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Controlling cyanobacterial blooms by managing nutrient ratio and limitation in a large hyper-eutrophic lake: Lake Taihu, China

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ABSTRACT

Excessive nitrogen (N) and phosphorus (P) loading of aquatic ecosystems is a leading cause of eutrophication and harmful algal blooms worldwide, and reducing nutrient levels in water has been a primary management objective. To provide a rational protection strategy and predict future trends of eutrophication in eutrophic lakes, we need to understand the relationships between nutrient ratios and nutrient limitations. We conducted a set of outdoor bioassays at the shore of Lake Taihu. It showed that N only additions induced phytoplankton growth but adding only P did not. Combined N plus P additions promoted higher phytoplankton biomass than N only additions, which suggested that both N and P were deficient for maximum phytoplankton growth in this lake (TN:TP = 18.9). When nutrients are present at less than 7.75–13.95 mg/L TN and 0.41–0.74 mg/L TP, the deficiency of either N or P or both limits the growth of phytoplankton. N limitation then takes place when the TN:TP ratio is less than 21.5–24.7 (TDN:TDP was 34.2–44.3), and P limitation occurs above this. Therefore, according to this ratio, controlling N when N limitation exists and controlling P when P deficiency is present will prevent algal blooms effectively in the short term. But for the long term, a persistent dual nutrient (N and P) management strategy is necessary.

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Introduction

Nitrogen (N) and phosphorus (P) are essential elements for the synthesis of nucleic acids, ATP and proteins, which are necessary for cell division and growth (Conley et al., 2009). The cycles of the key macronutrients N and P have been massively altered by anthropogenic activities (Canfield et al., 2010; Elser et al., 2007). N

and P over-enrichment accelerates eutrophication in aquatic ecosystems and has promoted harmful algal blooms worldwide (Conley et al., 2009; Paerl et al., 2011a; Schindler and Hecky, 2009). Some of our largest aquatic ecosystems are now experiencing severe cyanobacterial blooms (Paerl et al., 2011a). The strongest stimulation of algal productivity is usually observed when N and P enrichment occurs simultaneously (Elser et al., 2007; Lewis and

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Wurtsbaugh, 2008; Paerl et al., 2008). These results suggest that there is a need to reduce both N and P inputs to prevent further eutrophication and harmful algal bloom expansion (Paerl and Otten, 2013; Paerl and Paul, 2012; Xu et al., 2010).

Nutrient control strategies strongly rely on nutrient limitation studies. The TN:TP ratio has been widely proposed as a means to identify whether phytoplankton is N or P limited (Healey and Hendzel, 1980; Hecky et al., 1993; Smith, 2006; Verburg et al., 2013). Previous studies to predict which nutrient is limiting have often applied the hypothesis that there is an optimal ratio of N and P supply rates that promotes maximum rates of primary production (Redfield, 1958; Schanz and Juon, 1983). The most commonly cited ratio is the Redfield ratio, which states that marine algal cells contain N and P in a 16:1 (atomic) ratio on average (Redfield, 1958). Thus, assuming that nutrient concentrations in the water reflect supply rates and according to Liebig's law of the minimum (Liebig, 1842), algae should be limited by N if the water N:P ratio is lower than 16 on an atomic basis (TN:TP of 7.2). In contrast, they should be limited by P if the ratio is above that.

However, shifts from N to P limitation have often been found to occur at ratios different from the Redfield ratio in many field observations (Bergström, 2010; Elser et al., 2009; Guildford and Hecky, 2000; Morris and Lewis, 1988). In both lakes and oceans, N limitation was apparent at TN:TP < 9 whereas P limitation consistently occurred when TN:TP > 22.6, and either N or P could become deficient for ratios in the range 9 < TN:TP < 22.5 (Guildford and Hecky, 2000). In 106 oligotrophic lakes across Europe and North America, 72% of the variation in the response of algae to nutrient-enrichment assays revealed that phytoplankton growth shifted from N limitation at DIN:TP mass ratios < 1.5 to P limitation at DIN:TP mass ratios > 3.4 (Bergström, 2010). Schanz and Juon (1983) have reported N limitation of stream microphytobenthos at atomic N:P ratios < 10 and P limitation at N:P ratios > 20. Moreover, the N:P ratio also failed to predict the nutrient-limiting status in some cases (Francoeur et al., 1999; Wang et al., 2008).

To provide a rational strategy and predict future trends of eutrophication in eutrophic lakes, we need to understand the relationships between nutrient ratio, absolute nutrient concentration and nutrient limitation.

Lake Taihu, a large, shallow and hyper-eutrophic lake in China, has suffered annual cyanobacterial blooms from May to October and has changed from bloom-free to bloom-plagued conditions over the past 3 decades (Chen et al., 2003b; Paerl et al., 2011b). Approximately 40 million people live in cities and towns within the Taihu watershed. The lake is a key drinking water source for the local human population (estimated to be about 10 million), with tourism, fisheries, and shipping being additional important economic functions. Ironically, it is also a repository for waste from urban centers and nearby agricultural and industrial segments of the rapidly growing local economy (Qin et al., 2007).

Anthropogenic activities are likely to accelerate nutrient loading to Lake Taihu. Here, we analyzed the results of field experiments to evaluate the responses of phytoplankton biomass to manipulations of N and P availability in Lake Taihu water using a set of outdoor bioassays. Our goals were to examine the effects of individual and combined N and P additions on phytoplankton growth and determine the relationship between nutrient ratio and nutrient limitation in Lake Taihu, using a nutrient addition bioassay incubated under natural light and temperature conditions during the bloom period.

1. Materials and methods

1.1. Bioassay

A set of bioassays was conducted from 10 to 28 September, 2012 (Ma et al., 2014). Lake water samples were incubated

under natural light and temperature conditions at the Taihu Laboratory for Lake Ecosystem Research (TLLER), located on the shore of Lake Taihu, near Wuxi, China. We prepared 48 white plastic buckets (maximum volume of 35 L) and then pumped 30 L water from the lake into each bucket. N and P were added as NaNO₃ and KH₂PO₄ respectively as three treatments (N only, P only and N plus P). The added nutrient concentrations of treatments were 2, 4, 8, 16 and 32 times relative to the control (lake water with no additions) concentrations (Table 1). Each treatment was performed in triplicate. The pH, dissolved oxygen (DO), dissolved oxygen saturation (DO%), and electrical conductivity (EC) were measured in each bucket between 8:00 and 9:00 on days 0, 3, 6, 9, 12, 15, and 18 by a Yellow Springs Instruments (YSI) 6600 multi-sensor sonde (YSI Incorporated, Brannum Lane, Ohio, USA). We sampled 0.5 L water from each bucket in order to determine the chlorophyll *a* (Chl-*a*) concentration and total phytoplankton biovolume. Weather conditions and air temperature were recorded every day. Each bucket was stirred before sampling and twice daily at 7:00 and 19:00. Chl-*a* concentrations were determined spectrophotometrically after extraction in 90% hot ethanol (Párista et al., 2002). Phytoplankton samples were preserved with Lugol's iodine solution (2% final concentration) and sedimented in a plastic bottle for 48 hr. Cell density was measured with a Sedgwick-Rafter counting chamber under magnification of 200×–400×. Algal biovolumes were calculated from cell numbers and cell size measurements. Conversion to biomass was calculated by assuming that 1 mm³ of volume was equivalent to 1 mg of fresh weight biomass (Chen et al., 2003a).

1.2. Statistical analysis

Data are presented as means ± SD. Significant differences between control and treated samples were determined by analysis of variance with the Tukey post-hoc test. Statistical analyses were conducted with SPSS 17.0 (IBM, Armonk, State of New York, USA).

The growth rate (μ) under each set of treatment conditions was calculated according to the modified exponential growth equation,

$$\mu = \frac{\ln(X_2/X_1)}{T_2 - T_1}$$

where, X_1 (mg/L) is the concentration of Chl-*a* on day 0 (T_1), and X_2 (mg/L) is the concentration of Chl-*a* on day 18 (T_2).

2. Results

2.1. Environmental factors

The weather conditions were cloudy (days 0–2, day 4, day 11, days 17–18), rainy (day 3 and day 12) and sunny (days 5–10, days 13–16). Air temperature ranged from 16 to 28°C. Water temperature ranged from 18.8 to 22.5°C. The pH, dissolved oxygen (DO) concentration, dissolved oxygen saturation (DO%), and electrical conductivity (EC) in N plus P additions were greater than those observed in either N or P additions or controls (Table 2).

Table 1 – Nutrient concentrations (mg/L) and ratios in the experiment.

Buckets	TN	TDN	TP	TDP	TN:TP (+N)	TDN:TDP (+N)	TN:TP (+ P)	TDN:TDP (+ P)	TN:TP (+N plus P)	TDN:TDP (+N plus P)
Initial water	1.55	0.95	0.082	0.053	18.9	17.9	18.9	17.9	18.9	17.9
+2×	4.65	4.05	0.246	0.217	56.7	76.4	6.3	4.4	18.9	18.7
+4×	7.75	7.15	0.41	0.381	94.5	134.9	3.8	2.5	18.9	18.8
+8×	13.95	13.35	0.738	0.709	170.1	251.9	2.1	1.3	18.9	18.8
+16×	26.35	25.75	1.394	1.365	321.3	485.9	1.1	0.7	18.9	18.9
+32×	51.15	50.55	2.706	2.677	623.8	953.8	0.6	0.4	18.9	18.9

TN: total nitrogen; TP: total phosphorus; TDN: total dissolved nitrogen; TDP: total dissolved phosphorus; +N: nitrogen added; +P: phosphorus added; +N plus P: nitrogen and phosphorus added; +2× (4×, 8×, 16× and 32×):2 (4, 8, 16 and 32) times nitrogen, phosphorus or the both added.

2.2. N, P and N plus P addition results: TN:TP ratio changes and impact on phytoplankton growth

In the initial lake water, the TN:TP ratio was 18.9 (TDN:TDP = 17.9). The TN:TP ratios were 56.7–623.8 (TDN:TDP = 76.4–953.8) in N addition buckets, 0.6–6.3 (TDN:TDP = 0.4–4.4) in P addition buckets and remained at 18.9 in N and P addition buckets (Table 1). Biomass (Chl-*a*) in the initial lake water was (20.46 ± 0.82) $\mu\text{g/L}$. Chlorophyll *a* concentrations in N only additions were significantly higher than those in controls after 12 days of incubation ($p < 0.05$) but there was no significant difference between 2× and 32× (Fig. 1a). The biomass in P only additions was not significantly different from controls and there was no significant difference between 2× and 32× additions ($p > 0.05$, Fig. 1b). The biomass of N plus P additions was much higher than that of control and N only additions from day 3 ($p < 0.05$, Fig. 1c). The 2× N plus P addition promoted a 176% increase in biomass above all N only additions on day 18 ($p < 0.01$; Fig. 1a, c). The biomass of 4× N plus P addition was higher than that of 2×, with no significant difference ($p > 0.05$), and significantly lower than that of 8× addition ($p < 0.05$; Fig. 1c). The biomass response of the 8× N plus P addition (TN = 13.95 mg/L, TP = 0.74 mg/L in buckets) was the highest (281.6 ± 28.9 $\mu\text{g/L}$) on day 15 but showed no significant difference with 16× or 32× additions during the experimental period ($p > 0.05$; Fig. 1c).

The linear relationship between Chl-*a* and total phytoplankton biovolume in all treatments from day 0 to day 18 was clear and significant (Fig. 2). Genus *Microcystis* and *Cosmarium* were dominant in initial lake water and dynamic in the experiment.

Table 2 – Range of pH, DO, DO% and EC in the experiment.

Parameter	Control	N additions	P additions	N plus P additions
pH	8.91–9.41	8.79–9.46	8.97–9.54	9.45–11.28
DO (mg/L)	8.95–12.24	9.27–14.66	9.08–11.13	9.1–19.64
DO%	102.9–113.4	105.3–120.9	101.1–124.7	101.4–232.3
EC ($\mu\text{S/cm}$)	435–638	439–1211	428–698	488–1346

The pH, dissolved oxygen (DO), dissolved oxygen saturation (DO%), and electrical conductivity (EC) were measured between 8:00 and 9:00 on days 0, 3, 6, 9, 12, 15, and 18.

During the experiment, the growth rate of the phytoplankton assemblages in the control was (0.009 ± 0.008) day^{-1} . In the N only additions, growth rates ranged from 0.034 to

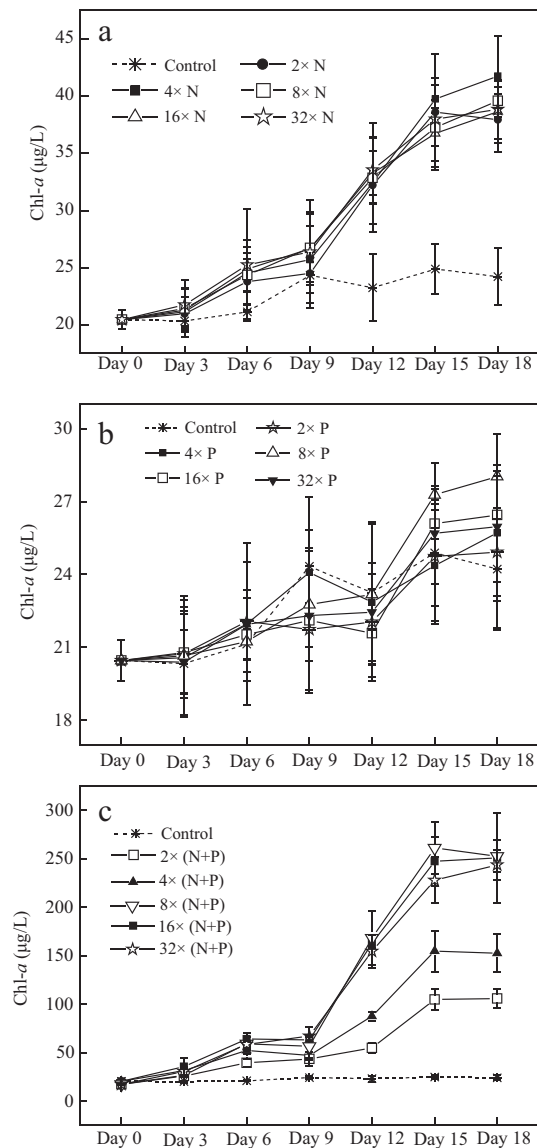


Fig. 1 – Phytoplankton biomass (Chl-*a*) changes in bioassays with added N (a), P (b) and N plus P (c). Controls were the same in all treatments.

0.039 day⁻¹, and had no significant differences with each other from 2× to 32× ($p > 0.05$) but were significantly higher than controls ($p < 0.01$). In P only additions, growth rates ranged from 0.008 to 0.015 day⁻¹, and showed no significant differences from controls and each other over the 2× to 32× range ($p > 0.05$). In N plus P additions, growth rates ranged from 0.096 to 0.143 day⁻¹. There were no significant differences between 8×, 16×, or 32× additions ($p > 0.05$), which were higher than 4× ($p < 0.05$) and 2× ($p < 0.01$). Growth rates of N plus P additions were about 3× higher than N additions and 10× higher than P additions or controls (Fig. 3).

The pH values for control and N only and P only additions were always below 10. However, due to the vigorous growth of phytoplankton in N plus P additions, values rose to above 10 (Fig. 4).

3. Discussion

N plus P additions promoted the highest biomass, suggesting that both N and P supplies were controlling algal production in the lake (TN:TP = 18.9). Both N and P input together promoted higher growth rates than N or P separately (Fig. 1a, c), which has been shown by Xu et al. (2010) and Paerl et al. (2011b). Compared to all N only additions, 8× N plus P addition (13.95 mg/L TN and 0.74 mg/L TP in buckets) promoted primary productivity about 5 times (Fig. 1a, c). More interestingly, phytoplankton biomass and growth rates showed no significant difference between 8×, 16× and 32× N plus P additions, which indicated that nutrient supply was sufficient for the maximum growth of phytoplankton in these conditions. Hence, when concentrations more than about 4×–8× N plus P additions (7.75–13.95 mg/L TN and 0.41–0.74 mg/L TP) were added, growth of phytoplankton assemblages in the bioassay was neither N nor P, nor N and P co-limited (Fig. 1). In response to these additions phytoplankton grew rapidly, which may have induced CO₂ limitation as indicated by the very high pH levels (Fig. 4) (Talling, 1976). There is preference for CO₂ over HCO₃⁻ and CO₃²⁻ as a photosynthetic carbon source, but under these conditions there is little free CO₂ in water when pH 10 is reached (Gavis and Ferguson, 1975; Paerl and Ustach,

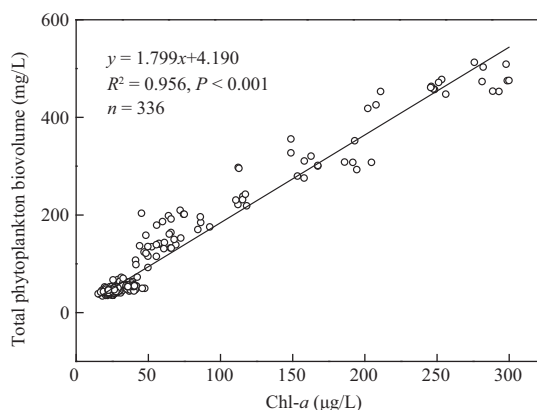


Fig. 2 – Relationship between Chl-a and total phytoplankton biovolume in all treatments from day 0 to day 18.

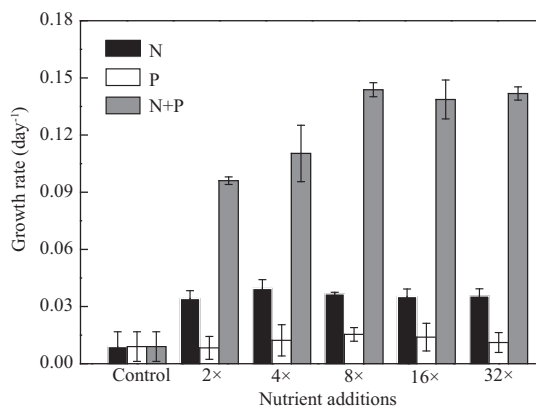


Fig. 3 – Growth rates of phytoplankton assemblages in experimental period. Water samples for bioassays were collected from buckets which had added N, P or N and P. Nutrient concentrations are relative to initial lake water (control). Error bars represent ± SD of triplicate samples. Controls were the same in all cases.

1982). Thus, the high pH conditions which were promoted by N plus P additions (Fig. 4) likely promoted inorganic CO₂ limitation.

N only additions (TN:TP = 56.7–623.8) promoted phytoplankton growth and P only additions (TN:TP = 0.6–6.3) did not (Table 1, Fig. 1), indicating that N limitation existed in the initial lake water (TN:TP = 18.9). Phytoplankton growth showed no significant difference between 2× to 32× N only additions, suggesting that 2× N additions to the initial lake water (TN:TP = 56.7) resulted in P limitation. Hence, N limitation occurred when TN:TP ≤ 18.9–56.7 and shifted to P limitation when TN:TP ≥ 18.9–56.7. We examined data from previous studies conducted in every season in Lake Taihu (Xu et al., 2010, 2013). It indicated that the TN:TP ratio threshold needed to obtain N- or P-limited conditions was between 21.5 and 24.7 (Table 3) in this lake when less than 7.75–13.95 mg/L TN and 0.41–0.74 mg/L TP were present. In addition, considering that phytoplankton cannot take up particulate nitrogen (PN) and particulate phosphorus (PP), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) may reflect the conditions in reality. The TDN:TDP ratio threshold shift for N or P limited conditions was 34.2–44.3 under 7.15–13.35 mg/L TDN, 0.381–0.709 mg/L TDP (Tables 1 and 3).

This is different from the Redfield ratio, and also different from numerous previous studies. Bioassay experiments of 19 Kansas (USA) reservoirs showed N limitation when TN:TP ratios were <8.1, co-limited by N and P when TN:TP ratios were between 9 and 20.8, and P limitation when TN:TP ratios >29.4 (Dzialowski et al., 2005). Elser et al. (2009) have reported that a large majority of experiments indicated N-limited phytoplankton growth when TN:TP was below ~20, and phytoplankton growth was consistently P-limited when above ~49.7.

Although numerous TN:TP ratio studies have been conducted, contradictory results have been noted in these works. Three factors may explain why these ratio shifts from N to P limitation were different. Firstly, a major reason could be

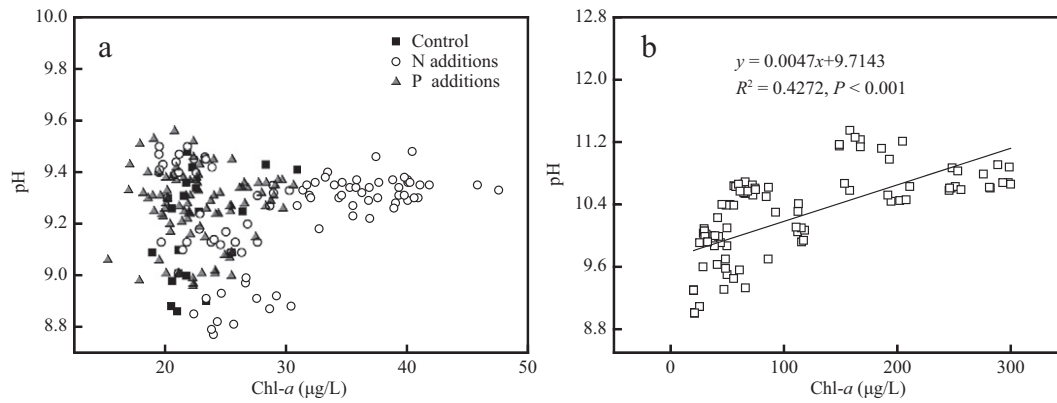


Fig. 4 – The relationship between pH and Chl-*a* concentration. In control, N only additions and P only additions, pH values were below 10 and had no significant relationship with Chl-*a* (a). However, a significant relationship occurred in N plus P additions (b, $n = 105$).

ascribed to the different forms of N and P present. TN and TP could not reflect the bioavailable N and P species in different lakes. As the bioavailable N and P species are dynamic and varying in different lakes, one is unlikely to obtain a common conclusion by measuring TN and TP. Secondly, our understanding of nutrient limitation is derived from Liebig's Law of the Minimum (Liebig, 1842). Liebig's Law of the Minimum only originally described the limitation on individual crop plants, but it has been extended to communities of plants and ecosystems (Danger et al., 2008). At the community level, a new layer of complexity exists because communities are comprised of many species, so that limiting ecological factors may be shared or unique adaptations (North et al., 2007). Thirdly, N or P limitation cannot be measured under conditions where another factor may control the growth of phytoplankton. For example, only the addition of Fe significantly stimulated *Microcystis* spp. growth, while addition of N and/or P had no effects on growth, indicating that Fe was a primary limiting nutrient in East Taihu (Xu et al., 2013).

Freshwater ecosystems tend to be P limited, but as the trophic state increases (i.e., becomes more eutrophic), these systems tend to show a higher frequency of N limitation or N

plus P co-limitation. TN:TP is high in oligotrophic lakes and very low in eutrophic lakes, declining with increased TP (Downing and McCauley, 1992). Competitive equilibrium favors greater allocation to P-poor resource-acquisition cell machinery, leading to a higher N:P ratio, and exponential growth favors greater allocation to P-rich assembly machinery, resulting in a lower N:P ratio (Klausmeier et al., 2004). N limitation is not only more frequent in lakes of low ambient TN:TP (TN:TP mass ratio ≤ 14) but is also more frequent in lakes with TP ≥ 0.03 mg/L (Downing and McCauley, 1992).

TN:TP ratios in Lake Taihu showed strong seasonal variations, with the highest values (52:1 to 212:1) during the low productivity season (winter) and lowest values (20:1) during the high productivity season (spring-fall). This pattern supports the seasonal bioassay results, which indicated that P limitation occurs in winter and spring, and, N limitation in summer and fall (Xu et al., 2010). When the TN:TP ratio exceeded 29:1 by weight, cyanobacteria tended to be rare, suggesting that modification of this ratio may provide a means of lake water quality management (Smith, 1983). Thus, the TN:TP ratio that we determine not only can be an effective tool for assessing potential nutrient limitations in

Table 3 – Bioassays in Lake Taihu suggesting the relationship between TN/TP, TDN/TDP ratio and nutrient limitation.

Source	TN (mg/L)	TP (mg/L)	TN:TP	TDN (mg/L)	TDP (mg/L)	TDN:TDP	Limitation
Xu et al. (2013)	1.89	0.2	9.5	0.8	0.05	16	N
Xu et al. (2010)	2.29	0.169	13.6	1.43	0.062	23.1	N
Xu et al. (2010)	1.63	0.103	15.8	0.98	0.035	28	N
Xu et al. (2013)	2.12	0.126	16.8	0.82	0.024	34.2	N
This research	1.55	0.082–2.706	≤ 18.9	0.95	0.053–2.677	0.6–18.9	N
Xu et al. (2013)	0.99	0.046	21.5	0.46	0.017	27.1	N
Xu et al. (2010)	2.37	0.096	24.7	1.55	0.035	44.3	P
Xu et al. (2010)	3.42	0.133	25.7	2.51	0.044	57	P
This research	4.65–51.15	0.082	≥ 56.7	4.05–50.55	0.053	56.7–623.8	P
Xu et al. (2013)	0.85	0.014	60.7	0.75	0.007	107.1	P
Xu et al. (2013)	0.76	0.012	63.3	0.62	0.005	124	P
Xu et al. (2013)	2.36	0.013	181.5	2.21	0.006	368.3	P

N: N limitation; P: P limitation.

Lake Taihu, but also can serve as a threshold to prevent cyanobacterial blooms. During winter and spring, TN:TP ratios ranged from 33:1 to 80:1 in Meiliang Bay, but in summer, this ratio dropped below 20:1. The ratios in the central lake varied between 30:1 and 64:1 during winter and spring and then declined in late summer to early fall below 20:1 (Xu et al., 2010). Hence, in summer and fall, controlling N will enhance N limitation. Conversely, controlling P in winter and spring will increase P limitation.

Overall, deficiency of either N or P or both is limiting to phytoplankton growth when less than 7.75–13.95 mg/L TN and 0.41–0.74 mg/L TP are present. In this study, high concentrations of N (with low P) or P (with low N), and low N plus P concentrations, could not promote continuing phytoplankton growth because of P, N or dual limitations. This supports most environmental policy that controls the input of nutrients into watersheds and maximizes its removal (Smith and Schindler, 2009). Phytoplankton biomass can be decreased substantially by lowering nutrient inputs (Brookes and Carey, 2011). Obviously, it does not matter whether N, P, or both are controlled since each strategy will inhibit the growth of algae if used properly. Therefore, different control strategies should be implemented according to the actual situation in different aquatic ecosystems. Previous studies in Lake Taihu (Xu et al., 2010; Paerl et al., 2011b) have suggested a dual N and P control strategy in the long term. Based on this strategy and according to the TN:TP or TDN:TDP ratio, controlling N when N is limiting and controlling P in case of P deficiency will prevent cyanobacterial blooms effectively in the short term.

It is worth noting that even bigger containers are different from the environment of natural lakes. For example, such bioassays change the hydrodynamics and the degree of mixing relative to light attenuation, and have no sediment. Hence, further research on the use of TN:TP and TDN:TDP ratios in lake management is needed.

4. Conclusions

When nutrients were present at less than 7.75–13.95 mg/L TN and 0.41–0.74 mg/L TP, the TN:TP ratio threshold to identify N or P limitation was 21.5–24.7 (TDN:TDP was 34.2–44.3) in experiments conducted on water from Lake Taihu. In addition, both N and P were below levels required for maximum phytoplankton growth in this lake (TN:TP = 18.9). The deficiency of either N or P or both limits the growth of phytoplankton. Based on the dual N and P control strategy of previous studies, controlling N when N is limiting and controlling P when P is deficient will prevent algal blooms effectively in the short term.

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