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Title: Total phytoplankton abundance is determined by phosphorus input: Evidence from an 18-month fertilization experiment in 4 subtropical ponds

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Abstract: There is a heated debate over the necessity of nitrogen (N) reduction, in addition to phosphorus (P) reduction, for the control of eutrophication. Whole-lake fertilization experiments and lake restoration practices in high latitudes have demonstrated that P is the primary factor regulating total phytoplankton. Recognizing the limited large-scale evidence in warmer climatic zones, a fertilization experiment was conducted in 4 ponds located in the subtropical Yangtze River Basin, China. Total phytoplankton abundance in a pond receiving P (+P) was similar to that in a pond receiving both N and P (+N+P). Both had higher phytoplankton than a pond receiving no additional nutrient (Control). Total nitrogen concentration (TN) in the +P pond increased with the appearance of N-fixing cyanobacteria. Total phytoplankton abundance was similar in the ponds without P addition (+N, Control) and both ponds had lower phytoplankton levels than the +N+P pond. These results showed that P, not N determines total phytoplankton abundance and that N deficiency is offset by N-fixation in subtropical lakes. This experiment supports the idea that attention should be mainly focused on P reduction in mitigating eutrophication.

Keywords: fertilization experiment, eutrophication, phosphorus, nitrogen, N-fixation

Introduction

Eutrophication of lakes is a global environmental problem, associated with algal blooms, fish kills, ecosystem degeneration, and toxic algae, and hence represents a hazard to drinking water supplies. Many whole-lake experiments and lake restoration practices suggest that phosphorus (P) is the key factor mitigating lake eutrophication (Schindler 2012), whereas it is also widely argued that nitrogen (N) should also be controlled as it can promote total phytoplankton abundance as well (Lewis and Wurtsbaugh 2008; Lewis et al. 2011). To control or not to control N is of great practical importance in lake management since dual control of N and P incurs substantial costs.

Combined N and P reduction costs approximately 4–15 times more than P reduction alone (Bryhn and Håkanson 2009; Schindler et al. 2012).

In the early stages of research on eutrophication, it was believed that overloading of several major and trace elements was responsible for lake eutrophication; however, attention later focused on N and P (Hutchinson 1973; Schindler 2006). By 1968, a multi-lake comparison showed that P loading from catchments was a key factor causing eutrophication (Vollenweider 1968). On the basis of the ratio of N to P (N:P) and small-scale bottle bioassays, some researchers argued that N was also factor promoting total phytoplankton abundance and should be controlled (White et al. 1985; Sanders and Cibik 1986). However, Hutchinson (1973) and Schindler (1977) believed that N loading reduction could stimulate N-fixing cyanobacterial blooms. To test this hypothesis, a long-term (1969–2009) fertilization experiment was conducted in Lake 227 of the Experimental Lakes Area (ELA) in Canada (Schindler et al. 2008; Paterson et al. 2011). The results suggested that reducing N input greatly favored N-fixing cyanobacteria, and that N-fixation was sufficient to enable phytoplankton to stay at a high level, given sufficient P and time. These findings suggested that total phytoplankton abundance was depended on P, not N. However, Scott and McCarthy (2010, 2011) reanalyzed the data and found that even though Lake 227 remained eutrophic after N addition was stopped, total nitrogen concentration (TN) and phytoplankton chlorophyll *a* (Chl *a*) decreased slowly. They suggested that the presence of massive heterocysts did not indicate that N-fixation can offset N shortage, and that more time was needed to reach a new steady state for the N pool in Lake 227. Lewis et al. (2011) provided some examples that suggested that N addition could stimulate phytoplankton to a similar (sometimes higher) degree as (than) P addition. Therefore, the role of N in regulating total phytoplankton abundance is still controversial.

The above-mentioned whole-lake fertilization experiments were carried out in high-latitude areas (46–63°N) and further tests at whole-ecosystem scale are needed in low-latitude areas. In the subtropical mid-lower Yangtze River Basin, there are a large number of lakes with a total area of 1.58×10^4 km^2; these lakes support a dense human population and rapidly developing economy. Most of the lakes are experiencing serious eutrophication and frequent algal blooms, with more than 40% in a eutrophic or hypertrophic state (Wang et al. 2009). Our multi-year investigations on more than 40 Yangtze lakes suggested that, in this area, total phosphorus (TP) was the primary factor regulating phytoplankton regardless of the ratio of total nitrogen to total phosphorus (TN:TP) (Wang et al. 2008). To test the relative role of N and P in regulating phytoplankton abundance in the subtropics at a whole-ecosystem scale, we conducted an 18-month fertilization experiment in 4 ponds located in the middle Yangtze Basin. Three treatments were used to represent various combinations of nutrient addition, namely, both N and P addition (+N+P), P addition (+P), and N addition (+N). The control treatment had no nutrient addition (Control).

Materials and methods

Study site and establishment of the experimental system

The experimental ponds (30°17′20″N, 114°43′45″E) are located to the northeast of Lake Bao’an, which is on the south bank of the middle Yangtze River (Fig. 1). In this region, a warm and humid subtropical monsoon climate dominates, with an average air temperature of ca. 19°C and precipitation of ca. 1030 mm (Fig. 2). Lake Bao’an is a meso-eutrophic lake with a surface area of ca. 48 km^2. According to our survey from 2011 through 2012, the lake averaged 19°C in water temperature (WT), 1.9 m in water depth (Z_M), 0.6 m in Secchi depth (Z_{SD}), 8.6 in pH, 474.8

μS cm^{-1} in conductivity (Cond), 1.41 mg L^{-1} in TN, 0.09 mg L^{-1} in TP, and 50.4 μg L^{-1} in Chl *a*. Measurement of these parameters was typically carried out between 09: 00 and 13: 00.

The experimental ponds used here were modified from a 0.3 ha pond that had been used to culture lotus (*Nelumbo nucifera*) for food. This pond was dredged to remove surface sediments rich in nutrients and organic matter and then separated into 4 equally sized compartments by the construction of embankments. Sediments and water were then introduced from Lake Bao'an with the aim of creating a natural lake-like system. The sediments were initially mixed and then placed in the ponds. The depth of the introduced sediments was approximately 10 cm. There was typically a small amount of inflow into the experimental ponds during periods of rainfall. In spring or summer when there was a large amount of rainfall, the water depths of the experimental ponds increased quickly. To ensure that the embankments remained safe and that the water in each of the experimental ponds was kept separate, we pumped equal amounts of water from each pond. Twelve gibel carp (*Carassius auratus*), with an average weight of 200 g, were introduced to each pond on Apr 28, 2012, the 16th month of the experiment, to inhibit the growth of benthic filamentous algae, which may compete with phytoplankton for nutrients. The experiment lasted for another 2 months after the gibel carp were introduced. Although the carp could increase TP and Chl *a*, their effects were assumed to be similar in the different ponds since equal number of fish with similar body mass were introduced. No submerged plants was intentionally cultivated in the ponds.

On Dec 22, 2010, one month after the establishment of the experimental system, an investigation of the initial status of the ponds was carried out. Average values for the measured parameters were as follows: water depth (Z_M), 1.3 m (range: 1.0–1.5 m); Secchi depth (Z_{SD}), 0.8 m

(0.6–1.0 m); water temperature (WT), 9.8°C (9.4–10.3°C); pH, 6.93 (6.89–6.97); conductivity (Cond), 294 μS cm^{-1} (282–311 μS cm^{-1}); total nitrogen concentration (TN), 0.37 mg L^{-1} (0.30−0.42 mg L^{-1}); total phosphorus concentration (TP), 0.05 mg L^{-1} (0.02−0.08 mg L^{-1}), and phytoplankton chlorophyll *a* (Chl *a*), 4.2 μg L^{-1} (2.4−5.6 μg L^{-1}). Data are provided in Table S1.

Fertilization

The background (initial) concentrations of the Control pond were used as the target concentrations (Table 1). Target concentrations of 2.0 mg TN/L and 0.2 mg TP/L were set according to the nutrient concentrations of Class V in the Environmental Quality Standards for Surface Waters (AQSIQ 2002a). In stage II, target TP concentration was raised to 0.3 mg/L to increase the difference between the treatments with and without P addition.

N and P fertilizers (NH_4Cl and $Na_2HPO_4 \cdot 12H_2O$, respectively) were added to meet the target concentrations (Table 1). The amount of fertilizer (F, g) added was calculated based on the difference between target concentrations and measured concentrations before fertilization in the ponds:

$$\mathrm{F} = (\mathrm{T} - \mathrm{M}) \times \mathrm{V},$$

where T is the target concentration in mg L^{-1}, M is the measured concentration in mg L^{-1}, V is the volume of pond water in m^3.

The first fertilization occurred on Dec 23, 2010, one day after the initial measurement of baseline conditions. In stage I (from Dec 23, 2010 through Jun 2011), nutrients were added twice per month, and half of the calculated amount of nutrients were added each time to simulate the escalation of nutrient concentrations in natural lakes undergoing eutrophication. In the +P pond, N was added at a N:P of 6:1 during stage I to create initial condition of N loading for the later simulation of N reduction. In Stage II (from Jul 2011 through Jun 2012), the calculated amount of nutrients were added once per month to meet target concentrations. Fertilizer was dissolved with pond water in polyethylene buckets before being added evenly to the ponds.

Sampling and analysis

Physical and chemical parameters were measured 2–4 times per month (but 8 times in Jan 2011). Density (D_{Phyt}), biomass (B_{Phyt}) and taxonomic composition of phytoplankton were analyzed once per month.

None of the ponds was stratified during the experiment. WT, pH, and Cond were measured at approximately 20 cm below the water surface using a PRO Plus device (Yellow Spring Instruments, USA) and were generally measured between 09: 00 and 12: 00. Z_M and Z_{SD} were measured using a sounding lead and a Secchi disc, respectively. Integrated water samples were collected using a tube sampler (1.5 m in height, 10 cm in diameter) at five randomly chosen locations within each pond before fertilization. One liter of mixed water was transferred to a polyethylene bottle for chemical analysis using national standards (Huang et al. 1999). TN was determined using the alkaline potassium persulfate digestion-UV spectrophotometric method (TU-1810) and TP was determined using the ammonium molybdate-ultraviolet spectrophotometric method (TU-1810). Chl *a* was determined spectrophotometrically after extraction in 90% acetone for 20–24 h at 4°C. The samples were read at two wavelengths, 665 nm and 750 nm, before and after acidification with 10% HCl. Chl *a* concentration was calculated using the method described by Huang et al. (1999). A further 1 L water was transferred to a polyethylene bottle for phytoplankton analysis, and preserved with Lugol's iodine solution (3–5% final conc.). After sedimentation for 48 h, phytoplankton samples were concentrated to 30–50 mL and taxa were identified and counted under a microscopic magnification of ×200 or 400. B_{Phyt} (mg L^{-1}) was calculated as follows:

$$B_{Phyt} = \sum_{i=1}^{S} \rho \times V_i \times D_{i,}$$

where S is the taxa number of phytoplankton, ρ is phytoplankton density in 1×10^{-9} mg μm^{-3}, V_i is the biovolume of taxa i in μm^3 (each taxa counted in the sample was regarded as a geometrical figure, excluding gelatinous envelopes and loricae), D_i is the density of taxa i in cells L^{-1}.

The heterocysts mentioned in our experiment were the sum of all the heterocysts in different N-fixing cyanobacteria. These were mainly *Dolichospermum* spp. [also known as *Anabaena* spp. (Wacklin et al. 2009)] with a small number of *Aphanizomenon* sp. and *Cylindrospermopsis* sp.

Data analysis

All data were averaged monthly except for Dec 2010. Excel 2010 and R were used for data analysis. As the treatments in the pond experiment have no replicate and the data cannot be normalized, a non-parametric test for repeated measures, the Friedman's test, was used to test difference among the ponds. Wilcoxon-Nemenyi-McDonald-Thompson post-hoc tests were used when the Friedman test gave a significant *p* value (< 0.05) (Hollander and Wolfe 1999).

Results

Variation in TN

Over the entire experimental period, average TN in the +P, +N+P, and +N ponds did not differ significantly, and they were all significantly higher than that in the Control pond (Fig. 3a) (n = 19) (the detailed p values are presented in Table S3 and S4 in the supporting information). A similar pattern was found when separating the experiment into two stages (stages I and II), but the difference among the ponds was not significant in stage I (Fig. 3a, Fig. S1, Table S3, Table S4).

Variation in TP

Over the entire experiment, average TP was higher in the two P addition ponds (+P, +N+P) than in the two ponds without P addition (+N, Control) (Fig. 3b, Fig. S1, Table S3, Table S4). In stage I, all ponds had similar and relatively low TP, whereas in stage II, TP in the two P addition ponds (+P, +N+P) increased and was obviously higher than that in the two ponds without P addition (+N, Control).

Variations in Chl *a*

Average Chl *a* varied among ponds in a pattern similar to TP. Over the entire experiment, the two P addition ponds (+P, +N+P) had higher average Chl *a* than the two ponds without P addition (+N, Control) (Fig. 3c, Fig. S1, Table S3, Table S4). In stage I, the Chl *a* of all ponds was at a similar level at the beginning but started to increase to a greater extent in the +P pond than in the other ponds in the late part of the stage. In stage II, Chl *a* averaged about 50% higher in the ponds receiving P (+P, +N+P) than in the Control and +N ponds. Regression analyses of summer $\log_{10}$ (Chl *a*) against spring $\log_{10}$ (TP) and spring $\log_{10}$ (TN) showed a much closer relationship between $\log_{10}$ (Chl *a*) and $\log_{10}$ (TP) ($n = 16$) ($R^2 = 0.50$, $p < 0.001$) than between $\log_{10}$ (Chl *a*) and $\log_{10}$ (TN) ($R^2 = 0.22$, $p < 0.001$).

Variations in D_{Phyt}

Over the entire experiment, D_{Phyt} averaged about 110% higher in the +P pond than in the other ponds (Fig. 4, Fig S1, Table S2). In late stage I and all of stage II, D_{Phyt} showed similar patterns to that observed in the experiment as a whole. All the ponds were dominated by Cyanobacteria (49.7–57.6%) (*Pseudoanabaena* sp., *Dolichospermum* sp., *Microcystis* sp., *Raphidiopsis* sp., and *Merismopedia* sp.) and Chlorophytes (0.5–95.2%) (*Scenedesmus* sp.) (Fig.

4). In autumn and winter, the +N and Control ponds were also dominated by Chrysophytes (0.3–98.9%) (*Dinobryon* sp., *Keqhyrion* sp., and *Chrysococcus* sp.) and Bacillariophytes (0–25%) (*Synedra* sp.). N-fixing cyanobacteria (mainly *Dolichospermum* spp.) appeared in all ponds successively from the beginning of the experiment. N-fixing cyanobacteria was highest in density in the +P pond [50.6% (0–79.4%)] [mean (range)] and endured the longest (11 months), with peaks in May–Jul 2011 and May–Jun 2012 (Fig. 4, Fig. S1).

Variation in B_{Phyt}

Over the entire experiment, B_{Phyt} averaged 110% higher in the two P addition ponds (+P, +N+P) than in the two ponds without P addition (+N, Control) (Fig. 5, Fig. S1, Table S2). B_{Phyt} in the +P pond started to increase in late stage I. In stage II, B_{Phyt} in the two P addition ponds (+P, +N+P) peaked in 2012 and became much higher than that in the two ponds without P addition (+N, Control). All ponds were dominated by Dinophytes (13.6–32.0%) (*Peridinium* sp. and *Ceratium* sp.) and Euglenophytes (0.0–89.8%) (*Trachelomonas* sp. and *Euglena* sp.) (Fig. 5). In autumn and winter, the +N and Control ponds were also dominated by Chrysophytes (0–99.7% of the total) (*Dinobryon* sp. and *Keqhyrion* sp.) and Bacillariophytes (0–68.3%) (*Synedra* sp.). The biomass of N-fixing cyanobacteria was much higher in the +P pond than in the other ponds (Fig. 5).

Heterocyst density related to TN:TP (mass ratio)

Over the entire experiment, the +P pond had higher average heterocyst density than the other ponds (Fig. 3e, Fig. S1, Table S2). No heterocysts was found when TN:TP was >35, with the sole exception that some heterocysts (0.03 million cells L^{-1}) occurred in the +N pond in Jun 2011 when TN:TP = 70. In general, high heterocyst densities were found mainly within a TN:TP range of between 5 and 20 (Fig. 3d, 3e) and with elevated P concentrations.

Discussion

P, not N input determines total phytoplankton abundance in subtropical lakes

Our experiment demonstrates that P is the primary factor regulating total phytoplankton abundance in subtropical lakes. Chl *a* and B_{Phyt} in the +N pond were similar to those in the Control, and were 59.5% and 47.6% of those in the +N+P pond, respectively (Fig. 3c, 5). Our multi-year investigations also showed similar results (Wang et al. 2008). Many other experiments can be found in other ecoregions that support the role of P as the limiting nutrient for eutrophication, such as the ELA of Canada. In Lake 302, no increases in Chl *a* and B_{Phyt} were found after HNO_3 fertilization; chrysophyte dominance gave way to chlorophytes and dinoflagellates due to the decreased pH (Findlay and Kasian 1990; Schindler 2012). In Lake 304, Chl *a* returned to a similar level as pre-fertilization when N alone was added after 2 years fertilization with both N and P. Chl *a* increased again when P was further added 2 years later (Schindler 1974; Schindler 2012). In Lake 226 during 1973−1980, phytoplankton biomass in the northeast basin (Lake 226 NE) increased 4−8 times when fertilized with both N and P. In the separated southwest basin (Lake 226 SW), where only N fertilizer was added, phytoplankton biomass also increased, but only by 2−4 times. Schindler argued that the increase in biomass was because of a leakage of P through the sea-curtain separating the 2 basins (Mills and Schindler 1987; Schindler 2012), but another possible explanation is that it was due to the addition of N (Findlay and Kasian 1987). Increases in phytoplankton biomass caused by N addition were also reported in other lakes (Lewis et al. 2011).

Many successful lake restoration practices in high-latitude areas (42–59°N) also suggested the vital role of P in determining total phytoplankton abundance (Schindler 2012; Dove and Chapra 2015). Additional cases can be found in subtropical areas, including Lake Xihu (30°N) in

Hangzhou, China, where treatment by water diversion and nutrient reduction from 1987 through 2013 decreased TP by 58.2–78.3% (0.12–0.04 mg L^{-1}), while decreasing TN by only 7.7–16.7% (2.6–2.3 mg L^{-1}). Chl *a*, however, decreased by 68.8–93.8% (160–30 μg L^{-1}) in concert with the decline in TP (You et al. 2015). Variations in Chl *a* have been shown to be highly positively correlated with TP but independent with TN (Wang and Wang 2009). Furthermore, Cyanobacteria dominance decreased, whereas Chlorophytes dominance increased, and phytoplankton diversity also increased after water treatment (Zhang et al. 2009). In Lake Apopka (28°N), USA, reduction of external nutrients decreased TP by 54% (0.23–0.11 mg L^{-1}) and TN by only 26% (5.3–3.9 mg L^{-1}), whereas Chl *a* decreased by 37% (94–60 μg L^{-1}). As in Lake Xihu, the Chl *a* decline was most closely related to TP (Coveney et al. 2005).

N deficiency is offset by N-fixation in subtropical lakes

Our pond experiment suggests that N deficiency is offset by N-fixation in subtropical lakes. Chl *a* and B_{Phyt} in the +P pond were similar to those in the +N+P pond, being 1.6–1.8 times higher than those in the +N pond and 2.7–3.6 times higher than those in the Control pond; D_{Phyt} was 2.1–3.0 times higher in the +P pond than in the other three ponds (Fig. 3c, 4, 5). The density and biomass of N-fixing cyanobacteria were 5.0 and 3.5 times higher in the +P pond than in the +N+P pond, and heterocyst density was 4.5 times higher in the +P pond than in the +N+P pond (Fig. 4, 5, 3e). Numerous heterocysts appeared when the TN:TP value was between 5 and 20. A similar TN:TP (20) was reported by Tõnno and Nõges (2003). The regression analysis of time versus TN in the +P pond showed a significant increase in TN from the beginning of the experiment (R^2 = 0.36, p = 0.005). TN in the +P pond was close to that in the +N+P pond from the 4th month (March 2011) (Fig 3a). Previous studies have demonstrated that denitrification is higher in lakes with higher TN or N loading (Seitzinger et al. 2006; Piña-Ochoa and Álvarez-Cobelas 2006). Thus, the rate of denitrification in the +P pond may be lower than that in the +N+P pond, although this was not measured. However, lower denitrification could only contribute to a reduction in the rate of loss of N from the pond, and not to increased N in the pond. Therefore, we suggest that the increased N in the +P pond was mainly induced by N-fixation.

We simultaneously conducted a long-term mesocosm (800-L tanks) experiment from Dec 2010 through Sep 2011 and obtained similar results to the pond experiment (Wang et al. 2016). In the mesocosm experiment, Chl *a* in the +P tank was similar to that in the +N+P tank in the first month. After that, a large amount of filamentous algae appeared in the tanks, and the biomass of the filamentous algae was the greatest in the +P tank. Although TN in the +P tank did not exceed that in the +N+P and +N tanks, it was higher than that in the Control tank for most of the duration of the experiment. Result of an N budget analysis showed that +P tank (6.80 g) had a higher natural supply than +N+P (4.90 g), +N (1.50 g), or Control tanks (3.00 g). Our multi-year investigations on the Yangtze lakes also showed that variation in Chl *a* was independent with TN at a given concentration of TP (Wang et al. 2008).

Many whole-lake nutrient addition or reduction experiments have been performed elsewhere. However, the results of these experiments are not consistent. In Seathwaite Tarn (54°N) in the English Lake District, Chl *a* increased slightly when P alone was added during 1992–1993 (May 1995). In Far Lake (63°N), Canada, following P addition, phytoplankton biomass did not change during the first year, minus 4% the second year, and plus 43% the third, but N-fixing cyanobacteria (periphyton and phytoplankton) were found during the experiment (Welch et al. 1989; Schindler 2012). In Lake 261 at ELA (49°N) of Canada, fertilization with P alone during

1973–1976, resulted in increases in Chl *a* and TN, which were at least twice as high as pre-fertilization values (Fee 1979; Schindler 2012). In Lake 227, Chl *a* and B_{Phyt} remained at high levels even after the N:P of added fertilizer was decreased from 12 (1969–1974) to 5 (1975–1989) and then to 0 (1990–2009). N-fixing cyanobacteria started to appear following N reduction and they became dominant in summers after the 1980s. The biomass percentage of N-fixing cyanobacteria was more than 50% after N additions were stopped entirely. N-fixation also increased with reductions of N loading and was sufficient to allow phytoplankton to be produced in proportion to P (Schindler et al. 2008; Paterson et al. 2011). However, this interpretation was later questioned by Scott and McCarthy (2010, 2011). They argued that the presence of massive heterocysts did not indicate efficient offset of N shortage by N fixation and some more years of P fertilization were further needed to determine if N reduction could result in significant decrease in phytoplankton.

There are also many researchers who support the idea of reducing N (Lewis and Wurtsbaugh 2008; Conley et al. 2009a, b; Abell et al. 2010; Scott and McCarthy 2010; Xu et al. 2010; Lewis et al. 2011; Paerl et al. 2011). Their opinions are mainly based on the following observations. First, seasonality of N and P limitation or co-limitation exists in some waterbodies. However, this finding was mostly based on bottle bioassays or mesocosm fertilization experiments. Such small short-term experiments fail to simulate some important processes in natural lakes, such as N-fixation and nutrient exchange across the sediment–water interface. In our tank and pond experiments, more than 3 months were needed for the TN to increase significantly in our +P treatments. Such a long process cannot be simulated by short-term experiments. Lewis et al. (2011) listed some whole-lake experiments supporting the view that N reduction mitigated eutrophication based on higher increases in phytoplankton after both N and P addition compared with either nutrient alone. However, even N-limitation may persist for a long time in some waterbodies, it can be transformed to P limitation by controlling P. This means that if we can sufficiently reduce P loading, the phytoplankton will no longer be limited by N, but by P. Second, N-fixation cannot compensate for N shortage in some waterbodies because denitrification may exceed fixation (Paerl and Scott 2010). However, in Lake 227 at ELA of Canada, when N addition was discontinued after several years fertilization with both N and P, TN and phytoplankton remained at a high level for years (Schindler et al. 2008). The increased TN and Chl *a* in our +P pond also suggest that N-fixation may offset N shortage. Third, it is difficult to reduce P in hypertrophic lakes with high internal loading. It is true that the decrease in internal P loading is a long process and 10–15 years are needed for a new equilibrium (Jeppesen et al. 2005). However, this should not be the reason to reduce N because N reduction may be offset by biological N-fixation if P is not controlled effectively. Therefore, the evidences supporting N reduction in mitigating eutrophication are not very persuasive.

Based on the whole-ecosystem fertilization experiment, in addition to our previous tank experiment and multi-year investigations, we conclude that P is the primary factor regulating total phytoplankton abundance in subtropical lakes, and that a lack of N could even induce massive growth of N-fixing cyanobacteria that offset TN deficiency. Evidences from these studies suggest that attention should be mainly focus on P reduction in mitigating eutrophication. Some lake restoration practices in subtropics also support this inference. Due to the fact that our tests were experiments about nutrient addition, these conclusions and inferences still need to be tested by nutrient reduction experiments.

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Figure and table captions.

Table 1. Target concentrations and the amount of fertilizer added to the experimental ponds

Fig. 1. Location of the experimental ponds.

Fig. 2. Precipitation and air temperature during the experiment.

Fig. 3. Total nitrogen (TN), total phosphorus (TP), mass ratio of TN to TP (TN:TP), phytoplankton chlorophyll *a* (Chl *a*), and heterocyst density in the experimental ponds, Dec 2010–Jun 2012. Fertilizer additions were generally half of the calculated amount in Stage I, and the full calculated amount in Stage II. Heterocyst data for Jan–Feb 2012 are missing.

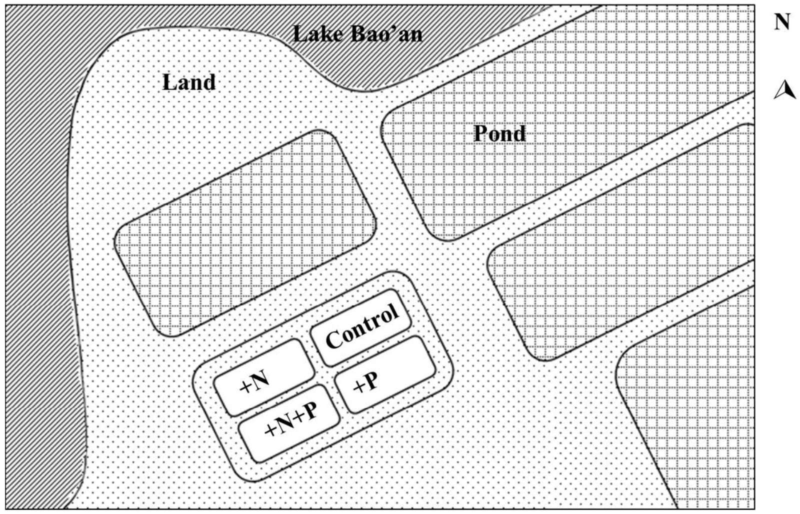
Fig. 4. Phytoplankton density (DPhyt) by algal group in the experimental ponds, Dec 2010–Jun 2012. Fertilizer additions were generally half of the calculated amount in Stage I, and the full calculated amount in Stage II. Phytoplankton data for Jan–Feb 2012 are missing.

Fig. 5. Phytoplankton biomass (B_{Phyt}) by algal group in the experimental ponds, Dec 2010–Jun 2012. Fertilizer additions were generally half of the calculated amount in Stage I, and the full calculated amount in Stage II. Phytoplankton data for Jan–Feb 2012 are missing.

Table 1. Target concentrations and the amount of fertilizer added to the experimental ponds

Pond	**Stage I (Dec 23, 2010–Jun 2011)**				**Stage II (Jul 2011–Jun 2012)**			
	Target concentration, mg L^{-1}		Fertilizer, mg L^{-1} mo^{-1}		Target concentration, mg L^{-1}		Fertilizer, mg L^{-1} mo^{-1}	
	TN	TP	N	P	TN	TP	N	P
+N+P	2.00	0.20	2.08	0.14	2.00	0.30	1.72	0.24
+P	1.00	0.20	0.57	0.10	-	0.30	0	0.17
+N	2.00	-	1.61	0	2.00	-	1.92	0
Control	-	-	0	0	-	-	0	0

“-”, background concentration



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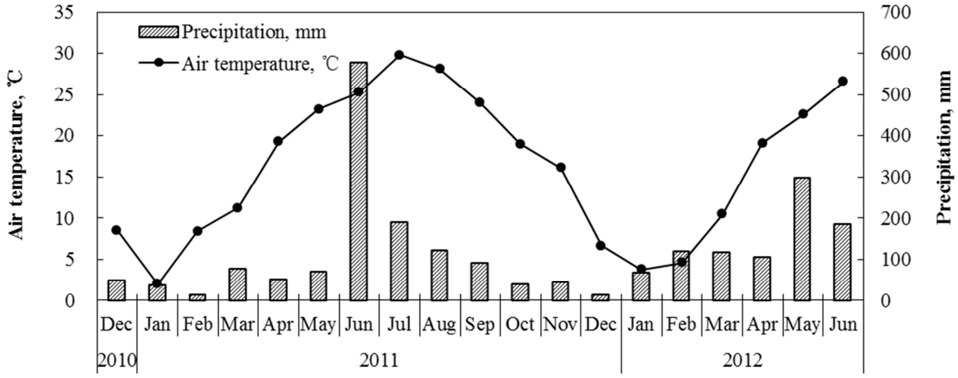

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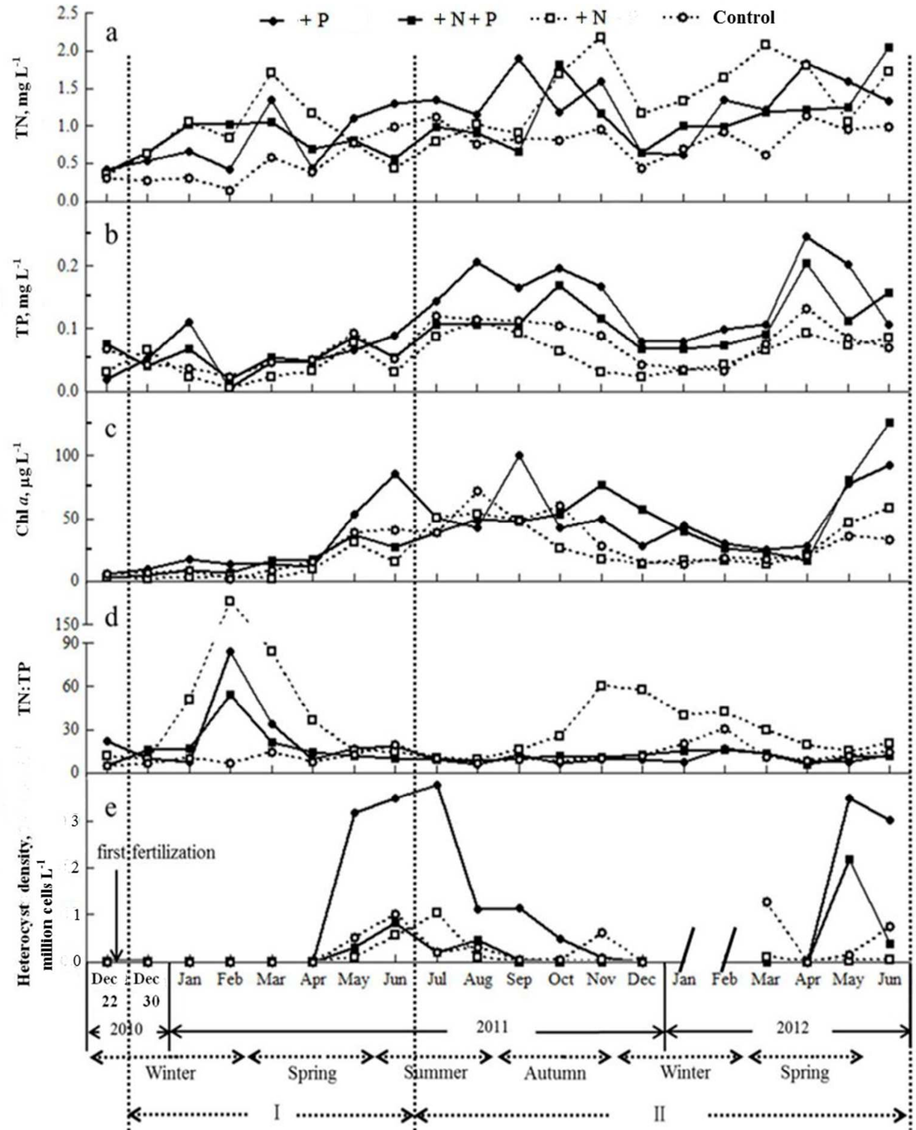

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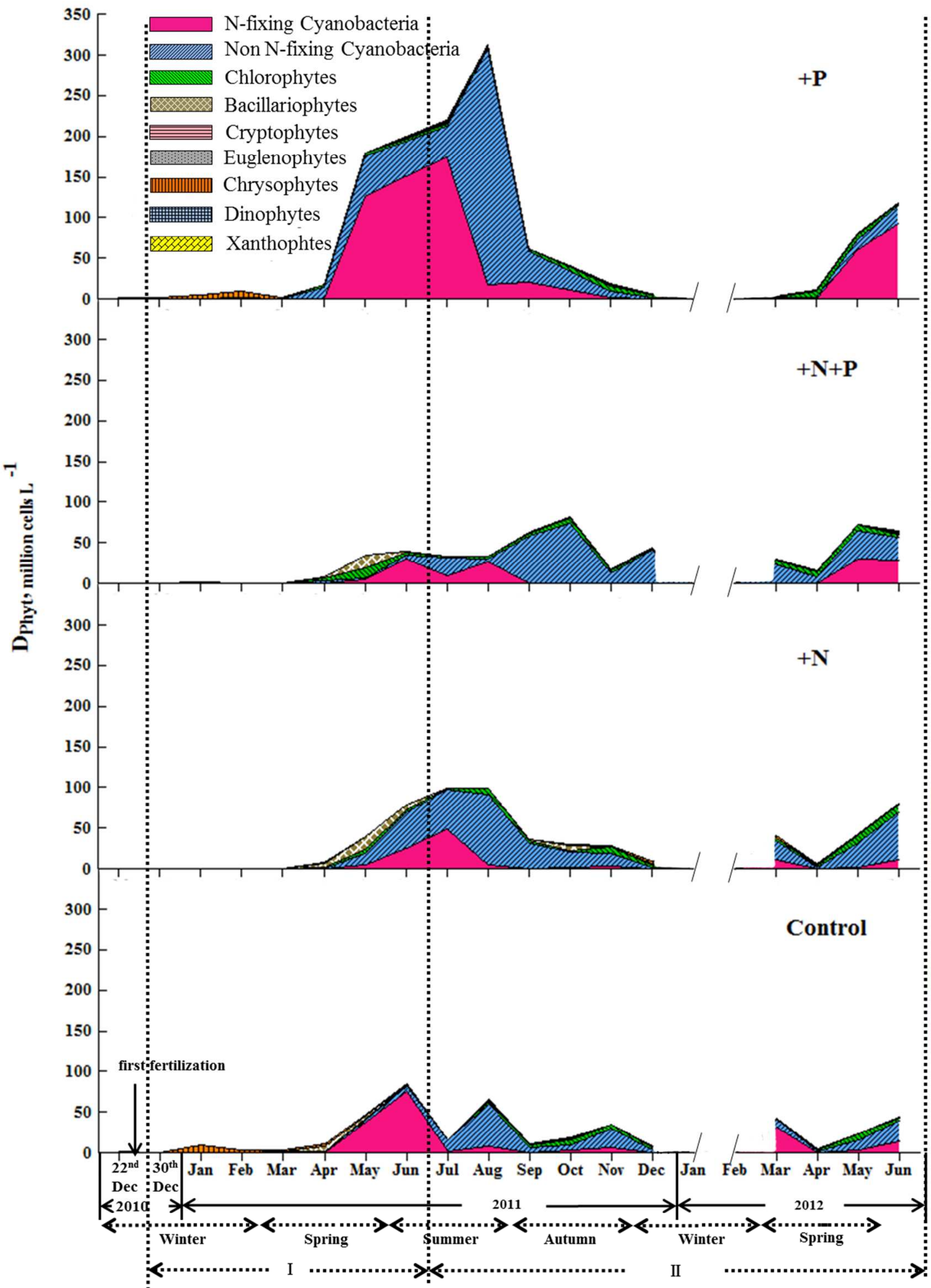

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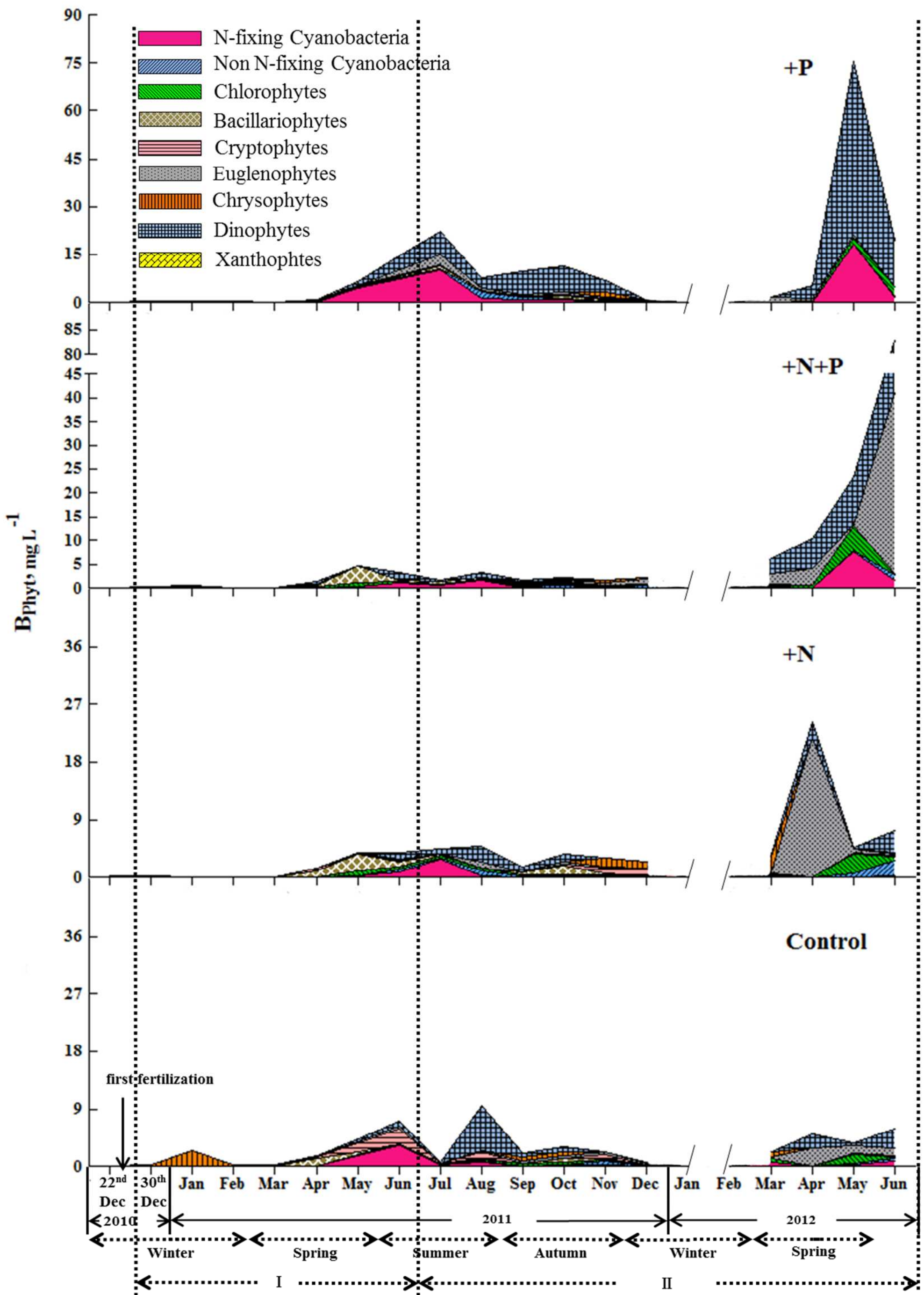

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