

# Stoichiometric imbalances complicate prediction of phytoplankton biomass in U.S. lakes: Implications for nutrient criteria

David L. Moon<sup>1</sup>,<sup>\*</sup> J. Thad Scott<sup>2</sup>,<sup>\*</sup> Tom R. Johnson<sup>1</sup>

<sup>1</sup>Water Quality Section, U.S. Environmental Protection Agency, Denver, Colorado

<sup>2</sup>Department of Biology and Center for Reservoir and Aquatic Systems Research, Baylor University, Waco, Texas

## Abstract

Using National Lakes Assessment data, we evaluated the influence of total N (TN), total P (TP), and other variables on lake chlorophyll-*a* concentrations. With simple linear regressions, high TN/TP samples biased predictions based on TN, and low TN/TP samples biased predictions based on TP. The bias problem was corrected, and correlation was improved, by splitting the dataset at the TN/TP ratio we estimated to be indicative of a balanced supply and developing separate regressions that predict chlorophyll-*a* based on TP, TN, dissolved inorganic N (DIN), dissolved organic carbon (DOC), nonalgal light attenuation, depth, area, latitude, elevation, and conductivity. Both nutrients were excellent predictors, and nonalgal light attenuation was the next most influential predictor. The regression analysis suggested that a potential for P only limitation (high TN/TP, 17% of samples) or N only limitation (low TN/TP, 14% of samples) can be inferred at the extremes of the TN/TP range. However, 69% of samples had an intermediate TN/TP ratio where it is difficult to infer anything about potential nutrient limitations (biomass could be N limited, P limited, N and P co-limited, or not limited by nutrients at all). Our results show that when developing phytoplankton response relationships using cross-lake datasets that span a wide range of trophic states, it is important to consider whether and how biomass is influenced by confounding factors—such as differences in the relative supply of N and P—so that biomass is not underestimated or overestimated, and nutrient criteria are not under-protective or over-protective.

To derive ambient water quality criteria for nutrients using empirical phytoplankton response relationships, state and tribal regulatory agencies in the U.S. are faced with the challenge of characterizing risks across a gradient of nutrient pollution. The goal is to appropriately minimize risks, consistent with the requirement to protect designated uses (U.S. Environmental Protection Agency or EPA 2017). In freshwater lakes, numerous factors may influence the relationship between nutrients and phytoplankton biomass (e.g., cyanobacteria, diatoms, green algae, dinoflagellates, etc.), particularly at large spatial scales (Wurtsbaugh et al. 2019). For example, the imbalance in phosphorus (P) and nitrogen (N) stoichiometry relative to phytoplankton demand often determines the limiting nutrient for lakes at continental scales, which can vary with trophic state (Scott et al. 2019). Thus, when P is strongly limiting (or any factor other than N), the yield of phytoplankton biomass per unit N will be less than expected based on the supply of N, and conversely when N is strongly limiting (or any factor other than P), the yield of biomass per unit of P

will be less than expected based on the supply of P (Carlson and Havens 2005).

A number of stoichiometric boundary conditions have been suggested to estimate whether phytoplankton biomass may be limited by P or N. For example, guidelines have been expressed as total nitrogen/total phosphorus (TN/TP) ratios (Forsberg and Ryding 1980; Guildford and Hecky 2000) and alternatively as DIN/TP ratios, where dissolved inorganic nitrogen or DIN is the sum of nitrate + nitrite and total ammonia (Morris and Lewis Jr. 1988; Bergström 2010). The rationale for using DIN rather than total dissolved N (TDN) is that TDN may include a large recalcitrant fraction of dissolved organic nitrogen (DON) that is less bioavailable (Lewis Jr. and Wurtsbaugh 2008). Conversely, dissolved inorganic forms of N and P are strongly bioavailable but often below reporting level concentrations during the warm growing season. Instead, the turnover rates of dissolved and particulate organic N and P influence the true availability of these nutrients, making total P and N useful indicators of stoichiometric imbalance (Dodds 2003).

Guildford and Hecky (2000) found that N-deficient growth was apparent at TN/TP mass ratios <9 (20 on a molar basis), P-deficient growth was apparent at TN/TP mass ratios greater than about 23 (50 on a molar basis), and at intermediate

\*Correspondence: moon.dave@epa.gov

Additional Supporting Information may be found in the online version of this article.

TN/TP ratios “either N or P can become deficient.” Stoichiometric boundaries such as these have often been treated as strict thresholds even though there is considerable variability in phytoplankton biomass per unit nutrient (i.e., chl-*a*:nutrient ratios) across a range of environmental conditions (Nürnberg 1996), and we understand that the diverse phytoplankton communities occurring in lakes also have diverse stoichiometric optima (Klausmeier et al. 2004). Forsberg and Ryding (1980) first recognized this issue and argued that stoichiometric indicators of potential nutrient limitations should also consider the lake trophic state that provides long-term selective pressure on phytoplankton species.

It is widely accepted that even though TN/TP ratios provide a means to assess the stoichiometric variability in lakes, they are imperfect indicators of potential nutrient limitations. For example, TN/TP ratios may provide a false indication when the ratio of the total concentrations does not represent the true difference in the bioavailable supply (Lewis Jr. and Wurtsbaugh 2008), when concentrations of N and P are both very high (Wetzel 1966), or when a nonnutrient factor (e.g., mineral turbidity, self-shading, time, etc.) limits phytoplankton growth (Bachmann 2001). Even considering these limitations, TN and TP concentrations can be useful if imperfect indicators of potential nutrient limitations and predictors of chlorophyll-*a* concentrations within and across lakes (Dolman et al. 2016).

Regression equations to predict phytoplankton biomass (often using chlorophyll-*a* as a surrogate) have been developed as functions of TP, TN, or both. In developing such equations, other growth limiting factors may need to be considered (Lewis Jr. and Wurtsbaugh 2008). In a study of German lakes, an approach was developed to predict chl-*a* based on TP for putatively P-limited samples, and based on TN for putatively N-limited samples (Dolman et al. 2016). In a study of U.S. reservoirs, the chl-*a* vs. TP relationship was shown to be strongly influenced by both nonalgal turbidity and N (Walker Jr. 1982).

Understanding how confounding factors affect nutrient-phytoplankton relationships at large spatial scales can inform ecosystem management decisions. Filstrup et al. (2014) demonstrated strong regional variability in the chl-*a* vs. total P relationship in lakes of the northeastern and midwestern U.S. Their study indicated that regional variation in pastureland use and percent wetland cover drastically changed the slope of the chl-*a*/TP relationship. Other studies have shown that the chl-*a* vs. nutrient (N or P) relationships in lakes can be influenced by nonalgal light attenuation (Dzialowski et al. 2005), zooplankton grazing (Mazumder and Havens 1998), salinity (Håkanson and Eklund 2010), alkalinity and/or lake depth (Spears et al. 2013), and even extreme total P and N concentrations in the water column (Filstrup and Downing 2017).

Quantile regression approaches have been used to characterize an upper limit on lake chl-*a* concentrations across TP and/or TN gradients (Abell et al. 2012). Brown et al. (2000)

used quantile regression to quantify the maximum chl-*a* response to TP observed in Florida lakes, and concluded that “the maximum curve describes P limitation when the CHL response falls on or near the line but indicates other limiting or co-limiting factors when the CHL response falls below the line.” They also noted that when predicting chl-*a* concentrations “other factors beyond nutrients need to be considered, particularly when applying population models to individual lakes.”

Here, we evaluate the chl-*a* vs. TP and chl-*a* vs. TN relationships as functions of stoichiometric variability in lakes of the contiguous U.S. using the 2007 and 2012 US EPA National Lakes Assessment data. Specific questions include: (1) what TN/TP ratio indicates a well-balanced nutrient supply (e.g., for use in sorting samples into putatively P-limited and putatively N-limited groups), (2) in addition to TN and TP, what other lake water quality variables have a significant influence on chlorophyll-*a* concentrations, and (3) is it reasonable to expect that the TN/TP indicative of a well-balanced supply will change with trophic state?

## Methods

### The 2007 and 2012 national lakes assessment surveys

The NLA is a collaborative project involving EPA, states, tribes, federal agencies, and other organizations, with a goal of producing condition estimates for lakes that are nationally and regionally representative. The stratified random design ensures that a range of conditions are sampled spanning a broad disturbance gradient. The authors did not conduct the surveys; we took the easier route of downloading the data from the U.S. EPA website for both the 2007 and 2012 surveys (U.S. EPA 2009, 2016).

NLA collaborators sampled a total of 1033 lakes in 2007 (July through October), and 1038 in 2012 (May through September). Approximately 400 of the lakes sampled in 2007 were re-visited in 2012. For each survey, approximately 10% of the sites were randomly selected and resampled during the same year. The timing of the second visit varied, but generally was within 6 weeks of the first sample. A stratified random design and an unequal probability selection process were used to allocate the number of lakes to be sampled in various categories. Lakes were categorized by size, by state, and by nine aggregated Omernik Level 3 ecoregions (Omernik 1987; U.S. EPA 2009). In addition, approximately 100 reference lakes (i.e., least disturbed and of good quality) were hand selected and sampled once during each survey (U.S. EPA 2009).

The National Hydrography Dataset was used to derive the list of lakes to sample for both survey years. Lakes were defined as natural and man-made freshwater lakes, ponds, and reservoirs >4 ha in 2007 and 1 ha in 2012. Lakes excluded were the Great Lakes, mine ponds, retention basins, cooling ponds, saline lakes, sewage ponds, and lakes <1 m in depth. See U.S. EPA (2009, 2016) for additional information,

e.g., how to access the data, field manuals, and laboratory protocols.

### The combined dataset

The combined dataset included 2482 near surface samples with results for TP and TN. This total included 2266 samples from randomly selected lakes (2071 from visit 1 and 195 from visit 2) and another 216 samples from hand-selected reference lakes. Duplicates were excluded but repeat visits to the same location on different dates were included. The sampled lakes spanned a wide spectrum of trophic states (Supporting information Table S1). Samples where certain parameters had an analytical result less than the reporting limit were included in the analysis and the concentration was estimated to be  $\frac{1}{2}$  the applicable reporting limit (Supporting information Table S2). Although nitrate + nitrite and ammonia were both measured, thereby allowing for estimation of dissolved inorganic nitrogen or DIN concentrations, dissolved forms of phosphorus (e.g., soluble reactive phosphorus) were not measured.

### Nonalgal light attenuation

Light attenuation in water is a function of both algal and nonalgal particles (or color). For this analysis, the nonalgal component of light attenuation was estimated with secchi depth observed/expected (*O/E*) ratios. The secchi depth vs. chl-*a* regression in Carlson 1977 ( $\ln \text{Secchi} = 2.04 - 0.68 \ln \text{Chl-}a$ ) was used as the denominator for each secchi depth *O/E* ratio (i.e., as the expected secchi depth). This line has been widely used to represent lakes where water transparency is dominated by phytoplankton. The premise is that when secchi depths deviate below Carlson's line, this indicates that nonalgal light attenuation is elevated. For example, at 10  $\mu\text{g/L}$  chl-*a*, Carlson's line predicts 1.6 m of secchi depth. Thus, if three different samples each have 10  $\mu\text{g/L}$  chl-*a* but secchi depths of 1.6, 0.8, and 0.4 m, the secchi *O/E* values would be 1.0, 0.5, and 0.25, indicating low, moderate, and high nonalgal light attenuation, respectively. Secchi *O/E* ratios can be translated to Carlson  $\text{TSI}_{\text{chl}} - \text{TSI}_{\text{secchi}}$  deviations with the equation  $\text{TSI}_{\text{chl}} - \text{TSI}_{\text{secchi}} = 14.416 \ln(\text{Secchi } O/E) + 0.0232$  ( $R^2 = 1$ ). Using this equation, secchi *O/E* values of 0.5 and 0.25 equate to TSI deviations of  $-10$  and  $-20$  TSI points, respectively.

### Comparing chl-*a*:TP and chl-*a*:TN yields

We used chl-*a*, TP, and TN concentrations as a basis for inferring, on a sample-by-sample basis, the potential phytoplankton-nutrient stoichiometric imbalance. Conceptually, the logic of our method is that P-deficient samples should have a greater yield of chl-*a* per unit P (since the supply of N is in excess), N-deficient samples should have a greater yield of chl-*a* per unit N (since the supply of P is in excess), and samples with a well-balanced nutrient supply should have similar yields of chl-*a* per unit of TP and TN.

One difficulty in comparing chl-*a*:nutrient yields in this way is that, like the cellular ratio in phytoplankton biomass, the TN concentration in a lake water sample typically is greater than its paired TP concentration (Downing and McCauley 1992). Across all samples, because the distributions of TN and TP concentrations do not occupy the same range, the corresponding chl-*a*:TP and chl-*a*:TN yields are not directly comparable. To work around this problem, we first calculated "high yield" chl-*a* vs. TP and chl-*a* vs. TN regression lines similar to Brown et al. (2000). To derive the high yield lines, all samples ( $n = 2472$ ) were ranked from low to high TP or TN and 200-sample moving 95th percentile chl-*a* and 50th percentile TP or TN concentrations were calculated. The paired percentile values were log-transformed and regressed to derive equations that estimate the 95th percentile chl-*a* as a function of 50th percentile TP or TN. We used 95th percentiles since the goal was to estimate a "high yield" benchmark chl-*a* concentration that could be observed in the absence of significant confounding influences (Jones et al. 2011). We used 50th percentile TP and TN concentrations to represent the mid-point for each subsampled group.

The high yield lines provide a simple framework for comparing chl-*a* yields on a sample-by-sample basis. The TP-based yield is expressed as the observed chl-*a* divided by the high yield of chl-*a* corresponding to the sample's TP ( $\text{fraction}_{\text{yield-TP}}$ ; Fig. 1a), and likewise the TN-based yield is expressed as the observed chl-*a* divided by the high yield of chl-*a* corresponding to the sample's TN ( $\text{fraction}_{\text{yield-TN}}$ ; Fig. 1b). This approach normalizes both values to the same scale and facilitates comparisons. A well-balanced nutrient supply is inferred when  $\text{fraction}_{\text{yield-TP}}$  and  $\text{fraction}_{\text{yield-TN}}$  values are the same, P-deficiency is inferred when  $\text{fraction}_{\text{yield-TP}}$  is substantially greater than  $\text{fraction}_{\text{yield-TN}}$ , and N-deficiency is inferred when  $\text{fraction}_{\text{yield-TN}}$  is substantially greater than  $\text{fraction}_{\text{yield-TP}}$  (Fig. 1c).

This provisional method of scaling chl-*a*:TP and chl-*a*:TN yields relative to the estimated 95th percentile chl-*a* concentrations is similar to the trophic state index (TSI) method described in Carlson (1977) for chl-*a*:TP response in selected temperate lakes, and in Kratzer and Brezonik (1981) for chl-*a*:TN response in selected Florida lakes. Comparison of TSI scores to infer potential nutrient limitations was described—and evaluated against National Eutrophication Study results—in Carlson and Havens (2005). In contrast to Carlson's method, key features of the provisional method used here include (1) chl-*a* concentrations are scaled relative to an estimated upper percentile of chl-*a* (as opposed to an expected mean), (2) nutrient and chl-*a* concentrations are evaluated together instead of independently, and (3) the same continental-scale dataset was used to develop both of the high yield chl-*a* regression lines.

### Inferring stoichiometric (im)balances from a tipping point N/P ratio

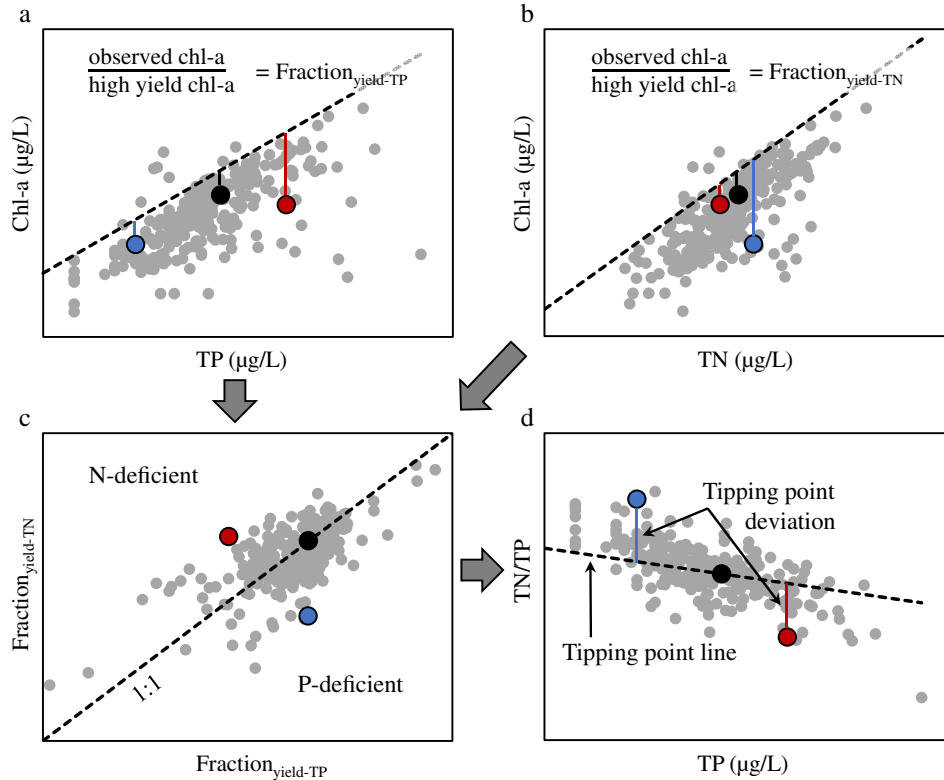
We estimated a "tipping point N/P ratio" to serve as a benchmark for identifying potential nutrient limitations. We

defined the tipping point N/P as the paired combinations of TP and TN where  $\text{fraction}_{\text{yield-TP}} = \text{fraction}_{\text{yield-TN}}$ . Thus, by definition the TN/TP ratio of a sample exactly equals the tipping point when the observed TP and TN concentrations correspond to the same 95th percentile high yield chlorophyll-*a* concentration. This assures identical fraction yields (e.g., 1:1 line in Fig. 1c). The utility of this method is that, by definition, any sample where the measured TN/TP ratio is greater than the tipping point N/P ratio will have a  $\text{fraction}_{\text{yield-TP}}$  greater than its  $\text{fraction}_{\text{yield-TN}}$  (putatively P-limited) and conversely any sample with a TN/TP ratio less than the tipping point N/P ratio will have a  $\text{fraction}_{\text{yield-TN}}$  greater than its  $\text{fraction}_{\text{yield-TP}}$  (putatively N-limited; Fig. 1d). Accordingly, we computed a “tipping point deviation” for each sample by dividing the measured TN/TP ratio by the corresponding tipping point N/P ratio so that values  $>1.0$  represent the magnitude of putative P limitation and values  $<1.0$  represent the magnitude of putative N limitation (Fig. 1d). For example, if the TN/TP ratio of a sample is  $\times 4$  the tipping point N/P, it would be a strong signal that P only limitation can be inferred, and if the TN/TP of a sample is  $\times 0.25$  the tipping point, it would be a strong signal that N only limitation can be inferred.

We emphasize that our method relies on a simplifying assumption that TP and TN concentrations adequately represent the true bioavailable nutrient supply. One problem with this assumption is that a portion of the total measurements—possibly a large portion—is not bioavailable. In such cases, TN/TP may provide a misleading indication about whether one, both, or neither nutrient could be a limiting factor. TN/TP may also be misleading if both concentrations are very high, or if a nonnutrient factor (e.g., light, time, or another element) limits phytoplankton biomass. These uncertainties apply to any recommendation/guideline based on a TN/TP ratio, and our estimated tipping point N/P lines are no exception. However, the continental scale of our analysis provides a probabilistic approach for capturing nutrient variability across a wide range of lake and reservoir conditions. At this scale, we simply assume that the relative turnover of N and P in lakes is likely proportional to the relative concentrations of TN and TP measured, and the relative instantaneous supply of N and P is captured by the TN/TP ratio.

#### Other methods

Chl-*a* regression models were developed using Microsoft Excel 2016 software. Predictor variables included TP, TN, DIN, DOC, secchi *O/E*, lake depth, lake area, latitude, elevation, and



**Fig. 1.** Conceptual summary of method. (a, b) Single samples and the 95th percentile chl-*a* line vs. TP and TN, respectively. (c)  $\text{Fraction}_{\text{yield-TN}}$  vs.  $\text{fraction}_{\text{yield-TP}}$ , with a 1:1 line overlaid. (d) TN:TP vs. TP, with the “tipping point” line overlaid. In each panel, one sample where a P-deficient, N-deficient, or intermediate nutrient supply can be inferred are shown as solid blue, red, and black dots, respectively.

conductivity. Consistent with Isles (2020), all variables except latitude and elevation were log transformed prior to being added to the regression. Likewise, for variables that were log-normally distributed (e.g., chl-*a*, TP, and TN), geometric means or medians were used instead of arithmetic averages to represent central tendency.

We also examined how various chemical (DIN/TN and DIN/TP), physical (lake depth, nonalgal light attenuation), and biological (phytoplankton community composition) lake characteristics change with increasing TP to establish a factual basis for assessing whether it is reasonable to expect the tipping point N:P ratio to change with trophic state.

## Results

### High yield and fractional yield chl-*a*

Strong positive relationships were found for the high yield chlorophyll-*a* lines by regressing moving (200 sample) 95th percentile chl-*a* against moving median TP and TN (Fig. 2) as follows:

$$(\text{Chl}95\text{th})_{\log} = 0.9647 * (\text{TP}50\text{th})_{\log} + 0.03627 \quad (R^2 = 0.978, n = 2,273) \quad (1)$$

$$(\text{Chl}95\text{th})_{\log} = 1.195 * (\text{TN}50\text{th})_{\log} - 1.758 \quad (R^2 = 0.993, n = 2,273) \quad (2)$$

The calculated 95th percentile high yield chl-*a* concentrations ranged from approximately 5 µg/L for relatively oligotrophic lakes with low nutrient concentrations to 400 µg/L for relatively hypereutrophic lakes with extremely high nutrient concentrations. We extrapolated Eqs. (1) and (2) to estimate high yield chl-*a* across the entire TP and TN range (i.e., two different high yield chl-*a* concentrations were calculated for each sample). The fraction<sub>yield-TP</sub> and fraction<sub>yield-TN</sub> values covered a wide range spanning four orders of magnitude. Using the higher of the two fraction<sub>yield</sub> values for each sample, 35% of samples had a fraction yield > 0.5, 33% of

samples had a fraction yield ranging from 0.25 to 0.5%, and 32% of samples had a fraction yield < 0.25. This distribution suggests that nutrient bioavailability and other site factors often result in chl-*a* concentrations substantially less than what could be observed based on Eqs. 1 and 2. Across the entire dataset, the median fraction<sub>yield-TP</sub> (0.3) was somewhat greater than the median fraction<sub>yield-TN</sub> (0.25).

### Tipping point N/P and tipping point deviations

The “tipping point N/P ratio” (Supporting information Fig. S1) declined with rising TP as follows:

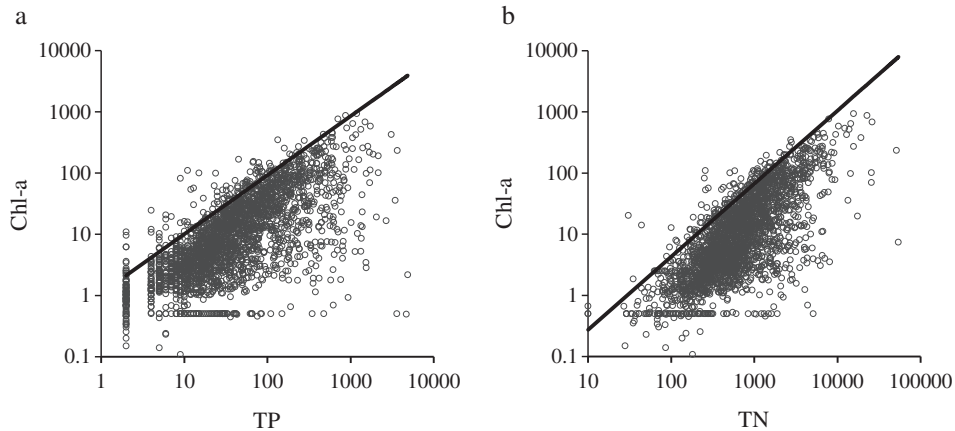
$$\text{Tipping point N/P}_{\log} = 1.5015 - 0.1927 * \text{TP}_{\log}, \quad (3)$$

or

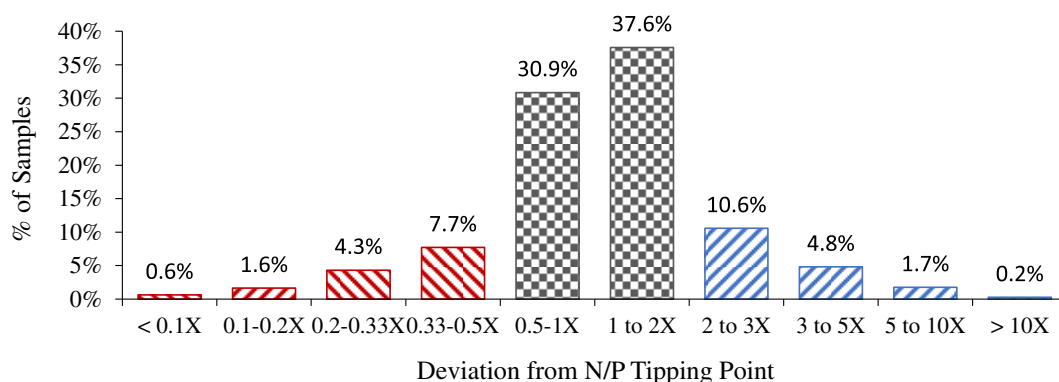
$$\text{Tipping point TN}_{\log} = 0.8073 * \text{TP}_{\log} + 1.5015 \quad (4)$$

“Tipping point deviations” (the measured TN/TP ratio divided by the tipping point N/P) indicated that a two-thirds majority of samples (69%) had a well-balanced intermediate TN/TP ratio where the deviation ranged from  $\times 0.5$  to  $\times 2$  (Fig. 3). This is consistent with meta-analyses showing that addition of both N and P often yields the greatest increase in phytoplankton biomass (Elser et al. 2007; Paerl et al. 2016). However, for the many samples with TN/TP in the intermediate range, phytoplankton biomass could be N limited, P limited, N and P co-limited, or not limited by nutrients at all (Guildford and Hecky 2000), and making inferences about potential nutrient limitations for these samples is difficult. Of the remaining samples, 17% had a measured TN/TP substantially above the tipping point (deviation  $> \times 2$ ), and 14% had a measured TN/TP substantially below the tipping point (deviation  $< \times 0.5$ ), suggesting that a strong stoichiometric imbalance can be inferred.

Figure 4 shows that samples with intermediate range TN/TP ratios ( $\times 0.5 < \text{deviation} < \times 2$ ) were dominant at the TP and



**Fig. 2.** Grabs and high yield (95%) chl-*a* as a function of (a) TP and (b) TN ( $n = 2472$ ).



**Fig. 3.** Distribution of samples based on deviation from the N/P tipping point.

TN concentrations that may be of primary interest for nutrient criteria and target setting (depending, of course, on what trophic state is selected as the target). Samples where P only limitation can be inferred (deviation  $> \times 2$ ) were more common at low TP and high TN, while samples where N only limitation can be inferred (deviation  $< \times 0.5$ ) were more common at high TP and low TN. Ecoregional differences in TN/TP ratios also were readily apparent (Supporting information Table S3 and Fig. S2).

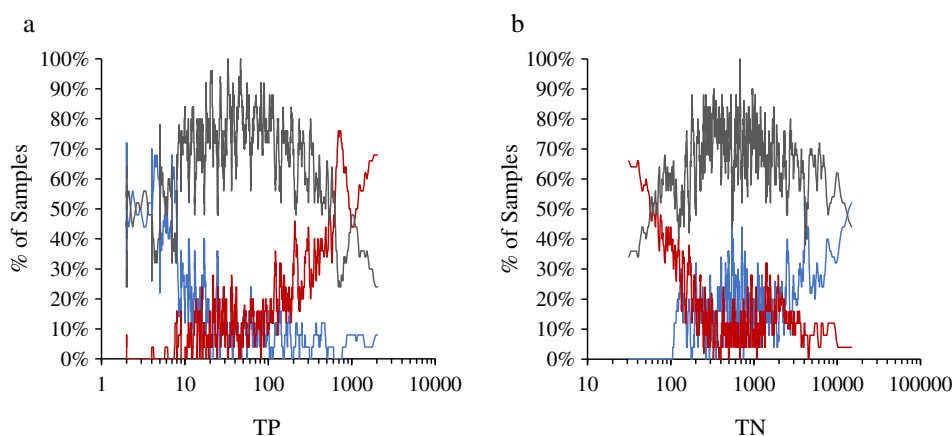
### Chl-*a* regression equations

A simple chl-*a* vs. TP regression overestimated chl-*a* concentrations for samples with low TN/TP ( $R^2 = 0.48$ , Supporting information Fig. S3a), while a simple chl-*a* vs. TN regression overestimated chl-*a* for samples with high TN/TP ( $R^2 = 0.53$ , Supporting information Fig. S3b). To illustrate the TN/TP bias problem, Fig. 5 shows the predicted/observed (P/O) ratios for individual samples as a function of the tipping point deviation. The P/O ratios were simply the quotient of the predicted and measured chl-*a* for any value of TP or TN. This comparison shows that prediction accuracy progressively deteriorates, with either predictor variable, as measured

TN/TP departs from the tipping point. The problem is especially evident at the extremes (outside a deviation between  $\times 0.5$  and  $\times 2$ ). Interestingly, chl-*a* was overestimated by the TP model when the tipping point deviation was  $< \times 0.5$  (i.e., putatively N-limited samples) and by the TN model when the tipping point deviation was  $> \times 2$  (i.e., putatively P-limited samples).

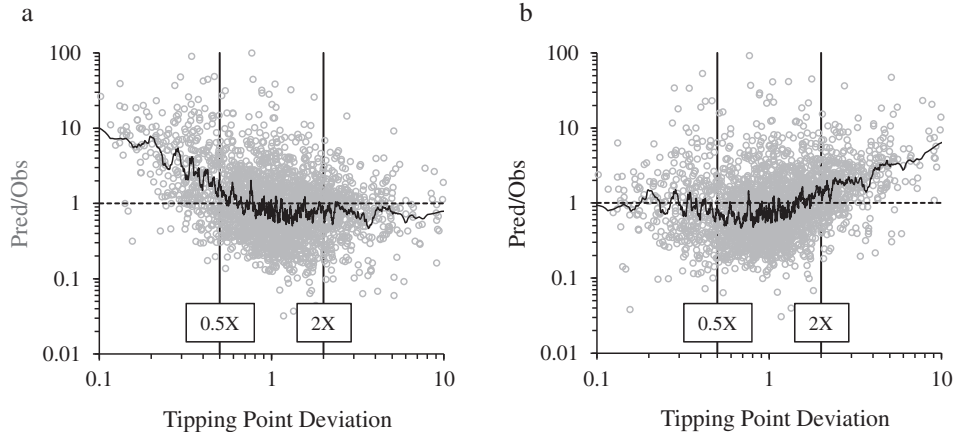
It is also worth noting that when considered from the opposite perspective, both models underestimated chl-*a* for many samples. For example, the TP-based model underestimated mean chl-*a* across the high TN/TP range (where P is more likely the limiting factor), while the TN-based model underestimated mean chl-*a* across much of the low TN/TP range (where N is more likely the limiting factor). Underestimation is problematic because nutrient criteria that are based on such predictions will not be adequately protective of the chl-*a* goal.

The TN/TP bias problem was partially resolved by splitting the dataset ( $n = 2472$ ) along the tipping point line and predicting chl-*a* with simple regression equations for (1) samples with TN/TP above the tipping point (TP was the predictor variable) and (2) samples with TN/TP below the tipping point



**Fig. 4.** % of samples with tipping point deviation  $< \times 0.5$  (red),  $\times 0.5$  to  $\times 2$  (grey), and  $> \times 2$  (blue) vs. (a) TP and (b) TN (25 sample moving counts,  $n = 2482$ ).





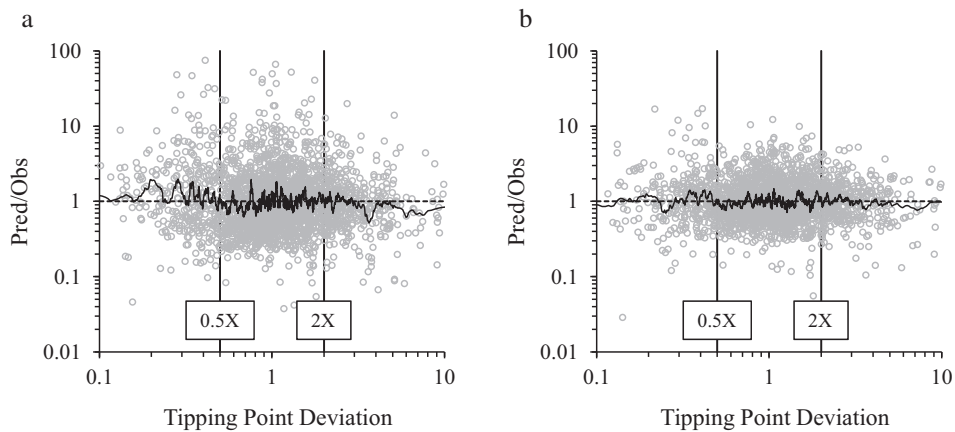
**Fig. 5.** Chl-*a* predicted/observed (P/O) ratios vs. tipping point deviation for grabs and the 25-sample moving geomean for (a) chl-*a* vs. TP and (b) chl-*a* vs. TN.

(TN was the predictor variable). The two-component modeling approach improved overall correlation ( $R^2 = 0.61$ , Fig. S3c) and diminished the bias problem, e.g., across the intermediate TN/TP range that is of heightened interest for purposes of nutrient criteria derivation (compare Fig. 6a to either panel of Fig. 5).

Refinement of the two-component model was achieved by including additional variables, including various predictors expected to be influential based on the scientific literature (Table 1). For example, TN was added to the “above tipping point” component and TP was added to the “below tipping point” component. In addition, DIN (the sum of ammonia and nitrate + nitrite), secchi *O/E* (as a surrogate for nonalgal light attenuation), lake depth, lake area, latitude and elevation (as surrogates for water temperature), and conductivity were added to both components. DOC was included only in the “below tipping point” component (it was not a significant predictor with the other component). Potentially important

confounding factors not represented in the two-component multiple linear regression (MLR) include community composition (e.g., % cyanobacteria), algal self-shading, and zooplankton grazing (though latitude and elevation may be useful surrogates for zooplankton grazing because of the linkage between water temperature and fish population diversity, as discussed in Moss et al. 2011).

With the two-component MLR that included 10 predictor variables (Table 1 and Supporting information Fig. S3d), overall correlation was maximized ( $R^2 = 0.81$ ) and P/O ratios, on average, showed improved alignment with the desired 1:1 ratio when plotted against tipping point deviation (Fig. 6b). All of the included predictor variables were highly significant ( $p < 0.01$ ). In the “above tipping point” component, TP was the stronger predictor based on *t* scores, but TN was also highly significant. In the “below tipping point” component, TN was the stronger predictor based on *t* scores, but TP was also highly significant. Aside from the TP and TN terms, and



**Fig. 6.** Chl-*a* predicted/observed (P/O) ratios vs. tipping point deviation for grabs and the 25-sample moving geomean for (a) the 2-component model and (b) the 2-component MLR.

**Table 1.** Summary statistics and coefficients for the multiple linear regression equations.  $y = \text{LOG Chl-}a$ ,  $p < 0.01$  for all variables.

Summary statistic/ variable	“Above tipping” component tipping point deviation > 1	“Below tipping” component tipping point deviation < 1	“Intermediate” TN/TP tipping point deviation $\times 0.5$ to $\times 2$
Observations	1235	999	1539
Adjusted $R$ square	0.81	0.79	0.82
Standard error	0.279	0.291	0.273
LOG TP	0.5751	0.1788	0.4803
LOG TN	0.4709	1.009	0.7218
LOG DIN	−0.1312	−0.1775	−0.1808
LOG DOC	N/A	−0.3353	−0.2352
LOG Secchi $O/E$	0.8630	0.6411	0.8180
LOG depth	−0.2264	−0.3988	−0.2932
LOG area	0.07184	0.07610	0.06368
Latitude	−0.01621	−0.01933	−0.01505
Elevation	−1.138E-04	−9.348E-05	−9.95E-05
LOG conductivity	−0.1089	−0.1379	−0.1196
Intercept	0.1031	−0.1594	−0.1747

for both components, the secchi  $O/E$  term was the next best predictor based on  $t$  scores. For example, a two-component model that included only TP and secchi  $O/E$  in the “above tipping” component and only TN and secchi  $O/E$  in the “below tipping” component (Supporting information Fig. S4) had an overall  $R^2$  of 0.72, i.e., addition of the secchi  $O/E$  term substantially improved accuracy, regardless of whether the primary independent variable was TP or TN.

Table 1 also includes a third multiple linear regression that borrows certain observations from each of the other two groups. It is specific to samples with intermediate range TN/TP ratios (tipping point deviations ranging from  $\times 0.5$  to  $\times 2$ ). For these samples, the same predictor variables were highly significant, and the  $\text{TP}_{\log}$  and  $\text{TN}_{\log}$  terms had very similar  $t$  scores, indicating that TP and TN exert a strong influence on chl- $a$ .

### Changes in trophic state

Various chemical, physical, and biological lake characteristics changed with trophic state along the TP gradient. With regard to lake water chemistry, the percentage of TN attributable to DIN declined with rising TP (Fig. 7a,  $n = 2481$ ). In addition, although DIN increased with TP, the slope of a log-log regression was  $< 0.5$ , which means that DIN/TP ratios also declined sharply with rising TP (Fig. 7b,  $n = 2481$ ). That many samples had ammonia and/or nitrate + nitrite concentration results less than the reporting limit is a source of uncertainty in both of these trends.

Turning to physical characteristics, as TP increased, lake depth decreased steadily, on average (Fig. 7c,  $n = 2380$ ) and there was an increasing likelihood of secchi  $O/E$  ratios indicating moderate/high levels of nonalgal light attenuation (Fig. 7d,  $n = 2329$ ). That TP levels in U.S. lakes would decrease as lake depth increases (and vice versa) is consistent with

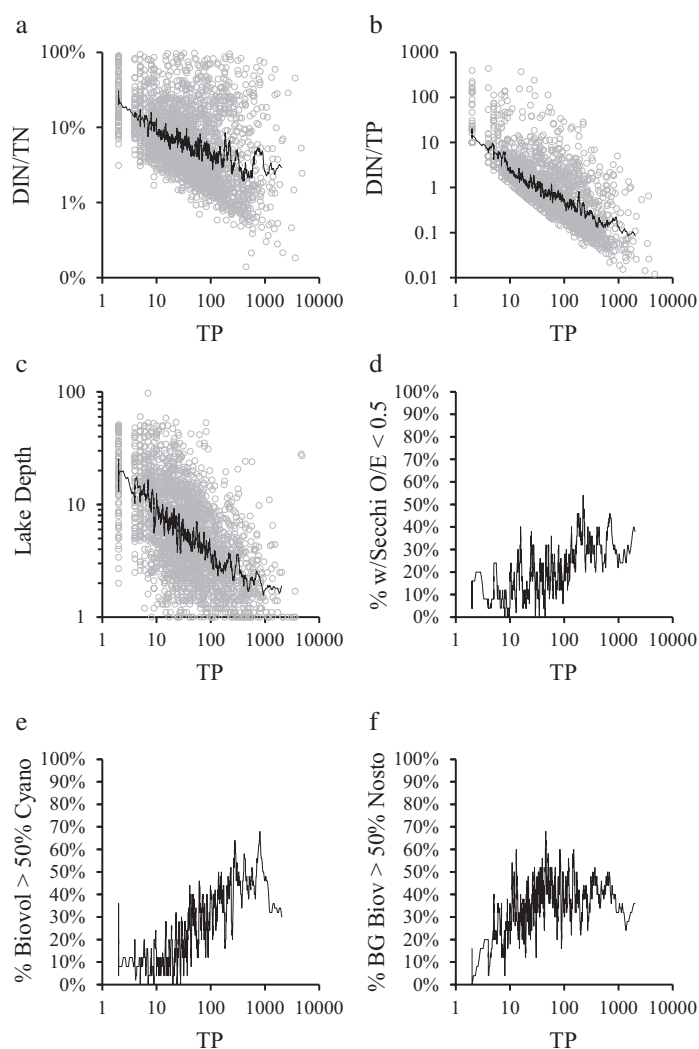
previous state-specific findings (Cross and Jacobson 2013, Minnesota lakes).

With regard to biological characteristics, as TP increased, there was an increasing likelihood of total biovolume being dominated by cyanobacteria (Fig. 7e,  $n = 2482$ ). By contrast, when looking only at samples where cyanobacteria were observed, the likelihood of total cyanobacteria biovolume being dominated by N-fixing Nostocales increased across the low TP range, but declined somewhat across the high TP range (Fig. 7f,  $n = 2417$ ).

### Discussion

Our study indicated that stoichiometric imbalances, e.g., P is in extremely low supply relative to N, or N is in extremely low supply relative to P, can control phytoplankton biomass in lakes. Our estimated tipping point N/P declined from about 23–28 (by mass) for the lowest TP lakes to about 6–10 (by mass) for the highest TP lakes, similar to the stoichiometric boundaries identified in Guildford and Hecky (2000). In evaluating whether this result is reasonable, it is important to consider that typical ambient lake water TN/TP ratios are higher than cellular N:P ratios, and that N and P bioavailability and phytoplankton community composition will vary across any large cross-lake dataset. Changes in community composition are important because of differences in species-specific N:P optima (Klausmeier et al. 2004). Thus the tipping point N/P might be expected to drop with trophic state if the phytoplankton community as a whole shifts from species with higher optimal N:P to those with lower optimal N:P in response to the relative long-term availability of these nutrients. Nutrient-rich eutrophic lakes likely have lower N:P ratios partly because the increased organic matter and the expanded volume of reduced conditions favor reactive N removal





**Fig. 7.** Changes across the TP gradient for: (a) DIN/TN, (b) DIN/TP, (c) depth (meters), (d) % of samples with secchi  $O/E < 0.5$ , (e) % of samples with biovolume > 50% cyanobacteria, and (f) % of samples with cyanobacteria biovolume > 50% Nostocales. Panels (a–c) show grabs and moving (25 sample) geomeans. Panels (d–f) use 25 samples for each moving probability calculation.

through denitrification (Finlay et al. 2013) that is not effectively compensated by ecosystem-scale N fixation (Scott and McCarthy 2010; Scott et al. 2019).

Interestingly, a two-thirds majority of samples had an intermediate TN/TP ratio similar to the estimated tipping point N/P (i.e., within a factor of  $\times 2$ , Fig. 3), which suggests that typically, N or P, both, or neither could be limiting (Paerl et al. 2016). From a management perspective, this is an important uncertainty and suggests a dual control strategy (i.e., limits on N and P) may be worth considering in many situations, particularly where the attainable condition is expected to include moderate (or higher) TP concentrations. That both TN and TP are strong predictors of phytoplankton

biomass, and of similar predictive value across the stoichiometrically balanced intermediate TN/TP range (Table 1) likewise suggests that to effectively control harmful algal bloom risks, a dual control approach would help to manage primary production, prevent excessive eutrophication, and better protect downstream waters (Paerl et al. 2016, 2019). Consider that high TN/TP samples where P only limitation can be inferred (i.e.,  $> \times 2$  the tipping point) were dominant only at the lowest TP concentrations (Fig. 4).

With regard to actually deriving the nutrient criteria, it is important to recognize that strongly N-limited conditions will result in reduced phytoplankton response to TP, and strongly P-limited conditions will result in reduced phytoplankton response to TN. Although the NLA did not definitively establish whether lake phytoplankton biomass was nutrient limited on the day of sampling, Fig. 5 suggests that P only and N only limitation may have an increased likelihood of occurrence at the extremes of the TN/TP spectrum (with the important caveat that single nutrient limitation can only be inferred, but is not assured). Accordingly, where nutrient criteria are to be developed based on phytoplankton biomass response relationships, it may be prudent to take steps such that the N and P criteria are defensible, protective, and unbiased by the confounding influence of the other nutrient.

Our results also indicate that nonnutrient variables may need to be accounted for and understood. For example, failure to incorporate the influence of nonalgal light attenuation would lead to overestimation of chl-*a* when lake conditions include heavy mineral turbidity, and underestimation when lake transparency is dominated by phytoplankton (Supporting information Fig. S4). Generally, the complexity of the problem suggests that to derive nutrient criteria that are protective of designated uses (e.g., aquatic life, recreation, and water supply), water quality requirements should be developed that will adequately minimize bloom risks during periods when lake conditions are especially favorable for growth (e.g., warm temperatures, less mineral turbidity, etc.), and include appropriate caveats or adjustments for situations where confounding factors preclude or suppress growth. Lake classification can be an important step toward addressing this need, so that appropriate nutrient criteria can be established for groups of lakes with similar phytoplankton response relationships. However, it may be necessary to account for the influence of confounding factors across the population of lakes assigned to each lake classification. Also, as with any criteria derivation problem, the need for scientific certainty must be balanced against the obligation to protect and restore water quality and beneficial uses.

The observed decline in DIN/TN as TP rises (Fig. 7a) may be a result of increased production, more complete DIN uptake, increased denitrification, and more frequent N limitation (Downing and McCauley 1992). The trend may be one reason why various studies have found that DIN/TP ratios predict nutrient limitations more accurately than TN/TP ratios (Morris

and Lewis Jr. 1988; Bergström 2010). It is also important to consider that TN and DIN concentrations will rise along with TP concentrations, and so at high TP—when indicator N:P ratios are low—there could be a surplus of both nutrients and an increased potential for nonnutrient factors to limit phytoplankton biomass (Klausmeier et al. 2004).

That DIN/TN declines as TP rises begs a question about whether the more bioavailable forms of P, as a percentage of TP, also change with trophic state. Although the NLA did not measure dissolved P (only total P), OECD's worldwide study (1982) reported that the percentage of TP that was dissolved orthophosphate increased with trophic state. This may have been driven by multiple factors, including differences in the sources of phosphorus (e.g., wastewater discharges, runoff from agricultural fields), internal loading and recycling of dissolved phosphorus to the water column, and more frequent nitrogen limitation in high TP lakes (Downing and McCauley 1992). As TP rises, if the bioavailable N decreases while the bioavailable P increases (as a percentage of TN and TP, respectively) this would indicate an increasing chance that N-deficient conditions will be observed. It would also suggest that nutrient limitation predictions based on TN/TP ratios should consider the trophic state of the lake water, as recommended in Forsberg and Ryding (1980).

Regarding physical habitat, geometric mean lake depth decreases from 16 m at 2  $\mu\text{g/L}$  TP to just 1.6 m at 1000  $\mu\text{g/L}$  TP (Fig. 7c). Although not shown, the trend is similar when natural and man-made lakes are evaluated separately. In combination with factors such as surface water temperature, the decrease in lake depth as TP rises is likely to be associated with shifts in water column stability/mixing and community composition (Huisman et al. 2004). Similarly, geometric mean secchi *O/E* ratio is reduced from 0.93 at 2  $\mu\text{g/L}$  TP to 0.63 at 1000  $\mu\text{g/L}$  TP, and there is an increasing frequency of samples where moderate/high levels of non-algal light attenuation is indicated (Fig. 7d). At high TP, reduced illumination of the water column may affect phytoplankton community composition (Havens et al. 2003) and the potential for nitrogen fixation by Nostocales (e.g., *Anabaena*, *Aphanizomenon*) since the fixation process can be limited by the supply of light within the water column (Lewis Jr. and Levine 1984).

At high TP there is also an increase in the frequency of samples where biovolume is dominated by cyanobacteria (Fig. 7e). This is consistent with Downing et al. (2001). Accordingly, it may be that the nutritional requirements of the algal community as a whole change with trophic state, as the portion of total biovolume contributed by cyanobacteria increases, and this influences the TN/TP ratio that is indicative of a well-balanced nutrient supply. This possibility is consistent with the concept that nutritional requirements and optimal N:P ratios vary by species (Smith 1982) such that at any given time, the limitation status for individual species may

differ from the limitation status for the community as a whole (Kolzau et al. 2014).

Also noteworthy is that across the high TP range, where low TN/TP conditions are more likely to be observed (Fig. 4a), there is a decreasing risk that cyanobacteria biovolume will be dominated by N-fixing Nostocales (Fig. 7). This indicates that community composition is influenced by numerous water quality and physical habitat factors, and that cyanobacteria not capable of N-fixation (e.g., *Microcystis*, *Oscillatoria*) may be dominant at the highest TP concentrations. This is consistent with Dolman et al. (2012) which found that, for example, the nostocalean fixer *Aphanizomenon gracile* often reached its highest biovolumes in lakes with abundant nitrogen (relative to phosphorus).

Finally, with regard to the use of lake water TN/TP ratios to identify potential nutrient limitations based on inferred stoichiometric imbalances, it should be considered that in a large cross-lake dataset, various lake characteristics may change across the TP gradient. For example, as TP rises across the NLA dataset, lakes are more likely to be shallow (Fig. 7c), turbid (Fig. 7d), dominated by cyanobacteria (Fig. 7e)—but not necessarily by N-fixers (Fig. 7f)—and (at least putatively) N-limited (Fig. 4a). These chemical, physical, and biological changes suggest that the ambient lake water TN/TP ratio “tipping point” may also change with increasing TP enrichment. Because only chl-*a*, TP, and TN measurements were used to develop the provisional tipping point lines, it would be instructive to evaluate how Eqs. (3) and (4) compare to the results of nutrient enrichment bioassays across a range of conditions to build a stronger basis for evaluating whether, on average, the ambient lake water TN/TP ratio “tipping point” changes with trophic state.

## Conclusion

Nutrient pollution can degrade lake water quality and impair recreation, water supply, and aquatic life uses. For the vast majority of U.S. lakes, water quality standards to protect against such impairments have not yet been established. Efforts to address this need may include compilation of cross-lake datasets and calculation of protective water quality criteria. One of the recommended approaches relies on derivation of empirical phytoplankton-nutrient response relationships (U.S. EPA 2010). It is important that such efforts consider whether and how phytoplankton biomass is influenced by confounding factors including—but not limited to—the potential for imbalances in lake water P and N stoichiometry relative to phytoplankton demand. Understanding the role of confounding factors will help explain why response to TP and TN varies within individual lakes, between lakes, and across nutrient ecoregions, and improve the accuracy of predictive tools so that biomass is neither overestimated nor underestimated, and nutrient criteria are neither over-protective nor under-protective.

## Author Contributions

David L. Moon contributed to data compilation, data analysis, and the drafting of the manuscript. J. Thad Scott contributed to the organization and drafting of the manuscript. Tom R. Johnson contributed to data compilation and the drafting of the manuscript.

## References

- Abell, J. M., D. Özkundakci, D. P. Hamilton, and J. R. Jones. 2012. Latitudinal variation in nutrient stoichiometry and chlorophyll-nutrient relationships in lakes: A global study. *Fund. Appl. Limnol.* **181**: 1–14.
- Bachmann, R. W. 2001. The limiting factor concept. What stops growth? *Lakeline* **21**: 26–28.
- Bergström, A.-K. 2010. The use of TN:TP and DIN:TP ratios as indicators for phytoplankton nutrient limitation in oligotrophic lakes affected by N deposition. *Aquat. Sci.* **72**: 277–281.
- Brown, C. D., M. V. Hoyer, R. W. Bachmann, and D. E. Canfield Jr. 2000. Nutrient-chlorophyll relationships: An evaluation of empirical nutrient-chlorophyll models using Florida and north-temperate lake data. *Can. J. Fish. Aquat. Sci.* **57**: 1574–1583.
- Carlson, R. E. 1977. A trophic state index for lakes. *Limnol. Oceanogr.* **22**: 361–369.
- Carlson, R. E., and K. E. Havens. 2005. Simple graphical methods for the interpretation of relationships between trophic state variables. *Lake Reserv. Manag.* **21**: 107–118.
- Cross, T. K., and P. C. Jacobson. 2013. Landscape factors influencing lake phosphorus concentrations across Minnesota. *Lake Reserv. Manag.* **29**: 1–12.
- Dodds, W. K. 2003. Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. *J N Am Benthol Soc* **22**: 171–181.
- Dolman, A. M., J. Rucker, F. R. Pick, J. Fastner, T. Rohrlack, U. Mischke, and C. Wiedner. 2012. Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. *PLoS One* **7**: e38757.
- Dolman, A. M., U. Mischke, and C. Wiedner. 2016. Lake-type-specific seasonal patterns of nutrient limitation in German lakes, with target nitrogen and phosphorus concentrations for good ecological status. *Freshw. Biol.* **61**: 444–456.
- Downing, J. A., and E. McCauley. 1992. The nitrogen:phosphorus relationship in lakes. *Limnol. Oceanogr.* **37**: 936–943.
- Downing, J. A., S. B. Watson, and E. McCauley. 2001. Predicting cyanobacteria dominance in lakes. *Can. J. Fish. Aquat. Sci.* **58**: 1905–1908.
- Dzialowski, A. R., S.-H. Wang, N.-C. Lim, W. W. Spotts, and D. G. Huggins. 2005. Nutrient limitation of phytoplankton growth in central plains reservoirs, USA. *J. Plankt. Res.* **27**: 587–595.
- Elser, J. J., and others. 2007. Global analysis of nitrogen and phosphorus limitation of primary production in freshwater, marine, and terrestrial ecosystems. *Ecol. Lett.* **10**: 1135–1142.
- Filstrup, C. T., and J. A. Downing. 2017. Relationship of chlorophyll to phosphorus and nitrogen in nutrient-rich lakes. *Inland Waters* **7**: 385–400.
- Filstrup, C. T., T. Wagner, P. A. Soranno, E. H. Stanley, C. A. Stow, K. E. Webster, and J. A. Downing. 2014. Regional variability among nonlinear chlorophyll-phosphorus relationships in lakes. *Limnol. Oceanogr.* **59**: 1691–1703.
- Finlay, J. C., G. E. Small, and R. W. Sterner. 2013. Human influences on nitrogen removal in lakes. *Science* **342**: 247–250.
- Forsberg, C., and S.-O. Ryding. 1980. Eutrophication parameters and trophic state indices in 30 Swedish waste-receiving lakes. *Arch. Hydrobiol.* **89**: 189–207.
- Guildford, 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.
- Håkanson, L., and J. M. Eklund. 2010. Relationships between chlorophyll, salinity, phosphorus, and nitrogen in lakes and marine areas. *J. Coast. Res.* **26**: 412–423.
- Havens, K., R. James, T. East, and V. H. Smith. 2003. N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. *Environ. Pollut.* **122**: 379–390.
- Huisman, J., J. Sharples, J. M. Stroom, P. M. Visser, W. E. A. Kardinaal, J. M. H. Verspagen, and B. Sommeijer. 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. *Ecology* **85**: 2960–2970.
- Isles, P. D. 2020. The misuse of ratios in ecological stoichiometry. *Ecology* **101**: e03153. doi:10.1002/ecy.3153
- Jones, J. R., D. V. Obrecht, and A. P. Thorpe. 2011. Chlorophyll maxima and chlorophyll: total phosphorus ratios in Missouri reservoirs. *Lake Reserv. Manag.* **27**: 321–328.
- Klausmeier, C. A., E. Litchman, T. Daufrense, and S. A. Levin. 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature* **429**: 171–174.
- Kolzau, S., C. Wiedner, J. Rucker, J. Köhler, and A. Köhler. 2014. Seasonal patterns of nitrogen and phosphorus limitation in four German lakes and the predictability of limitation status from ambient nutrient concentrations. *PLoS One* **9**: e96065.
- Kratzer, C. R., and P. L. Brezonik. 1981. A Carlson-type trophic state index for nitrogen in Florida lakes. *Water Resour. Bull. Am Water Resour. Assoc.* **17**: 713–715.
- Lewis, W. M., Jr., and S. N. Levine. 1984. The light response of nitrogen fixation in Lake Valencia, Venezuela. *Limnol. Oceanogr.* **29**: 894–900.
- Lewis, W. M., Jr., and W. A. Wurtsbaugh. 2008. Control of lacustrine phytoplankton by nutrients: Erosion of the phosphorus paradigm. *Int. Rev. Hydrobiol.* **93**: 446–465.

- Mazumder, A., and K. Havens. 1998. Nutrient-chlorophyll-secchi relationships under contrasting grazer communities of temperate versus subtropical lakes. *Can. J. Fish. Aquat. Sci.* **55**: 1652–1662.
- Morris, D. P., and W. M. Lewis Jr. 1988. Phytoplankton nutrient limitation in Colorado mountain lakes. *Freshw. Biol.* **20**: 315–327.
- Moss, B., and others. 2011. Allied attack: climate change and eutrophication. *Inland Waters* **1**: 101–105.
- Nürnberg, G. K. 1996. Trophic state of clear and colored, soft- and hardwater lakes with special consideration of nutrients, anoxia, phytoplankton, and fish. *Lake Reserv. Manag.* **12**: 432–447.
- OECD. 1982. Eutrophication of waters. Monitoring, assessment, and control. Paris: Organization for Economic Cooperation and Development (OECD), p. 154.
- Omernik, J. M. 1987. Ecoregions of the conterminous United States. *Ann. Assoc. Am. Geogr.* **77**: 118–125.
- Paerl, H. W., J. T. Scott, M. J. McCarthy, S. E. Newell, W. S. Gardner, K. E. Havens, D. K. Hoffman, S. W. Wilhelm, and W. A. Wurtsbaugh. 2016. It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. *Environ. Sci. Tech.* **50**: 10805–10813.
- Paerl, H. W., and others. 2019. Mitigating eutrophication and toxic cyanobacterial blooms in large lakes: The evolution of a dual nutrient (N and P) reduction paradigm. *Hydrobiologia* **847**: 4359–4375. doi:[10.1007/s10750-019-04087-y](https://doi.org/10.1007/s10750-019-04087-y)
- 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.
- Scott, J. T., M. J. McCarthy, and H. W. Paerl. 2019. Nitrogen transformations differentially affect nutrient-limited primary production in lakes of varying trophic state. *Limnol. Oceanogr. Lett.* **4**: 96–104.
- Smith, V. H. 1982. The nitrogen and phosphorus dependence of algal biomass in lakes: An empirical and theoretical analysis. *Limnol. Oceanogr.* **27**: 1101–1112.
- Spears, B. M., L. Carvalho, B. Dudley, and L. May. 2013. Variation in chlorophyll a to total phosphorus ratio across 94 UK and Irish lakes: Implications for lake management. *J. Environ. Manage.* **115**: 287–294.
- U.S. Environmental Protection Agency. 2009. National Lakes Assessment: A Collaborative Survey of the Nation's Lakes. EPA 841-R-09-001. Washington, D.C.: Office of Water and Office of Research and Development.
- U.S. Environmental Protection Agency. 2010. Using stressor/response relationships to derive numeric nutrient criteria. EPA820-S-15-001
- U.S. Environmental Protection Agency. 2016. National Lakes Assessment 2012: A Collaborative Survey of Lakes in the United States. EPA 841-R-16-113.
- U.S. Environmental Protection Agency. 2017. Water quality standards handbook. Chapter 3: Water quality criteria (updated 2017). EPA823-B-27-001.
- Walker, W. W., Jr. 1982. An empirical analysis of phosphorus, nitrogen, and turbidity effects on reservoir chlorophyll-*a* levels. *Can. Water Resour. J.* **7**: 88–107.
- Wetzel, R. G. 1966. Variations in productivity of Goose and hypereutrophic Sylvan Lakes, Indiana. *Investig. Indiana Lakes Streams* **7**: 147–184.
- Wurtsbaugh, W. A., H. W. Paerl, and W. K. Dodds. 2019. Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. *WIREs Water* **6**: 6Le1373. doi:<https://doi.org/10.1002/wat2.1373>

#### Acknowledgments

We thank Lori Sprague, Mike Suplee, and Wayne Wurtsbaugh for providing helpful comments on an earlier draft of the manuscript, Andrew Todd for improving a presubmission version of the manuscript, and everyone that contributed to the National Lakes Assessment surveys. We also thank two anonymous reviewers for providing many inciteful comments that greatly improved the manuscript. JTS was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under award number 1P01ES028942. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the U.S. Environmental Protection Agency. The authors dedicate this work to the memory of David Rathke, who was the Clean Lakes Program Coordinator during his tenure at the Denver, Colorado office of the U.S. Environmental Protection Agency.

#### Conflict of Interest

None declared.

Submitted 19 May 2020

Revised 26 February 2021

Accepted 02 May 2021

Associate editor: John Downing