measured rates were among the highest reported in the scientific literature. Nevertheless, annually variable factors such as precipitation and subsequent hydraulic loading to lakes and reservoirs can affect the magnitude of internal N cycle processes (Seitzinger et al. 2002, 2006; Scott et al. 2008). Multiyear mass balance studies have also found that N and P retention in lentic systems can vary interannually and that a reservoir can be either a nutrient source or sink, depending on the time scale (Garnier et al. 1999; Ferris and Lehman 2006). More work is needed to extend our reservoir nutrient budgets to include multiple years of simultaneous denitrification and N_2 fixation measurements that coincide with the full suite of internal and external reservoir loading measurements.

Although important, these limitations do not detract from our study's implications. The P-limitation paradigm

holds lakes and reservoirs to be inherently P limited because N₂ fixation can hypothetically add N to the system and overcome stoichiometric N deficits as fixed N inputs accumulate over time (Schindler 1977, 2012; Schindler and Hecky 2009). However, for this mechanism to be widely relevant for highly productive lakes and reservoirs, N and P retention, storage, and subsequent internal recycling rates must occur in proportions that correspond to strict P deficiency. In the study reservoirs, the stoichiometry of these system fluxes instead almost always indicated strict N deficiency or N + P co-deficiency. Rates of retention, storage, and internal recycling vary between lentic systems, and more stoichiometric studies of ecosystem fluxes are needed to further test our observations. However, patterns in ecosystem stoichiometry clearly show that the three study reservoirs are unlikely to develop strict P deficiency over time and that factors associated with eutrophication increased the reservoirs' propensity to remain perpetually N deficient by simultaneously enhancing denitrification efficiency and reducing N₂ fixation efficiency.

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