Critical nutrient thresholds needed to control eutrophication and synergistic interactions between phosphorus and different nitrogen sources

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Abstract Eutrophication is one of the greatest threats to global freshwater ecosystems. The phytoplankton responses to nutrient inputs vary in different water bodies, so it is particularly important to determine the nutrient thresholds and synergistic interactions between nutrients in different freshwater ecosystems. Field sampling and bioassay experiments were conducted to determine the thresholds of soluble reactive phosphorus (SRP), nitrate-nitrogen ($\text{NO}_3\text{-N}$), and ammonium-nitrogen ($\text{NH}_4\text{-N}$) in Miyun Reservoir. A separate nutrient addition bioassay was designed to assess the synergistic interactions between these nutrients. Chlorophyll a (Chl a) concentrations were used to estimate phytoplankton biomass. The results showed the following: (1) nutrient threshold bioassay indicated that eutrophication thresholds of SRP, $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ should be targeted at below 0.04 mg P L^{-1} , 0.5 mg N L^{-1} , and 0.3 mg N L^{-1} , respectively, to limit the growth of phytoplankton. (2) The stimulatory effect of “ $\text{NH}_4\text{-N}$ plus P” on phytoplankton biomass was greater than “ $\text{NO}_3\text{-N}$ plus P” at the same N concentration, and “ $\text{NH}_4\text{-N}$ plus $\text{NO}_3\text{-N}$ ” did not

show such associated stimulatory effect as “ $\text{NH}_4\text{-N}$ plus P” or “ $\text{NO}_3\text{-N}$ plus P”. (3) The average concentrations of total phosphorus (TP), $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ in Miyun Reservoir were $0.017 \text{ mg P L}^{-1}$, $0.620 \text{ mg N L}^{-1}$, and $0.143 \text{ mg N L}^{-1}$, respectively. The reservoir-wide average Chl a is below $20 \mu\text{g L}^{-1}$ on an annual basis. (4) Ammonium was an important factor for the growth of phytoplankton and inputs of both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ should be reduced to control bloom formation. Our findings imply that although P load reduction is important, appropriate reductions of all forms of N in watershed is recommended in the nutrient management strategy for Miyun Reservoir.

Keywords Nutrient threshold · Synergistic interaction · Multiple nutrient limitations · Ammonium · Eutrophication · Bioassays

Introduction

Eutrophication is one of the most common and greatest threats to freshwater ecosystems worldwide (Donald et al. 2011; Smith 2003). The primary symptom of eutrophication is excessive growth of aquatic autotrophs, including phytoplankton, periphyton, and macrophytes (Lewis et al. 2011). These blooms represent a serious threat to drinking water supplies and strongly affect the ecological and economic sustainability of our freshwater ecosystems (Paerl et al. 2011). The reduction of anthropogenic nutrient inputs has been widely recognized as necessary to mitigate the negative effects of eutrophication (Smith 2003). The phytoplankton responses to nutrient inputs vary in different water bodies, so it is particularly important to determine the limiting nutrient factors and nutrient thresholds in freshwater ecosystems (Yang et al. 2009).

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The concept of ecological threshold emerged in the 1970s and is defined as the point at which there is an abrupt change in an ecosystem quality, property, or phenomenon or where small changes in an environmental driver may produce large responses in the ecosystem (Groffman et al. 2006). An important application of ecological thresholds is to determine the critical loads of pollutant that an ecosystem can safely assimilate before there is a change in ecosystem state (Groffman et al. 2006). One of the best-studied state shifts is the sudden loss of water transparency and submerged plants when human-induced eutrophication occurs (Scheffer et al. 2001). Nutrient threshold is defined as the critical levels of N and P that control an abrupt change or regime shift such as sudden and protracted dominance by phytoplankton (Xu et al. 2014). Previous studies have shown that eutrophication thresholds of total phosphorus (TP) for freshwaters are from 0.02 to 0.10 mg P L⁻¹ and of total nitrogen (TN) are from 0.50 to 1.00 mg N L⁻¹ (Xu et al. 2010). Abundant evidence has proven that understanding and applying ecological thresholds is the key to successful environmental management (Groffman et al. 2006). Keeping a nutrient concentration below its threshold is a more practical and economical basis for setting nutrient criteria than reducing nutrient concentrations to as low levels as possible.

Our historical understanding of nutrient limitation is single-nutrient limitation according to Liebig's Law of the Minimum (von Liebig and Gregory 1842). It is generally accepted that in marine and estuarine systems, phytoplankton tend to be nitrogen-limited, whereas in freshwater systems, phytoplankton tend to be phosphorus-limited (Chaffin et al. 2014; Hecky and Kilham 1988; Smith 1984). Recent work has begun to call attention to synergistic interactions between limited supplies of N and P across aquatic and terrestrial systems (Elser et al. 2007; Harpole et al. 2011; Paerl et al. 2014). Quantifying the significance of N and P to phytoplankton growth informs decisions about whether one nutrient should be controlled preferentially, or both nutrients should be managed with equal emphasis (Lewis et al. 2011). Multinutrient colimitation of growth rates can occur when two nutrients are below the threshold of uptake and phytoplankton growth is stimulated by the simultaneous addition of both nutrients (North et al. 2007). Furthermore, the nutrient limitation is classified into four categories according to Harpole et al. (Fig. S1) (Harpole et al. 2011; Kolzau et al. 2014): (1) single limitation: response to only a single resource when added individually (+N or +P) and the response to the +NP treatment is no different (Fig. S1-A). (2) Serial limitation: response to only a single nutrient treatments (+N or +P) but a larger response to the combined treatment (+NP) (Fig. S1-B). (3) Independent colimitation: response to both single nutrient treatments when added individually and a larger response to the +NP treatment (Fig. S1-C). (4) Simultaneous colimitation: response only occurs if both resources are added simultaneously (Fig. S1-D).

The need of algae for inorganic nitrogen is satisfied by ammonium and nitrate together. Despite ambient ammonium concentrations, one or more orders of magnitude lower than ambient nitrate concentrations, in a previous study, phytoplankton showed preference for ammonium (Gardner et al. 2004). This is mainly because less energy is required to incorporate and assimilate reduced forms of N (ammonium and dissolved free amino acids) than oxidized N forms (nitrate and nitrite) (Donald et al. 2013; Gardner et al. 2004). In addition, ammonium (NH₄⁺) recycling may be equally rapid with phosphate (PO₄³⁻) recycling (Paerl et al. 2011). However, in most studies, the eutrophication threshold of N referred to the threshold of nitrate-nitrogen (NO₃-N), and little information is available about the threshold range of ammonium-nitrogen (NH₄-N) (Wang et al. 2006; Xu et al. 2010, 2014). What is more, the nutrient colimitation bioassays were generally conducted with phosphorus and nitrate-nitrogen addition and little is known about the colimitation and synergistic interactions among ammonium-nitrogen, nitrate-nitrogen, and phosphorus.

The Miyun Reservoir has become the most important source of drinking water for Beijing since 1981. It is an integrative water conservancy hub used primarily for municipal water supply, flood control, and irrigation of agricultural land (Wang et al. 2008). Furthermore, it serves as the receiving water system of the middle route of the South-to-North Water Transfer Project (SNWTP). Considering these situations, the water quality of Miyun Reservoir has received much attention.

The purpose of this study was to determine the eutrophication thresholds of P, NO₃-N, and NH₄-N in Miyun Reservoir. Nutrient addition bioassays were conducted to gain a better understanding of the colimitation and synergistic interactions among NH₄-N, NO₃-N, and P. Three hypotheses were proposed, including (a) the utilization efficiency of NH₄-N is higher than the utilization efficiency of NO₃-N; (b) "NH₄-N plus P" addition has a greater associated stimulatory effect on phytoplankton growth than "NO₃-N plus P" addition does at the same N concentration; and (c) inputs of both NH₄-N and NO₃-N should be reduced to control bloom formation. The results of this study will help managers to establish more practical and economical nutrient criteria for Miyun Reservoir.

Materials and methods

Study area

Miyun Reservoir, located in the northeast part of Beijing City (longitude 116° 56' E, latitude 39° 54' N), is the largest reservoir in northern China, with an average storage volume of 1.062 billion m³. The water surface covers an area of 188 km², and the catchment area is 15,788 km². The annual

freshwater input into the reservoir is about 0.286 billion m^3 , and the average water residence time of Miyun Reservoir is 3.7 years. Miyun Reservoir is a mountain valley reservoir with a maximum water depth of approximately 40 m. Bottom elevations are higher in the north and lower in the west and south (Zeng et al. 2015). Two major inflows to Miyun Reservoir are the Bai River and the Chao River. Two primary outflows are the Bai River Dam and the Chao River Dam (Fig. 1). Water transferred from the middle route of the SNWTP finally enters Miyun Reservoir from Bai River Dam (Site 1). Agricultural and industrial activities are strictly limited in the watershed, and the reservoir has been kept in a mesotrophic state for years. The phytoplankton taxa present in the reservoir is shown in Fig. S2 (see Supporting Information).

Nutrient limitation bioassay experiments

Water samples containing natural phytoplankton assemblages were collected from 0.2 m below the surface with pre-cleaned 25-L polyethylene carboys at site 1 (Fig. 1). Zooplanktons were removed from the water samples by screening through a 200- μm mesh to minimize the confounding effects of zooplankton grazing (Paerl et al. 2011; Turner and Rabalais 2013). The integrated water sample was thoroughly mixed before division, making the properties of the water sample as homogeneous as possible within each treatment. Water treatments were incubated in a 10-L transparent glass tank in the laboratory within 2 °C of the in situ temperature. The light

intensity was kept at 100 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ with a 14:10 light-dark cycle. Both the temperature and light intensity were close to the mean values of the sampling sites (0.2 m under the surface). The initial physical, chemical, and biological properties of the water collected from site 1 for nutrient bioassay experiments are shown in Table S1 (see Supporting Information). Soluble reactive phosphorus (SRP), $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ were added as $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, NaNO_3 , and NH_4Cl in the experiment.

The nutrient threshold bioassay experiment was conducted in July 2015 to determine the nutrient threshold levels of SRP, $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$. Chlorophyll a (Chl a) concentrations were used to estimate phytoplankton biomass (Lewis et al. 2011; Paerl et al. 2011; Xu et al. 2010). The upper limit of these nutrients needed to control phytoplankton growth and bloom formation (Chl a concentration exceeding 20 $\mu\text{g L}^{-1}$) could be established using this approach. The SRP threshold experiment was conducted with various P concentrations (0, 0.01, 0.02, 0.03, 0.04, 0.06, 0.08, 0.1, 0.5, 1.0, 1.5 mg P L^{-1}) and with a fixed $\text{NO}_3\text{-N}$ level (10 mg N L^{-1}). The $\text{NO}_3\text{-N}$ threshold experiment was conducted with various $\text{NO}_3\text{-N}$ concentrations (0.4, 0.5, 0.6, 0.7, 0.8, 1.0, 1.2, 1.5, 2.0, 3.0 mg N L^{-1}) and with a fixed P level (5 mg P L^{-1}). The $\text{NH}_4\text{-N}$ threshold experiment was conducted with various $\text{NH}_4\text{-N}$ concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, 1.0, 1.5, 3.0 mg N L^{-1}) and with a fixed P level (5 mg P L^{-1}).

A separate nutrient addition bioassay experiment was conducted in August 2015 with treatments of reservoir water

Fig. 1 Study area (water monitoring sites: 1 Bai River Dam, 2 Kuxi, 3 Henghe, 4 Kudong, 5 Jingou, 6 Chao River, and 7 Shuijiu)

containing individual or combined SRP, $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ concentrations. Using this approach, the individual or combined effects of these nutrients on phytoplankton biomass could be assessed. Three scenarios and a total of 30 treatments were designed to test the above hypotheses. Initial reservoir water treatments served as controls. The full experimental design is shown in Table 1. The final concentrations of SRP, $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ in the treatments were obtained by analyzing the background nutrient concentrations in the reservoir and adding a supplemental amount to this background level. The lower bound of the setting concentrations was supposed to be higher than the initial (ambient) concentrations of the water collected (Table S1). The upper bound of the setting concentrations were designed to reflect the relatively high values in the reservoir and to saturate algal growth. In addition, the concentrations of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ in the treatments were designed the same so that the utilization efficiency between different nitrogen sources can be compared easily.

All treatments were performed in triplicate. After nutrient additions, water in the tanks was agitated during the incubations. The incubation experiments were performed for 15 days due to its low initial phytoplankton biomass and slow growth rates. This incubation period provided sufficient time to measure phytoplankton growth responses. Water was sampled at intervals of 3 days at 9 a.m. for Chl *a* and nutrient analyses. Each tank was fully stirred before sampling. Nutrients were measured according to standard methods (Huang et al. 2000). Chl *a* concentrations were determined spectrophotometrically after extraction in 90 % hot ethanol (Párista et al. 2002). Phytoplankton species were identified according to freshwater algae in China (Hu and Wei 2006). P, $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ were supplemented in a timely manner based on the analytical results to maintain the setting concentrations throughout the experiment.

Field method

Water samples were collected from the surface of Miyun Reservoir at monthly intervals from April to November, when algal bloom occasionally occurred in shallow water. These nutrient concentrations were measured to help reflect the whole nutrient conditions of the reservoir and served as background information for the eutrophication state of Miyun Reservoir. A sample of approximately 1 L of water was taken from each site to measure nutrient concentrations. Water samples were transferred and kept cool in incubators. The samples were measured within 24 h in the laboratory after collection. TP, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and Chl *a* were analyzed according to standard methods (Huang et al. 2000; Párista et al. 2002).

Statistical analyses

The growth rate (μ) for each set of treatment conditions was estimated as the slope of the log-transformed data ($\log X_t$) versus time, where X_t represents the concentration of Chl *a* at day *t*, respectively. Response curves were established, and the change points on the curves were estimated as the eutrophication threshold (Wang et al. 2006; Xu et al. 2010, 2014). The changepoint analyzer 2.3 (Taylor Enterprises, Inc.) was used for examining the change plots on the response curves. The maximum growth rate ($\mu_{\max}$) and the half saturation constant (K_u) were calculated using the Monod kinetic equation (Monod 1950).

The thresholds of TP and TN can be calculated by the thresholds of SRP and dissolved inorganic nitrogen (DIN) based on the ratio of SRP/TP and DIN/TN (Xu et al. 2014),

$$\text{Total nutrient threshold} = \frac{\text{Dissolved nutrient threshold}}{\text{Dissolved nutrient concentration} / \text{Total nutrient concentration}} \quad (1)$$

Significant differences among the various treated samples were analyzed by one-way ANOVA with Tukey's post hoc test. Statistical analysis was conducted with SPSS 19.0. The box plot of mean monthly nutrient concentrations in Miyun Reservoir was conducted with Origin 9.0.

Results

Nutrient thresholds of phytoplankton growth

To specify nutrient threshold levels of SRP, $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$, growth rate responses to different SRP, $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ supplies were examined. Growth curves fitted by nonlinear

regression are shown in Fig. 2. The results of the changepoint analysis of growth rate response curves are shown in Table S2 (see Supporting Information). Figure 2a showed that the phytoplankton growth rate increased proportionately with SRP additions from 0 to 0.04 mg P L^{-1} , while from 0.04 to 1.5 mg P L^{-1} , the growth rate stopped increasing and remained constant. Changepoint analysis further demonstrated that 0.04 mg P L^{-1} was the change point on the response curve (Table S2). The abrupt change with phytoplankton growth rate around 0.04 mg P L^{-1} in the growth curves indicated that phytoplankton growth was no longer P limited when SRP enrichment was $\geq 0.04 \text{ mg P L}^{-1}$. Therefore, 0.04 mg P L^{-1} could be regarded as the threshold of SRP. For $\text{NO}_3\text{-N}$ utilization, although only two to three

Table 1 Basic schemes for nutrient addition experiments (bioassays) used in this study

Scenarios	No.	Nutrient addition	
Control	0	–	
A	1-1	0.5 mg N/L NH_4Cl	Without P addition
	1-2	1.0 mg N/L NH_4Cl	
	1-3	2.0 mg N/L NH_4Cl	
	1-4	4.0 mg/L NH_4Cl	
	2-1	0.5 mg/L NH_4Cl	5.0 mg P/L, K_2HPO_4
	2-2	1.0 mg/L NH_4Cl	
	2-3	2.0 mg/L NH_4Cl	
	2-4	4.0 mg/L NH_4Cl	
	1-1	0.5 mg N/L NaNO_3	Without P addition
	1-2	1.0 mg N/L NaNO_3	
	1-3	2.0 mg N/L NaNO_3	
	1-4	4.0 mg N/L NaNO_3	
	2-1	0.5 mg N/L NaNO_3	5.0 mg P/L, K_2HPO_4
	2-2	1.0 mg N/L NaNO_3	
	2-3	2.0 mg N/L NaNO_3	
	2-4	4.0 mg N/L NaNO_3	
C	1-1	0.02 mg P/L K_2HPO_4	Without N addition
	1-2	0.5 mg P/L K_2HPO_4	
	1-3	1.0 mg P/L K_2HPO_4	
	1-4	2.0 mg P/L K_2HPO_4	
	2-1	0.02 mg P/L K_2HPO_4	10 mg N/L, $\text{NH}_4\text{-N}$
	2-2	0.5 mg P/L K_2HPO_4	
	2-3	1.0 mg P/L K_2HPO_4	
	2-4	2.0 mg P/L K_2HPO_4	
	3-1	0.02 mg P/L K_2HPO_4	10 mg N/L, $\text{NO}_3\text{-N}$
	3-2	0.5 mg P/L K_2HPO_4	
	3-3	1.0 mg P/L K_2HPO_4	
	3-4	2.0 mg P/L K_2HPO_4	
	4	2.0 mg P/L K_2HPO_4	5.0 mg N/L NH_4Cl + 5.0 mg N/L NaNO_3

concentrations at the range where a rapid change in the growth rate took place, it was obvious that algal growth rate was promoted greatly from 0 to 0.5 mg N L⁻¹. From 0.5 to 3.0 mg N L⁻¹, the growth rate stopped increasing and remained stable (Fig. 2b). Phytoplankton growth was no longer limited when $\text{NO}_3\text{-N}$ enrichment was ≥ 0.5 mg N L⁻¹. The result of the changepoint analysis further confirmed that 0.5 mg N L⁻¹ was the change point on the response curve (Table S2). Therefore, 0.5 mg N L⁻¹ could be regarded as the threshold for a $\text{NO}_3\text{-N}$ effect on the growth rate of phytoplankton. A similar approach was used for $\text{NH}_4\text{-N}$ and the threshold of $\text{NH}_4\text{-N}$ was determined as 0.3 mg N L⁻¹ (Fig. 2c).

Microcystis spp. and *Scenedesmus spp.* accounted for about 26.9 and 20.6 % of the initial total phytoplankton biomass, and they sustain dominance throughout the duration of the bioassays. At the end of the experiment, *Microcystis spp.* and *Scenedesmus spp.* accounted for 19.7–33.6 % and 28.0–

52.5 % of the total phytoplankton biomass, respectively. The main phytoplankton genera identified in nutrient threshold experiment is shown in Table S3 (see Supporting Information).

The Monod equation was used to describe the relationship between algal growth rates and nutrient concentrations (Xu et al. 2014). The results are shown in Table S4 (see Supporting Information). Growth kinetics data showed that the maximum growth rates (μ_{max}) of phytoplankton for SRP, $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ were very close. The values were 0.344, 0.313, and 0.270 day⁻¹, respectively. $\text{NO}_3\text{-N}$ has the highest half-saturation constant value (0.268 mg L⁻¹), then followed by $\text{NH}_4\text{-N}$ (0.087 mg L⁻¹) and SRP (0.028 mg L⁻¹). The ratio μ_{max}/K_u was used as a better index than K_u values alone to indicate the advantage in the nutrient competition, and a higher μ_{max}/K_u ratio indicates a higher rate at the lowest nutrient concentrations (Healey 1980). Hence, the utilization

Fig. 2 Growth kinetics of natural phytoplankton assemblages in response to **a** SRP concentrations, **b** $\text{NO}_3\text{-N}$ concentrations, and **c** $\text{NH}_4\text{-N}$ concentrations. Curves were fitted by nonlinear regression. Error bars represent ± 1 SD of triplicate samples

efficiency of $\text{NO}_3\text{-N}$ was expected to be lower than the utilization efficiency of $\text{NH}_4\text{-N}$, with a lower μ_{max}/K_u value than the μ_{max}/K_u value of $\text{NH}_4\text{-N}$. The results also indicated a congruent relationship between the μ_{max}/K_u value and the nutrient thresholds. A higher μ_{max}/K_u value suggests a higher utilization efficiency of the nutrient, thus corresponding to a relatively lower nutrient threshold.

Fig. 3 Phytoplankton biomass (as Chl a) responses to various concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and SRP additions. Differences between treatments are shown on the basis of the ANOVA post hoc tests ($a < b < c$; $p < 0.05$)

Phytoplankton growth responses to nutrient addition bioassays

The phytoplankton biomass (as Chl a) responses to an incremental concentration of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and SRP additions are shown in Fig. 3. Generally, $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$ addition alone had little effect on the Chl a compared with the control ($p > 0.05$). When 5.0 mg P L^{-1}

K_2HPO_4 was supplied, the Chl *a* increased gradually with the increasing concentration of NH_4 -N. As for NO_3 -N, phytoplankton biomass with 0.5 mg N L^{-1} NO_3 -N addition appeared almost the same compared with the control ($p > 0.05$). Furthermore, phytoplankton biomass was much higher than the control and showed no significant differences over the range of NO_3 -N additions from 1.0 to 4.0 mg N L^{-1} ($p > 0.05$). Like the results obtained with N, there were no significant differences between treatments with SRP addition alone compared with the control ($p > 0.05$). However, with NH_4 -N addition, phytoplankton growth remained high and showed no significant differences over the range of SRP additions from 0.5 to 2.0 mg P L^{-1} ($p > 0.05$). In addition, NH_4 -N addition had a larger stimulatory effect on phytoplankton growth than NO_3 -N addition did at the same N concentration. Phytoplankton growth rate showed patterns similar to phytoplankton biomass in response to individual or combined SRP, NO_3 -N, and NH_4 -N additions (Fig. S3) (see Supporting Information).

Harpole et al. summarized the interactive responses of phytoplankton communities to N and P addition from 641 published studies in aquatic ecosystems. Overall, 17.8 and 10.1 % of the studies appeared to be simultaneously and independently colimited by N and P, respectively (Harpole et al. 2011). According to the nutrient limitation categories, the results of our bioassay fall into the category of simultaneous colimitation. There is increasing evidence that simultaneous colimitation by N and P occurs in both natural lake phytoplankton communities and most of the genera in the community (Elser et al. 2007; Kolzau et al. 2014; Müller and Mitrovic 2015; Ma et al. 2015b). Multiple nutrient limitations of phytoplankton growth can occur because phytoplankton species differ in their optimum nutrient ratios for growth (Hecky and Kilham 1988). However, simultaneous multiple nutrient limitation has not been shown for any unialgal culture, since the macronutrients cannot substitute for each other in their biochemical functions (de Vries and Klapwijk 1987; Droop 1974; Hecky and Kilham 1988; Rhee 1978).

The nutrient addition bioassays showed stimulation of algal biomass production (as Chl *a*) and phytoplankton growth rate in response to individual or combined SRP, NO_3 -N, and NH_4 -N additions (Fig. 4). Phytoplankton response to NH_4 -N alone was no different than with the control ($p > 0.05$), and the response was significantly larger when P was added in the treatments. The same trend was observed for the NO_3 -N addition treatments with and without P, yet the stimulatory effect of NO_3 -N on phytoplankton biomass was less than the effect of NH_4 -N at the same N concentration. P addition alone showed little stimulation of Chl *a* compared with the control ($p > 0.05$). From the perspective of the phytoplankton growth rate, the impact

Fig. 4 The synergistic interactions among ammonium-nitrogen, nitrate-nitrogen, and phosphorus. Differences between treatments are shown on the basis of the ANOVA post hoc tests ($a < b < c$; $p < 0.05$)

of P addition alone appeared slightly higher compared with the control ($p < 0.05$). Both NH_4 -N and NO_3 -N addition helped stimulate the growth of phytoplankton with the presence of P, and the stimulatory effect of NH_4 -N plus P on phytoplankton biomass was greater than of NO_3 -N plus P at the same N concentration ($p < 0.05$). The highest value of Chl *a* ($294.04 \pm 34.82 \text{ µg L}^{-1}$) and the highest growth rate ($0.47 \pm 0.01 \text{ day}^{-1}$) appeared at the “ 2.0 mg P L^{-1} and $10.0 \text{ mg NH}_4\text{-N L}^{-1}$ ” treatment. However, phytoplankton biomass and growth rate with “ $2.0 \text{ mg P L}^{-1} + 5.0 \text{ mg NH}_4\text{-N L}^{-1} + 5.0 \text{ mg NO}_3\text{-N L}^{-1}$ ” showed no significant differences from “ $2.0 \text{ mg P L}^{-1} + 10.0 \text{ mg NO}_3\text{-N L}^{-1}$ ” ($p > 0.05$). “ NH_4 -N plus NO_3 -N” did not show such associated stimulatory effect on phytoplankton growth as “ NH_4 -N plus P” or “ NO_3 -N plus P”. Bioassays also illustrated that the utilization efficiency of NH_4 -N is higher than the utilization efficiency of NO_3 -N at the same N concentration. Per amount of N added, NH_4 -N stimulated significantly more algal biomass formation than NO_3 -N did.

Discussion

How to maintain nutrient concentrations at the setting values

Nutrient addition methods were summarized from previous nutrient addition bioassays. The most common way of adding nutrient is adding a finite supply of dissolved nutrients as the setting concentrations at the beginning of the experiment and adding no more nutrients during the process of phytoplankton incubation (Domingues et al. 2011; Ma et al. 2015a, b; Paerl et al. 2011; Xu et al. 2010, 2015). However, there are some problems with this approach. In the bioassays, the water samples are incubated in closed containers, and the concentrations of the dissolved nutrients will tend to decline as the nutrients are utilized by the phytoplankton. The dissolved nutrients being taken up metabolically from the solution by phytoplankton aggregate into organic particles, and organisms can be decomposed into inorganic nutrients through the mineralization process (Halemejkó and Chrost 1984). However, the effects of the mineralization process are estimated to be small and it was generally not taken into consideration in the nutrient limitation bioassays (Bloesch et al. 1977; Gao et al. 2000, 2006; Gardner et al. 1989; Kagami et al. 2013; Kim et al. 2006; Kolzau et al. 2014). In this case, a particular nutrient could limit the growth of the phytoplankton once its concentration declined to a certain degree. While in the field, nutrients were potentially replenished and balanced by continual allochthonous sources. Therefore, bioassays with a finite initial supply of dissolved nutrients might not necessarily reflect limitation by the same nutrient concentrations in the field due to exhaustion of nutrient supplies (Xu et al. 2010).

In later bioassay experiments, dissolved nutrients were supplied every few days to simulate pulsed nutrient inputs (Paerl et al. 2015; Xu et al. 2014). The loss of nutrients absorbed by the phytoplankton can be replenished to some extent in this way. However, the biggest uncertainty of this approach is that it is unknown how much nutrient has been absorbed. At the beginning of the bioassays, phytoplankton biomass is small and the added dissolved nutrients might not be consumed completely before the next replenishment. The final nutrient content is the sum of the remaining nutrients and the pulsed input nutrients, which may exceed the setting concentrations. As a result, the thresholds of dissolved nutrients might have been underestimated in the bioassays.

To address this potential problem, the remaining dissolved nutrient concentrations in each tank were analyzed at intervals of 3 days in our study. SRP, $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ were supplemented in a timely manner to the setting concentrations based on the analytical results. The remaining nutrient concentrations in the nutrient threshold bioassay are shown in Fig. S4 (see Supporting Information). Nutrient consumptions were different between different time intervals with the increase of

algal biomass, and the supplements of nutrient were adjusted according to the remaining concentrations. Nutrients supplied in this way can retain the dissolved nutrient concentrations at the setting levels to the utmost extent. The downside of this method is that the analysis and supplementation processes are very troublesome. Moreover, although dissolved nutrients were supplemented every 3 days, the concentrations still decreased between the intervals due to the phytoplankton uptake. Actually, the best way to keep dissolved nutrient concentrations at the setting levels is real-time monitoring and supplement. Taking the feasibility and workload into consideration, 3 days of interval was selected. Even though the results cannot be used to derive true nutrient-growth kinetics, they can provide an upper-bound estimate for dissolved nutrient concentrations that saturate algal growth (Xu et al. 2014). Nevertheless, this method still served as the best way to keep nutrient concentrations to the setting levels at present.

In conclusion, if the initial phytoplankton biomass in the original water samples was high enough, phytoplankton biomass would be able to respond to different dissolved nutrient addition treatments in a short time. The incubating time is supposed to be correspondingly short to minimize the impact of nutrient reduction. In this case, dissolved nutrients can be given one time at the beginning of the bioassays. On the other hand, if the initial phytoplankton biomass was very low in the original water samples, just like the situation in our case, short-time incubation was not sufficient for the phytoplankton biomass to show identifiable changes for each treatment. Therefore, dissolved nutrients should be supplemented in a timely manner based on residual concentration analysis to maintain the setting nutrient levels.

Except for the issues discussed above, results from these bioassays might not necessarily reflect all of the ecological interactions of the system (Hecky and Kilham 1988). Bottle bioassays or mesocosm studies are often conducted on a relatively small scale and cannot properly account for important long-term processes such as atmospheric exchange, changes in the grazer community, and nutrient exchange with sediments (Schindler et al. 2008). Therefore, generalizations from bioassay experiments to the real situation in the field must be drawn with caution (Hecky and Kilham 1988).

Seasonal variation of nutrients and chlorophyll a in Miyun Reservoir

The seasonal trends of mean monthly TP, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and Chl a concentrations are shown in Fig. 5. The TP concentrations ranged from 0.005 to 0.040 mg P L^{-1} , with an average of 0.017 mg P L^{-1} . SRP accounted for 56.52 % of TP in Miyun Reservoir; thus, the calculated result for the TP threshold was 0.071 mg L^{-1} . The mean monthly concentrations of both TP and SRP in Miyun Reservoir were below their thresholds. As for N, $\text{NO}_3\text{-N}$ concentrations varied between 0.04 to 1.40 mg

Fig. 5 Mean monthly nutrient concentrations in Miyun Reservoir. The red dotted lines are the thresholds of TP, NO₃-N, and NH₄-N and the criteria of Chl a for bloom formation, respectively

N L⁻¹ with peak in spring and declined in summer and autumn. The average concentration of NO₃-N was 0.620 mg N L⁻¹ and was higher than the NO₃-N threshold (0.5 mg N L⁻¹). NH₄-N concentrations ranged from 0.025 to 0.580 mg N L⁻¹, with an average of 0.143 mg N L⁻¹. The average concentrations of NH₄-N were below 0.3 mg N L⁻¹ throughout the year, which was also no more than its threshold (0.3 mg N L⁻¹). Chl a concentrations were used to estimate phytoplankton biomass. From April to November, Chl a gradually increased and showed a peak in September. The Chl a concentrations changed from a minimum of 0.55 μg L⁻¹ to a maximum of 16.38 μg L⁻¹, with an average of 4.09 μg L⁻¹. Chl a concentration exceeding 20 μg L⁻¹ is considered as blooms (Xu et al. 2014), while the reservoir-wide average Chl a is below 20 μg L⁻¹ on an annual basis.

Nutrient thresholds needed for controlling algal blooms in the Miyun Reservoir

The eutrophication thresholds of TP for freshwaters vary from 0.02 to 0.10 mg P L⁻¹, while those for TN range from 0.50 to 1.00 mg N L⁻¹ (Xu et al. 2010). In our experiments, the calculated result for the TP threshold was 0.071 mg L⁻¹, which is consistent with the previous research. In addition to nutrient loading, sediment release is also an important source of

nutrients in natural conditions. Factors that affect the release of P from sediment include temperature, pH, oxygen condition, bioturbation, and sediment type (Boers 1991; Holdren and Armstrong 1980; North et al. 2015). In shallow waters, P released from the sediment can soon be mixed into the entire water column, while in the deep stratified Miyun Reservoir, the released P is trapped in the hypolimnion and is unavailable to the phytoplankton to a large extent (Kolzau et al. 2014). Moreover, many phytoplankton species are able to store enough cellular P through “luxury uptake,” and with this intracellular storage, they are able to support several rounds of cell division when ambient P concentration is insufficient (Reynolds 2006). In our experiment, phytoplankton growth was no longer P limited when ambient TP concentration was ≥0.071 mg P L⁻¹; hence, the setting criteria of TP should be targeted lower than this value.

In our bioassay, NH₄-N addition has a larger stimulatory effect on phytoplankton growth than NO₃-N addition has at the same N concentration. This observation helped explain why the threshold of NH₄-N was lower than the threshold of NO₃-N. What is more, the relative preference for ammonium or nitrate is phytoplankton group-specific. In particular, cyanobacteria, chlorophytes, and chrysophytes prefer ammonium, while nitrate is preferred by diatoms, cryptophytes, and dinoflagellates (Chaffin and Bridgeman

2014; Domingues et al. 2011; Donald et al. 2011, 2013; Ohashi et al. 2011). Cyanophytes and chlorophytes account for a very large proportion in Miyun Reservoir in July and August (Fig. S2 and Table S3, see supporting information). Therefore, phytoplankton growth response in $\text{NH}_4\text{-N}$ addition bioassays has been stronger than $\text{NO}_3\text{-N}$ addition bioassays. In fact, the thresholds of TN calculated by “ $\text{NO}_3\text{-N}$ threshold and $\text{NO}_3\text{-N}/\text{TN}$ ratio” or calculated by “ $\text{NH}_4\text{-N}$ threshold and $\text{NH}_4\text{-N}/\text{TN}$ ratio” were different. For example, the thresholds of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ were 0.5 and 0.3 mg N L^{-1} in the Miyun Reservoir. $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ accounted for 55.66 and 17.15 % of TN, respectively. The threshold for TN calculated by the $\text{NO}_3\text{-N}$ threshold was 0.898 mg L^{-1} , while the value calculated by the $\text{NH}_4\text{-N}$ threshold was 1.749 mg L^{-1} . As a result, 0.898 mg L^{-1} was selected as the eutrophication threshold for TN in Miyun Reservoir. However, in areas with a higher $\text{NH}_4\text{-N}/\text{TN}$ ratio, the threshold value for TN calculated by the $\text{NH}_4\text{-N}$ threshold might be lower than the threshold value calculated by the $\text{NO}_3\text{-N}$ threshold. In this case, the lower value should be chosen as the threshold of TN. This is why it is important to measure the threshold of $\text{NH}_4\text{-N}$ as well as the threshold of $\text{NO}_3\text{-N}$. Otherwise, the critical value of TN might have been overestimated if it was calculated simply by $\text{NO}_3\text{-N}$.

Environmental implications

In aquatic ecosystems, biologically available N can be supplied through nitrogen (N_2) fixation by cyanobacteria (Paerl 2009). Therefore, some research argue that P is the ultimate limiting nutrient overtime since N_2 fixation helps satisfy N requirements (Schindler et al. 2008). In fact, N limitation has been observed in whole-lake experiments and many studies have indicated that N_2 fixation by phytoplankton cannot fully compensate for nitrogen deficiency (Kolzau et al. 2014; Lewis and Wurtsbaugh 2008; Paerl 2009; Scott and McCarthy 2010). This is mainly because the process is controlled by lots of physical-chemical factors except for N/P ratio (Paerl 2009). Furthermore, the dominant species in Miyun Reservoir, *Microcystis spp.*, belong to a non-nitrogen-fixing cyanobacterial genus (Paerl et al. 2001), making their proliferation highly dependent on exogenous nitrogen sources.

Results from this study showed that phytoplankton grew better in treatments with ammonium-nitrogen as the nitrogen source than in treatments with nitrate-nitrogen (Figs. 3 and 4). This pattern of nitrogenous nutrient preference was similar to previous research, suggesting that the ammonium-nitrogen utilization is preferred by phytoplankton than the nitrate-nitrogen utilization (Baldia et al. 2007; Dokulil and Teubner 2000).

When ammonia, nitrate, and urea are offered in combination, ammonia is used first, then urea becomes the next

choice, and finally nitrate (Chaffin and Bridgeman 2014; Takamura et al. 1987). Despite ambient ammonium concentrations one or more orders of magnitude lower than ambient nitrate concentrations, ammonium utilization is preferred by phytoplankton (Ahlgren et al. 1994). The assimilation of nitrate is inhibited by the presence of ammonium over a wide range of concentrations (Ohashi et al. 2011). Theoretically, utilization of other forms of nitrogen occurs only when the ammonium concentration is insufficient to meet the nitrogen requirements of the phytoplankton (Gu and Alexander 1993). Phytoplankton prefer reduced forms of N (ammonium or organic N) mainly because the energetic costs for incorporating and assimilating these forms are less than the energetic costs for oxidized N forms (nitrate and nitrite) (Donald et al. 2013; Gardner et al. 2004). From Fig. 4, the “2.0 mg P L^{-1} + 10.0 $\text{mg NH}_4\text{-N L}^{-1}$ ” treatment is nearly two times the biomass of the “2.0 mg P L^{-1} + 5.0 $\text{mg NH}_4\text{-N L}^{-1}$ + 5.0 $\text{mg NO}_3\text{-N L}^{-1}$ ” treatment, which would be consistent with primarily using ammonium. The experiment results showed that when given nitrate and ammonium, plus P, less biomass was produced presumably because the algae used up the ammonium first, then exhibited lower efficiency with nitrate.

Nutrient threshold bioassays and nutrient addition bioassays proved that ammonium was also an important factor in causing algal blooms. It is likely that any N form that enters into the water column has the potential to be reduced to ammonium, and appropriate reductions of all forms of N in watershed are recommended.

Conclusions

The results of this study showed that the reservoir-wide average Chl a in Miyun Reservoir is below 20 $\mu\text{g L}^{-1}$ on an annual basis. When SRP enrichment is $\geq 0.04 \text{ mg P L}^{-1}$, $\text{NO}_3\text{-N}$ enrichment $\geq 0.5 \text{ mg N L}^{-1}$, and $\text{NH}_4\text{-N}$ enrichment $\geq 0.3 \text{ mg N L}^{-1}$, the growth of phytoplankton was not nutrient limited. Ammonium was an important factor for the growth of phytoplankton. $\text{NH}_4\text{-N}$ plus P had a larger stimulatory effect on phytoplankton growth than $\text{NO}_3\text{-N}$ plus P did at the same N concentration. Our findings imply that although P input reductions are important, appropriate reductions of all forms of N in watershed is necessary for eutrophication management in Miyun Reservoir.

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References

- Ahlgren I, Sörensson F, Waara T, Vrede K (1994) Nitrogen budgets in relation to microbial transformations in lakes. *Ambio* 23
- Baldia S, Evangelista A, Aralar E, Santiago A (2007) Nitrogen and phosphorus utilization in the cyanobacterium *Microcystis aeruginosa* isolated from Laguna de Bay, Philippines. *J Appl Phycol* 19:607–613
- Bloesch J, Stadelmann P, Bühner H (1977) Primary production, mineralization, and sedimentation in the euphotic zone of two Swiss lakes. *Limnol Oceanogr* 22:511–526
- Boers PC (1991) The influence of pH on phosphate release from lake sediments. *Water Res* 25:309–311
- Chaffin JD, Bridgeman TB (2014) Organic and inorganic nitrogen utilization by nitrogen-stressed cyanobacteria during bloom conditions. *J Appl Phycol* 26:299–309
- Chaffin JD, Bridgeman TB, Bade DL, Mobilian CN (2014) Summer phytoplankton nutrient limitation in Maumee Bay of Lake Erie during high-flow and low-flow years. *J Great Lakes Res* 40:524–531
- de Vries PJ, Klapwijk SP (1987) Bioassays using *Stigeoclonium tenue* Kütz. and *Scenedesmus quadricauda* (Turp.) Bréb. as testorganisms; a comparative study. *Hydrobiologia* 153:149–157
- Dokulil MT, Teubner K (2000) Cyanobacterial dominance in lakes. *Hydrobiologia* 438:1–12
- Domingues RB, Barbosa AB, Sommer U, Galvão HM (2011) Ammonium, nitrate and phytoplankton interactions in a freshwater tidal estuarine zone: potential effects of cultural eutrophication. *Aquat Sci* 73:331–343
- Donald DB, Bogard MJ, Finlay K, Bunting L, Leavitt PR (2013) Phytoplankton-specific response to enrichment of phosphorus-rich surface waters with ammonium, nitrate, and urea. *PLoS One* 8:e53277
- Donald DB, Bogard MJ, Finlay K, Leavitt PR (2011) Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. *Limnol Oceanogr* 56:2161–2175
- Droop M (1974) The nutrient status of algal cells in continuous culture. *J Mar Biol Assoc U K* 54:825–855
- Elser JJ et al (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett* 10:1135–1142
- Gao G, Gao X, Qin B (2000) Experimental study on the PO_4^{3-} -P threshold of the alkaline phosphatase activity in Taihu lake. *J Lake Sci* 12: 353–358
- Gao G, Zhu G, Qin B, Chen J, Wang K (2006) Alkaline phosphatase activity and the phosphorus mineralization rate of Lake Taihu. *Sci China Series D* 49:176–185
- Gardner WS, Chandler JF, Laird GA (1989) Organic nitrogen mineralization and substrate limitation of bacteria in Lake Michigan. *Limnol Oceanogr* 34:478–485
- Gardner WS, Lavrentyev PJ, Cavaletto JF, McCarthy MJ, Eadie BJ, Johengen TH, Cotner JB (2004) Distribution and dynamics of nitrogen and microbial plankton in southern Lake Michigan during spring transition 1999–2000. *J Geophys Res Oceans* 109:178–2012:109
- Groffman PM et al (2006) Ecological thresholds: the key to successful environmental management or an important concept with no practical application? *Ecosystems* 9:1–13
- Gu B, Alexander V (1993) Dissolved nitrogen uptake by a cyanobacterial bloom (*Anabaena flos-aquae*) in a subarctic lake. *Appl Environ Microbiol* 59:422–430
- Halemejkó GZ, Chrost RJ (1984) The role of phosphatases in phosphorus mineralization during decomposition of lake phytoplankton blooms. *Archiv für Hydrobiologie Stuttgart* 101:489–502
- Harpole WS et al (2011) Nutrient co-limitation of primary producer communities. *Ecol Lett* 14:852–862
- Healey F (1980) Slope of the Monod equation as an indicator of advantage in nutrient competition. *Microb Ecol* 5:281–286
- Hecky R, Kilham P (1988) Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. *Limnol Oceanogr* 33:796–822
- Holdren GC, Armstrong DE (1980) Factors affecting phosphorus release from intact lake sediment cores. *Environ Sci Technol* 14:79–87
- Hu H, Wei Y (2006) Freshwater Algae in China—system, classification and ecology. (in Chinese)
- Huang X, Chen W, Cai Q (2000) Survey, observation and analysis of lake ecosystem. China Standards Press, Beijing (in Chinese)
- Kagami M, Hirose Y, Ogura H (2013) Phosphorus and nitrogen limitation of phytoplankton growth in eutrophic Lake Inba, Japan. *Limnology* 14:51–58
- Kim C, Nishimura Y, Nagata T (2006) Role of dissolved organic matter in hypolimnetic mineralization of carbon and nitrogen in a large, monomictic lake. *Limnol Oceanogr* 51:70–78
- Kolzau S, Wiedner C, Rucker J, Köhler J, Köhler A, Dolman AM (2014) Seasonal patterns of nitrogen and phosphorus limitation in four German lakes and the predictability of limitation status from ambient nutrient concentrations. *PLoS One* 9:1–14
- Lewis WM Jr, Wurtsbaugh WA, Paerl HW (2011) Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. *Environ Sci Technol* 45:10300–10305
- Lewis WM, Wurtsbaugh WA (2008) Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. *Int Rev Hydrobiol* 93:446–465
- Müller S, Mitrovic SM (2015) Phytoplankton co-limitation by nitrogen and phosphorus in a shallow reservoir: progressing from the phosphorus limitation paradigm. *Hydrobiologia* 744:255–269
- Ma J et al (2015a) Green algal over cyanobacterial dominance promoted with nitrogen and phosphorus additions in a mesocosm study at Lake Taihu, China. *Environ Sci Pollut Res* 22:5041–5049
- Ma J, Qin B, Wu P, Zhou J, Niu C, Deng J, Niu H (2015b) Controlling cyanobacterial blooms by managing nutrient ratio and limitation in a large hyper-eutrophic lake: Lake Taihu, China. *J Environ Sci* 27:80–86
- Monod J (1950) Theory and applications of continuous culture technique. *Ann Inst Pasteur Paris* 79:390–410
- North R, Guildford S, Smith R, Havens S, Twiss M (2007) Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. *Limnol Oceanogr* 52:315–328
- North RL et al (2015) Evidence for internal phosphorus loading in a large prairie reservoir (Lake Diefenbaker, Saskatchewan). *J Great Lakes Res* 41:91–99
- Ohashi Y et al (2011) Regulation of nitrate assimilation in cyanobacteria. *J Exp Bot* 62:1411–1424
- Párista É, Ács É, Böddi B (2002) Chlorophyll-a determination with ethanol—a critical test. *Hydrobiologia* 485:191–198
- Paerl HW (2009) Controlling eutrophication along the freshwater–marine continuum: dual nutrient (N and P) reductions are essential. *Estuar Coasts* 32:593–601
- Paerl HW, Fulton RS, Moisaner PH, Dyble J (2001) Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *Sci World J* 1:76–113
- Paerl HW, Gardner WS, McCarthy MJ, Peierls BL, Wilhelm SW (2014) Algal blooms: noteworthy nitrogen. *Science* 346:175–175
- Paerl HW, Xu H, Hall NS, Rossignol KL, Joyner AR, Zhu G, Qin B (2015) Nutrient limitation dynamics examined on a multi-annual scale in Lake Taihu, China: implications for controlling eutrophication and harmful algal blooms. *J Freshw Ecol* 30:5–24
- Paerl HW, Xu H, McCarthy MJ, Zhu G, Qin B, Li Y, Gardner WS (2011) Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): the need for a dual nutrient (N & P) management strategy. *Water Res* 45:1973–1983
- Reynolds CS (2006) The ecology of phytoplankton. Cambridge University Press

- Rhee G-Y (1978) Effects of N: P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. *Limnol Oceanogr* 23:10–25
- Scheffer M, Carpenter S, Foley JA, Folke C, Walker B (2001) Catastrophic shifts in ecosystems. *Nature* 413:591–596
- Schindler DW et al (2008) Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. *Proc Natl Acad Sci* 105:11254–11258
- Scott JT, McCarthy MJ (2010) Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. *Limnol Oceanogr* 55:1265–1270
- Smith S (1984) Phosphorus versus nitrogen limitation in the marine environment. *Limnol Oceanogr* 29:1149–1160
- Smith VH (2003) Eutrophication of freshwater and coastal marine ecosystems a global problem. *Environ Sci Pollut Res* 10:126–139
- Takamura N, Iwakuma T, Yasuno M (1987) Uptake of ^{13}C and ^{15}N (ammonium, nitrate and urea) by *Microcystis* in Lake Kasumigaura. *J Plankton Res* 9:151–165
- Turner RE, Rabalais NN (2013) Nitrogen and phosphorus phytoplankton growth limitation in the northern Gulf of Mexico. *Aquat Microb Ecol* 68:159–169
- von Liebig JF, Gregory W (1842) Animal chemistry; or, Organic chemistry in its application to physiology and pathology. London, Taylor and Walton
- Wang X et al (2006) Probability and threshold values for recognizing eutrophication in Lake Songhua. *Acta Ecol Sin* 26:3989–3997 (in Chinese)
- Wang Z, Hong J, Du G (2008) Use of satellite imagery to assess the trophic state of Miyun Reservoir, Beijing, China. *Environ Pollut* 155:13–19
- Xu H, Paerl HW, Qin B, Zhu G, Gao G (2010) Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. *Limnol Oceanogr* 55:420–432
- Xu H, Paerl HW, Qin B, Zhu G, Hall NS, Wu Y (2014) Determining critical nutrient thresholds needed to control harmful cyanobacterial blooms in eutrophic Lake Taihu, China. *Environ Sci Technol* 49:1051–1059
- Xu S, Wang Y, Huang B, Wei Z-B, Miao A-J, Yang L-Y (2015) Nitrogen and phosphorus limitation of phytoplankton growth in different areas of Lake Taihu, China. *J Freshw Ecol* 30:113–127
- Yang L, Wang X, Wang Z, Jia D, Wu Z, Wu J (2009) Dynamic AGP modeling analysis of phosphorus threshold in eutrophication waters. *Ecol Environ Sci* 18:1625–1630 (in Chinese)
- Zeng Q, Qin L, Li X (2015) The potential impact of an inter-basin water transfer project on nutrients (nitrogen and phosphorous) and chlorophyll a of the receiving water system. *Sci Total Environ* 536:675–686