

Watershed vs. within-lake drivers of nitrogen: phosphorus dynamics in shallow lakes

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Abstract. Research on lake eutrophication often identifies variables affecting amounts of phosphorus (P) and nitrogen (N) in lakes, but understanding factors influencing N:P ratios is important given its influence on species composition and toxin production by cyanobacteria. We sampled 80 shallow lakes in Minnesota (USA) for three years to assess effects of watershed size, proportion of watershed as both row crop and natural area, fish biomass, and lake alternative state (turbid vs. clear) on total N : total P (TN : TP), ammonium, total dissolved phosphorus (TDP), and seston stoichiometry. We also examined N:P stoichiometry in 20 additional lakes that shifted states during the study. Last, we assessed the importance of denitrification by measuring denitrification rates in sediment cores from a subset of 34 lakes, and by measuring seston $\delta^{15}\text{N}$ in four additional experimental lakes before and after they were experimentally manipulated from turbid to clear states. Results showed alternative state had the largest influence on overall N:P stoichiometry in these systems, as it had the strongest relationship with TN : TP, seston C:N:P, ammonium, and TDP. Turbid lakes had higher N at given levels of P than clear lakes, with TN and ammonium 2-fold and 1.4-fold higher in turbid lakes, respectively. In lakes that shifted states, TN was 3-fold higher in turbid lakes, while TP was only 2-fold higher, supporting the notion N is more responsive to state shifts than is P. Seston $\delta^{15}\text{N}$ increased after lakes shifted to clear states, suggesting higher denitrification rates may be important for reducing N levels in clear states, and potential denitrification rates in sediment cores were among the highest recorded in the literature. Overall, our results indicate lake state was a primary driver of N:P dynamics in shallow lakes, and lakes in clear states had much lower N at a given level of P relative to turbid lakes, likely due to higher denitrification rates. Shallow lakes are often managed for the clear-water state due to increased value as wildlife habitat. However, our results indicate lake state also influences N biogeochemistry, such that managing shallow lakes for the clear-water state may also mitigate excess N levels at a landscape scale.

Key words: alternative stable states; denitrification; eutrophication; N:P ratio; nutrient limitation; nutrient stoichiometry.

INTRODUCTION

Lake eutrophication is a global issue threatening biodiversity, drinking water, and recreational use of freshwater ecosystems (Smith and Schindler 2009). Research on lake eutrophication has often focused on identifying primary sources of phosphorus (P) and nitrogen (N) in

efforts to control and mitigate the overall levels of nutrients in lentic ecosystems. However, understanding factors that influence the ratio of total N to total P (TN : TP) is also important because this ratio influences and reflects competitive interactions among algal species (Fujimoto et al. 1997), and low TN : TP is often associated with blooms of noxious cyanobacteria (Kosten et al. 2012, Beaulieu et al. 2013). Furthermore, high levels of N can stimulate production of toxins in cyanobacteria (Gobler et al. 2016). High N:P values in unproductive systems may be an outcome that is due to low organic carbon constraints on an important feedback in the N cycle, denitrification (Finlay et al. 2013). Though eutrophication research has historically focused on P, excessive reactive N in terrestrial and aquatic

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ecosystems at the global scale (Rockström et al. 2009) makes understanding principal drivers of N concentrations and denitrification rates a pressing issue.

Understanding TN : TP dynamics in smaller, shallow lakes is especially important given their large numbers (Downing et al. 2006) and the large quantities of nutrients and organic matter they receive from their watersheds (Downing 2010). Additionally, many shallow lakes in the U.S. Midwest and elsewhere are found in areas of heavy row crop agricultural with high use of industrial fertilizer, increasing their vulnerability to eutrophication but also their ability to mitigate high N input rates via high denitrification rates. This is especially true in the Prairie Pothole Region (PPR) of North America, a 715,000 km² area of North America comprised of thousands of shallow lakes in a historically grassland landscape, but with uplands that have now been largely converted to agricultural use (Euliss et al. 1999).

Understanding TN : TP dynamics in shallow lakes is also important given these systems exhibit alternative stable states (Scheffer 2004). Alternative stable states have been documented in shallow lakes of the PPR, with one stable state dominated by submerged macrophytes and the other dominated by phytoplankton (Zimmer et al. 2009). Research on eutrophication and alternative stable states in shallow lakes has focused on the influence of nutrients (especially P) on the stability of turbid and clear states, and has shown that P is a primary determinant of lake state and bifurcation points where lakes shift from one state to the other (reviewed by Scheffer and van Nes [2007]). It is well established that nutrient levels influence state, but it is also possible that the opposite is true; that ecosystem state influences nutrient dynamics in shallow lakes. Submerged aquatic macrophytes have been shown to increase denitrification rates by excreting dissolved oxygen from roots, which results in tightly coupled nitrification-denitrification in sediments (Weisner et al. 1994, Ottosen et al. 1999), and by supporting epiphytic biofilms with high rates of denitrification (Bourgues and Hart 2007). Thus, it is plausible that higher abundance of submersed aquatic plants in lakes in clear-water states vs. turbid states facilitates increased denitrification rates relative to systems without rooted plants, contributing to lower TN : TP ratios relative to lakes in turbid states. Though it is well documented that submersed aquatic plants facilitate higher denitrification rates, it is unknown whether this influence is sufficient to cause ecosystem-scale differences in TN : TP relationships between lakes in turbid vs. clear water states.

TN : TP dynamics in shallow lakes and other lentic systems are a result of the combined influences of factors influencing input, storage, and loss rate of each element. Land use in watersheds has been shown to be an important driver of TN : TP in deeper lakes, with watersheds dominated by use of industrial fertilizer increasing TN : TP ratios and watersheds used for animal production lowering the TN : TP ratio (Arbuckle and Downing 2001). Sediments are the largest internal storage pool for nutrients in shallow lakes (Bowden 1987), and several

processes can influence long-term storage vs. internal loading from sediments. Oxygen concentrations at the sediment–water interface are especially important as they influence decomposition rates (Sobek et al. 2009) and redox reactions that determine the solubility of PO₄ in water (Gunnars and Blomqvist 1997). As discussed above, oxygen from macrophyte roots may also facilitate loss of N in lake sediments due to stronger coupling between nitrification and denitrification.

In shallow lakes, benthivorous fish may also have strong influences on N:P dynamics. Biomass of benthivorous fish is often higher in shallow lakes relative to deeper lakes (Jeppesen et al. 2003), and feeding activities of fish in sediments translocate nutrients from sediments to the water column (reviewed by Vanni 2002). Moreover, the fathead minnow (*Pimephales promelas*) is a detritivorous fish common in shallow lakes throughout the southern portion of the PPR and 55% of its diet can be comprised of lake detritus (Herwig and Zimmer 2007). Bioenergetics modeling has shown that excretion rates from fathead minnows in PPR shallow lakes can equal external loading from lake watersheds, and that these fish may reduce lake water N:P ratios due to low excretion N:P ratios (Zimmer et al. 2006). Planktivorous fish can also influence N:P dynamics at the whole-lake scale via trophic cascades that influence the abundance and nutrient concentrations of seston (Elser et al. 2000).

Though several studies have documented the importance of within-lake factors (e.g., fish biomass) and watershed-scale variables (e.g., land use) on N:P ratios in lakes, their relative importance is poorly known. Additionally, to date, the influence of alternative stable states on N:P relationships in shallow lakes has not been evaluated. In this study we assessed the relative importance of land use patterns, fish biomass, and alternative stable states on N:P dynamics in shallow lakes, and examined the potential role of denitrification as a driver of N dynamics in these systems.

METHODS

This study was conducted in 100 shallow lakes selected from four ecoregions of Minnesota (based on Omernik 1987). We selected 22 lakes in the Western Corn Belt ecoregion (hereafter Prairie region), 18 lakes in the North Central Hardwood Forest on the edge of the Minneapolis, Minnesota, USA, metropolitan area (Metro region), 23 lakes in the Northern Glaciated Plains (Parkland region), 15 lakes in the Northern Lakes and Forest (Forest region), and 22 additional lakes also in the Northern Lakes and Forest but with most of the sites in Itasca State Park (State Park region). The Prairie and Parkland regions were located within the broader PPR. Lakes were selected from the National Wetlands Inventory GIS database (<https://www.fws.gov/wetlands/>) using a stratified-random procedure based on the five regions.

Farm Service Agency color digital orthophoto quadrangles from 2008 (hereafter “air photos”) and ArcGIS

(Environmental Systems Research Institute, Redlands, CA, USA) were used to estimate surface area of each lake. Watershed areas were manually delineated following the methods of Minnesota Department of Natural Resources' statewide Lake Watershed Project (Minnesota Department of Natural Resources 2017). ArcGIS (ESRI, Redlands, California, USA) was then used to estimate watershed size for each study site. Land use within the watershed of each lake was classified into 13 categories using standardized on-screen digitizing procedures using 2008 air photos as primary references and existing digital land cover layers (GAP Land Cover 1991-1993, NLCD 2001) as corroboratory references. For this analysis, we used row crop agriculture (hereafter Row Crop), comprised largely of soybeans and corn, and Natural Areas, which consisted of the summed area of grasslands, forests, and shrub lands in each watershed. Row Crop and Natural Area were both expressed as proportion of the watershed area for each lake.

Within lake characteristics were sampled in July of 2009, 2010, and 2011, with all variables measured for each site visit. The exception was particulate carbon (PC), particulate nitrogen (PN), and particulate phosphorus (PP), which were sampled in 2009 and 2011 only. Abundance of planktivorous and benthivorous fish was sampled with two types of nets deployed in each lake for 24 h. One experimental gill net (61.0-m multifilament net with 19-, 25-, 32-, 38-, and 51-mm bar meshes) was deployed along a 2 m deep contour or at the maximum depth in lakes <2 m deep, while three mini-fyke nets (6.5 mm bar mesh with four hoops, one throat, 7.62 m lead, and a 0.69 × 0.99 m rectangular opening) were set perpendicular to shore (Herwig et al. 2010). Fish were identified to species and total biomass was recorded for each species in each lake. We summed all of the biomass of all non-piscivorous fish (all fish excluding walleye, *Sander vitreus*, northern pike, *Esox lucius*, and largemouth bass, *Micropterus salmoides*) to create a planktivores + benthivores variable (hereafter Fish Biomass). Submerged aquatic macrophytes were sampled with methods modified from Deppe and Lathrop (1992). A cast was made with a weighted plant rake at 15 stations uniformly spaced across each lake, with the rake dragged across 3 m of the sediment surface for each cast. Total wet biomass of macrophytes collected was recorded for each throw, and the average biomass across the 15 throws was used as an index of macrophyte abundance.

Water chemistry was assessed using two water samples collected from near the center of each lake. For each sample, phytoplankton abundance was estimated by filtering water through a GF/F filter, freezing the filter, and then analyzing it for chlorophyll *a* (chl *a*) by acetone extraction and fluorometric analysis. A subset of whole water was frozen for analysis of TP, while samples for total dissolved phosphorus (TDP) were first filtered with a GF/F filter and then frozen. TP and TDP samples were both analyzed with persulfate oxidation followed by ascorbic acid colorimetry. Another subset of whole

water was frozen for analysis of TN and subsequently analyzed with persulfate oxidation and second derivative spectrophotometry. Ammonium samples (NH_4^+ , hereafter NH_4) were filtered in the field through GF/F filters, frozen, and analyzed with an OI Analytical Flow Solution IV automated analyzer (OI Analytical, College Station, TX, USA) using the phenol-hypochlorite method. In 2009 and 2011 we sampled PC, PN, and PP by collecting particles on GF/F filters. One filter was used for PP, while the other was used for both PN and PC. The PN-PC filter was rinsed with 1% HCl followed by nanopure water to remove inorganic carbon. The filters were frozen, and the PP filters were analyzed with the same methods used for TP and TDP. PC and PN filters were analyzed with a Thermo Electron Flash EA1112 Series CN analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Chl *a* is expressed as $\mu\text{g/L}$, while all other water chemistry variables are in $\mu\text{mol/L}$.

We used *K*-means cluster analysis to classify each lake ($n = 100$) in each year into one of two groups (turbid vs. clear) based on abundance of submerged macrophytes and chl *a* concentrations (Zimmer et al. 2009). That resulted in three categories of lakes: clear lakes (lakes in clear states all three years), turbid lakes (lakes in turbid states all three years), and shifting lakes (lakes that shifted states at least once). We then analyzed the stable lakes ($n = 80$) and shifting lakes ($n = 20$) separately.

For the stable lakes, our interest was focused on assessing the influence of within-lake factors (lake State [turbid or clear] and Fish Biomass) and watershed-level factors (Watershed Size, proportion Row Crop, proportion Natural Area) on TN : TP, PN : PP, PC : PN, PC : PP, NH_4 : TN, and TDP : TP. We used the denominator of each ratio in our regression models (sensu Downing and McCauley 1992) to avoid the pitfalls of using a ratio as our response variable (Berges 1997). In this approach, parameter estimates for additional variables beyond the denominator indicated the effect each variable has on the overall ratio. For example, a positive parameter estimate for Row Crop in the model $\text{TN} = \text{Row Crop} + \text{TP}$ indicates Row Crop increases TN at a given level of TP. Though the numerator is the response variable in our models, we refer to the ratio in our analyses as our focus was understanding how within lake and watershed factors influence the relationship between the numerator and denominator.

We used an information theoretic approach (Anderson et al. 2000) to find the most parsimonious model for predicting TN : TP, PN : PP, PC : PP, NH_4 : TN, and TDP : TP. We first used restricted maximum likelihood and AIC_c to estimate the most parsimonious covariance model for our repeated measures design using our most complex fixed-effects model (State, Row Crop, denominator, Region, and Year; Wolfinger 1993). In the second step, we used maximum likelihood to assess model fit of Year and Region based on AIC_c , and Year and Region were used in subsequent analyses only when their AIC_c value was smaller than the denominator-only model.

We refer to results of this second step, where models are composed of only combinations of denominator, Year, and Region, as our “base model.” In the third step, we again used maximum likelihood to assess whether base models could be improved by adding within lake- and watershed-level variables. Here we added each predictor variable (State, Fish Biomass, Watershed Size, Row Crop, Natural Area) to the base model individually, then tested a sixth model composed of the within-lake variable and watershed-level variable with the lowest individual AIC_c scores. Given the complexity of our models, we did not fit any interaction terms. Overall parsimony of models in our third step was assessed using AIC_c , Δ_i , w_i (Akaike weights), and evidence ratios (Burnham and Anderson 2002). AIC_c values were used to generate w_i (proportion of support for a given model relative to all other models being considered), Δ_i (difference in AIC_c values between each model and the most parsimonious model), and evidence ratios (multiplicative support of the top ranked model relative to all other models, equal to w_i ratios). Models within six AIC_c units of the model with the smallest AIC_c value ($\Delta_i = 6$) were considered plausible (Richards 2008). The exception was more complex models (e.g., Row Crop + State) required smaller AIC_c values than simpler nested models (e.g., State) to be retained as a plausible model (Richards 2008, Arnold 2010). We also present 95% confidence intervals for parameter estimates to help clarify the importance of individual variables in models. We report results for the top three models plus the base model for each analysis, and to simplify our tables we give parameter estimates for our within lake and watershed variables only. Parameter estimates for Year and Region are given in our Supporting Information (Appendix S1). We also show Redfield ratios (C:N:P = 106:16:1 by moles) in figures for TN : TP, PN : PP, PC : PN, and PC : PP as a reference for relative nutrient limitation for P and N.

We also assessed potential denitrification rates in a subset of 34 lakes in stable turbid and clear states in 2011 (8 Parkland lakes, 11 Metro, 8 State Park, and 7 Prairie) using the acetylene inhibition method (Yoshinari and Knowles 1976). Although this method has well-documented limitations in inferring in situ denitrification rates (Seitzinger et al. 1993), it is useful for assessing spatial variability (Groffman et al. 2006). Sediment cores were collected during the summer of 2011 from the center of each lake using an HTH gravity corer (Renberg and Hansson 2008). Approximately 40 g of sediment from the upper 5 cm of sediment was added to 315 mL Wheaton incubation bottles. Unamended potential denitrification assays were conducted by adding 40 mL of study site lake water. We also conducted amended potential denitrification assays, in which overlying lake water was amended with 100 mg organic carbon/L (as glucose), 100 mg NO_3^- -N/L, and 14 mg PO_4 -P/L. We used amended and unamended assays as the former is designed to give us a maximum potential denitrification rate (under resource-replete conditions) while the later gives a potential denitrification rate with ambient water chemistry.

For both techniques, bottle headspace was flushed with N_2 for 3 min to remove oxygen, and 10 mL of acetylene (C_2H_2) was injected into each bottle. We assumed constant denitrification rates over the course of the incubation, and bottles were incubated for 2.5 h. Incubations occurred in the dark, at room temperature ($22^\circ C$), and bottles were shaken several times throughout the incubations to ensure homogeneity. Headspace samples were collected at initial and final time points. At each sampling time, 10 mL of headspace gas was removed through bottle septa, and 5 mL of this sample was injected into a helium-flushed, 10 mL Agilent headspace vial (Agilent, Santa Clara, CA, USA). Gas samples were analyzed within 1 week using an HP5890 Series II gas chromatograph (Hewlett-Packard, Wilmington, DE, USA) with a headspace autosampler and electron capture device, flame ionization detector, and thermal conductivity detector. Potential denitrification rates were determined as the production of N_2O during the incubations on the basis of dry sediment mass. Assays were typically performed in duplicate for each lake; means of replicate samples were used in our analyses. To facilitate comparison with other studies, amended and unamended potential denitrification rates were used to estimate areal denitrification rates using sediment bulk density measurements (Richardson et al. 2004), based on the assumption that all denitrification occurs within the top 5 cm of sediment. Our estimates of denitrification rates were not measured in situ, so they should be considered potential rates of denitrification.

Our interest was in assessing denitrification rates in our study sites relative to estimates from other lakes. Thus, we present our results as the mean and range of estimates observed in each study region, and compare our unamended rates to unamended rates for lake sediments summarized in Piña-Ochoa and Álvarez-Cobelas (2006). We did not test whether rates differed between lakes in turbid vs. clear-water states, as macrophytes increase denitrification rates by supporting epiphytic biofilms and by their roots excreting oxygen into sediments, which results in enhanced coupling of nitrification–denitrification (Ottosen et al. 1999, Bourgues and Hart 2007). Both of these effects are eliminated when sediment samples are removed from lakes.

We used a matched-pairs approach (Wiens and Parker 1995) to assess the amount of change in N and P in the lakes that shifted states during the study ($n = 20$) using log-transformed TN, TP, NH_4 , and TDP data. Each lake was paired with itself across the adjacent years when the shift occurred, and the TN, TP, NH_4 , and TDP value when the lake was clear was subtracted from the value when the lake was turbid. We then used a t test to determine whether the degree of change differed between TN and TP, and between TDP and NH_4 . The difference of log values is equivalent to the ratio of geometric means in the original units, thus the t test amounts to testing for differences in the ratio of change between states.

Denitrification increases $\delta^{15}\text{N}$ in seston due to preferential conversion of ^{14}N to N_2 by denitrifying bacteria (Lehmann et al. 2004). Thus, we assessed the potential role of denitrification in N dynamics by measuring $\delta^{15}\text{N}$ in seston before and after lakes shifted from turbid to clear states. For this analysis we used two consistently turbid lakes that were part of our larger 100 lake study, plus six additional lakes not used in the 100-lake analysis that were all turbid in 2010. In fall of 2010, four of the lakes not used in our 100-lake study were treated with the fish toxicant rotenone to reduce fish densities and induce shifts to clear-water states (hereafter “experimental lakes”), while the other four lakes remained in turbid states and served as controls. Seston $\delta^{15}\text{N}$ was sampled in July of both 2010 and 2011 in all eight lakes by collecting two water samples near the center of each lake, and filtering water onto GF/F filters. The filters were frozen until analysis of $\delta^{15}\text{N}$ by the Stable Isotope Biogeochemistry Laboratory at Stanford University, and the mean value for each lake was used for statistical analysis. We used a matched-pairs design where the $\delta^{15}\text{N}$ of each lake in 2010 was subtracted from its $\delta^{15}\text{N}$ value in 2011, and we then used a *t* test to test for a significant difference in the change in $\delta^{15}\text{N}$ between control and experimental lakes between years.

All variables for all analyses (excluding Row Crop and Natural Area) were log-transformed to normalize the data, and statistical analyses were done using SAS 9.4 (SAS Institute 2013) or JMP 10 (SAS Institute 2012). All ratios are molar unless specified otherwise.

RESULTS

K-means cluster analysis indicated 69 lakes remained in the stable clear-water state during the three years of the study, 11 were in the stable turbid-water state, and 20 lakes shifted states at least once. Phytoplankton and submerged macrophyte abundance differed sharply between stable turbid and stable clear lakes, and lakes that shifted states exhibited major changes in phytoplankton and submersed macrophyte abundance during the one year between state shifts. Chl *a* values in shifting lakes averaged 14 $\mu\text{g/L}$ while in the clear state and 112 $\mu\text{g/L}$ when turbid. Submersed macrophyte biomass averaged 556 g/throw and 109 g/throw when in the clear and turbid states, respectively. Though macrophytes were present in some turbid-state systems, their distribution was largely limited to shallow water in the lake periphery. This indicates lakes can develop characteristics of the alternative state within one year of shifting, with little lag in changes in abundance of phytoplankton or submerged macrophytes. Non-shifting turbid and clear lakes both exhibited substantial variability of within-lake and watershed-level characteristics (Table 1).

Results for TN : TP in the 80 stable lakes indicated the base model was TP + Region, and the best-supported model was State + TP + Region (Table 2, Fig. 1a). State had strong support, with evidence ratios

TABLE 1. Within lake and watershed characteristics of non-shifting turbid and clear study sites during 2009–2011.

Characteristic	Clear-state lakes (<i>n</i> = 69)	Turbid-state lakes (<i>n</i> = 11)
Average depth (m)	0.68 (0.80)	0.41 (0.69)
Surface area (ha)	22.4 (35.9)	25.5 (16.4)
Total phosphorus ($\mu\text{mol/L}$)	1.9 (2.1)	6.7 (3.9)
Total nitrogen ($\mu\text{mol/L}$)	89.2 (43.7)	294.5 (129.0)
Particulate carbon ($\mu\text{mol/L}$)	135.1 (111.6)	1,127.5 (752.9)
Particulate nitrogen ($\mu\text{mol/L}$)	22.8 (13.9)	197.4 (138.5)
Particulate phosphorus ($\mu\text{mol/L}$)	1.0 (1.0)	4.9 (2.9)
Total dissolved phosphorus ($\mu\text{mol/L}$)	0.94 (1.24)	1.51 (0.98)
Ammonium ($\mu\text{mol/L}$)	4.31 (3.00)	8.57 (7.52)
Chlorophyll <i>a</i> ($\mu\text{g/L}$)	7.47 (6.9)	89.2 (44.9)
Macrophyte biomass (CPUE g)	653.1 (644.1)	93.3 (185.1)
Planktivore + benthivore biomass (kg)	5.6 (10.6)	26.1 (17.9)
Natural areas (proportion of watershed)	0.64 (0.29)	0.27 (0.20)
Row crop (proportion of watershed)	0.11 (0.21)	0.34 (0.19)
Watershed size (ha)	479 (1,173)	1,504 (3,336)

Note: Values are means with SD in parentheses. Particulate carbon, nitrogen, and phosphorus are averages from 2009 and 2011.

>10,000 relative to models lacking State as a predictor. The effect size of State was also large, as back-transforming the state parameter estimate showed turbid lakes had 1.9-fold more TN at a given level of TP compared to clear lakes. Moreover, the TP slope of 0.2 indicated TN did not increase at the same proportional rate as TP across the study sites, giving an overall pattern of decreasing TN : TP as TP increased. Given their large range of TP, TN : TP in clear lakes ranged from potentially intense P limitation at low TP (TN : TP = 458) to sub-Redfield ratio (N:P < 16:1) at high TP (TN : TP = 7). TN : TP on average was lower in turbid lakes given their higher average TP levels, but at a given TP value, TN : TP ratios were higher in turbid lakes. The only other variable with marginal support among the top models for influencing TN : TP was Fish Biomass, and results indicated fish increase TN : TP in these systems. However, the effect size and support for the Fish Biomass model was much less than that for State. Row Crop was a component of the second-best supported model, but its slope parameter was not significantly different from zero.

The base model for PN : PP was PP + Region + Year, and the top supported model was State + Row Crop + PP + Region + Year (Table 3, Fig. 1b). The top model had strong support relative to all others, with over 600-fold more support than the second best model and >10,000-fold more support than all other models. Parameter estimates showed State had an even larger effect on

TABLE 2. Performance of the top three models and the base model for TN : TP.

Model and parameters	AIC _c	Δ_i	w_i	Evidence ratio	Parameter estimate
State + TP + Region	-175.4	0	0.52		
State: clear					-0.28 (-0.35, -0.20)
TP					0.20 (0.14, 0.27)
Row Crop + State + TP + Region	-175.2	0.2	0.48	1.1	
Row crop					0.11 (-0.04, 0.26)
State: clear					-0.28 (-0.35, -0.20)
TP					0.20 (0.14, 0.27)
Fish Biomass + TP + Region	-139.7	35.7	<0.01	>10,000	
Fish Biomass					0.06 (0.01, 0.11)
TP					0.25 (0.18, 0.32)
TP + Region (base model)	-136.8	38.6	<0.01	>10,000	
TP					0.26 (0.19, 0.33)

Notes: Models are sorted in order of increasing values of the Akaike information criterion corrected for sample size (AIC_c), with smaller AIC_c values indicating more parsimonious models. Δ_i is the difference in AIC_c values between each model and the model with lowest AIC_c value, w_i are Akaike weights and represent weight of evidence (out of 1.00) that each model is the best model in the set, and the evidence ratio shows multiplicative improvement of the best model over all other models. Also shown are the estimates and 95% confidence intervals for model parameters. The state parameter is the difference between clear and turbid states (clear – turbid), and fish biomass stands for the total CPUE (catch per unit effort) of planktivores and benthivores in each lake. Values in parentheses are 95% CI. Parameter estimates for region for the top model are given in Appendix S1.

PN than it did on TN, as PN was 3.7-fold higher in turbid lakes at a given level of PP relative to clear lakes. Moreover, 22% of the clear-lake years were sub-Redfield, compared to 10% of the turbid-lake years. Row crop showed a positive effect on PN, and also exhibited a large effect size, with PN increasing 2.1-fold as proportion of watershed used for Row Crop increased from 0 to 1.0 and other factors remained constant.

Results for PC indicated State was an important factor for both PC : PN and PC : PP, and that seston had higher concentrations of PN in the turbid-water state and higher concentrations of PP in the clear-water state at a given level of PC. For PC : PN, the base model was PN + Region + Year, and the best-supported model was

State + PN + Region + Year (Table 4). The best-supported model had 403-fold more support than the base model, and no other non-nested models performed better than the base model. The State effect was relatively weak on PC : PN, with clear lakes having just 1.1-fold higher PC at a given level of PN compared to turbid lakes, and most lakes showed PC : PN values close to the Redfield ratio of 6.6:1 (Fig. 2a). For PC : PP, the base model was PP + Year, and the best-supported model was State + Row Crop + PP + Year (Table 5). However, the top model had just 8-fold more support than the State + PP + Year model, indicating marginal support for a Row Crop effect. The state effect on PC : PP was much stronger than that observed for

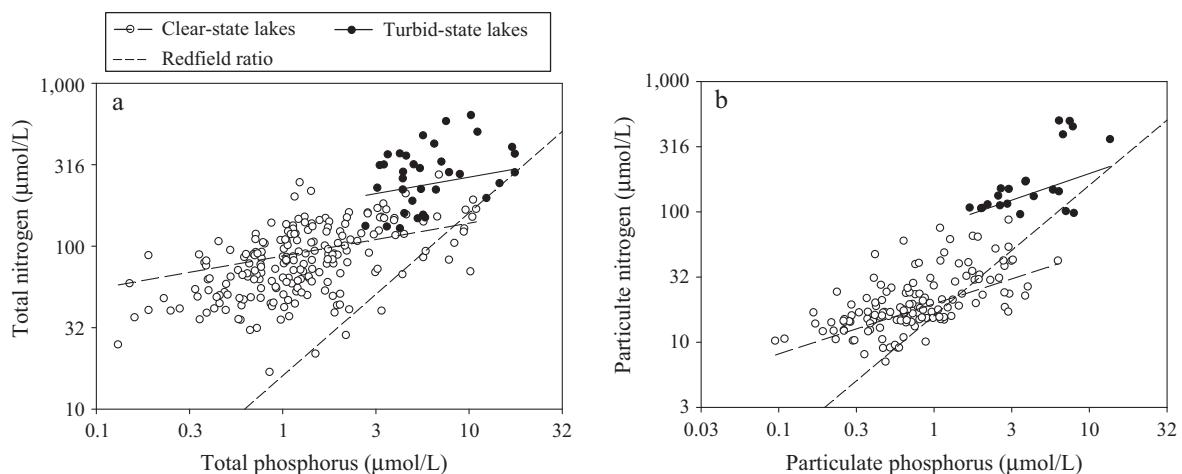


FIG. 1. (a) Relationship between total nitrogen and total phosphorus in 80 shallow lakes during 2009–2011. The best-fit lines are from the best-supported model and show predicted values for lakes in turbid vs. clear-water states in the Prairie Region of North America. (b) Relationship between particulate nitrogen and particulate phosphorus in 80 shallow lakes during 2009 and 2011. The best-fit lines are from the best-supported model and show predicted values for lakes in turbid vs. clear-water states in the Prairie Region in 2011 at the average value of proportion of watershed used for row crop agriculture.

TABLE 3. Performance of the top three models and the base model for PN : PP.

Model and parameters	AIC _c	Δ_i	w_i	Evidence ratio	Parameter estimate
State + Row crop + PP + Region + Year	-115.8	0	0.99		
State: clear					-0.57 (-0.65, -0.48)
Row crop					0.32 (0.16, 0.48)
PP					0.41 (0.32, 0.50)
State + PP + Region + Year	-103.0	12.8	<0.01	602	
State: clear					-0.58 (-0.68, -0.49)
PP					0.40 (0.31, 0.50)
Fish Biomass + PP + Region + Year	-27.9	87.9	<0.01	>10,000	
Fish Biomass					0.17 (0.10, 0.25)
PP					0.57 (0.45, 0.68)
PP + Region + Year (base model)	-11.3	104.5	<0.01	>10,000	
PP					0.59 (0.47, 0.70)

Notes: Models are sorted in order of increasing AIC_c values, with smaller AIC_c values indicating more parsimonious models. PN, particulate nitrogen; PP, particulate phosphorus. Statistical parameters and acronyms are defined in Table 2. Values in parentheses are 95% CI. Parameter estimates for Region and Year for the top model are given in Appendix S1.

TABLE 4. Performance of the top three models and the base model for PC : PN.

Model and parameters	AIC _c	Δ_i	w_i	Evidence ratio	Parameter estimate
State + PN + Region + Year	-247.3	0	0.52		
State: clear					0.16 (0.08, 0.25)
PN					1.27 (1.19, 1.36)
Watershed + State + PN + Region + Year	-247.1	0.2	0.47	1.1	
Watershed					-0.02 (-0.04, 0.01)
State: clear					0.16 (0.08, 0.25)
PN					1.29 (1.20, 1.27)
PN + Region + Year (base model)	-235.3	12	<0.01	403	
PN					1.15 (1.09, 1.20)
Watershed + PN + Region + Year	-235.0	12.3	<0.01	469	
Watershed					-0.02 (-0.05, 0.01)
PN					1.16 (1.10, 1.22)

Notes: Models are sorted in order of increasing AIC_c values, with smaller AIC_c values indicating more parsimonious models. PN, particulate nitrogen; PC, particulate carbon. Statistical parameters and acronyms are defined in Table 2. Values in parentheses are 95% CI. Parameter estimates for Region and Year for the top model are given in Appendix S1.

PC : PN (Fig. 2b), with PC 3.7-fold higher in turbid lakes at a given level of PP compared to clear lakes, and PC at a given level of PP doubled as Row Crop went from 0 to 1.0 proportion of the watershed.

The base model for TDP : TP was TP + Region, while the best-supported model was State + TP + Region and it had 233-fold more support than the next best non-nested model based on Fish Biomass (Table 6). Results indicated TDP was likely less limiting in clear-state lakes, as TDP was 1.7-fold higher in clear lakes relative to turbid lakes at a given level of TP (Fig. 3a). For NH₄ : TN, NH₄ was not related to TN levels, making Region the base model. The best-supported model overall was again State (Table 7), with NH₄ on average 1.4-fold higher in turbid lakes (Fig. 3b).

Our estimates of amended and unamended denitrification rates were both highly variable within each study region (Table 8). As a general trend, amended and unamended rates in the Metro study region tended to be higher than other regions, but the large range of estimates

observed in each region resulted in considerable overlap across regions. Averaged across all study sites, amended rates ($\bar{x} = 216 \text{ mg N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) were 21-fold higher than unamended rates ($\bar{x} = 10 \text{ mg N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$), and amended rates were higher than unamended rates in 94% of our study sites. Comparing our unamended rates to published estimates of unamended, single-time estimates for lake sediments suggests our rates are among the highest reported in the literature. Denitrification rates reported in Piña-Ochoa and Álvarez-Cobelas (2006) using a variety of methods ranged from 0.02 to 4.4 $\text{mg N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, while estimates from studies using the same acetylene inhibition method we used ranged from 0.03 to 0.8 $\text{mg N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$. Thus, our mean unamended rate of 10 $\text{mg N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ is substantially higher than the range of values reported for lake sediments in Piña-Ochoa and Álvarez-Cobelas (2006).

Results from the 20 lakes that shifted states during the study indicated N was more responsive to state shifts than was P. The ratio of change in NH₄ as lakes shifted states was greater than the change observed in TDP,

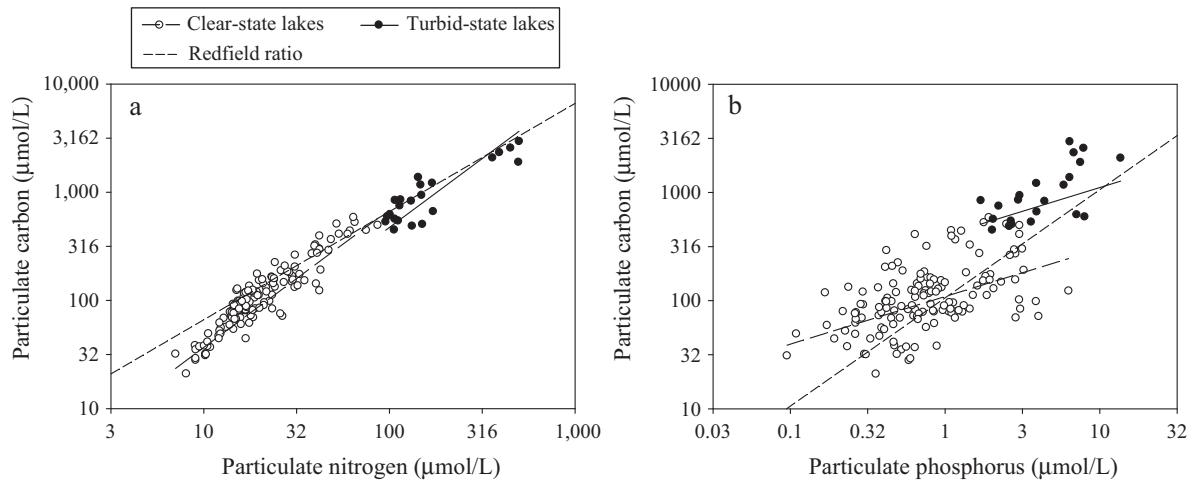


FIG. 2. (a) Relationship between particulate carbon and particulate nitrogen in 80 shallow lakes during 2009 and 2011. The best-fit lines are from the best-supported model and show predicted values for lakes in turbid vs. clear-water states in the Prairie Region in 2011. (b) Relationship between particulate carbon and particulate phosphorus in 80 shallow lakes during 2009 and 2011. The best-fit lines are from the best-supported model and show predicted values for lakes in turbid vs. clear-water states in 2011 at the average value of proportion of watershed used for row crop agriculture.

though neither variable showed multiplicative change significantly different from one (Fig. 4a). TN and TP were both significantly higher in turbid lakes, but the ratio of change between states was significantly higher for TN than TP (Fig. 4b). $\delta^{15}\text{N}$ in seston also changed as lakes shifted states, as seston $\delta^{15}\text{N}$ showed a significant enrichment in the four experimental lakes that were shifted to a clear state while no change was observed in the turbid control lakes (Fig. 5).

DISCUSSION

Our results show that lake alternative state had the largest influence on all stoichiometric variables we measured, and had a much stronger influence on N:P relationships than did watershed-level variables and fish

biomass. The overall pattern was turbid lakes had higher concentrations of TN and PN at a given level of P, higher concentrations of NH_4 , and higher concentrations of PN per unit C in seston relative to lakes in clear states. In contrast, lakes in clear states had higher levels of TDP at given levels of TP and higher concentrations of PP per unit C. Absolute concentrations of all dissolved and particulate fractions (C, N, P) were much lower in clear-state lakes than turbid-state lakes. However, the differential effects on N species were stronger than those for P species resulting in a lower overall N:P in clear-state lakes compared with turbid-state lakes.

Alternative state consistently had stronger support as a predictor of lake stoichiometry relative to fish biomass. However, changes in fish biomass are often associated with state shifts in shallow lakes (reviewed by Scheffer

TABLE 5. Performance of the top three models and the base model for PC : PP.

Model and parameters	AIC _c	Δ_i	w_i	Evidence ratio	Parameter estimate
State + Row crop + PP + Year	10.7	0	0.86		
State: clear					-0.57 (-0.71, -0.42)
Row crop					0.25 (0.05, 0.44)
PP					0.44 (0.32, 0.56)
State + PP + Year	14.8	4.1	0.11	8	
State: clear					-0.59 (-0.73, -0.44)
PP					0.49 (0.37, 0.61)
Fish Biomass + PP + Year	54.6	43.9	<0.01	>10,000	
Fish Biomass					0.17 (0.10, 0.25)
PP					0.57 (0.45, 0.68)
PP + Year (base model)	66.5	55.8	<0.01	>10,000	
PP					0.76 (0.64, 0.88)

Notes: Models are sorted in order of increasing AIC_c values, with smaller AIC_c values indicating more parsimonious models. Statistical parameters and acronyms are defined in Table 2. Values in parentheses are 95% CI. Parameter estimates for Year for the top model are given in Appendix S1.

TABLE 6. Performance of the top three models and the base model for TDP : TP.

Model (parameters)	AIC _c	Δ_i	w_i	Evidence ratio	Parameter estimate
State + TP + Region	12.1	0	0.74		
State: clear					0.24 (0.13, 0.35)
TP					0.76 (0.66, 0.87)
State + Natural cover + TP + Region	14.2	2.1	0.26	2.9	
State: clear					0.24 (0.13, 0.35)
Natural cover					-0.03 (-0.18, 0.13)
TP					0.76 (0.66, 0.87)
Fish Biomass + TP + Region	23.0	10.9	<0.01	233	
Fish Biomass					-0.08 (-0.15, -0.01)
TP					0.70 (0.60, 0.80)
TP + Region (base model)	26.4	14.3	<0.01	1,274	
TP					0.67 (0.57, 0.76)

Notes: Models are sorted in order of increasing AIC_c values, with smaller AIC_c values indicating more parsimonious models. Statistical parameters and acronyms are defined in Table 2. Values in parentheses are 95% CI. TDP, total dissolved phosphorus. Parameter estimates for Region for the top model are given in Appendix S1.

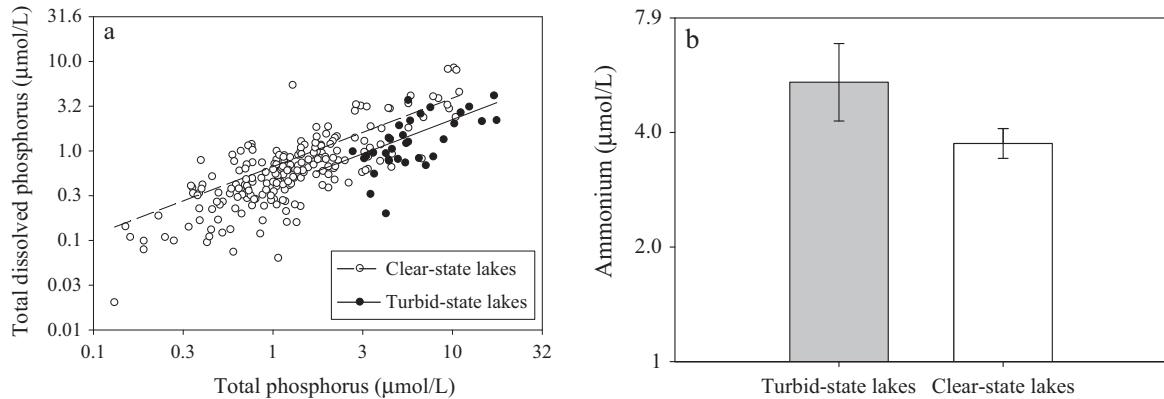


FIG. 3. (a) Relationship between total dissolved phosphorus and total phosphorus in 80 shallow lakes during 2009–2011. The best-fit lines are from the best-supported model and show predicted values for lakes in turbid vs. clear-water states in the Prairie Region. (b) Ammonium concentrations in turbid and clear lakes during 2009–2011 (mean and 95% CI). Lake state was the best-supported model for predicting ammonium levels in shallow lakes.

TABLE 7. Performance of the top three models and the base model for NH₄.

Model (parameters)	AIC _c	Δ_i	w_i	Evidence ratio	Parameter estimate
State + Region	72.7	0	0.46		
State: clear					-0.16 (-0.27, -0.05)
State + Natural cover + Region	73.0	0.3	0.40	1.2	
State: clear					-0.14 (-0.25, -0.03)
Natural cover					-0.12 (-0.29, 0.05)
Natural cover + Region	76.9	4.2	0.06	8.1	
Natural cover					-0.17 (-0.34, 0.01)
Region (base model)	78.3	5.6	0.03	16	

Notes: No relationship was detected between NH₄ and TN, so TN was not included in the base model. Models are sorted in order of increasing AIC_c values, with smaller AIC_c values indicating more parsimonious models. Statistical parameters and acronyms are defined in Table 2. Values in parentheses are 95% CI. Parameter estimates for Region for the top model are given in Appendix S1.

TABLE 8. Amended and unamended potential rates of denitrification in sediments from lakes in the four study regions.

Region	Denitrification rates ($\text{mg N}_2\text{O-N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$)					
	Amended			Unamended		
	Mean	SE	Range	Mean	SE	Range
Metro	412	104	14–976	18	10	0.04–107
Prairie	144	59	8–461	1.3	0.6	0.07–5
Parkland	127	59	11–461	1.6	0.8	0.06–6
State Park	97	40	2–319	14	9	0.3–67

2004), potentially confounding whether differences in lake stoichiometry observed in this study were driven by state shifts or large changes in fish biomass. Thus, we did a subsequent analysis to try to clarify the effects of state shifts vs. changes in fish biomass. We first determined the 20 lakes in stable clear-states that exhibited the largest change in fish biomass between years. We then determined the difference in log transformed fish biomass between years (year of higher fish biomass – year of lower fish biomass), and the difference between the same years for macrophyte biomass, chl *a*, TN, and TP. We then used a matched-pairs *t* test to determine whether the change from year of low fish biomass to year of high fish biomass differed from zero for all five variables. The difference of log values is equivalent to the ratio of geometric means in the original units, thus the *t* test amounts to testing whether the multiplicative change between years differs from one. We did the same analysis for all 11 lakes in stable turbid states. Finally, we did a similar analysis for the 20 lakes that shifted states during the study except we subtracted the value when each lake was in a clear state from the value when the lake was in the turbid state to maintain the pattern of subtracting the year with smaller fish biomass (when the lake was clear) from year of

larger fish biomass (when the lake was turbid). The stable clear and stable turbid lakes show the effects of large changes in fish biomass but no state shift, while the shifting lakes show the effects of large changes in fish biomass and state shifts.

Results showed that fish biomass increased in all three groups of lakes between years (Fig. 6a–c), and a one-way ANOVA ($P = 0.015$) and subsequent Tukey tests indicated the change between years was higher in clear lakes than shifting lakes, while change in turbid lakes was moderate and did not differ from the clear nor shifting lakes. Despite significant increases in fish biomass, no changes were detected for macrophytes, chl *a*, TN, or TP in either clear lakes (Fig. 6a) or turbid lakes (Fig. 6b). In contrast, lakes that shifted states showed significant changes in macrophytes, chl *a*, TN, and TP (Fig. 6c), supporting the notion that changes in lake stoichiometry were driven by the state shift and not by changes in fish biomass.

It is also possible that nutrient changes occur prior to state shifts, such that TP, TN, and TN : TP increase in clear-state lakes, and the higher TP induces a shift to the turbid state characterized by the higher TN : TP we observed in this study. However, TN : TP in the 20 clear-state lakes with the largest change in TP between years was significantly higher in the low TP year (TN : TP $\bar{x} = 102$) compared to the high TP year (TN : TP $\bar{x} = 30$) (paired *t* test, $P < 0.001$), opposite the pattern we observed where TP and N:P both increase when lakes shift to turbid states. Though it is difficult to isolate specific mechanisms in this study, our results indicate differences in lake stoichiometry between turbid and clear states were due to effects associated with alternative states (such as domination by different primary producers) rather than changes in fish biomass or changes in P and N:P prior to state shifts. The emerging pattern appears to be that state shifts are caused by

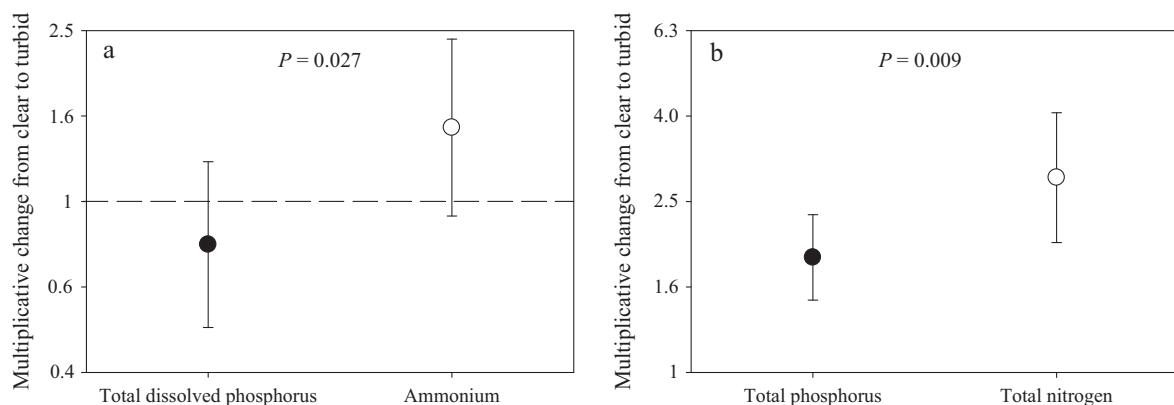


FIG. 4. (a) Multiplicative change in total dissolved phosphorus vs. ammonium in 20 shallow lakes as they shifted states during 2009–2011 (mean and 95% CI). The *P* value shows results of a matched-pairs *t* test testing whether the multiplicative change differed between total dissolved phosphorus and ammonium. (b) Multiplicative change in total nitrogen vs. total phosphorus concentrations in 20 shallow lakes that shifted states during 2009–2011 (mean and 95% CI). The *P* value shows results of a matched-pairs *t* test testing whether the multiplicative change differed between total nitrogen and total phosphorus.

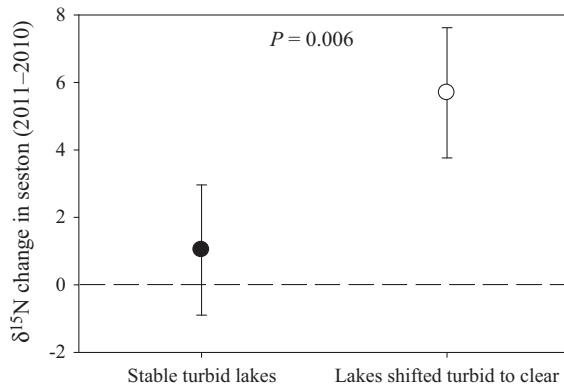


FIG. 5. Change (mean and 95% CI) in $\delta^{15}\text{N}$ in seston between 2010 and 2011 in four turbid lakes and four experimental lakes that were shifted from turbid in 2010 to clear in 2011 with biomanipulation. The P value is from a t test testing whether the change between years for individual lakes differed between turbid and experimental lakes.

complex interactions among changes in fish biomass, changes in TP, lake depth, and lake size (Scheffer and van Nes 2007), and subsequent effects associated with the state shift influence shallow lake stoichiometry.

From a management perspective, our results show that managing lakes for the clear-water via water-level draw-downs or application of piscicides (Hanson et al. 2017) can be an effective tool to mitigate excessive N at the landscape scale. Potential N inputs for these study sites included atmospheric deposition, watershed runoff, groundwater inflow, and N fixation, while outputs included long-term sediment burial, surface effluent, groundwater outflow, and denitrification. Based on our estimates for TN, shifting a lake from turbid to clear could reduce the amount of N in groundwater outflow and surface water effluent by approximately 68%, while TP would decrease 46%. However, a key difference is P does not have a significant gaseous phase. Thus, even though TP levels are lower in the water column in clear lakes, shifting the lake to a clear-water state may not reduce the total amount of P in the lake, but instead shift P from water column pools to benthic primary producers and potentially the sediment (Zimmer et al. 2001). N also shifts from water column to benthic pools when lakes shift to clear states, but N also has the potential to be permanently lost to the atmosphere via higher denitrification rates. Estimates from our sediment cores indicate denitrification rates in these systems are likely high enough to influence overall N dynamics (Seitzinger 1988). Moreover, $\delta^{15}\text{N}$ in seston increased rapidly when lakes shifted to clear states, a result consistent with increased denitrification rates in clear-water states. Though we suggest denitrification played a key role in our results, there are several other potential explanations for the much greater reduction in N relative to P as lakes shifted to clear states.

First, significantly higher $\delta^{15}\text{N}$ in seston as lakes shift to clear states could also be explained by reduced N fixation, which could also cause lower N levels in lakes in

clear states. However, lower N fixation rates in the clear state seem unlikely because shifts from turbid to clear-water states in these systems are often accompanied by blooms of *Aphanizomenon* (Ginger et al., *personal observation*), a cyanobacteria capable of N fixation. This observation is consistent with that of Downing and McCauley (1992) who suggested that lakes with N:P ratios <31 should experience N-limitation. This value is close to what we observed in the clear lakes. Another potential mechanism leading to heavier ^{15}N could be volatilization of NH_3 . However, the pH in the clear lakes was always lower by about 0.5 pH units than that observed in the turbid lakes when we had measurements of both types of lakes in the same region. Thus, it seems more likely that N fixation rates actually increased when lakes shifted from turbid to clear due to lower TN : TP ratios, but denitrification rates likely increased at an even higher rate, resulting in a net reduction of TN in the water column and increased $\delta^{15}\text{N}$ in seston. Second, it is possible that anammox and/or coupled nitrate reduction and sulfur oxidation may play important roles in N_2 losses from these systems (Burgin and Hamilton 2007), but the net effect on N cycling would be similar to that of denitrification. Third, lower overall N:P in clear-state lakes could be explained by higher N:P uptake by submersed macrophytes and periphyton relative to phytoplankton. Duarte (1992) examined N:P ratios in phytoplankton, freshwater angiosperms, macroalgae, and sea grasses, and found that N:P showed a strong linear relationship across all groups and N:P averaged 27 by moles. However, there was a tendency for freshwater angiosperms to have higher N:P ratios relative to phytoplankton, so it is possible that higher N:P ratios in macrophytes plays a role in our results. Differential uptake of N by phytoplankton could also be responsible for the higher TN : TP and PN : PP and lower C:N in turbid lakes relative to clear lakes. Light limitation is likely more intense in turbid systems, favoring higher chl a requirements and subsequently more N rich seston in turbid lakes (Stramski et al. 2002). Last, it is possible that changes in relative storage rates of P and N in lake sediments are responsible for the lake state effect on N:P. Yet Zimmer et al. (2016) showed in a subset of lakes used in this study that organic C burial rates did not differ between clear, turbid, and shifting lakes, and a one-way ANOVA done for this study on the same sites showed no difference in sediment C:N among clear ($\bar{x} = 12.3$), turbid ($\bar{x} = 12.2$), or shifting lakes ($\bar{x} = 12.1$) ($P = 0.960$, data not shown). Similarly, there was no clear relationship between sediment P burial rate and lake state in a lake that shifted states four times over a 27-yr period (Hobbs et al. 2012). Thus, we feel that higher denitrification rates, perhaps coupled with increased N uptake by aquatic macrophytes, are reasonable explanations for much lower N in clear lakes relative to P. Jeppesen et al. (1998) also observed sharp reductions in N concentrations after shallow lakes shifted from turbid to clear states, and concluded higher

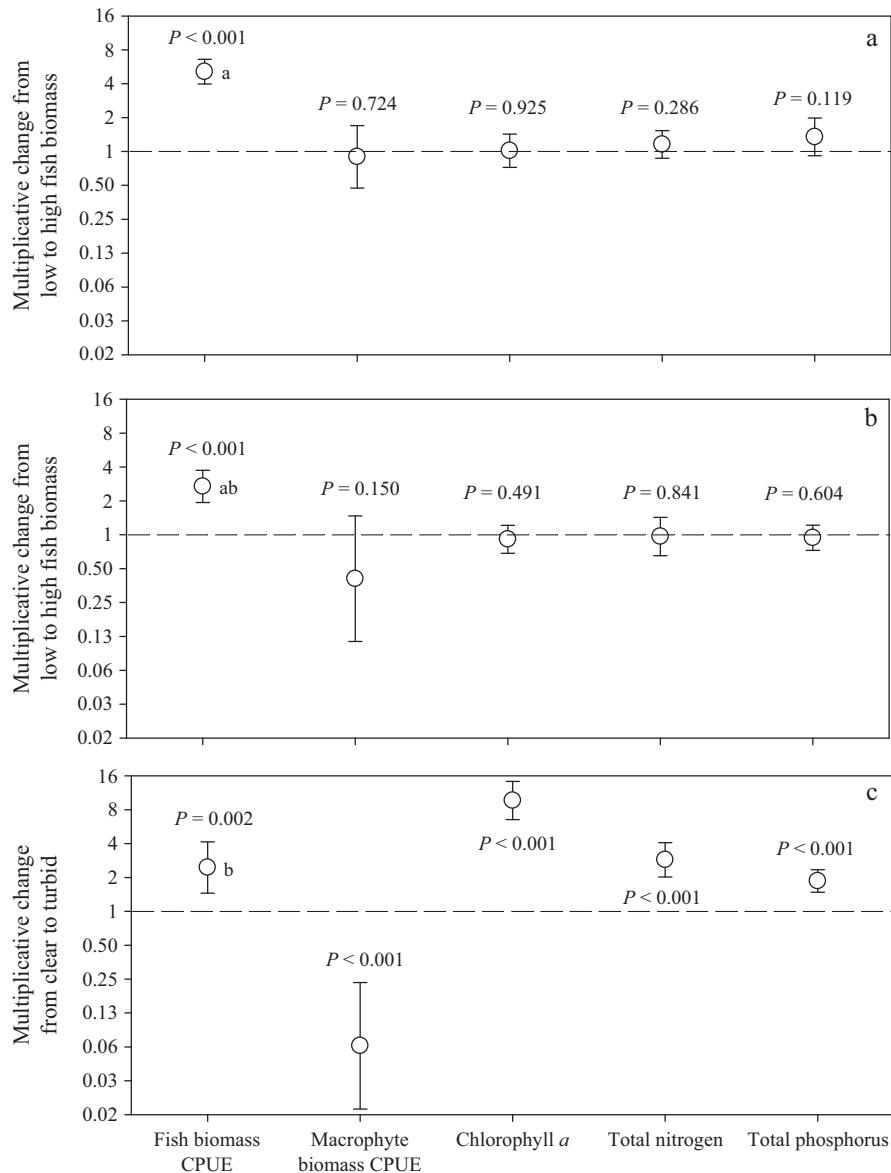


FIG. 6. Multiplicative change (mean and 95% CI) in fish biomass, submerged macrophyte biomass, chlorophyll *a*, total nitrogen, and total phosphorus in (a) the 20 stable clear-water state lakes with the largest interannual change in fish biomass, (b) the 11 stable turbid-water state lakes in years with the greatest inter annual change in fish biomass, and (c) the 20 lakes that shifted states during the study. Values in panels a and b represent the multiplicative change from year of low to year of high fish biomass for each variable, while values in panel c are multiplicative change from year of clear state to year of turbid state. *P* values are from matched pairs *t* tests for whether the multiplicative change between years differed from 1 for each variable. Letters to the right of the estimate for multiplicative change in fish biomass for panels a–c show results of one-way ANOVA and Tukey tests to determine whether change in fish biomass differed among a–c, where means with a common letter are not significantly different. CPUE, catch per unit effort.

rates of denitrification could be important. However, our evidence is indirect and additional research is clearly needed that directly assesses the processes responsible for reduced N in clear states. No matter the process, it is clear that managing lakes for the clear state will reduce N levels in surface and groundwater outflow to an even greater degree than it does P.

Our data also indicate several broad patterns regarding N vs. P limitation in phytoplankton. First, the variability

in TN : TP in these study sites was large (7–458) and approximately equal to the range observed by Downing and McCauley (1992) in a study of 221 lakes from 14 countries. This variability in a relatively small geographic area of our study suggests that biological processes and differences in nutrient limitation may be important to the biogeochemical behavior of these systems. Similar to other studies (Arbuckle and Downing 2001, Sterner et al. 2008), slopes for both TN : TP and PN : PP were <1,

indicating the probability of N limitation increases at higher P levels. Given their wide range of P, clear lakes exhibited N:P values ranging from strong P limitation at low P (TN : TP = 459) to likely N limitation at high P (TN : TP = 7). Though all turbid lakes had high levels of P, N limitation was less likely in these systems compared to clear lakes given their much higher N at a given P. Lower NH_4 concentrations and higher TDP in clear lakes also indicated higher probability of N limitation there and P limitation in turbid lakes. Numerous studies have shown that P plays a major role in influencing the stability of alternative states in shallow lakes (Jeppesen et al. 1990, Bayley et al. 2007, Zimmer et al. 2009), but our results indicate that alternative states also influence the biogeochemistry of these systems, and may influence the likelihood of P vs. N limitation.

It is difficult to compare estimates of denitrification rates due to different methodologies and limitations with various methods (Seitzinger et al. 1993), but our estimates of unamended denitrification rates appear to be among the highest reported in Piña-Ochoa and Álvarez-Cobelas (2006). High denitrification rates in our study sites were likely influenced by shallow depths and warm water temperatures, large amounts of organic C due to high productivity, and high quantities of reactive N (Knowles 1982). These high rates suggest most of these lakes had the potential for very high denitrification rates, and the rapid increase in the $\delta^{15}\text{N}$ seston signature in our four experimental lakes indicates shifting lakes to clear states allowed these systems to better realize this potential. Amended rates averaged 21-fold higher than unamended rates across our study sites, indicating unamended rates were limited by some combination of organic carbon C, nitrate, or P. Our previous work has shown these systems bury large amounts of organic C in their sediments (Zimmer et al. 2016) and that P concentrations in sediments are also high (Hobbs et al. 2012, 2014), suggesting organic C or P limitation is unlikely. If unamended rates were limited by nitrate, it indicates the enhanced coupling of nitrification–denitrification likely associated with roots of submerged macrophytes could be very important for maximizing rates of denitrification in these systems.

We also failed to find any strong relationships between land use in watersheds and N:P dynamics despite a wide range of land use patterns among lakes. This observation may be due to the fact that most of these systems are very productive with high levels of organic C and nutrient storage in sediments, enabling highly autochthonously driven dynamics. This observation could also be obfuscated by changes in the landscape that have occurred in the last few hundred years. Almost all of the lake watersheds have been impacted by development since European settlement in this region, complicating the “legacy” of each lake (Ramstack Hobbs et al. 2016). Indeed, much of the watershed cover now classified as “natural cover” in our analysis is the result of watershed restoration, particularly in the Prairie, Parkland, and

Metro study regions. In these regions, natural cover often consisted of native grasses planted as habitat in Waterfowl Production Areas (WPA) by the U.S. Fish and Wildlife Service. However, Hobbs et al. (2014) analyzed sediment cores from one of the sites used in this study and showed lake responses to watershed conversion to WPAs did not return watershed impacts to pre-development levels. Hence, though land use in watersheds varied a great deal in our study, past land use complicates these responses, making it difficult to detect relationships between current land use and N:P dynamics. It is important to note that our results may not translate well to other areas with different patterns of historic and contemporary land use, and in some cases present-day land use in watersheds may have strong influences on lake stoichiometry.

Our results show that alternative state was the primary driver of N:P dynamics in our study sites. Shallow lakes are actively managed for the clear-water state at the global scale due to their increased value as wildlife habitat, human recreational use, and improved water quality (Moss et al. 1996). Previous water quality value associated with clear-water states focused on the benefits of reduced levels of P, but our results show benefits of a clear state are even greater for managing N than they are for P. Given the global issue of excessive reactive N in the biosphere (Rockström et al. 2009), the large number of shallow lakes at the global scale (Downing et al. 2006), and the large reduction in surface water N observed in this study, managing shallow lakes for the clear-water state appears to be an important tool for mitigating N at a landscape scale.

ACKNOWLEDGMENTS

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SUPPORTING INFORMATION

Additional supporting information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/eap.1599/full>

DATA AVAILABILITY

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.6b650>