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Effects of nitrogen and phosphorus on chlorophyll a in lakes of China: a meta-analysis

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Abstract

Whether or not chlorophyll a (Chla) is limited by nitrogen (N) or phosphorus (P), or both, remains highly debated. In part this is due to the lack of a robust statistical rarely considered using a statistical method in studies of peer-review research of lakes. Individual studies only used the use of the relationship between Chla and nutrients to judge the effects of N and P, ignoring the spatial heterogeneity of the ecological environment. Here, we evaluated the effects of nitrogen and phosphorus on Chla via a meta-analysis. The dataset consists of 1024 observations from 34 lakes in China. The results show that Chla is one of the most important indicators of water eutrophication, both nutrients were related Chla trophic state, and that TP plays an important role over TN, especially in the hypereutrophic Chla conditions. With the increase of TN/TP, Chla concentration is decreased, which may be the co-limited of TN and TP. These three values largely explained the increment of eutrophication Chla, although the relative effects differed across nutrients, with changes in the TN total contributing 5.2% of the increment in Chla, while the TP change contributed 272.0% of the increment in Chla. At spatial scales, logarithm-transformed response ratio (ln RR) of Chla indicated that the lake shifts from N limitation (64.0% > 28.0%) to oligo-mesotrophic to P limitation (170.0% > 99.0%) in eutrophic and co-limitation (66.0% and 46.0%) by N & P in hypereutrophic. These results demonstrate that TP reduction can mitigate eutrophication in most large lakes but a dual TN and TP reduction may be needed in eutrophic lakes, especially in hypereutrophic states. This study highlights the differential effects of the three factors concentration, which may offer important implications for better management of the response of the eutrophication lakes to nutrients (N and P) in the future.

1. Introduction

Eutrophication is a widespread phenomenon in lake waters worldwide and is one of the greatest environmental problems facing humanity (Schindler et al 2016, Liang et al 2020, Wang et al 2020). Diffusive N and P pollution is the main driving force for eutrophication (Beusen et al 2015). The excessive nutrient input can stimulate algal blooms, leading to decreased light permeability and low oxygen levels (Jetoo et al 2015), which then generate large numbers of harmful algal bloom and accelerate the process of eutrophication in the water column (Kast et al 2021). Therefore, it is necessary to quantify...
the impact of the nutrient increase on phytoplankton biomass and determine which nutrients restrict phytoplankton growth is an important step in formulating effective strategies for lake and watershed management (Ding et al. 2021, Janssen et al. 2021).

In various aquatic habitats, there is a consistent relationship between total phosphorus (P) and phytoplankton biomass (using chlorophyll-a as an indicator) (Schindler 1977, Smith 2003, Liu 2017, Liu et al. 2019, Wang et al. 2019, Rao et al. 2021). Additionally, in some freshwater ecosystems, particularly in the diverse large lake ecosystems, N can be the primary limiting nutrient for phytoplankton biomass (Xu et al. 2010, Liu 2017, Yao et al. 2018). Chlorophyll-a (Chla) concentration is a widely used phytoplankton biomass measurement method (Oliver et al. 2017, Li et al. 2018). The parallel microscopic measurement of phytoplankton biomass is consistent with the results of Chla (Zhao et al. 2002, Yan et al. 2003). Chla is a good indicator of phytoplankton biomass response in bioassays. Therefore, Chla is often used to reflect the true growth of phytoplankton biomass (Zhao et al. 2002, Yan et al. 2003). The most common way to control eutrophication is to reduce the nutrient load inside and outside the reservoir, especially P and N, to reduce algal biomass (Vinçon-Leite and Caseneuve 2019).

For a long time, phosphorus has been considered the main nutrient that restricts the growth of phytoplankton in freshwater ecosystems, and management efforts generally focus on controlling the phosphorus load (Smith 2003). Algae chlorophyll in freshwater systems is mainly affected by total phosphorus (Liu et al. 2021). Algae chlorophyll in coastal and marine systems is generally controlled by TN, and some aquatic systems are jointly restricted, especially in high mountain areas (Smith and Schindler 2009, Dodds and Smith 2016). For example, based on 37 years of experimental data, Smith and Schindler (2009) showed that nitrogen is the main factor controlling the eutrophication of freshwater lakes and cannot be controlled by reducing nitrogen input.

However, other studies have shown that the N limitation and the joint limitation of nitrogen and phosphorus are more common than previously thought (Elser et al. 1990, Maberly et al. 2002, Paerl et al. 2016, Hampel et al. 2018). Among them, Howarth et al. (2006) proposed that nitrogen is the main factor controlling eutrophication. In a review of freshwater bioassay experiments in North America, Elser et al. (1990) reported that the co-limitation of nitrogen and phosphorus is the most common response of phytoplankton communities to nutrient addition which occurs more frequently than limiting nitrogen or phosphorus alone. Similarly, Maberly et al. (2002) reported that the percentage frequency of N, P, and co-limitation was 13%, 24%, and 63%. Most authors acknowledge that nitrogen may restrict the growth of phytoplankton, but the predictions of what nitrogen restriction will happen are inconsistent and even vague (Lewis and Wurtsbaugh 2008). The conversion range of nitrogen restriction and phosphorus restriction has also given different ratios of nitrogen and phosphorus in many studies (Paerl et al. 2016, Mamun et al. 2020).

Generally, debate focuses on whether or not lakes are solely P limited or N limited or co-limited by P and N. Ma et al. (2015) showed that N limitation then takes place when the TN:TP ratio is less than 21.5–24.7, and P limitation occurs above this. Xu et al. (2015) found that TN and TP concentration thresholds should be targeted at below 0.80 mg l$^{-1}$ and 0.05 mg l$^{-1}$, respectively, to limit intrinsic growth rates of microcystis dominated blooms. Therefore, it is crucial to understand the nutrient ratios of eutrophic lakes, clarify the relationship between nitrogen and phosphorus and nutrient limits, and quantify the drivers of lake eutrophication.

In this study, our objectives are (a) to examine how Chla is affected by TN, TP, and TN/TP; (b) to quantify the interaction between nitrogen/phosphorus and Chla, and (c) to explore the nutrient limitation of eutrophication on a macro scale. Here, we evaluated the effects of TN and TP on Chla of 34 lakes in China and investigated the relationships between different nutrients (TN, TP) thresholds and Chla. The analysis will assist in the establishment of the relationship between reducing phosphorus (or reducing nitrogen) and Chla concentration, so policymakers can familiarize themselves with the influencing factors of eutrophication. The findings provide a scientific basis and certain theoretical guidance for future water environmental management. It was especially formulating a reasonable nutrient input.

2. Materials and methods

2.1. Data collection

We selected experimental studies that assessed the effects of nutrients (total phosphorus, total nitrogen), TN/TP on Chla of the lake in China. Firstly, we conducted a literature search using Web of Science with the following sequence of key terms: (TS = (phosphorus* OR ‘Total phosphorus’ OR TP OR nitrogen* OR ‘Total nitrogen’ OR TN) AND TS = eutrophication AND TS = (Chl-a OR ‘Chlorophyll a’) AND AD = China). This search yielded 805 hits.

We complied with the following criteria to avoid bias in selecting publications. First, we included only field experiment data. Second, all nitrogen limits in eutrophication contained at least TN and TP. Third, Chla had to be explicitly reported with the published data. If the data were presented in a graph, we digitized the data using the GetData
Figure 1. Flow chart showing the preliminary selection criteria and the refined selection criteria used for determining meta-analysis. Numbers (k values) correspond to the number of journal articles. See SI references for a full list of studies included in the meta-analysis.

Graph Digitizer (version 2.24, Russian Federation). Finally, we included only reports on the lake in China. For accuracy, our analysis required the following details in the selected publications: location (longitude/latitude), the research location, nutrition (TN, TP, and TN/TP). A total of 61 peer-reviewed papers were produced during the screening process based on abstracts, charts, tables, and full text, as well as the removal of duplicates (figure 1, supplementary information (SI) references). Representing 42 sites made up the final dataset (figure 2, SI table S1).

2.2. Statistical analysis
In a meta-analysis, results in the form of one or more effect sizes are extracted from each study. Effect sizes are designed to combine the results of different studies on the same scale, and use a set of indicators for comparison (Gurevitch et al 2018). This is essential for past effect size indicators. In this study, we used the response ratio (RR) as the effect size. The natural-logarithm-transformed response ratio lnRR is generally used as an indicator in the field of ecology (Hedges et al 1999). By using the lnRR, we can give a more robust prediction of how Chla varies with a change in a given nutrient driver. All statistical analyses were performed with R v3.5.4 (R Core Team 2020).

2.3. Response ratio
According to (Lajeunesse 2015), calculate the response rate lnRR and its variance (v) of the natural logarithmic transformation of individual experimental units:

\[
\ln(RR) = \ln \left( \frac{Y_t}{Y_r} \right) + \frac{1}{2} \left[ \frac{S_t^2}{N_t \cdot Y_t^2} - \frac{S_r^2}{N_r \cdot Y_r^2} \right]
\]

\[
v = \frac{S_t^4}{N_t \cdot Y_t^4} + \frac{S_r^4}{N_r \cdot Y_r^4} + \frac{1}{2} \left[ \frac{S_t^4}{N_t \cdot Y_t^4} - \frac{S_r^4}{N_r \cdot Y_r^4} \right]
\]

where \(Y\) denotes mean Chla, \(S\) is the standard deviation of that mean, and \(N\) is the sample size for the treatment (subscript t) and the reference (subscript r) groups.

We calculated the weighted mean lnRR using the mixed-effects model with the R package metaphor. The weighted mean (\(M\)) and its variance (\(V\)) were calculated as

\[
M = \frac{\sum_{j=1}^{k} W_j M_j}{\sum_{j=1}^{k} W_j}
\]

\[
V = \frac{1}{\sum_{j=1}^{k} W_j}
\]

where \(k\) is the total number of experimental units, \(M_j\) is effect size lnRR in experimental unit \(j\), and \(W_j\) is the weighting factor, which is the inverse of the variance. The 95% confidence interval (CI) for the weighted mean was computed as:

\[
CI = M \pm 1.96 \times \sqrt{V}.
\]

In the presentation of this article, the value of lnRR is reversely converted, and the percentage change of Chla caused by nutrients was calculated based on the weighted response rate (equation (6))

\[
E(\%) = \frac{X_e - X_r}{X_r} \times 100\% = \left( \frac{X_e}{X_r} - 1 \right) \times 100\% = \left( e^{\ln(RR)} - 1 \right) \times 100\%.
\]

They are considered statistically significant if the 95% CI do not overlap with zero.

3. Results

3.1. Effects of TN and TP on Chla in lakes of China
Our results indicated that the response of TN and TP to Chla was positive in the lakes of China (figure 3)
Figure 3. Effect of elevated N/P on eutrophication of water bodies. The figure showed the external loading and cycling of N and P, and how these nutrients cause an increase in phytoplankton (Paerl et al 2016, Wurtsbaugh et al 2019).

with RR values of 5.5% and 272.0% respectively. As shown in the figure 3, TN and TP mainly enter the ecosystem through runoff from land, which leads to water blooms, or great concentrations of algae, and even fish die. This study revealed the effect of TN and TP on Chla at three concentration levels: lower, medium to high (TN: 64.0%, 99.0%, and 66.0%, TP: 28.0%, 170.0%, and 46.0%).

3.1.1. Effects of TN on Chla
The average effect in the TN of the lakes in China was 5.5% (figure 3). Our model quantified the response of RR to changes in TN was positive and significant across studies (figure 4(b)). We observed a positive relationship between the TN and Chla (figure 5(a), SI figure S1) (Phillips et al 2008, Magumba et al 2014). Whilst analyzing individual studies, we observed that TN (0.75 < TN ≤ 1.4 mg l\(^{-1}\)) have the largest RR flexibility (r = 99% [60, 147], n = 602, P < 0.001) followed by TN > 1.4 mg l\(^{-1}\) (r = 66% [34, 106], n = 250, P < 0.001) and TN (0.75 < TN ≤ 1.4 mg l\(^{-1}\)) (r = 64% [27, 113], n = 172, P < 0.001) in lake (figure 4(b), SI table S2). Generally, the effect of TN on Chla in the lakes showed a positive relationship trend in China. Detailed RR of Chla in TN from different concentrations were listed in table S2.

Figure 4. Overall the effect of TN, TP, and TN/TP on Chla in lake. Dots show means, error bars represent are 95% confidence intervals. The number of mean are displayed in parentheses on the right-hand side of the figure (a), respectively. The number of observations are displayed in parentheses on the right-hand side of the figures (b)–(d), respectively.

3.1.2. Effects of TP on Chla
In the nutrients collected from the lakes in China, Chla was the most effective in the group of TP. Especially, the TP concentration in the middle level (0.025 < TP ≤ 0.05 mg l\(^{-1}\)) was 170%, and the
total impact even reached 272%. Compared with low (TN \leq 0.025 mg l^{-1}, n = 197) or high levels (TP > 0.05 mg l^{-1}, n = 154), research on the middle level (n = 673) was more for the lakes of China. In the range TP > 0.05 mg l^{-1} the changes in Chla was 46% (46% [19, 78], P < 0.001) in lake. However, the TN < 0.025 mg l^{-1} (0.28, [-3.89, 0.71], n = 197, P > 0.05, table S2) was not statistically significant in lake. With increases TP concentration, we found that the impact on Chla will be greater (figure 5(b), SI figure S2), while the RR of high concentration (TP > 0.05 mg l^{-1}, 46%) is smaller than the RR middle concentration (0.025 < TP \leq 0.05 mg l^{-1}, 170%). It is in agreement with the earlier finding that chlorophyll concentration increased slowly at high TP concentrations (Filstrup et al. 2014, Filstrup and Downing 2017). For different concentrations of TP, the changes in Chla showed variation between low, middle, and high levels. The RR showed a decreasing (46% < 154%) trend with increasing TP concentration, which is consistent with the TP and Chla concentration data fitted by Filstrup et al. (2014).

3.1.3. Effects of TN/TP on Chla

TN/TP is one of the widely used indicators to explain the nutrient limitation of phytoplankton. As shown in figure 4(a), the total of the response RR to changes in TN/TP is negative (−0.5%, [−0.7, −0.3], P < 0.05) (table S2), TN/TP \geq 16 have a largest RR (213%, [161, 275], n = 663, P < 0.001), and negative to TN/TP < 16 (−12%, [−23, −5], n = 361, P < 0.05) in lake.

To quantify the interactive effects of TN and TP on variations in Chla in the China lakes, TN and TP were used as the predictor variables, and Chla was chosen as the response variable in regression analysis (figure 5). The same pattern was observed for TN and TP in lakes (TN: R^2 = 0.16, p < 0.001; TP: R^2 = 0.34, p < 0.001, n = 1024). As shown in table 1, the results of our study are consistent with those of previous studies of eutrophic water bodies at the macroscopic scale (Spears et al. 2013, Filstrup and Downing 2017, Zou et al. 2020). For example, the results of Filstrup and Downing (2017) study of 139 lakes in the Midwestern United States and Zou et al. (2020) study of 39 lakes in Eastern Plains ecoregion China are consistent with our findings. However, there are differences with some individual studies, which may be due to spatial and temporal differences, and the amount of data (Mingliang et al. 2012, Huo et al. 2013, Chunni 2018).

3.2. The concentration of TN and TP affects Chla in main lakes of China

Response of Chla trophic states under different combinations of TN and TP concentrations are shown in figure 6. Firstly, we compare the response of the Chla trophic state when both nutrients are at low levels (figure 6(a)) and when both nutrients are at middle levels (figure 6(e)) or high levels of nutrient states (figure 6(i)). When both TN and TP are at low levels of nutrient states, the ln R of Chla Eutrophic is high (2.41) and the ln R of Chla oligo-mesotrophic is low (0.83). However, if the concentration state of TN becomes middle or high levels, the ln R of Chla oligo-mesotrophic decreases greatly to 0.65 and 0.64, respectively. On the one hand, the response of oligo-mesotrophic Chla is 0.66 when both nutrients (TN and TP) are at middle levels and 1.28 for eutrophic Chla. On the other hand, when the TN and TP are at high levels the ln R increased with the Chla increased (figure 6(i)). Therefore, the levels of TN and TP are very important for the effect of lakes Chla. This shows that Chla is limited by nutrients at macroscales.

Next, we discuss the question of whether or not a single (TN or TP) or both (TN and TP) nutrient affects Chla trophic state, because we have established that the nutrients are important for the response of Chla. We kept the levels of the other nutrient constant so that we can explore the effect of one nutrient independent of the other. For example, we can determine the effect of TN on Chla by comparing figures 6(a)–(c). When we set the TP levels to be lower, changing the TN levels from lower (figure 6(a)) to the middle (figure 6(b)) or high (figure 6(c)) will lead to a decrease in the ln R of Chla (a decline from 0.83 when TP is lower to 0.64 when TN is high). Concurrently, we see an increase in the ln R of Chla (an increase from 2.41 when TN is lower to 2.49 when TN is middle), while the data is less convincing when TN concentration is at a high level. When we keep the TP state at the middle (figures 6(d)–(f)) or hyper (figures 6(g)–(i)) levels, we obtain similar results to the results that the effect of TN on Chla.

To determine the effect of TP on Chla, we hold the TN concentration levels. If TN concentration levels are lower (figures 6(a), (d) and (g)), changing the levels of TP from lower to middle or hyper will cause a change effect to Chla based on oligo-mesotrophic and eutrophic trophic states (0.83 when TP is at lower levels, 0.91 when TP is middle levels, and 0.10 when TP is high levels). If TN concentration levels are middle (figures 6(b), (e) and (h)), the oligo-mesotrophic trophic states of Chla changes are little (0.65 when TP is at lower levels, 0.66 when TP is at middle levels, and 0.77 when TP is high levels). If
Table 1. Comparison of our study with other studies.

| References                  | Region and data                  | Parameter | $R^2$  | p-value |
|-----------------------------|----------------------------------|-----------|--------|---------|
| Filstrup and Downing (2017) | Midwestern United States         | TN        | 0.39   | <0.001  |
|                             | 139 lakes                        | TP        | 0.29   | <0.001  |
| Spears et al (2013)         | UK                               | TN        | N/A    | N/A     |
|                             | 95 lakes                         | TP        | 0.81   | <0.001  |
| Chunni (2018)               | Northern lake Taihu, China       | TN        | 0.48   | <0.01   |
|                             | 1 lake                           |           | 0.58   | <0.01   |
| Zou et al (2020)            | Eastern Plains ecoregion, China  | TN        | 0.24   | <0.001  |
|                             | 39 lakes                         | TP        | 0.43   | <0.001  |
| Mingliang et al (2012)      | Poyang lake, China               | TN        | 0.04   | N/A     |
|                             | 1 lake                           | TP        | 0.06   | N/A     |
| Huo et al (2013)            | Eastern plain ecoregion lakes, China | TN   | 0.09   | <0.01   |
|                             | 7 lakes                          | TP        | 0.18   | <0.01   |
| Present study               | China                            | TN        | 0.34   | <0.001  |
|                             | 34 lakes                         | TP        | 0.16   | <0.001  |

N/A = Not applicable.

Note: The bold value stand for our results in present study.

Figure 6. Effects of the Chla trophic state under different combinations of TN and TP (table S3). Significance codes: ‘L’ means low levels of nutrient states, ‘M’ means middle levels of nutrient states, ‘H’ means high levels of nutrient states, ‘NTS’ means nutrients trophic states, ‘Oligo-meso’ = oligo-mesotrophic, ‘Eutro’ = eutrophic, ‘Hyper’ = hyper-eutrophic.

TN concentration levels are high (figures 6(c), (f) and (i)), the eutrophic trophic states of Chla changes are decreased (2.49 > 1.28 > 1.21). Therefore, according to the effect of TN or TP on different Chla trophic states, both TN and TP could influence the Chla lnR, showing that both TN and TP could be limiting.

To determine the relative importance of TN and TP, as both nutrients can affect Chla. We assume that both TN and TP concentrations are at lower levels, and then shift either concentration levels to middle or high concentration. The changes in the TP concentration to a middle or high level will to 72.9% (0.825–0.096) of the oligo-mesotrophic trophic states of Chla and 88.0% (2.41–1.53) of the eutrophic trophic states of Chla (figures 6(a), (d) and (g)). In contrast, the lnR of the TN concentration to a middle or high level only causes a change for 18.5% (0.825–0.64) and 8.0% (2.49–2.41) of the Chla (figures 6(a)–(c)). Moreover, we do not have enough data to evaluate changes in lnR for hyper-eutrophic trophic states of Chla. Therefore, although TN and TP influence the Chla trophic state, TP (72.9% > 18.5%, and 88.0% > 8.0%) is more important than TN. Considering the huge changes of lnR between the TN and TP effect and the large effect of TP on Chla, TP generally dominates in determining Chla trophic state, indicating that TP is more important than TN in limiting Chla.

Finally, we examined whether TN and TP could interactively affect the Chla trophic state. We found that when the TN concentration was at lower levels, changing the TP concentration from lower to middle or high levels would cause an increase in the change of Chla being hypereutrophic (figures 6(a), (d) and (g)). However, when the TN concentration levels were middle or high, the response of Chla being eutrophic or hypereutrophic increased when changing the TP concentration levels from lower to middle or high (figures 6(d)–(f)) in a middle level, figures 6(g)–(i) in high levels). That is, the impact of the TP concentration on the Chla is much larger when the TN concentration levels are middle or high, indicating that TN and TP have a positive interaction in determining the eutrophic or hypereutrophic of Chla. There is a relative decrease in the change to Chla being hyper-eutrophic when the TN or TP changes from middle or high levels to lower levels. Therefore, both nutrients (TN, TP) are important and suggest co-limitation, when the Chla is eutrophic or hypereutrophic.

4. Discussion

4.1. The effect of TN and TP on Chla

We summarized whether the Chla of lakes in China is limited by TN, TP, or both on a macro scale. Although
both the TN and TP affect the Chla, TP generally plays a more important role (272% > 5.5%), which is consistent with most of the earlier synthesis studies (Xu et al 2013, 2015, Schindler et al 2016). Early stages of eutrophication in lakes can be managed by reducing phosphorus alone (Guildford and Hecky 2000, Ma et al 2015). Our results showed that the effects of N are limited when Chla trophic state is oligo-mesotrophic (TP: 64.4% vs TN: 9.6%) and co-limitation of P and N when Chla trophic state is eutrophic (TP: 110% vs TN: 121%) or hypereutrophic (TP: 240% vs TN: 173%). As shown in table 2, a finding consistent with the findings of many earlier studies.

As a representative index of eutrophication, the Chla level has always been a crucial indicator monitored by environmental managers. Importantly, TN/TP is one of the most widely used indicators to explain the nutrient limitation for phytoplankton (Liebig 1842, Redfield 1958, Liang et al 2020). In most cases, the relationships between Chla and TN/TP are fundamental to guiding the lake's eutrophication management. Our results showed a significant decreasing trend of the TN/TP with increasing Chla concentration for the lakes in our database (linear regression line fitted to figure 7). As shown in figure 7, ln(Chla) and ln(TN/TP) have a linear decay relationship. The linear relationships between logarithmically transformed data of TN/TP and Chla have been widely used by environmental managers seeking eutrophication governance (Tong et al 2018). The nutrient limitation condition shifts from P-limitation to that of P and N co-limitation, when the TN/TP approaches the Redfield Ration (7.2 by mass, as shown in figure 7 dashed horizontal line). Three eutrophication trophic were digitized with two solid lines which set the Chla concentration to be 7 µg l$^{-1}$ and 30 µg l$^{-1}$ (oligo-mesotrophic and eutrophic, eutrophic and hypereutrophic). We estimated TN/TP is 16.7 (oligo-mesotrophic and eutrophic), which is close to the proposal of 16 by (Tong et al 2018). At this time, the estimated TN/TP is 24.4 (eutrophic and hypereutrophic), which is close to the proposal of 22 by (Guildford and Hecky 2000).

Generally, P and N are most related to the Chla trophic state in the lake. The release of P from the sediment and decomposition of phytoplankton was the key process to the release of P. However, the N cycle has natural removal mechanisms at the water-body, such as denitrification, which can decrease N inputs. The denitrification increases with increased water eutrophication because of the increased organic matter. So, P inputs are often retained at higher proportions than N inputs. Globally, TN/TP is decreasing trend with increasing Chla concentration. Yan et al (2016) found a similar negative relationship between the TN/TP and Chla from 157 publications data. In addition, Liang et al (2020) found a similar negative relationship between the TN/TP and Chla analyzed data from 1382 lakes in 17 US states with a probabilistic machine learning method, Bayesian Network. Similarly, the TN/TP decreased with the eutrophication in lakes such as Taihu, Dianchi, and

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**Table 2.** Comparison of our study with other eutrophic TN and TP limitation studies.

| References          | Chla state       | Limiting nutrient | Lake name          |
|---------------------|------------------|-------------------|--------------------|
| Yan et al (2019)    | Oligo-mesotrophic| TP                | Lake Chenghai      |
| Xu et al (2013)     |                  |                   | Lake Taihu         |
| Paerl et al (2011)  | Eutrophic or Hypereutrophic | TP&TN | Lake Taihu         |
| Wu et al (2017)     |                  |                   | Lake Dianchi       |
| Ma et al (2015)     | Oligo-mesotrophic| TP                | 34 lakes, China    |
| Present study       | Eutrophic        | TP &TN            | 34 lakes, China    |
|                     | Hypereutrophic   | TP &TN            | 34 lakes, China    |

TP: P limitation; TP & TN: N & P co-limitation; (TP: 64.4% TN: 9.6%).

* Effect of TN and TP on chlorophyll.
The effect of TP or TN on different Chla trophic states (kept the state of other nutrient, tables S4 and S5).

Figure 8. The effect of TP or TN on different Chla trophic states (kept the state of other nutrient, tables S4 and S5).

some American or Canadian lakes (Dove and Chapra 2015, Yan et al 2016, Tong et al 2018).

Overall, our meta-analysis was performed using 61 individual research data that a wide range of trophic states, and because our results are supported by single lake studies. Therefore, our findings are significant to understand the limitations of TN or TP and provide some reference to eutrophication managers. For example, we can decide whether to limit N or P under different Chla trophic states according to the concentration threshold of TN and TP.

4.2. Significance for management of lake eutrophication

As shown in figure 8, we quantitatively analyzed the response of Chla to different TN and TP concentrations. However, considering the temporal and spatial variations of lake information, it is impossible to have a strategy that can be suitable for the eutrophication management of all lakes. Therefore, our results are not omnipotent but can apply to general guidelines for many lakes. It can provide important prior information for eutrophication management, especially for lakes that have little data.

On the one hand, when we keep the TN concentration unchanged. Our results showed, decreasing the concentration of TN and TP is useful for the recovery of hypereutrophic lakes. The response of Chla being hypereutrophic reduced a large when TP concentration from high to middle level (54.0%, 2.4–1.56, figure 8(a)). Similarly, the lnR of Chla being eutrophic reduced a large when TP concentration from high to middle level (29.0%, 1.39–1.1, figure 8(a)). For the recovery of hypereutrophic lakes, decreasing concentrations of TN also can reduce the lnR of Chla being hypereutrophic when we keep the TP concentration unchanged. The lnR of Chla being hypereutrophic would be reduced by 17.0% (1.73–1.56, figure 8(b)) when the TN concentration becomes middle level. In other words, the eutrophic or hypereutrophic lake is more suitable for simultaneously controlling the input of N and P, or reducing the input of P alone.

4.3. The importance large dataset and the use of a meta-analysis

The novelty of our research is examining effects of N and P across 41 lakes (61 studies) in China. We emphasize the importance of using a dataset with a large number of lakes and a variety of ecological contexts, and then quantified the effect of TN and TP on Chla with meta-analysis methods. The analysis of our national lake eutrophication database revealed that different concentrations of TN and TP influences Chla status, although the consistency and magnitude of the effect varies across the lake.

Our work also highlights the innovative application of meta-analysis in exploring the effect of TN and TP on Chla at macroscales. As shown in figures 4 and 5, under certain concentration levels, the lnR of Chla is not deterministic. Fortunately, meta-analysis can obtain quantitative results by collecting peer-reviewed articles. As Hedges et al (1999) said, meta-analysis is the quantitative, scientific synthesis of research results. We also argue that the meta-analysis could be encouraged as an effective tool for use in macrosystem studies. Firstly, the variance between groups in the mixed-effects model explains the impact of the spatial heterogeneity of the ecological environment and reduces the bias. Secondly, our findings can provide targeted strategies for eutrophication management, but we still need to be cautious because some data are rare. Finally, open science and big data have emphasized unbiased access to research data, which is of longstanding importance in meta-analysis. On this basis, we can get more peer-review meta-data, so that the results are more robust. This will provide an important reference for formulating eutrophication strategies from a macro perspective.

Despite its current utility and future potential, meta-analysis has various limitations as a tool. For example, this study evaluated the effects of TN, TP, and TN/TP on the Chla trophic state in lakes at macroscales in China. The predictions of their net effects are difficult to generalize because these factors may depend on the Secchi depth, vegetation type/composition, and time scale. Our analysis did not address the impact of these additional factors. Therefore, future work needs to be carried out more factors.

5. Conclusion

The findings of this study have extensive implications for the evaluation and management of water quality in lakes. Based on the results of this meta-analysis, we conclude that the effects of TP on Chla are significantly greater than that of TN in 34 lakes in China. In this study, the average response of Chla to TP (272.0%), was higher than TN (5.5%) and
TN/TP (−0.5%) collected from the metadata. Especially when the Chla trophic state change from hyper-eutrophic to eutrophic, compared with TN, reducing TP can significantly reduce Chla (54.0% vs. 17.0%). The relative effect of the three factors varied temporally and differed across Chla concentrations. TN has the greatest impact on the Chla of the lakes at lower concentrations, and the input of nitrogen should be controlled first for eutrophication management. The change in TP is the dominant contributor to eutrophication (170.0%) in middle concentrations. In addition, both TN and TP contributed to eutrophication (TN 66.0% and TP 46.0%) in high concentrations, and then need control of TN and TP input in eutrophication management. These results suggest that eutrophication management may have to consider the effect of N and P interactions on Chla and for eutrophication management in various water bodies. Our findings enhance the understanding of nutrient limitation on Chla, which could facilitate the formulation of management strategies for eutrophication and provide prior information for the eutrophication management in various water bodies.

Data availability statement

The data that support the findings of this study are openly available at the following URL/DOI: https://github.com/GhuangHui/article-code-and-data.

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