LETTER

Nitrogen transformations differentially affect nutrient-limited primary production in lakes of varying trophic state[a][b]

J. Thad Scott [ORCID],[1]* Mark J. McCarthy,[2] Hans W. Paerl[3]
[1]Department of Biology and Center for Reservoir and Aquatic Systems Research, Baylor University, Waco, Texas;
[2]Department Earth and Environmental Sciences, Wright State University, Dayton, Ohio; [3]Institute of Marine Sciences,
University of North Carolina at Chapel Hill, Morehead City, North Carolina

Scientific Significance Statement

The idea that P limitation "evolves" in lakes due to slow, but continuous, inputs of fixed nitrogen (N) over long-time scales has persisted in limnology for decades. This study uses a simple model, along with literature values of nutrient loading, denitrification, and N fixation, to suggest that fixed N may not accumulate in many eutrophic lakes because denitrification removes more N on an annual basis than is replenished through N fixation. This result suggests that eutrophic lakes become increasingly N-limited with time and that increased N inputs will further degrade water quality in those ecosystems.

Abstract

The concept of lakes "evolving" phosphorus (P) limitation has persisted in limnology despite limited direct evidence. Here, we developed a simple model to broadly characterize nitrogen (N) surpluses and deficits, relative to P, in lakes and compared the magnitude of this imbalance to estimates of N gains and losses through biological N transformations. The model suggested that approximately half of oligotrophic lakes in the U.S.A. had a stoichiometric N deficit, but 72–89% of eutrophic and hypereutrophic lakes, respectively, had a similar N deficit. Although reactive N appeared to accumulate in the most oligotrophic lakes, net denitrification perpetuated the N deficit in more productive lakes. Productive lakes exported reactive N via biological N transformations regardless of their N deficit. The lack of N accumulation through N fixation underscores the need for a modern eutrophication management approach focused on reducing total external nutrient loads, including both N and P.

*Correspondence: thad_scott@baylor.edu

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The capacity of living organisms to modify the chemistry of their environment has been a central theme in aquatic sciences since Redfield (1958) described the biological influence on ocean stoichiometry. Schindler (1977) expanded this concept using whole-lake experiments to suggest that biological N fixation compensates for nitrogen (N) deficiency relative to phosphorus (P), causing primary production in lakes to "evolve" P limitation. The evolution of P limitation concept has inspired a global view of gradual N accumulation in lakes (Schindler 2012) and oceans (Tyrell 1999), and even influenced the concept of proximate vs. ultimate nutrient limitation of terrestrial primary production (Vitousek et al. 2010). One of the few experiments testing this idea directly is the whole-lake fertilization of Lake 227 in the Experimental Lakes Area of Canada. Lake 227 was fertilized with N and P for 20 yr, and then P only for another 20+ years, to test the effects of these nutrients on primary production. A recent analysis of the Lake 227 experiment suggested

that short-term fixed N inputs may balance the N pool relative to P on a seasonal basis, but without any new N carryover from one year to the next (Higgins et al. 2017). Thus, rather than evolving P limitation through a decades-long, gradual accumulation of fixed N (Schindler 2012), Lake 227 requires new N via N fixation each year to partially resupply a N pool that has continually declined due to denitrification and/or N burial (Scott and McCarthy 2010; Higgins et al. 2017).

In simple terms, the apparent lack of fixed N carryover in lakes from one growing season to the next can occur if annual denitrification (DNF) rates exceed annual N fixation (Nfix) rates (Paerl et al. 2016). Although the net effects of DNF and Nfix on the N budget in lakes vary on many different temporal and spatial scales, longer-term effects of N transformations on primary production may be considered over annual scales that capture seasonal variability of relevant biogeochemical processes. On an annual scale, DNF (g N m^{-2} lake surface area yr^{-1}) represents N loss from the total N pool (inorganic N + organic N) to the atmosphere as N_2. Conversely, annual Nfix (g N m^{-2} lake surface area yr^{-1}) represents the gain of N into the total N pool from atmospheric N_2. Thus,

$$\text{net } N_2 \text{ flux} = \text{DNF} - \text{Nfix} \qquad (1)$$

represents the direction and magnitude of N transformations into and out of the total N pool. Lakes with DNF rates exceeding Nfix have a positive net N_2 flux (i.e., N_2 efflux from total N), and lakes with Nfix rates exceeding DNF have a negative net N_2 flux (i.e., N_2 influx into total N). Unfortunately, there are very few lakes globally where both DNF and Nfix rates have been quantified simultaneously over a range of temporal scales (but see Grantz et al. 2014), so net N_2 flux is an exceedingly rare measurement. Annual DNF rates are typically greater than Nfix rates in diverse lakes worldwide, with higher DNF rates typically measured in lakes with high total N concentrations

(Seitzinger 1988), and higher Nfix rates typically measured in lakes with low total N concentrations and, in some instances, low N:P (Howarth et al. 1988; Bunting et al. 2010). Although N-fixing (diazotrophic) cyanobacteria can dominate seasonally in some lakes and provide new N when external N inputs are low (e.g., Beversdorf et al. 2013; Baker et al. 2018), eutrophic lakes have higher DNF rates than less productive lakes due to high organic matter availability and anoxic conditions in sediments and/or stratified water columns (Finlay et al. 2013). Relating net N_2 flux to the stoichiometric balance of N and P may be useful for understanding the causes of N- or P-limitation, or N + P colimitation (i.e., balanced growth), of primary production in lakes (Paerl et al. 2016).

If we assume that primary production has a stoichiometric optimum (but see Klausmeier et al. 2004), then the imbalance of N relative to P (N_I) for primary production can be quantified as either a concentration or flux:

$$N_I = N_x - (P_x * N : P_{opt}) \qquad (2)$$

where N_x is the concentration or flux of total N in or to a lake, P_x is the concentration or flux of total P in or to a lake, and $N:P_{opt}$ is the stoichiometric optimum of the N:P ratio for primary production. Regardless of whether the N_I for primary production is computed as a concentration or flux, negative N_I values indicate a N deficit relative to P, and positive N_I values indicate a N surplus relative to P. Considering the relationship between N_I and net N_2 flux, most lakes are likely either P-limited and losing total N due to a positive net N_2 flux (i.e., N_2 efflux because DNF > Nfix; quadrant I, Fig. 1A) or are N-limited but gaining total N due to a negative net N_2 flux (i.e., N_2 influx because DNF < Nfix; quadrant III, Fig. 1A). Focusing on watershed inputs of total N and P explicitly, N_I can have units identical to net N_2 flux (g N m^{-2} lake surface area yr^{-1}). Using this approach, lakes
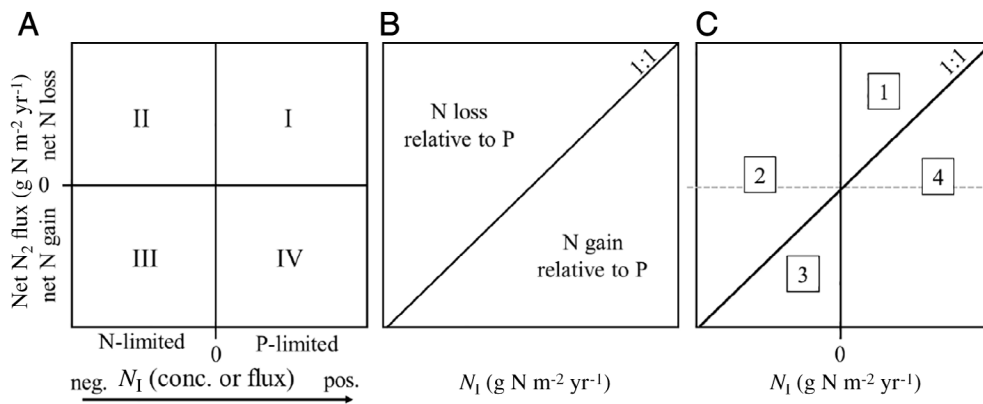
Fig. 1. The relationship between N_I and net N_2 flux into and out of the total N pool in lakes: (**A**) quadrats represent lakes with a N surplus losing reactive N to atmosphere (I), lakes with a N deficit losing N to atmosphere (II), lakes with a N deficit gaining N from atmosphere (III), and lakes with a N surplus gaining N from atmosphere (IV), (**B**) lakes falling above 1:1 line are experiencing N loss relative to P due to net N_2 flux exceeding N_I and lakes falling below the 1:1 line are gaining N relative to P due to N_I exceeding net N_2 flux, and (**C**) four categories of lakes emerge by combining (**A**) and (**B**): (1) lakes with a N surplus that are losing N relative to P, (2) lakes with a N deficit that are losing N relative to P, (3) lakes with a N deficit that are gaining N relative to P, and (4) lakes with a N surplus that are gaining N relative to P.

with N_I > net N_2 flux are gaining N relative to P annually, while lakes with N_I < net N_2 flux are losing N relative to P (Fig. 1B).

By combining these two classification approaches (Fig. 1A, B), the two dimensional space can be divided into four categories of lakes (Fig. 1C) based on their N_I and net N_2 flux: (1) lakes with a N surplus from external sources and losing N relative to P; (2) lakes with a N deficit from external sources and losing N relative to P; (3) lakes with a N deficit from external sources and gaining N relative to P; and (4) lakes with a N surplus from external sources and gaining N relative to P. The combined approach is important because many lakes may have a negative net N_2 flux, but still not gain N at a rate equivalent to the N_I from external sources (and vice versa).

We used this simple stoichiometric model with continental-scale data from 1964 USA lakes (USEPA 2009, 2016) to explore the potential variability in N_I and net N_2 flux across lakes of differing trophic states. The probabilistic nature of this data set was useful in understanding patterns for lakes in diverse landscapes across the USA (Yuan and Pollard 2018). We developed and calibrated the model using data from a subset of lakes, with a global distribution, for which external N and P input data, as well as DNF and/or Nfix measurements, were available. The overall goal of the study was to explore the role of net N_2 flux in influencing the long-term, gradual accumulation of fixed N in lakes, which theoretically causes the "evolution" of P limitation (Schindler 1977).

Methods

The Vollenweider (1968) model was used to derive external P and N input (P_s and N_s, respectively) estimates from measurements of total P and N concentrations (P_c and N_c, respectively) for the 33 lakes that had measured values for nutrient concentrations and loads (hereafter, "measured lakes"; Supporting Information Table S1), and for 1964 lakes from the 2007 and 2012 US EPA National Lakes Assessment database that had measured data only for nutrient concentrations (hereafter, "modeled lakes"; Supporting Information Table S2). Briefly, the Vollenweider equation used here required the hydraulic residence time (τ_w) and annual water loading rate (q_s) for each lake. The τ_w was unknown for all lakes but was expected to vary between 0.1 and 10 yr. The q_s was also unknown for all lakes but is a linear transformation of τ_w and the mean depth ($\bar{z}$) of a lake. The $\bar{z}$ for the 33 measured lakes was known (Supporting Information Table S1), but the $\bar{z}$ for the modeled lakes was unknown. However, the maximum depth (z_{max}) of the modeled lakes was known (Supporting Information Table S2), and several studies have shown a direct relationship between z_{max} and $\bar{z}$ for lakes at landscape scales. We used the empirical relationship reported by Chow-Fraser (1991) to estimate $\bar{z}$ from z_{max} for each of the modeled lakes.

We conducted a two-step calibration and sensitivity analysis to identify the best choice for τ_w for all lakes and to determine the effect of this choice and the prediction of $\bar{z}$ from z_{max} in modeled lakes. That analysis revealed that a τ_w of 1 yr minimized the root mean square error (RMSE) of prediction for both P_s and N_s in measured lakes and resulted in the most accurate prediction of external nutrient loads. Further, modeled $\bar{z}$ estimates had a minor effect on the variation of P_s and N_s estimates when compared to the variation in nutrient concentrations and ratios in the measured data for both measured and modeled lakes. The supplemental material provides a full description of model development, calibration, and sensitivity analysis in deriving P_s and N_s estimates for all lakes.

The N_I for each lake was computed assuming an $N:P_{opt}$ of 23 (by mass), which is the reported N:P threshold for strictly P-limited lakes in a global analysis of N and P limitation of phytoplankton (Guildford and Hecky 2000). We conservatively used the upper range of colimitation (N:P = 23 by mass), rather than the lower range (N:P = 9), for strict N limitation, because we assumed that even moderate N deficiency experienced by N + P colimited phytoplankton (N:P between 9 and 23) could result in Nfix, which could supplement the N pool and increase the N:P within a year. The annual Nfix for each lake was assumed to be equivalent to the rate of N deficit (negative N_I) multiplied by an efficiency factor (EF). We assumed that EF, which is the proportion of N_I that could be fixed annually, could vary from 1% to 100% and tested this assumption using measured and modeled Nfix rates for measured lakes (Supporting Information Table S1). Calibration analysis revealed that the RMSE of Nfix predictions decreased with decreasing EF, and we chose a 10% EF as the optimal value for the full analysis (*see* Supporting Information for details). The annual DNF for each lake was estimated as a proportion of N_s based on Seitzinger et al. (2006). The efficacy of this model was also tested with measured and predicted values from the measured lakes (Supporting Information Table S1). A complete description of the full modeling approach, as well as a description of the calibration, sensitivity analysis, and model optimization decisions are presented in the Supporting Information.

Results

P_c and N_c concentrations in measured and modeled lakes each varied by 4–5 orders of magnitude. As shown previously (Downing and McCauley 1992), increasing N_c were not stoichiometrically balanced with increasing P_c (Fig. 2A). The variability in nutrient concentrations was proportional to the variability in N_s and P_s for both the measured and modeled lakes (Table 1). Furthermore, the statistical distributions of $\bar{z}$, P_c, N_c, P_s, N_s, N_I, Nfix, DNF, and net N_2 flux were similar across the measured and modeled lake data sets (Table 1). Model development and calibration analysis (Supporting Information) demonstrated that assumptions used to derive N_I and net N_2 flux for modeled lakes were valid, and that the resulting model estimates reflect reasonable estimates of these biogeochemical fluxes across the continental USA.
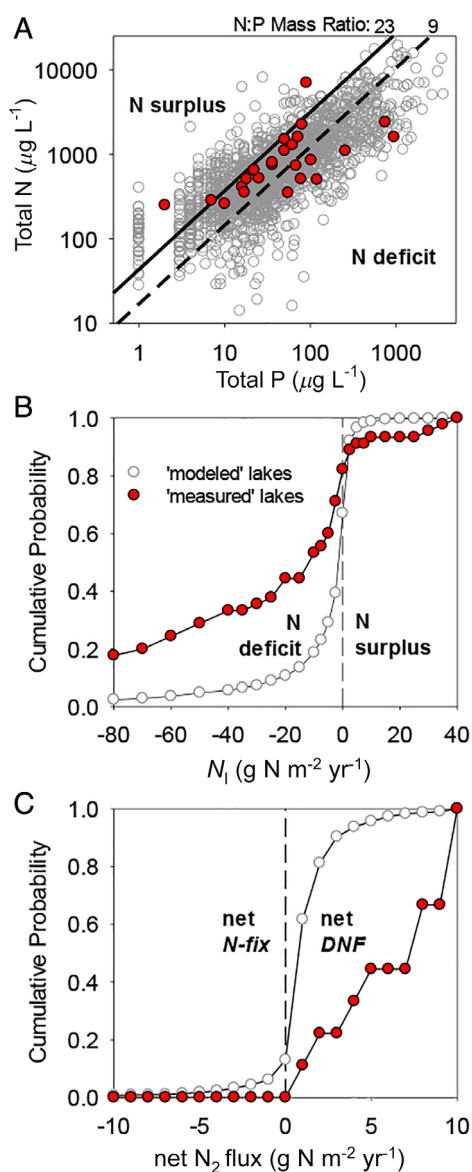
Fig. 2. (**A**) Relationship between total P and total N for measured (red circles) and modeled lakes (gray circles). The reference lines in (**A**) represent the boundaries for strict N limitation (N:P < 9 by mass) and strict P limitation (N:P > 23 by mass) as described by Guildford and Hecky (2000), (**B**) the cumulative probability distribution of N_I for measured and modeled lakes, and (**C**) the cumulative probability distribution of net N_2 flux for measured and modeled lakes.

The N_I for most lakes ranged from −80 to 40 g N m^{-2} yr^{-1} (Fig. 2B). The distribution of N_I across all lakes was skewed toward a N deficit (Table 1), but this skewness was more apparent for measured lakes than modeled lakes (Fig. 2B). The net N_2 flux for most lakes ranged from −3 to 8 g N m^{-2} yr^{-1} (Fig. 2C). The distribution of net N_2 flux across all lakes was strongly skewed toward a positive net N_2 flux (i.e., N_2 efflux because DNF > Nfix; Table 1), with zero measured lakes, and less than 20% of modeled lakes, exhibiting a negative net N_2 flux (i.e., N_2 influx because DNF < Nfix; Fig. 2C).

Sixty percent of the most oligotrophic lakes had some degree of N deficit, but the proportion of oligotrophic lakes (chlorophyll *a* [Chl *a*] < 3.5 *μ*g L^{-1}) with a N deficit decreased as Chl *a* increased from 1 to 3 *μ*g L^{-1} (Fig. 3). Thus, oligotrophic lakes approaching the oligotrophic-mesotrophic boundary were evenly distributed between N deficit and N surplus. This pattern reversed as Chl *a* increased from 3 to 1000 *μ*g L^{-1}, and the cumulative proportion of lakes with a N deficit increased from 50% to almost 70% (Fig. 3). According to the model output, and using trophic categories identified by Smith et al. (1999), 51% of oligotrophic lakes (Chl *a* < 3.5 *μ*g L^{-1}), 60% of mesotrophic lakes (Chl *a* = 3.5–9 *μ*g L^{-1}), 72% of eutrophic lakes (Chl *a* = 9–25 *μ*g L^{-1}), and 89% of hypereutrophic lakes (Chl *a* > 25 *μ*g L^{-1}) were experiencing a N deficit. The average N deficit observed in lakes with a negative N_I (14.6 ± 38.8 g N m^{-2} yr^{-1}) was approximately six times greater than the average N surplus observed in lakes with a positive N_I (2.4 ± 7.1 g N m^{-2} yr^{-1}).

Surprisingly, the distribution of lakes in the four categories of N_I vs. net N_2 flux were skewed toward category 2 lakes, which had a N deficit from external sources and were losing N relative to P (Fig. 4A). The 1314 category 2 lakes had, on average, the highest total P and Chl *a* concentrations (Fig. 4B). By contrast, the 371 category 4 lakes had a N surplus from external sources, were gaining N relative to P (Fig. 4A), and had the lowest average total P and Chl *a* concentrations (Fig. 4B). Another 279 lakes in category 1 (Fig. 4A) had a N surplus from external sources and were losing N relative to P, with moderate total P and Chl *a* concentrations. No lakes fell into category 3 because this condition was mathematically impossible; Nfix rates would have to exceed the N deficit for a lake, which is impossible because Nfix will not occur when reactive N is available from other sources (Baker et al. 2018).
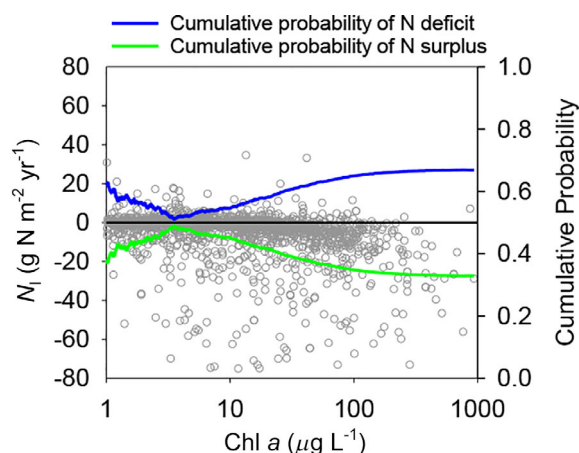
Although very few lakes worldwide had sufficient data to graph measured N_I vs. net N_2 flux (Fig. 4A), all of these lakes fell into category 2, and none had a negative net N_2 flux. However, some lakes had estimates of N_I and either Nfix or DNF rates (Supporting Information Table S1). DNF rates were strongly proportional to N_s (Fig. 4C) and showed a strong fit with the Seitzinger et al. (2006) model. Similarly, Nfix rates were strongly proportional to the ratio of N_s:P_s (Fig. 4D), especially when N:P of nutrient loads was less than the threshold of 23 for strict P limitation (Guildford and Hecky 2000).

Discussion

The results of this analysis indicate that biological N transformations, particularly in more productive lakes, may perpetuate N deficient conditions for primary production. This result suggests the potential need for a paradigm shift in limnology. Although some oligotrophic lakes may accumulate N as described by Schindler (1977, 2012), eutrophic lakes may exhibit perpetual N limitation (Scott and Grantz 2013; Grantz et al. 2014; Paerl et al. 2016) caused by biogeochemical transformations that convert biologically

Table 1. Summary statistics and distributions of mean depth ($\bar{z}$), nutrient concentrations, external nutrient loading estimates (P_s, N_s), N imbalance (N_I), N fixation (Nfix), and denitrification (DNF) rates from measured (Supporting Information Table S1) and modeled (Supporting Information Table S2) lakes.

| Source/statistic	$\bar{z}$ (m)	Total P (mg L^{-1})	Total N (mg L^{-1})	P_s (g P m^{-2} yr^{-1})	N_s (g N m^{-2} yr^{-1})	N_I (g N m^{-2} yr^{-1})	Nfix (g N m^{-2} yr^{-1})	DNF (g N m^{-2} yr^{-1})	Net N$_2$ flux (g N m^{-2} yr^{-1})
Measured data—Supporting Information Table S1 ($n = 33$)									
Minimum	0.1	0.002	0.05	0.03	0.05	−600	0.00	0.05	0.05
25th percentile	4.5	0.020	0.50	0.61	7.90	−56	0.02	6.6	3.6
Median	5.9	0.050	0.73	2.40	23.0	−11	0.63	18	7.3
Mean	25	0.105	1.13	4.13	44.0	−51	2.7	18	7.0
75th percentile	9.8	0.085	1.29	4.30	80.0	−2.0	3.0	24	11
Maximum	290	0.950	7.00	27.0	300	190	18	47	14
Modeled data—EPA/NLA 2007, Supporting Information Table S2 ($n = 841$)									
Minimum	0.3	0.001	0.01	< 0.01	0.09	−510	0.00	0.03	−32
25th percentile	1.2	0.009	0.28	0.06	1.66	−4.7	0.00	0.50	0.31
Median	2.3	0.025	0.57	0.17	3.65	−0.2	0.02	1.1	0.73
Mean	3.8	0.126	1.37	0.64	6.67	−8.1	0.89	2.0	1.1
75th percentile	4.9	0.110	1.24	0.55	7.94	0.8	0.47	2.4	1.7
Maximum	22	2.67	26.1	25.2	95.3	31	51	29	24
Modeled data—EPA/NLA 2012, Supporting Information Table S2 ($n = 1123$)									
Minimum	0.4	0.004	0.01	0.01	0.06	−650	0.00	0.02	−57
25th percentile	1.1	0.021	0.33	0.12	1.82	−7.3	0.01	0.55	0.18
Median	1.8	0.043	0.65	0.27	3.78	−2.0	0.20	1.1	0.63
Mean	3.0	0.121	1.18	0.70	6.36	−9.8	1.1	3.9	0.86
75th percentile	3.5	0.107	1.29	0.61	7.46	−0.1	0.73	2.2	1.5
Maximum	22	3.64	54.0	29.5	164	160	65	49	49

Fig. 3. Relationship between Chl *a* concentrations and the N_I for the modeled lakes. The cumulative probability of a N deficit (negative N_I) or N surplus (positive N_I) are also shown as a function of the lake Chl *a* concentration.

reactive N forms to relatively unreactive N_2 gas. Oligotrophic lakes may "evolve" P limitation due to three conditions that increase the N:P ratio: (1) nutrient inputs from pristine landscapes have a

relatively high N:P compared to the demands of phytoplankton (Downing and McCauley 1992; Bunting et al. 2010); (2) Nfix adds reactive N seasonally when N deficiencies occur (Scott et al. 2008; Higgins et al. 2017); and (3) DNF does not remove adequate N to induce N limitation due to organic matter limitation and/or lack of anoxic conditions (Finlay et al. 2013).

Our study suggests that these conditions may reverse as lakes become increasingly eutrophic (Paerl et al. 2016). The net N_2 flux may rarely, if ever, support N accumulation relative to P in productive lakes. Insufficient N accumulation relative to P even occurs in lakes with extremely low N_I, where Nfix can represent a significant contribution to the annual N budget. Rather than accumulating fixed N, as many as 80% of USA lakes (categories 1 and 2; Fig. 4A,B) may experience N losses relative to P due to biogeochemical N transformations. Both measured and modeled data support the idea that most lakes experience a positive net N_2 flux (i.e., N_2 efflux because DNF > Nfix; Fig. 2C), even when external inputs are imbalanced toward N deficiency. Although the large proportion of lakes falling into category 2 lakes was surprising, these results highlight the importance of DNF as a keystone ecosystem process and support the explanation that DNF is responsible for the long-term decline of total N in Lake 227 (Scott and McCarthy 2010; Higgins et al. 2017). Our results
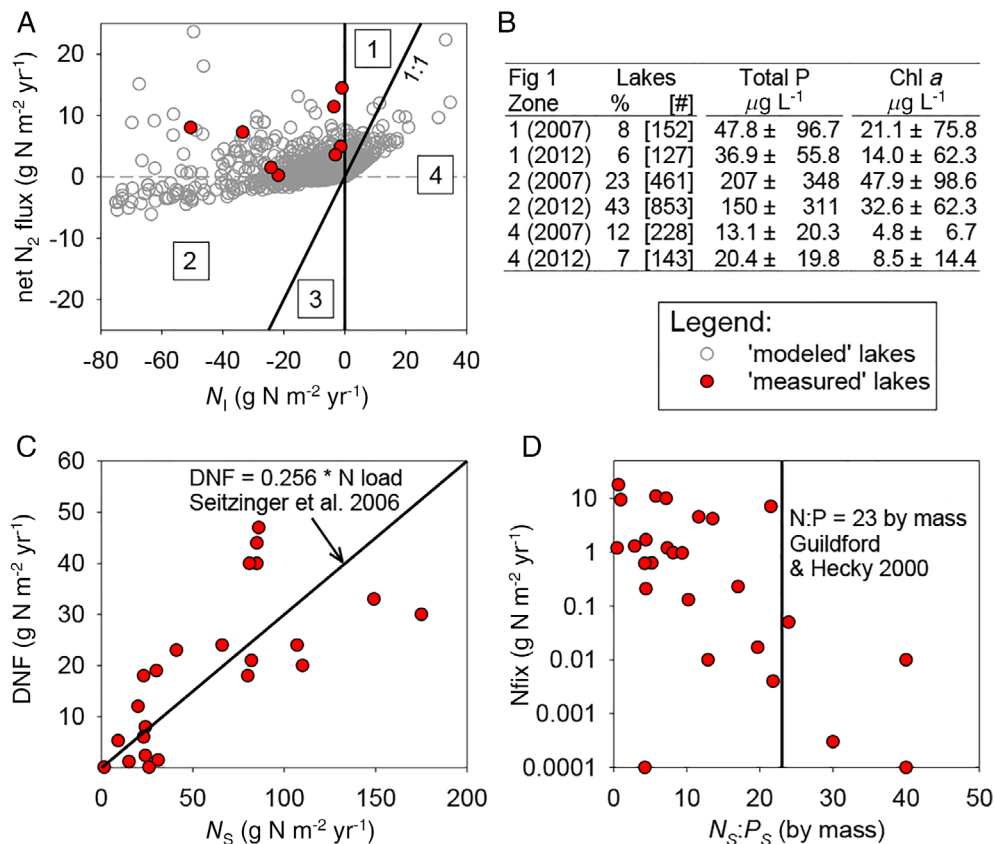
Fig. 4. (**A**) The relationship between N_I and net N_2 flux for measured and modeled lakes, (**B**) the distribution of lakes across the categories proposed in Fig. 1C, and the total P and Chl *a* concentrations of the modeled lakes in each category, (**C**) the relationship between N_s and DNF for measured lakes, and (**D**) the relationship between the N:P ratio of external inputs and the Nfix for measured lakes.

also provide a biological mechanism for explaining why P accumulates faster than N in lakes experiencing accelerated eutrophication (Yan et al. 2016).

The most accurate calibration of the model in this study used an EF assumption of 10% Nfix efficiency. The relatively low EF could have been caused by assuming $N{:}P_{opt}$ = 23, a global threshold separating strict P limitation and N + P colimitation (Guildford and Hecky 2000). Using that threshold, rather than the threshold for strict N limitation (N:P = 9 by mass), could have over-estimated potential Nfix, which therefore required a lower EF for accurate calibration. The N:P from measured lakes was also more skewed toward N deficiency than modeled lakes (Fig. 2B), indicating that studies focused on Nfix were more likely conducted in lakes with a N deficit.

However, the low efficiency of Nfix observed here can also be explained by ecological constraints other than P availability, such as water column turbulence (Moisander et al. 2002), oxygen supersaturation (Paerl 1990, 2018), light (Scott and Grantz 2013), micronutrient availability (Glass et al. 2010), and/or zooplankton grazing (Hambright et al. 2007). Nfix is an energy intensive process requiring a lengthy genetic cascade, which culminates in heterocyst differentiation and nitrogenase production; indeed, Nfix in cyanobacteria is initiated only when cells detect severe N deficiency (Thiel and Pratte 2014). The biomass yield of phytoplankton relying heavily on Nfix is reduced compared to those growing on combined N sources (Baker et al. 2018). These constraints can also explain the relatively low Nfix efficiency observed in this study and magnify the reality that Nfix may only rarely compensate for N deficiency on annual scales (Scott et al. 2008; Higgins et al. 2017). However, more research is needed to understand the relative importance of each of these constraints and how they may be manifested in lakes across diverse landscapes.

If biological N transformations contribute to perpetual N limitation in productive lakes, then this finding has important implications for our understanding of lake ecology and eutrophication management. Annually recurring N limitation can promote large, often colonial, heterocystous cyanobacteria (Levine and Schindler 1999), which have decreased palatability for zooplankton grazers (DeMott et al. 2001; Ghadouani et al. 2003), resulting in decreased efficiency in the trophic transfer of energy and nutrients. However, many lakes also exhibit annually recurring N limitation but remain dominated by non-N-fixing cyanobacterial taxa (e.g., Paerl et al. 2011; Chaffin et al. 2013; McCarthy et al. 2013), which also have decreased palatability and energy transfer efficiency through the ecosystem (Vanderploeg et al. 2001). Our results support previous analyses (e.g., Paerl et al. 2016) showing that high external N inputs, particularly in lakes with moderate to high P inputs, will support higher biomass production than lakes relying on N fixation (Paerl et al. 2016).

The N:P of some anthropogenic nutrient sources, such as human and animal waste, can be very low relative to demands for primary production (Downing and McCauley 1992). However, the N:P of nonpoint source loading from row-crop agricultural watersheds can be quite high relative to demands for primary production (e.g., Stow et al. 2015), and both N and P reductions are needed to control the harmful effects of accelerated eutrophication in these ecosystems (e.g., Paerl et al. 2016). Our study focused on fluxes based on total N and P concentrations, without considering differences in the reactivity and ecological importance of different chemical forms of N and P, which have important consequences for primary production, phytoplankton community structure, and cyanobacteria toxicity (McCarthy et al. 2009; Donald et al. 2011; Davis et al. 2015; Glibert and Burford 2017; Schoffelen et al. 2018). More research is needed to understand the dynamic relationship between dominant chemical forms, internal recycling mechanisms, and nutrient stoichiometry in lakes. Because P does not have a major gaseous component, legacy P accumulated in lakes may fuel elevated production even after excess P inputs are reduced (Song and Burgin 2017). Likewise, legacy N accumulated in lakes and watersheds may also inhibit recovery from eutrophication in aquatic systems (Duff et al. 2009; Van Meter et al. 2018). Our study shows that reducing N inputs, in addition to P inputs, will likely yield a faster decrease in primary production and phytoplankton biomass because DNF counteracts the accumulation of legacy N. Denitrification efficiency, however, decreases at high external N loading rates (e.g., Mulholland et al. 2008; Gardner and McCarthy 2009), further illustrating the need to reduce external N loads to aquatic systems.

References

Baker, B. C., A. E. Wilson, and J. T. Scott. 2018. Phytoplankton N₂ fixation efficiency and its effect on harmful algal blooms. Freshw. Sci. **37**: 264–275. doi:10.1086/697530

Beversdorf, L. J., T. R. Miller, and K. D. McMahon. 2013. The role of nitrogen fixation in cyanobacterial bloom toxicity in a temperate, eutrophic lake. PLoS One **8**: e56103. doi:10.1371/journal.pone.0056103

Bunting, L., P. R. Leavitt, R. P. Weidman, and R. D. Vinebrookeb. 2010. Regulation of the nitrogen biogeochemistry of mountain lakes by subsidies of terrestrial dissolved organic matter and the implications for climate studies. Limnol. Oceanogr. **55**: 333–345. doi:10.4319/lo.2010.55.1.0333

Chaffin, J. D., T. B. Bridgeman, and D. L. Bade. 2013. Nitrogen constrains the growth of late summer cyanobacterial blooms in Lake Erie. Adv. Microbiol. **3**: 16–26. doi:10.4236/aim.2013.36A003

Chow-Fraser, P. 1991. Use of the morphoedaphic index to predict nutrient status and algal biomass in some Canadian lakes. Can. J. Fish. Aquat. Sci. **48**: 1909–1918. doi:10.1139/f91-227

Davis, T. W., G. S. Bullerjahn, T. Tuttle, R. M. McKay, and S. B. Watson. 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during *Planktothrix* blooms in Sandusky Bay, Lake Erie. Environ. Sci. Technol. **49**: 7197–7207. doi:10.1021/acs.est.5b00799

DeMott, W. R., R. D. Gulati, and E. Van Donk. 2001. Daphnia food limitation in three hypereutrophic Dutch lakes: Evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. Limnol. Oceanogr. **46**: 2054–2060. doi:10.4319/lo.2001.46.8.2054

Donald, D. B., M. J. Bogard, K. Finlay, and P. R. Leavitt. 2011. Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. Limnol. Oceanogr. **56**: 2161–2175. doi:10.4319/lo.2011.56.6.2161

Downing, J. A., and E. McCauley. 1992. The nitrogen:phosphorus relationship in lakes. Limnol. Oceanogr. **37**: 936–945. doi:10.4319/lo.1992.37.5.0936

Duff, J. H., K. D. Carpenter, D. T. Snyder, K. K. Lee, R. J. Avanzino, and F. J. Triska. 2009. Phosphorus and nitrogen legacy in a restoration wetland, Upper Klamath Lake, Oregon. Wetlands **29**: 735–746. doi:10.1672/08-129.1

Finlay, J. C., G. E. Small, and R. W. Sterner. 2013. Human influences on nitrogen removal in lakes. Science **342**: 247–250. doi:10.1126/science.1242575

Gardner, W. S., and M. J. McCarthy. 2009. Nitrogen dynamics at the sediment–water interface in shallow, subtropical Florida Bay: Why denitrification efficiency may decrease with increased eutrophication. Biogeochemistry **95**: 185–198. doi:10.1007/s10533-009-9329-5

Ghadouani, A., B. Pinel-Alloul, and E. E. Prepas. 2003. Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. Freshw. Biol. **48**: 363–381. doi:10.1046/j.1365-2427.2003.01010.x

Glass, J. B., F. Wolfe-Simon, J. J. Elser, and A. D. Anbar. 2010. Molybdenum-nitrogen co-limitation in freshwater and coastal heterocystous cyanobacteria. Limnol. Oceanogr. **55**: 667–676. doi:10.4319/lo.2010.55.2.0667

Glibert, P. M., and M. A. Burford. 2017. Globally changing nutrient loads and harmful algal blooms: Recent advances, new paradigms, and continuing challenges. Oceanography **30**: 58–69. doi:10.5670/oceanog.2017.110

Grantz, E. M., B. E. Haggard, and J. T. Scott. 2014. Stoichiometric imbalance in rates of nitrogen and phosphorus retention, storage, and recycling can perpetuate nitrogen deficiency in highly-productive reservoirs. Limnol. Oceanogr. **59**: 2203–2216. doi:10.4319/lo.2014.59.6.2203

Guildford, S. J., and R. E. Hecky. 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? Limnol. Oceanogr. **45**: 1213–1223. doi:10.4319/lo.2000.45.6.1213

Hambright, K. D., N. G. Hairston, W. R. Schaffner, and R. W. Howarth. 2007. Grazer control of nitrogen fixation: Phytoplankton taxonomic composition and ecosystem functioning. Fundam. Appl. Limnol. **170**: 103–124. doi:10.1127/1863-9135/2007/0170-0103

Higgins, S. N., M. J. Paterson, R. E. Hecky, D. W. Schindler, J. J. Venkiteswaran, and D. L. Findlay. 2017. Biological nitrogen fixation prevents the response of a eutrophic lake to reduced loading of nitrogen: Evidence from a 46-year whole-lake experiment. Ecosystems. **21**: 1088–1100. doi:10.1007/s10021-017-0204-2

Howarth, R. W., R. Marino, J. Lane, and J. J. Cole. 1988. Nitrogen fixation in freshwater, estuarine, and marine systems: Biogeochemical controls. Limnol. Oceanogr. **33**: 688–701. doi:10.4319/lo.1988.33.4part2.0688

Klausmeier, C. A., E. Litchman, T. Daufresne, and S. A. Levin. 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature **429**: 171–174. doi:10.1038/nature02454

Levine, S. N., and D. W. Schindler. 1999. Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria and phytoplankton species composition in the Experimental Lakes Area, Canada. Can. J. Fish. Aquat. Sci. **54**: 451–466. doi:10.1139/f98-183

McCarthy, M. J., R. T. James, Y. Chen, T. L. East, and W. S. Gardner. 2009. Nutrient ratios and phytoplankton community structure in the large, shallow, eutrophic, subtropical lakes Okeechobee (Florida, USA) and Taihu (China). Limnology **10**: 215–227. doi:10.1007/s10201-009-0277-5

McCarthy, M. J., W. S. Gardner, M. F. Lehmann, and D. F. Bird. 2013. Implications of water column ammonium uptake and regeneration for the nitrogen budget in temperate, eutrophic Missisquoi Bay, Lake Champlain (Canada/USA). Hydrobiologia **718**: 173–188. doi:10.1007/s10750-013-1614-6

Moisander, P., J. L. Hench, K. Kononen, and H. W. Paerl. 2002. Small-scale shear effects on heterocystous cyanobacteria. Limnol. Oceanogr. **47**: 108–119. doi:10.4319/lo.2002.47.1.0108

Mulholland, P. J., and others. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nat. Lett. **452**: 202–206. doi:10.1038/nature06686

Paerl, H. W. 1990. Physiological ecology and regulation of N_2 fixation in natural waters. *In* K. C. Marshall [ed.], *Advances in microbial ecology*, v. **11**: 305–344. Plenum Publishing Corporation.

Paerl, H. W. 2018. Why does N-limitation persist in the world's marine waters? Mar. Chem. **206**: 1–6. doi:10.1016/j.marchem.2018.09.001

Paerl, H. W., H. Xu, M. J. McCarthy, G. Zhu, B. Qin, Y. Li, and W. S. Gardner. 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N & P) management strategy. Water Res. **45**: 1973–1983. doi:10.1016/j.watres.2010.09.018

Paerl, H. W., and others. 2016. It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. Environ. Sci. Technol. **50**: 10805–10813. doi:10.1021/acs.est.6b02575

Redfield, A. C. 1958. The biological control of chemical factors in the environment. Am. Sci. **46**: 205–221.

Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. Science **46**: 260–262. doi:10.1126/science.195.4275.260

Schindler, D. W. 2012. The dilemma of controlling cultural eutrophication of lakes. Proc. Biol. Sci. **279**: 4322–4333. doi:10.1098/rspb.2012.1032

Schoffelen, N. J., W. Mohr, T. G. Ferdelman, S. Littman, J. Duerschlag, M. V. Zubkov, H. Ploug, and M. M. M. Kuypers. 2018. Single-cell imaging of phosphorus uptake shows that key harmful algae rely on different phosphorus sources for growth. Nat. Sci. Rep. **8**: 17182. doi:10.1038/s41598-018-35310-w

Scott, J. T., R. D. Doyle, S. J. Prochnow, and J. D. White. 2008. Are watershed and lacustrine controls on planktonic N_2 fixation hierarchically structured? Ecol. Appl. **18**: 805–819. doi:10.1890/07-0105.1

Scott, J. T., and M. J. McCarthy. 2010. Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. Limnol. Oceanogr. **55**: 1265–1270. doi:10.4319/lo.2010.55.3.1265

Scott, J. T., and E. M. Grantz. 2013. N_2 fixation exceeds internal nitrogen loading as a phytoplankton nutrient source in perpetually nitrogen-limited reservoirs. Freshw. Sci. **32**: 849–861. doi:10.1899/12-190.1

Seitzinger, S., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, C. Tobias, and G. Van Drecht. 2006. Denitrification across landscapes and waterscapes: A synthesis. Ecol. Appl. **16**: 2064–2090. doi:10.1890/1051-0761(2006)016[2064:DALAWA]2.0.CO;2

Seitzinger, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. Limnol. Oceanogr. **33**: 702–724. doi:10.4319/lo.1988.33.4part2.0702

Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: Impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ. Pollut. **100**: 179–196. doi:10.1016/S0269-7491(99)00091-3

Song, K., and A. J. Burgin. 2017. Perpetual phosphorus cycling: Eutrophication amplifies biological control on internal phosphorus loading in agricultural reservoirs. Ecosystems **20**: 1483–1493. doi:10.1007/s10021-017-0126-z

Stow, C. A., Y. K. Cha, L. T. Johnson, R. Confesor, and R. P. Richards. 2015. Long-term and seasonal trend decomposition of Maumee River nutrient inputs to western Lake Erie. Environ. Sci. Technol. **49**: 3392–3400. doi:10.1021/es5062648

Thiel, T., and B. S. Pratte. 2014. Regulation of three nitrogenase gene clusters in the cyanobacterium *Anabaena variabilis* ATCC 29413. Life **4**: 944–967. doi:10.3390/life4040944

Tyrell, T. 1999. The relative influences of nitrogen and phosphorus on oceanic primary production. Nature **400**: 525–531. doi:10.1038/22941

USEPA. 2009. National lakes assessment 2007: A collaborative survey of the nation's lakes. EPA 841-R-09-001. US Environmental Protection Agency.

USEPA. 2016. National lakes assessment 2012: A collaborative survey of lakes in the United States. EPA 841-R-16-113. US Environmental Protection Agency.

Van Meter, K. J., P. Van Capellen, and N. B. Basu. 2018. Legacy nitrogen may prevent achievement of water quality goals in the Gulf of Mexico. Science **360**: 427–430. doi:10.1126/science.aar4462

Vanderploeg, H. A., J. R. Liebig, W. W. Carmichael, M. A. Agy, T. H. Johengen, G. L. Fahnenstiel, and T. F. Nalepa. 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. Can. J. Fish. Aquat. Sci. **58**: 1208–1221. doi:10.1139/f01-066

Vitousek, P. M., S. Porder, B. Z. Houlton, and O. A. Chadwick. 2010. Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen-phosphorus interactions. Ecol. Appl. **20**: 5–15. doi:10.1890/08-0127.1

Vollenweider, R. A. 1968. Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. Technical report. DAS/CSI/68.27. OECD.

Yan, Z., W. Han, J. Penuelas, J. Sardans, J. J. Elser, E. Du, P. B. Reich, and J. Fang. 2016. Phosphorus accumulates faster than nitrogen globally in freshwater ecosystems under anthropogenic impacts. Ecol. Lett. **19**: 1237–1246. doi:10.1111/ele.12658

Yuan, L. L., and A. I. Pollard. 2018. Changes in the relationship between zooplankton and phytoplankton biomasses across a eutrophication gradient. Limnol. Oceanogr. **63**: 2493–2507. doi:10.1002/lno.10955