The role of phosphorus and nitrogen on chlorophyll *a*: evidence from hundreds of lakes

Zhongyao Liang*[1], Patricia A. Soranno†[2], and Tyler Wagner‡[3]

[1]Pennsylvania Cooperative Fish and Wildlife Research Unit, 407 Forest Resources Building, Pennsylvania State University, University Park, Pennsylvania 16802, USA
[2]Department of Fisheries and Wildlife, Michigan State University, 480 Wilson Road, East Lansing, Michigan 48824, USA
[3]U.S. Geological Survey, Pennsylvania Cooperative Fish and Wildlife Research Unit, Pennsylvania State University, 402 Forest Resources Building, University Park, Pennsylvania 16802, USA

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*zvl5373@psu.edu, +1-571-523-9800, corresponding author
†soranno@msu.edu
‡txw19@psu.edu

Abstract

The effect of nutrients on phytoplankton biomass in lakes continues to be a subject of debate by aquatic scientists. However, determining whether or not chlorophyll *a* (CHL) is limited by phosphorus (P) and/or nitrogen (N) is rarely considered using a probabilistic method in studies of hundreds of lakes across broad spatial extents. Several studies have applied a unified CHL-nutrient relationship to determine nutrient limitation, but pose a risk of ecological fallacy because they neglect spatial heterogeneity in ecological contexts. To examine whether or not CHL is limited by P, N, or both nutrients in hundreds of lakes and across diverse ecological settings, a probabilistic machine learning method, Bayesian Network, was applied. Spatial heterogeneity in ecological context was accommodated by the probabilistic nature of the results. We analyzed data from 1382 lakes in 17 US states to evaluate the cause-effect relationships between CHL and nutrients. Observations of CHL, total phosphorus (TP), and total nitrogen (TN) were discretized into three trophic states (oligo-mesotrophic, eutrophic, and hypereutrophic) to train the model. We found that although both nutrients were related to CHL trophic state, TP was more related to CHL than TN, especially under oligo-mesotrophic and eutrophic CHL conditions. However, when the CHL trophic state was hypereutrophic, both TP and TN were important. These results provide additional evidence that P-limitation is more likely under oligo-mesotrophic or eutrophic CHL conditions and that co-limitation of P and N occurs under hypereutrophic CHL conditions. We also found a decreasing pattern of the TN/TP ratio with increasing CHL concentrations, which might be a key driver for the role change of nutrients. Previous work performed at smaller scales support our findings, indicating potential for extension of our findings to other regions. Our findings enhance the understanding of nutrient limitation at macroscales and revealed that the current debate on the limiting nutrient might be caused by failure to consider CHL trophic state. Our findings also provide prior information for the site-specific eutrophication management of unsampled or data-limited lakes.

Keywords: Bayesian Network, eutrophication, limiting nutrient, macroscale, data-limited lakes

Introduction

Nutrients, including phosphorus (P) and nitrogen (N), are considered as main drivers of phytoplankton growth (Conley et al. 2009). However, which nutrient is the primary limiting nutrient remains a topic of substantial debate. Generally, debate focuses on whether or not lakes are solely P limited or co-limited by P and N. Some researchers propose that P is the only limiting nutrient, based on results of whole-lake experiments and historical observations (Schindler 1974, Correll 1999, Schindler et al. 2016). They found that N fixation was sufficient for phytoplankton growth in proportion to P (Schindler et al. 2008). Other researchers challenged the P control paradigm, mainly based on results of bottle or mesocosm experiments, in which they found that the addition of N could also significantly promote phytoplankton growth (Elser et al. 2007, Xu et al. 2009). While these small-scale experiments of short duration were criticized to give spurious and confusing results (Schindler 2012), a few recent studies used long-term observations to reveal N limitation in summer, which was believed to support the notion of co-limitation by P and N (van Gerven et al. 2019, Shatwell and Köhler 2019). However, short-term N limitation as the evidence for controlling N has long been doubted (Schindler et al. 2008, Carpenter 2008).

Although whole-lake experiments or historical observations provide useful information for informing lake eutrophication management (Schindler et al. 2016), previous studies typically focused on a few, selected lakes, e.g. lakes in the Experimental Lakes Area of Canada (Schindler 2012), the Laurentian Great Lakes (Chaffin et al. 2013, Dove and Chapra 2015), and Lake Taihu (Xu et al. 2009, Paerl et al. 2011). However, several studies have shown that the spatial heterogeneity of ecological contexts, including lake characteristics and phytoplankton and fish community structure, could impact the relationship between chlorophyll *a* (CHL) and nutrients (Malve and Qian

2006, Phillips et al. 2008, Wagner et al. 2011). The CHL-nutrient relationship could vary among lakes even in the same ecoregion and under the same trophic conditions (Liang et al. 2019). The variation of CHL-nutrient relationships might further change relative limitation strength of nutrients (Kolzau et al. 2014). Moreover, the spatial heterogeneity of other factors, e.g. climate and residence time, could determine the availability of nutrients and thereby impact the limiting nutrient for phytoplankton (Genkai-Kato and Carpenter 2005, Lewis et al. 2011, Maranger et al. 2018). Therefore, inferences deduced from a limited numbers of lakes might be constrained to certain ecological contexts.

Large datasets of lakes located across varied ecological contexts have long been used to explore CHL-nutrient relationship (Dillon and Rigler 1974, Canfield and Bachmann 1981, Rast et al. 1983, Oliver et al. 2017). A few studies also determined the limiting nutrient based on the performance of CHL-nutrient log-linear regressions. For example, Seip (1994) explored the limiting nutrient of 46 north temperate lakes based on the predictive ability of the CHL-nutrient model. Abell et al. (2012) found that the CHL-nutrient relationship varied with latitude and further explored the nutrient limitation patterns based on the statistical significance of regression coefficients. Similarly, Zou et al. (2020) determined the limiting nutrient of lakes in the Chinese Eastern Plains. These aforementioned studies always spatially aggregated data and then developed a unified CHL-nutrient relationship (space-for-time substitution) that was believed to be suitable for all lakes in the analysis. As such, the deduced limiting nutrient(s) for aggregated lakes are the same. However, because of the spatial heterogeneity of ecological contexts of lakes, the regional relationship might be not applicable for some lakes. More importantly, as revealed in some recent studies (Qian et al. 2019, Liang et al. 2020), the regional relationship might entirely over- or under-estimate the nutrient effect of all the lakes, which is a typical phenomenon of ecological fallacy (Maashebner et al. 2015). The deduced regional limiting nutrient could be thereby misleading.

Classifying lakes into several types based on the ecological contexts, e.g. lake characteristics, land use, meteorological factors, and phytoplankton community structure, could improve CHL-nutrient model performance (Phillips et al. 2008, Yuan and Pollard 2014, Hayes et al. 2015) and thereby provide more accurate information for the limiting nutrient deduction. However, the number of potential factors effecting nutrient limitation could be large. In practice, it is extremely difficult to collect data for many drivers and across hundreds of lakes. As such, if only a limited number of drivers are included in the modeling exercise, there is still no guarantee that ecological fallacy won't occur. Therefore, it is critical to apply effective tools to accommodate the spatial heterogeneity in ecological contexts that exists for inland lakes, and at the same time, help to overcome the data-limitation often present when exploring nutrient limitation of lakes at macroscales.

As a probabilistic machine learning method, Bayesian Network (BN) can implicitly reflect the impacts of drivers in a probabilistic manner (Rigosi et al. 2015), rather than including many potential drivers in the model. BN is therefore suitable for handling the spatial heterogeneity of ecological contexts and does not require additional data for potential drivers. In a BN, it is straightforward to conduct an analysis that provides easily communicated probability distributions of the response given the predictors' conditions. BN is also capable of accommodating nonlinear relationships (Chen and Pollino 2012). In this study, our objective was to examine whether or not CHL is limited by P, N or both nutrients in hundreds of lakes located across diverse ecological settings. We applied BN to analyzed data from a temporally and spatially extensive database for lakes in 17 Northeastern and Midwest US states (LAGOS-NE; Soranno et al. 2017). The usage of BN in developing CHL-nutrient relationships of one or multiple lakes is not new (Nojavan et al. 2017), but its application as a tool to explore nutrient limitation of lakes that span a range of ecological contexts at macroscales is novel.

Methods

Although nutrients have many different forms, total phosphorus (TP) and total nitrogen (TN) were used here as the indicators of nutrients due to data availability. Also, TP and TN are the most widely used indicators in determining the limiting nutrient of phytoplankton (Cha et al. 2016, Søndergaard et al. 2017, Liang et al. 2019). In north temperate lakes, summer is the most sensitive season for phytoplankton growth, so we focused our analysis on the summer period (June 15 to September 14) (Wagner and Schliep 2018). Because there might be interannual dynamics of nutrient limitation even in the same lake, we averaged TP, TN, and CHL concentrations in the summer period of each year to obtain yearly lake-summer average values. This resulted in 6424 average values of TP, TN, and CHL from 1382 lakes. The lake-summer average values were then used to determine the trophic state of TP, TN, and CHL, according to the classification method of the National Lake Assessment (NLA) (Table 1) (USEPA 2009).

`Table 1 should be here.`

Bayesian Network

A BN model is a probabilistic machine learning method. It is defined in terms of a directed acyclic graph and conditional distributions (Aguilera et al. 2011). BN models are based on a relatively simple causal graphical structure, making it easy to build and understand (Chen and Pollino 2012). In addition, the probabilistic representation of a BN model enables it to be a proper method to deal with uncertainties (Aguilera et al. 2011). Moreover, the belief propagation makes BN models an effective tool for reasoning, which makes them useful for helping to inform and support decision making (Chen and Pollino 2012). As such, BN models have been increasingly used in modeling ecological systems (McLaughlin and Reckhow 2017, Yuan and Pollard 2018, Marcot and Penman 2019).

To build a credible BN model, three key steps should be included, namely the determination of model structure, learning of model parameters, and model evaluation. In this study, the model structure was very simple and was determined based on the basic understanding of lake ecosystems. As shown in Figure 1, TP and TN were drivers (parents nodes) and CHL was the response variable (child node). Note that although we gave the prior that both TN and TP could impact CHL when determining the BN model structure, that did not have to lead to the conclusion that both nutrients must have effects on the CHL trophic state. If a nutrient has no effect on the CHL state, the change of that nutrient will not cause any change on the distribution of the CHL state. Distributions of the trophic state of the three variables are also shown in Figure 1.

`Figure 1 should be here.`

The categorized data (data that represent the trophic state of TP, TN, and CHL) were used as the input and output of the BN model. Although there are many other supervised or unsupervised methods to discretize nutrients and CHL concentrations to build the BN model (Beuzen et al. 2018), our NLA-guided data discretization method (Table 1) is management-oriented and thus was expected to provide useful information for lake eutrophication management. Parameter estimation was based on Bayes' theorem, which is embedded in the bnlearn package (Scutari 2010) in the R software. We conducted a 10-fold cross-validation for the BN model, in which the model was fitted 10 times to 90% of the observations while the remaining 10% was retained for out of sample prediction (Wagner and Schliep 2018). We used classification accuracy to evaluate model performance. The classification accuracy was calculated by comparing highest probability predictions to observed real outcomes (Marcot 2012). The classification accuracy was very high (76.4%), ensuring the reliability of model results and corresponding inferences.

The 'top-down' reasoning of the calibrated BN model allows us to determine the probability of a CHL tropic state under certain trophic states of TP and TN. For example, we can obtain the probability of CHL being oligo-mesotrophic ('O') when setting TP to be oligo-mesotrophic ('O') and TN to be eutrophic or hypereutrophic ('E' or 'H'), as expressed by: P(CHL = O | TP = O, TN = (E or H)). Expressions before and after the vertical bar ("|") represent the event and evidence, respectively. And we obtain the probability of the event (the trophic state of CHL) under the evidence (trophic states of nutrients) via 'top-down' reasoning.

Note that we aggregated data from a large number of lakes located across diverse ecological contexts. A unified deterministic CHL-nutrient relationship (e.g. a linear regression model) to determine nutrient limitation could be misleading because of ecological fallacy (Qian et al. 2019). In our study, the key advantage of the application of BN is the implicit accounting of the effects of potential drivers by the probabilistic results of CHL state given the trophic state of the nutrients. We emphasize that the probability of a CHL trophic state should be interpreted as the proportion of lakes whose CHL concentration is in that certain state rather than the possibility of that certain CHL state in a given lake. For example, P(CHL = O) = 0.3 means there are 30% of the lakes whose CHL state is oligo-mesotrophic – rather than that for a certain lake the probability of CHL being oligo-mesotrophic is 0.3.

By comparing probabilities of the CHL trophic state under different combinations of nutrient trophic states, we can explore the role of TP and TN on phytoplankton. Specifically, we addressed the following three questions:

1) Is CHL limited by nutrients? Although the answer to this question seems to be well established, it is rarely discussed based on the results of analyses that examine hundreds of lakes using a BN, in which the potential effects of spatial heterogeneity of ecological contexts are implicitly accounted for.
2) If CHL is limited by nutrients, is CHL limited by both nutrients or only one?
3) If CHL is limited by both nutrients, is there one nutrient that is more important than the other one?

While there are many combinations of TP and TN trophic state that are used as the evidence in the BN to calculate the probability of the CHL trophic state, we focused on the nutrient trophic state combinations which were helpful to answer the above questions. All the computations were conducted in R software (Version 3.6.0) (R Core Team 2019). We developed the BN using `bnlearn` package (Scutari 2010).

Results

Probabilities of CHL trophic states under different combinations of TP and TN trophic states are shown in Figure 2. To answer the question of whether nutrients affect the CHL trophic state, we can compare the results of the CHL trophic state when both nutrients are oligo-mesotrophic (Figure 2g) and when both nutrients are eutrophic (Figure 2e) or hypereutrophic (Figure 2c). When both nutrients are oligo-mesotrophic, the probability of CHL being oligo-mesotrophic is high (0.793) and the probability of CHL being eutrophic or hypereutrophic is small (0.201 and 0.006, respectively). However, if nutrient trophic state becomes eutrophic or hypereutrophic, the probability of CHL being oligo-mesotrophic decreases greatly to 0.104 and 0.023, respectively. That is, on one hand, the trophic state of 68.9% (0.793 − 0.104) of lakes will shift to a more enriched CHL trophic state when both nutrients become eutrophic and 78.7%

(0.793 – 0.006) of lakes will shift to a more enriched CHL state when both nutrients become hypereutrophic. On the other hand, when nutrient trophic state becomes oligo-mesotrophic from eutrophic or hypereutrophic the proportion of lakes being classified as eutrophic and hypereutrophic based on the CHL concentrations is greatly reduced (e.g., compare Figure 2b, e, and h). Therefore, TP and TN are indeed very important for determining the CHL trophic state of lakes, indicating that CHL is limited by nutrients at macroscales.

`Figure 2 should be here.`

Next, we address the question of whether or not a single nutrient or both nutrients affect CHL trophic state – given that we have established that nutrients are important determinants of CHL trophic state. To explore the effect of one nutrient independent of the other, we kept the trophic state of the other nutrient constant. For example, we can determine the effect of TP on CHL trophic state by comparing Figures 2a, d, and g. When setting the TN trophic state to be oligo-mesotrophic, changing the TP trophic state from oligo-mesotrophic (Figure 2g) to eutrophic (Figure 2d) or hypereutrophic (Figure 2a) will lead to a large decrease of the probability of CHL being oligo-mesotrophic (a decline from 0.793 when TP is oligo-mesotrophic to 0.056 when TP is hypereutrophic). Concurrently, we see an increase in the probability of CHL being eutrophic and hypereutrophic (Figure 2g, d, & a). When holding the TN state constant at eutrophic (Figure 2b, e, & h) or hypereutrophic (Figure 2c, f, & i), we obtain similar results to the results for TP on the probability change of the CHL trophic state. To determine the effect of TN on CHL trophic state, we compare plots holding the TP trophic state constant. If the TP state is oligo-mesotrophic (Figures 2g, h, & i), changing the trophic state of TN from oligo-mesotrophic to eutrophic or hypereutrophic will cause a decrease in the probability of CHL being oligo-mesotrophic (from 0.793 when TN is oligo-mesotrophic to 0.609 when TN is hypereutrophic) and an increase of the probability of CHL being eutrophic and hypereutrophic (Figures 2g, h, & i). If the TP state is eutrophic (Figure 2d, e, & f) or hypereutrophic (Figure 2a, b, & c), changing the trophic state of TN from oligo-mesotrophic to eutrophic or hypereutrophic will primarily lead to the shift of the CHL state from eutrophic to hypereutrophic, since the probability of CHL being oligo-mesotrophic is already very small. Therefore, according to the change of the probability of different CHL trophic states, both TP and TN could influence the CHL trophic state, showing that both nutrients could be limiting.

To determine the relative importance of nutrients – since both nutrients could influence the CHL trophic state – we assume that both nutrients are oligo-mesotrophic, and then shift either nutrient to a more nutrient enriched trophic state. The shift of the TP trophic state to a eutrophic state will lead to 67.7% ($0.793 - 0.126$) of the lakes transferring from an oligo-mesotrophic trophic state to a eutrophic or hypereutrophic state and 74.7% (0.793 – 0.056) of lakes transferring from an oligo-mesotrophic trophic state to a eutrophic or hypereutrophic state if TP shifts to a hypereutrophic state (Figures 2g, h, & i). In contrast, the shift of TN to a eutrophic state or hypereutrophic state will only cause such a change for 12.7% ($0.793 - 0.664$) and 18.4% (0.793 – 0.609) of the lakes, respectively (Figures 2g, d, & a). In addition, the shift of the TP state to a hypereutrophic state will lead to a larger proportion of lakes being classified as hypereutrophic based on CHL (0.374), a much larger proportion than that resulting from the shift of TN (0.015). Moreover, when TP trophic state is hypereutrophic the probability of CHL being oligo-mesotrophic is as small as 0.006 (Figure 2a) and changing the TN trophic state from oligo-mesotrophic to hypereutrophic has little influence on that probability (Figure 2c). However, when the TN trophic state is hypereutrophic, changing the TP trophic state from oligo-mesotrophic to hypereutrophic results in a large decrease in the probability of CHL being oligo-mesotrophic (from 0.609 to 0.023)

(Figure 2i & c). Therefore, although TN has an influence on the CHL state, TP is substantially more important than TN. Considering the huge difference between the TP and TN effect and the large effect of TP on CHL trophic state, TP generally plays a dominant role in determining the CHL state, indicating that TP seems more important for limiting CHL compared to TN.

Finally, we examined if TP and TN could interactively impact the CHL trophic state. We found that when the TP trophic state was oligo-mesotrophic, changing the TN trophic state from oligo-mesotrophic to eutrophic or hypereutrophic would only cause a small increase in the probability of CHL being hypereutrophic (Figure 2g, h & i). However, when the TP state was eutrophic or hypereutrophic, the probability of CHL being hypereutrophic increased substantially when changing the TN trophic state from oligo-mesotrophic to eutrophic or hypereutrophic (Figure 2d, e & f for TP in a eutrophic state and Figure 2a, b, & c when TP is in a hypereutrophic state). That is, the impact of the TN state on the CHL state is much larger when the TP state is eutrophic or hypereutrophic, indicating that there is a positive interaction between TP and TN in determining the hypereutrophic state of CHL. When either TP or TN goes to oligo-mesotrophic from being eutrophic or hypereutrophic, this will lead to a relatively large decrease in the probability of CHL being hypereutrophic. Therefore, when the CHL state is hypereutrophic, both nutrients are likely important and suggests potential co-limitation by TP and TN.

We further checked the robustness of the above results by changing the sampling period, lake depth, and thresholds to determine the CHL state. We set the sampling years < 2000, < 2005, < 2010, ≥ 1995, ≥ 2000, and ≥ 2005, and the mean lake depth < 3 m, < 5 m, ≥ 3 m, and ≥ 5 m. We tried another popular method determining the CHL state proposed by Smith et al. (1999), by which the lake is oligo-mesotrophic when CHL < 9 μg/L, is eutrophic when CHL > 9 μg/L but < 25 μg/L, and is hypereutrophic when CHL > 25 μg/L. Note that thresholds in Smith et al. (1999) are similar to those in USEPA (2009). We found that all the results had a similar pattern. Therefore, our results are very robust, which gives strength to the reliability of the analysis.

Discussion

The role of TP and TN on limiting CHL

We summarized whether or not CHL is limited by TP, TN, or both nutrients in inland lakes at macroscales. Although both nutrients affect CHL trophic state, TP generally plays a dominant role. However, when the CHL trophic state is hypereutrophic, both TP and TN are important. Our findings on the role of nutrients indicate P-limitation when the CHL trophic state is not hypereutrophic and the co-limitation of P and N when the CHL trophic state is hypereutrophic.

The TN/TP ratio is one of the most widely used indicators to explain the nutrient limitation for phytoplankton (Redfield 1958, Cha et al. 2016, Liang et al. 2018). It is well recognized that a higher TN/TP ratio indicates a higher possibility of P-limitation. We found a significant decreasing trend (the fitted linear regression line in Figure 3) of the TN/TP ratio with increasing CHL concentration for the lakes in the LAGOS-NE database (Figure 3). As the TN/TP ratio approaches to the Redfield Ratio (7.2 by mass, the dashed horizontal line in Figure 3), the nutrient limitation condition shifts from P-limitation to that of co-limitation by P and N. Average values of the TN/TP ratio are 54.1, 26.8, and 18.1 (white points in Figure 3), when the CHL state is oligo-mesotrophic, eutrophic, and hypereutrophic, respectively. According to the linear regression line between log(TN/TP) and log(CHL), we can set the CHL concentration to be 30 μg/L (the threshold to determine the eutrophic and hypertrophic state) and calculate the corresponding TN/TP ratio as a rough estimation of the TN/TP ratio to classify the P-limitation and co-limitation of P and N. The estimated TN/TP ratio is 20.2, which is close to the proposal of 22 by Guildford and Hecky (2000).

`Figure 3 should be here.`

There are several mechanisms that may influence the TN/TP ratio in lakes, such as watershed nutrient input and atmospheric deposition of nutrients (Downing and McCauley 1992, Elser et al. 2009). Two processes that are most related to the CHL trophic state are the release of P from the sediment and the denitrification of N in the waterbody (Cottingham et al. 2015, Zhang et al. 2018). The decomposition of phytoplankton was identified as the key process to the release of P from the sediment by providing the low dissolved oxygen and proper pH environment supporting the Iron(II)-P coupling (Chen et al. 2018). Moreover, lake eutrophication often leads to nuisance blooms of some phytoplankton species (e.g. Cylindrospermopsis raciborskii) which are able to regulate their metabolism to accommodate conditions of low dissolved inorganic phosphorus (Wu et al. 2012, Figueredo et al. 2014, Araujo et al. 2018). P thereby would increase faster in the waterbody than N. Meanwhile, the decomposition of phytoplankton leads to the increase of total organic carbon which could fuel the potential denitrification rate (Zhang et al. 2018). Moreover, N-fixation usually cannot compensate for the loss of N caused by denitrification (Hayes et al. 2018, van Gerven et al. 2019). At the continental scale, net denitrification will lead to a larger N deficit in more productive lakes (Scott et al. 2019). As such, it appears that P accumulates faster than N in more eutrophic lakes that are heavily impacted by anthropogenic activities (Yan et al. 2016).

Globally, a decreasing trend of the TN/TP ratio with the increase of CHL concentration has also been shown. Yan et al. (2016) found a similar negative relationship between the TN/TP ratio and CHL using worldwide data compiled from

157 publications. Some studies also found a decreasing trend of the TN/TP ratio with the increase of TP concentration based on compiled datasets (Downing and McCauley 1992, Sterner 2008), which also indicated the negative correlation of the TN/TP ratio with CHL concentrations – considering the high positive correlation between CHL and TP. Across a larger latitudinal range (from 70 °S to 83 °N), Abell et al. (2012) found that TN/TP ratios were smaller in lakes with a higher trophic state. The same negative relationship between the TN/TP ratio and CHL has also been shown in the same lake over time. For example, the TN/TP ratio decreased with the increasing eutrophication in lakes such as Dianchi, Taihu, and Okeechobee (Yan et al. 2016). Similarly, the TN/TP ratio increased during the lake recovery period in the Laurentian Great Lakes (Dove and Chapra 2015) and some Chinese lakes (Tong et al. 2018). Besides, the TN/TP ratio would be smaller in summer than that in the other seasons because of the higher CHL concentration in summer (Ding et al. 2018).

Our findings on the role of limiting nutrients are deduced from cross-sectional data. However, our results are supported by several case studies (i.e., non compiled, cross-sectional databases) worldwide (Table 2). For example, for some lakes in the Experimental Lakes Area of Canada (Schindler et al. 2008, Schindler 2012) and the Laurentian Great Lakes (Dove and Chapra 2015), whose CHL states were oligo-mesotrophic or eutrophic, the limiting nutrient was identified as TP. In some hypereutrophic lakes (e.g. Lake Dianchi and Lake Taihu) in China, both TN and TP were determined as limiting nutrients (Xu et al. 2009, Wu et al. 2017). In addition, Søndergaard et al. (2017) found that CHL was generally more strongly related to TP than to TN, but TN could be important to the variability of CHL at high TP concentrations (> 107 μg/L) based on the observations of 817 Danish lakes. Similarly, Filstrup and Downing (2017) revealed that CHL was weakly related to TN when TP concentration was low, but displayed a much stronger response to TN at higher

TP concentrations (> 100 μg/L) for lakes located in an agricultural region in the Midwestern US. These high TP concentrations in both studies always corresponded to the hypereutrophic state of CHL. Considering the high correlation between CHL and TP, the importance of TN at high TP concentrations indicates the importance of TN when the CHL state is hypereutrophic. These studies also support the dominant role of TP when the CHL state is not hypereutrophic and the dual role of TP and TN when CHL is hypereutrophic.

`Table 2 should be here.`

Because our analysis was performed using data from over 1300 lakes that spanned a wide range of trophic states and ecological contexts, and because our results are supported be several single-lake and multi-lake studies from across the globe, we believe that our findings have great potential for generalizing to other lakes. Therefore, our findings are helpful to better understand the role of limiting nutrients and provide further insight to the current controversy on limiting nutrients. For example, the debate over limiting nutrients might be caused by trying to answer the same question, but under two different CHL trophic state conditions. Researchers insisting on P-limitation might focus on the lakes with oligo-mesotrophic or eutrophic CHL trophc state, while researchers finding evidence of co-limitation by P and N might have focused efforts in lakes with hypereutrophic CHL conditions (Table 2). It appears likely that the difference in CHL state was neglected in previous studies.

Implications for management of lake eutrophication

It is impossible to propose a unique strategy for lake eutrophication management that is applicable for all lakes, given the spatial and temporal variability of ecological contexts (Wagner et al. 2011, Moal et al. 2019, Qian et al. 2019). Although deductions based on our findings cannot be generalized to all lakes, since our findings are deduced from spatially aggregated data, they are suitable for providing some general guidance for lake eutrophication management for many lakes. Considering the impossibility of a unified law, general guidance suitable for a large number of lakes is critical. Our findings would provide important prior information for site-specific eutrophication management, particularly for unsampled or data-limited lakes.

Firstly, for the recovery of hypereutrophic lakes, decreasing concentrations of both TP and TN would likely be advantageous. The probability of CHL being hypereutrophic reduced by a large proportion when the TP state changed from hypereutrophic to eutrophic or oligo-mesotrophic state (left panel in Figure 4). The probability of CHL being hypereutrophic would be reduced by more than a half (from 25.6 % and 25.2 % to 11.4 %) when the TN state becomes oligo-mesotrophic (right panel in Figure 4).

`Figure 4 should be here.`

Secondly, note that the co-limitation by P and N when CHL is hypereutrophic does not have to lead to the strategy that both nutrients should be controlled in practice (Harpole et al. 2011), because the reduction of either nutrient would be helpful. However, we should be aware that controlling TP solely imposes a high risk of causing a hypereutrophic state of CHL (Figure 2c & e) if, for example, there is an abrupt TP concentration increase caused by a sudden or extreme event. In other words, an oligo-mesotrophic or eutrophic lake with a higher TN concentration has less resiliency (the ability to keep the original state) to an abrupt increase in TP concentration than a lake with a lower TN concentration.

Finally, to maintain the oligo-mesotrophic state of a lake, maintaining an oligo-mesotrophic TP state will be important. Maintaining an oligo-mesotrophic TP state would result in a more than 70 % of lakes being in an oligo-mesotrophic CHL state.

However, the change of TN trophic state will not lead to a large proportion change of CHL oligo-mesotrophic state. Similarly, to further recover a lake to the oligo-mesotrophic state, a decrease in TP will be more effective than a decrease in TN.

Importance of a large dataset and the use of a Bayesian Network

The novelty of our research is due to two primary factors: examining effects of N and P across hundreds of lakes and the application of BN at macroscales. We emphasize the importance of using a dataset with large numbers of lakes and with a wide-range of different ecological contexts, rather than using a limited number of lakes to explore the role of TP and TN as potential limiting nutrients. The extension of results deduced from a limited number of lakes to a broader population of lakes might improperly identify the limiting nutrient and misinform lake eutrophication management. For example, as shown by our probabilistic results, there is a proportion of lakes whose CHL trophic state will be oligo-mesotrophic when the TP state is hypereutrophic (left panel in Figure 4). If research focused only on these lakes we might conclude that TP is not related to the CHL trophic state (i.e., that CHL is not limited by TP), while TP is in fact very important for many other lakes. The extension of the corresponding strategy for lake eutrophication control might be also ineffective for other lakes. Similarly, there a large proportion of lakes whose CHL state is eutrophic or hypereutrophic when TP state is hypereutrophic (left panel in Figure 4). The generalization of nutrient limitation deduction from these lakes to other lakes could be misleading.

Our work also highlights the novel application of BN in exploring the role of P and N on CHL at macroscales. As shown in Figure 2 and Figure 4, under certain nutrient states, the CHL state is not deterministic but probabilistic, reflecting impacts of spatial heterogeneity of drivers that were not included in the analysis. We argue that the application of BN could be encouraged as an effective tool for use in macrosystem studies. Firstly, BN implicitly accounted for impacts of spatial heterogeneity of ecological contexts and avoided the risk of ecological fallacy. Secondly, although classifying ecosystems is useful for improving our understanding of ecological processes, data used for classification could be rare, particularly for many systems at macroscales. Under this circumstance, BN allows the probabilistic exploration of response-drivers relationship. Finally, if we are also interested in the effect of other factors or the data of potential drivers become available, adding other factors as predictors in BN is straightforward.

In the future, it will be critical to identify drivers of the limiting nutrient at both the regional and site-specific scales so the limiting nutrient of a lake can be determined more accurately according to its ecological context. This would enable predicting limiting nutrients to unsampled (or data-limited) lakes which could better inform the site-specific eutrophication management at macroscales.

Conclusions

We explored the TP vs. TN limitation in inland lakes at macroscales. The novel application of BN allowed us to directly build CHL-nutrient relationships without collecting extra data of potential drivers of nutrient limitation. Results showed that TP generally played a more important role on driving phytoplankton biomass than TN. When CHL is in a hypereutrophic state, both TP and TN are important. We revealed that the current debate on the limiting nutrient might be caused by failure to consider CHL trophic state. Our findings enhance the understanding of nutrient limitation at macroscalea, which could also facilitate eutrophication management of unsampled or data-limited lakes.

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Figure Legends:

Figure 1: Structure of BN model. Horizontal bars show the proportion of lakes classified into each of the three trophic states

Figure 2: Probabilities of the CHL trophic state under different combinations of nutrient trophic states

Figure 3: The relationship between $\log_e$ TN/TP ratio and $\log_e$ CHL for lakes in the LAGOS-NE database

Figure 4: Probablities of CHL state given the state of one nutrient

Table 1: Concentration thresholds used to determine the trophic state of TP, TN, and CHL (modified from USEPA (2009). Please refer to Figure 1 for the distributions of lake trophic states in our study). For our analysis, we combined the oligotrophic and mesotrophic states into a single category (USEPA 2009).

trophic state	**TP (μg/L)**	**TN (mg/L)**	**CHL (μg/L)**
Oligo-mesotrophic (‘O’)	≤ 25	≤ 0.75	≤ 7
Eutrophic (‘E’)	> 25 & ≤ 50	> 0.75 & ≤ 1.4	> 7 & ≤ 30
Hypereutrophic (‘H’)	> 50	> 1.4	> 30

Table 2: Documented cases of nutrient limitations. The CHL state is determined by the average CHL concentration.

CHL state	Limiting nutrient	Lake name	Country	Location	Reference
oligo-mesotrophic or Eutrophic	TP	Lake 227	Canada	50°N, 94°W	Schindler et al. (2008)
		Lake 261		50°N, 94°W	Schindler (2012)
		Lake 303		50°N, 94°W	
		Lake 304		50°N, 94°W	
		Lake Erie	Canada, US	42°N, 81°W	Dove and Chapra (2015)
		Lake Ontario	US	44°N, 78°W	
		Lake Huron		44°N, 82°W	
		Lake Michigan		44°N, 87°W	
		Scharmützelsee	Germany	52°N, 14°E	Kolzau et al. (2014)
		Untere Havel		52°N, 13°E	
		Lake Chenghai	China	26°N, 100°E	Yan et al. (2019)
Hypereutrophic	TP & TN	Langer See	Germany	52°N, 14°E	Kolzau et al. (2014)
		Müggelsee		52°N, 14°E	
		Lake Taihu	China	31°N, 120°E	Xu et al. (2009), Paerl et al. (2011)
		Lake Dianchi		24°N, 102°E	Wu et al. (2017)

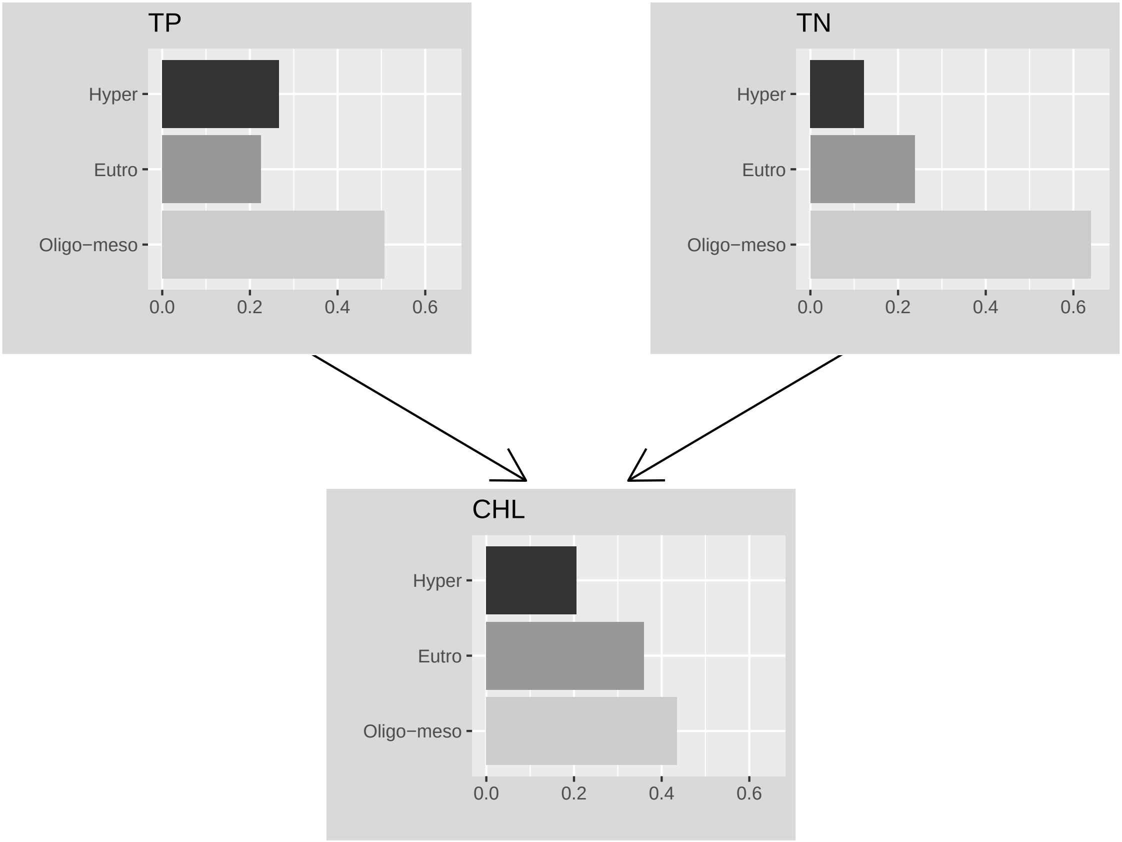

Figure 1: Structure of the Bayesian Network model. Horizontal bars show the proportion of lakes classified into each of the three trophic states. TP = total phosphorus, TN = total nitrogen, CHL = chlorophyll a.

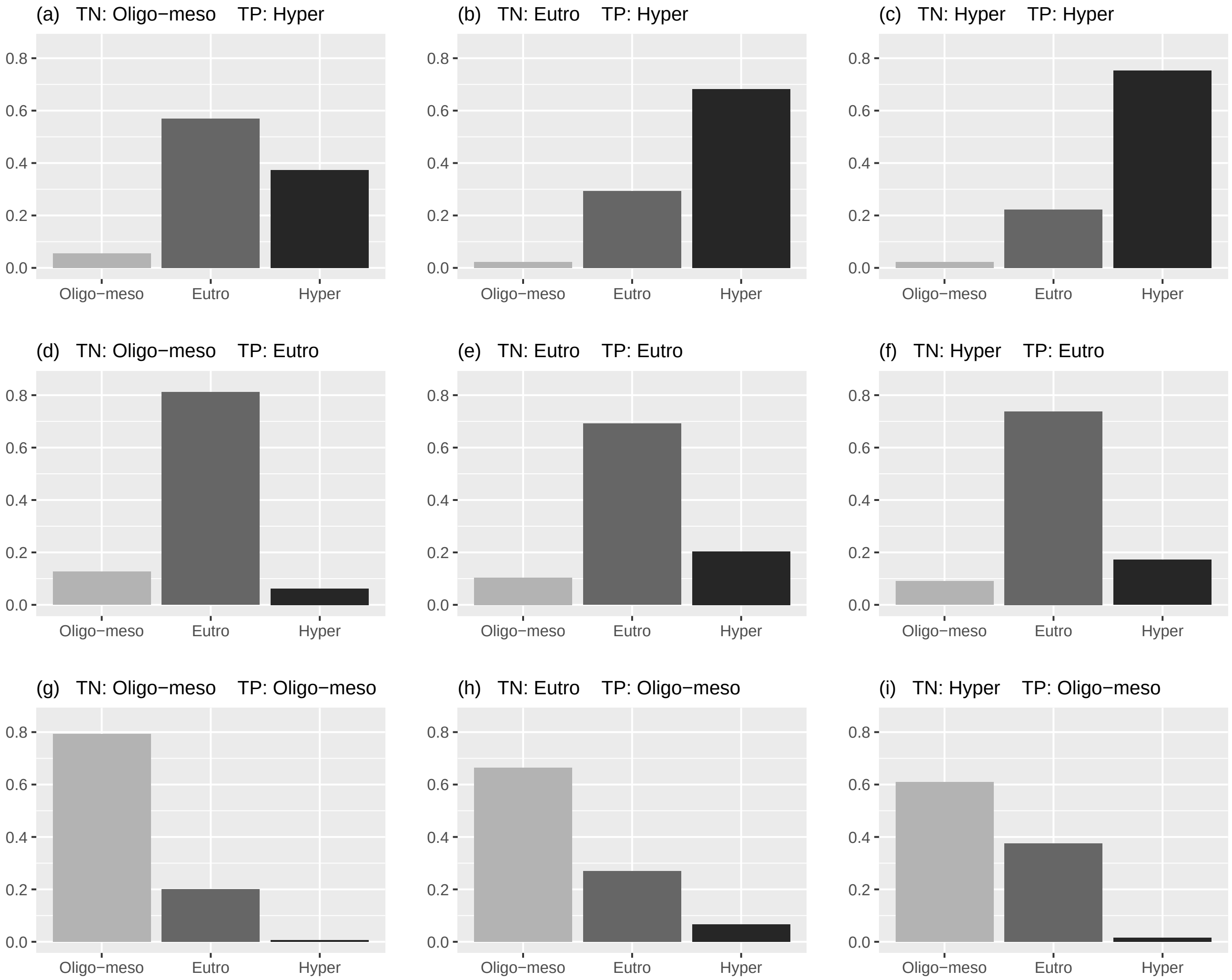

Figure 2: Probabilities of the CHL trophic state under different combinations of nutrient trophic states. 'Oligo-meso' = oligo-mesotrophic, 'Eutro' = eutrophic, 'Hyper' = hypereutrophic, TP = total phosphorus, TN = total nitrogen.

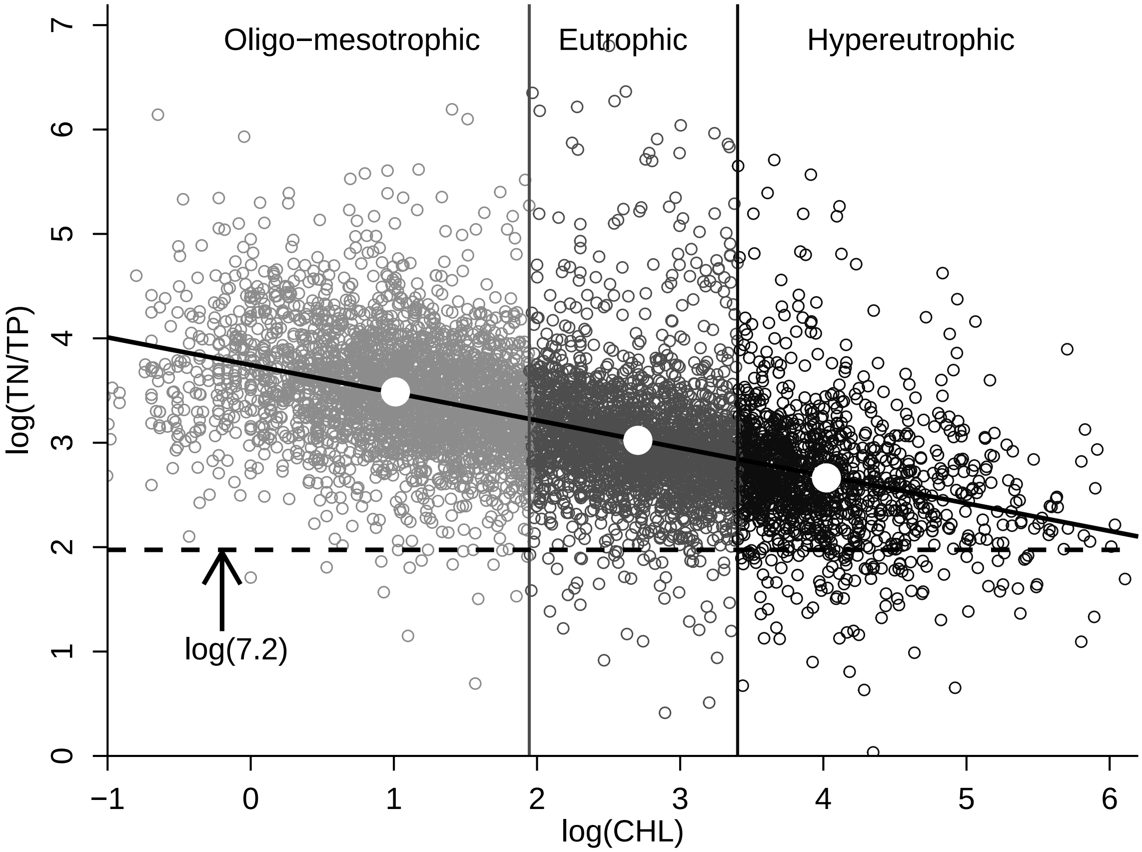

Figure 3: The relationship between $\log_e$ TN/TP ratio and $\log_e$ CHL for lakes in the LAGOS-NE database. Solid line is fitted regression line ($R^2 = 0.262$, $p < 0.001$), vertical lines indicate breaks in trophic status (indicated at the top of the figure), white points are average TN/TP for each trophic state, and dashed horizontal line indicates the Redfield Ratio on a $\log_e$-scale.

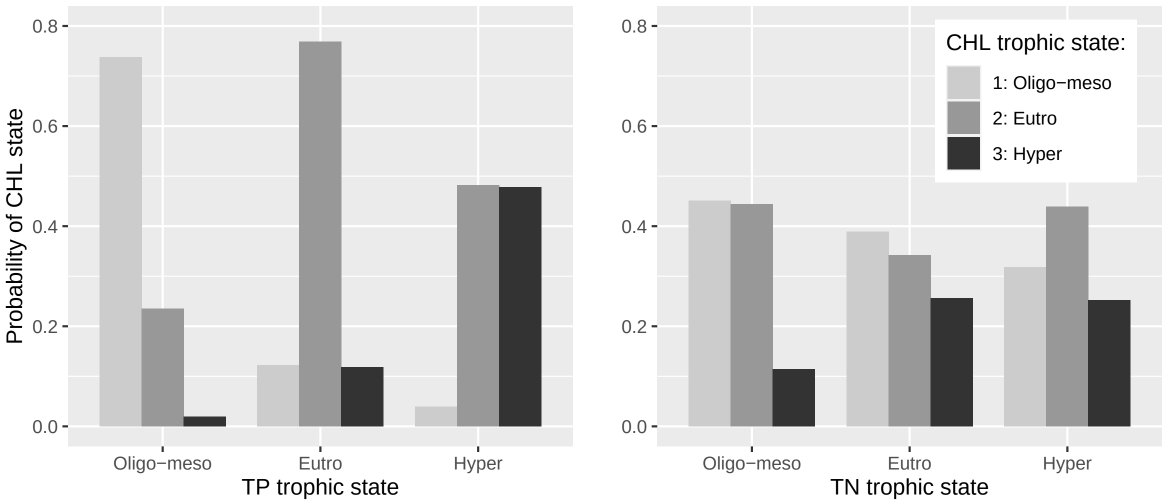

Figure 4: Probablities of CHL state given the state of one nutrient.

Lakes with variable ecological contexts + **Bayesian Network (BN)** →

- **Distributions of CHL state given nutrients states**
- **Deduce whether CHL is limited by TP and TN**