

## Can short-term and small-scale experiments reflect nutrient limitation on phytoplankton in natural lakes?\*

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**Abstract** Whether it is necessary to reduce nitrogen (N) and/or phosphorus (P) input to mitigate lake eutrophication is controversial. The controversy stems mainly from differences in time and space in previous studies that support the contrasting ideas. To test the response of phytoplankton to various combinations of nutrient control strategies in mesocosms and the possibility of reflecting the conditions in natural ecosystems with short-term experiments, a 9-month experiment was carried out in eight 800-L tanks with four nutrient level combinations (+N+P, -N+P, +N-P, and -N-P), with an 18-month whole-ecosystem experiment in eight ~800-m<sup>2</sup> ponds as the reference. Phytoplankton abundance was determined by P not N, regardless of the initial TN/TP level, which was in contrast to the nutrient limitation predicted by the N/P theory. Net natural N inputs were calculated to be 4.9, 6.8, 1.5, and 3.0 g in treatments +N+P, -N+P, +N-P, and -N-P, respectively, suggesting that N deficiency and P addition may promote natural N inputs to support phytoplankton development. However, the compensation process was slow, as suggested by an observed increase in TN after 3 weeks in -N+P and 2 months in -N-P in the tank experiment, and after 3 months in -N+P and ~3 months in -N-P in our pond experiment. Obviously, such a slow process cannot be simulated in short-term experiments. The natural N inputs cannot be explained by planktonic N-fixation because N-fixing cyanobacteria were scarce, which was probably because there was a limited pool of species in the tanks. Therefore, based on our results we argue that extrapolating short-term, small-scale experiments to large natural ecosystems does not give reliable, accurate results.

**Keyword:** eutrophication; nutrient control; extrapolation; mesocosm experiment

### 1 INTRODUCTION

The eutrophication of lakes from excessive nutrient inputs is becoming a global environmental problem. One of the main problems arising from eutrophication is phytoplankton overgrowth, which leads to bad taste and odor, toxic algal blooms, exclusion of submersed aquatic vegetation, fish kills, and increased turbidity (Dodds, 2002). Controlling nutrient inputs is fundamental to inhibiting phytoplankton development. However, whether it is necessary to reduce nitrogen (N) and/or phosphorus (P) input is controversial (Schindler, 2012). Deciding which nutrient control strategy to employ is of practical importance

(Schindler, 2012). The cost of controlling both elements would be much higher than that of controlling P alone. It was 7.2 to 14.8 times to meet the European Union's Water Framework Directive in the Baltic Sea (Bryhn and Håkanson, 2009) and 4 to 8 times in the city of Winnipeg, Manitoba, Canada sewage treatment (Schindler et al., 2012). If less

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**Table 1 Literature supporting phosphorus (P) control versus dual nitrogen (N) and P control to mitigate lake eutrophication**

	Lakes	Location	Duration	Approach	Reference
P control only	45 shallow lakes	Subtropical, China (28°–31°N)	1–3 years (2001–2005)	Multi-lake comparison	Wang et al. (2008)
	Lake Apopka	Subtropical, USA (28°N)	15 years (1988–2003)	Practical management	Coveney et al. (2005)
	West Lake	Subtropical, China (30°N)	9 years (1999–2007)	Practical management	Deng et al. (2009)
	8 ponds	Subtropical, China (30°N)	2 years (2011–2012)	whole-ecosystem fertilization	Li et al. (in revision)
	4 shallow lakes and 5 deep lakes	Temperate (45°–56°N)	10–20 years (1970s–2000s)	Practical management	Jeppesen et al. (2005)
	Lake 227 in ELA	Temperate, Canada (49°N)	37 years (1969–2005)	Multi-year whole-lake fertilization	Schindler et al. (2008)
	Lake 304	Temperate, Canada (49°N)	31 years (1965–1996)	Multi-year whole-lake fertilization	Schindler (2012)
	Lake Washington	Temperate, USA (47°N)	60 years (1933–1994)	Practical management	Edmondson (1994)
	Lake Tiefwareensee	Subfrigid, Germany (53°N)	9 years (1998–2006)	Practical management	Mehner et al. (2008)
	Lake Alchichica	Trophic, Mexico (19°N)	6–8 days once a month (2004–2005)	In situ bioassays (1 L)	Ramírez-Olvera et al. (2009)
Dual control of N and P	Taihu Lake	Subtropical, China (31°N)	4 or 6 days once a month (May, July, October and December of 2008)	In situ bioassays (1 L)	Xu et al. (2010)
	12 volcanic lakes	Subtropical-temperate, New Zealand (38°–41°S)	2 h once (4 times in 1982)	Laboratory bioassays (about 30 L)	White et al. (1985)
	Patuxent River	Subtropical, USA (39°N)	2–4 weeks once (June 1983–November 1984)	Laboratory bioassays (500 L)	Sanders et al. (1987)
	Narragansett Bay	Temperate, USA (41°N)	9 weeks (1988)	13 m <sup>3</sup> mesocosm experiment	Oviatt et al. (1995)
	Lake El Tejo	Subtropical, Spain (39°N)	8 days (1997)	1 L glass bottles	Camacho et al. (2003)
	4 lakes	Subfrigid, Germany (52°N)	3 days every two weeks (March–September in 2011)	Laboratory bioassays (0.15 L)	Kolzau et al. (2014)
	Lake Stigsholm	Subfrigid, Denmark (55°N)	1 month or 10 days once a month (May, June, August, and September in 1993, and 10 days in 1994)	Six mesocosm experiment (5 m in diameter, 0.6 m in height)	Schittüter et al. (1997)

emphasis were placed on controlling N, much more energy could be focused on P control and the cost to mitigate eutrophication would be considerably less, thus solving water crises in advance. N control has, however, long been considered a necessary practice. Massive funds have been invested in N-removal during wastewater treatment.

When looking at the evidence supporting the contrasting nutrient control strategies, it can be seen that the above-mentioned controversy mainly results from the differences in time and space in previous studies (Table 1). In terms of P-only control, as reviewed by Schindler (2012), there have been many long-term, whole-ecosystem experiments and lake recovery case histories that prove it is a successful strategy (also refer to Table 1). There are some more lake recovery cases in China, e.g., Lake Xihu, Hangzhou (Deng et al., 2009). In this lake, a diversion project was conducted to introduce water from the Qiantangjiang River, which is nutrient rich (3.08 mg TN/L, 0.13 mg TP/L), to flush and dilute this highly

eutrophic lake. After the introduced water was subjected to P reduction treatment, TP was reduced to a much lower level of 0.04 mg/L, compared with a TN level of 2.07 mg/L. Thereafter, phytoplankton abundance was effectively controlled, reducing phytoplankton chlorophyll *a* (Chl *a*) by 11%–82%.

However, there is a lack of reliable evidence to support either N-only or P and N control strategies. Short-term, small-scale nutrient addition experiments have traditionally been the main approach to test N control strategies (White et al., 1985; Oviatt et al., 1995; Camacho et al., 2003; Kolzau et al., 2014), assuming that some nutrient can be reduced by controlling it over a long period, if this nutrient stimulates phytoplankton during the experiment (Schindler, 2012; see also in Table 1). Most nutrient addition experiments were >30 L, ranging from 0.15 to 13 000 L (Table 1). The problems associated with extrapolating small-scale experiments to natural ecosystems by comparing them with whole-lake nutrient addition experiments has been reported

previously (Hecky and Kilham, 1988; Carpenter, 1996; Schindler, 2012). In terms of time scale, most of the experiments were less than 2 weeks, ranging from 2 h to 9 weeks (Table 1). Some have criticized the shortage of short-term experiments in reflecting long-term situations in natural ecosystems (Wang and Wang, 2009). However, whether the validity of extrapolation increases by extending the duration of experiments has not been tested to date.

Dual control of N and P is usually supported by nutrient limitation of phytoplankton development assessed by the ratio of N to P (N/P). Various N/P thresholds have been proposed to discriminate the limitations of N and P, such as 10–17 (Sakamoto, 1966) and 10–30 (Huber et al., 1988). A lake is believed to be limited by P when the ratio exceeds the high threshold, by N if the ratio is under the low threshold, and co-limited by N and P in the case of an intermediate ratio. Through logical reasoning and empirical analysis based on regional comparative limnological studies, Wang and Wang (2009) pointed out that the N/P hypothesis is a subjective assumption without conclusive evidence. Actually, the problem with the N/P hypothesis is also one of temporal and geographical scales. Phytoplankton nutrient limitation is usually assessed through a combination of N/P ratio and short-term, small bioassay experiments.

The aim of this study was to test the hypothesis that short-term experiments cannot reflect real situations in natural ecosystems with regard to the response of phytoplankton to various combinations of nutrient control strategies. A 9-month mesocosm experiment was carried out in eight 800-L tanks with various nutrient addition treatments. The results were compared with our 18-month whole-ecosystem experiment in eight ~800-m<sup>2</sup> ponds (Li et al., in revision).

## 2 MATERIAL AND METHOD

The experiment was carried out in eight cylindrical tanks (1.0 m in both height and diameter) (30°17'20"N, 114°43'45"E) located to the northeast of Lake Bao'an (48 km<sup>2</sup> in surface area, 1.9 m in mean depth) in the middle Changjiang (Yangtze) Basin. A warm, humid subtropical climate dominates, with an annual mean air temperature of approximately 19°C and 1 030 mm precipitation.

The water used for the experiment was introduced from the southeast sub-area (6.25 km<sup>2</sup>, 2.5 m) of Lake Bao'an to the tanks on 2 December 2010. One day before the water was introduced, an investigation was

**Table 2 Basic limnological characteristics of Lake Bao'an and the experimental tanks**

	Southeast of Lake Bao'an (1 December 2010) (n=4)		Experimental tanks (22 December 2010) (n=8)	
	Mean	s. d.	Mean	s. d.
Water depth (m)	0.55	0.06	0.85	0.00
pH	7.38	0.07	7.98	0.23
Conductivity (μS/cm)	470	6.8	316	2.8
Dissolved oxygen (mg/L)	10.8	0.81	13.3	0.30
Turbidity (NTU)	15.8	4.8	3.6	0.2
Total nitrogen (mg/L)	0.44	0.06	0.36	0.07
Total phosphorus (mg/L)	0.04	0.01	0.06	0.02
Phytoplankton chlorophyll <i>a</i> (μg/L)	19.7	1.9	1.9	1.5

carried out in this area at four sampling sites (Table 2). No sediment was introduced. *Potamogeton crispus* is the only submersed macrophyte in this sub-area. No macrophyte was present when the water was pumped for the experiment. To create the same initial environment in the experimental systems, one extra tank was prepared and the water was introduced to this tank before being evenly distributed to the eight tanks used for experiment.

Four combinations of target total nitrogen (TN) and total phosphorus (TP) concentration were set to represent all of the possible nutrient control strategies. Each treatment was run in duplicate. For the no control on either nutrient treatments (expressed as +N+P), the target TN was set at 2.0 mg/L and TP at 0.2 mg/L. The target TN and TP concentrations are the corresponding values of standards for Class V according to the "Environmental Quality Standards for Surface Waters, GB 3838-2002." (GAQSIQ, 2002). For the N only control (-N+P) treatment, target TP was set at 0.2 mg/L and no N fertilizer was added. For the P only (+N-P) treatment, target TN was set at 2.0 mg/L and no P fertilizer was added. To control both nutrients (-N-P), no fertilizer was added. The first fertilization was performed on 23 December 2010, 1 day after the first investigation. The last fertilization was performed on 24 August 2011. Analytical reagents NH<sub>4</sub>Cl (≥98.5% in concentration) and Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O (>99%) (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) were added. The fertilizer was dissolved before being evenly poured into the tanks. To maintain the target concentrations, fertilizer was added every month according to the difference between target concentration and measured concentration:

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

$F$ , weight of N or P fertilizer to be added in g;  $T$ , target concentration in mg/L;  $M$ , measured concentration in mg/L;  $V$ , measured volume of pond water in m<sup>3</sup>.

The experiment lasted for approximately 10 months, covering winter, spring, summer, and early autumn. An investigation on the initial status was performed on 22 December (Table 2). After the first fertilization, comprehensive investigations were carried out every week for the first 3 weeks, once per day for 5 days after 3 weeks, and one to four times per month after the first month. The simulation experiment was ceased after the last investigation on 25 September, 2011.

Similar methods were applied in investigations on Lake Bao'an and the tanks, with the exception of water depth, which was measured by a sounding lead in the lake but by reading the water level from the outer wall for the tanks. Water temperature, pH, conductivity, and dissolved oxygen were measured in situ with an YSI ProPlus (Yellow Spring Incorporated., Ohio, USA). Turbidity was measured with a portable turbidity meter (2100Q, Hach Company, Loveland, USA). Water samples were taken from the lake at 0.5 m below the surface and at half the water depth at each site, and mixed for analysis. Water samples were only taken from the tanks only at half the water depth. TN, TP, and phytoplankton Chl *a* analysis were carried out according to the Chinese Water Analysis Methods Standards (Huang et al., 1999). TN was determined by an alkaline potassium persulfate digestion-UV spectrophotometric method (TU-1810, Persee, Beijing, China). TP was determined by an ammonium molybdate-ultraviolet spectrophotometric method after being digested with K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> solution (TU-1810, Persee, Beijing, China). Chl *a* was extracted using 90% acetone (at 4°C for 24 h) after being filtered with Whatman GF/C filters (0.45 μm, GE Healthcare UK Limited, Buckinghamshire, UK), and absorbance was then read at 665 nm and 750 nm, both before and after acidification with 10% HCl by spectrophotometry.

A total of 20 L of water was concentrated to 30 mL by filtering it through a 0.064-mm nylon net to collect zooplankton. The samples were fixed with a formaldehyde solution (3%–5% final conc.) and identified microscopically at a magnification of either ×100 or ×200. The biomass of the dominant zooplankton groups was obtained by multiplying the averaged density and individual dry mass. Zooplankton dry weight ( $W$ , μg) was calculated from

length ( $L$ , μm) as follows:

$$\text{Copepoda: } W=1.1 \times 10^{-7} \times L^{2.59} \text{ (Dumont et al., 1975);}$$

$$\text{Cladocera: } W=1.0 \times 10^{-6} \times L^{2.30} \text{ (Dumont et al., 1975);}$$

$$\text{Rotifera: } W=3.9\% \times 0.13 \times 10^{-6} \times L^3 \text{ (Zhang and Huang, 1991; Dumont et al., 1975).}$$

A total of 1 L of water was fixed with Lugol's iodine solution (3%–5% final conc.) and concentrated to 30–50 mL to collect phytoplankton after sedimentation for 48 h. The phytoplankton species were identified microscopically at a magnification of either ×200 or ×400. Phytoplankton biomass was calculated by multiplying the average density and individual dry mass. Phytoplankton dry weight ( $W$ , μg) was calculated from biovolume ( $V$ , μm<sup>3</sup>) as follows:

$$W=4.7 \times 10^{-7} \times V \text{ (Reynolds, 1984).}$$

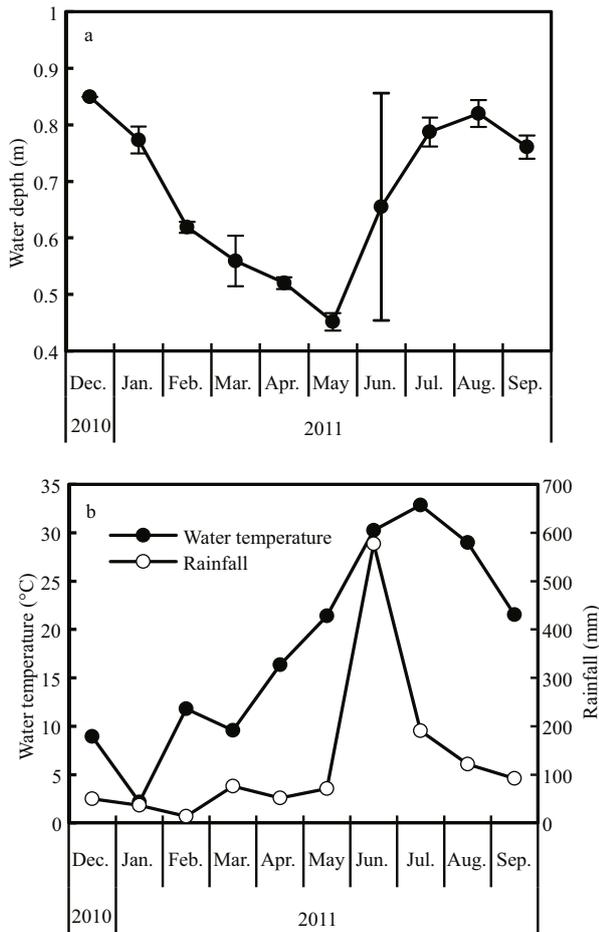
At end of the experiment, the nutrients contained in various components were measured to estimate the nutrient budget for each tank. First, the filaments were air-dried to measure the TN and TP concentrations after removing them with a nylon net and scraping from the walls of tanks with a plastic brush when necessary. After this, the nutrient concentrations in the water column were measured before the water was removed until it was 20 cm from the bottom. The remaining water and detritus were then moved to a 25-L polyethylene bucket. After a week of sedimentation, the water 10 cm from the bottom was removed and the remaining mixture of water and detritus was cleared and dried to measure the nutrient concentrations. TN and TP in filamentous algae and detritus were determined by the Kjeldahl method and an ammonium molybdate-ultraviolet spectrophotometric method, respectively. The methods for determining TN and TP in water are the same to those applied to the regular samples described above.

The N (in g) budgets during the experiment were estimated as follows:

$$N_0 + N_{\text{Fer}} + N_{\text{Fix}} - N_{\text{Den}} = N_{\text{Fil}} + N_{20 \text{ cm}} + N_{10 \text{ cm}} + N_{\text{Det}}$$

$$\text{Therefore, } N_{\text{Fix}} - N_{\text{Den}} = N_{\text{Fil}} + N_{20 \text{ cm}} + N_{10 \text{ cm}} + N_{\text{Det}} - N_0,$$

where,  $N_0$  is the amount in the water at the beginning of the experiment calculated based on TN concentration and water volume;  $N_{\text{Fer}}$ , N from added fertilizers;  $N_{\text{Fix}}$ , N fixed during the experiment;  $N_{\text{Den}}$ , N lost through denitrification;  $N_{\text{Fil}}$ , N contained in filamentous algae;  $N_{20 \text{ cm}}$ , N in water 20 cm from the bottom of tank at the end of the experiment;  $N_{10 \text{ cm}}$ , N in water 10 cm from the bottom of bucket (25 L) at the end of the experiment;  $N_{\text{Det}}$ , N contained in



**Fig.1** Water depth (mean±standard error) and temperature in tanks (mean), and rainfall recorded at the Lake Bao'an Meteorological Station

detritus. Therefore,  $N_{\text{Fix}} - N_{\text{Den}}$  is the net natural input.

Microsoft Excel 2010 and STATISTICA 8.0 were used to process and analyze data. The Mann-Whitney U test was used to test the differences between treatments.

### 3 RESULT

#### 3.1 Environmental conditions

The measured water depth and temperature in the tanks, and rainfall are given in Fig.1. Because of sampling, the depth decreased over time and reached its lowest in May, it then started to increase and peaked in August as result of the high precipitation.

The initial concentrations of both nutrients (22 December 2011) differed very little among treatments (Table 2). TN averaged 0.36 mg/L, ranging from 0.27 to 0.45 mg/L. TP averaged 0.06 mg/L, ranging from 0.04 to 0.09 mg/L.

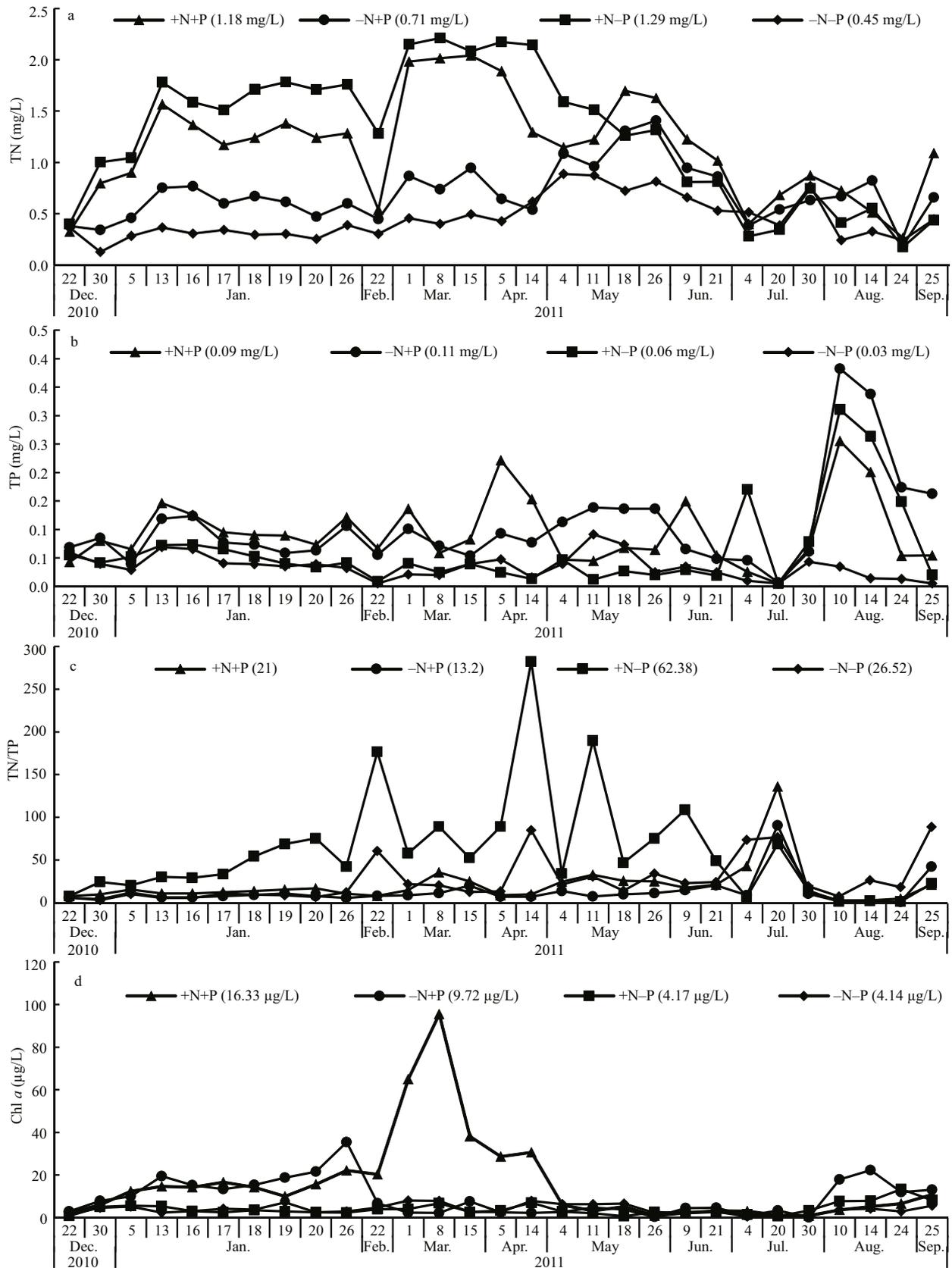
TN averaged over the entire experimental period

(from 30 December after the first fertilization through to 25 September, 2011) was significantly higher in the two N-addition treatments (+N+P, averaged 1.18 mg/L and ranged between 0.26–2.04 mg/L; +N–P, 1.29 (0.17–2.21) mg/L) than in the two without N-addition (–N+P, 0.71 (0.19–1.41) mg/L; –N–P, 0.45 (0.13–0.82) mg/L) ( $n=10$ ;  $P<0.001$ ) (Fig.2a) (treatments were compared using the monthly means of pooled values for duplicate tanks). The difference between the two N-addition treatments ( $n=10$ ;  $P=0.36$ ) was minor compared with that between the two treatments without N-addition ( $n=10$ ;  $P<0.001$ ). Two periods can be easily identified by the variations in the differences among treatments, before and after May 2011. Prior to May, the differences were much more apparent, with the highest TN concentration in treatment +N–P, followed by +N+P, –N+P, and –N–P. After May, various treatments overlapped. Although no N fertilizer was added, increases in TN can be clearly seen in treatments –N+P and –N–P. In –N+P, TN started to increase from 13 January and values as high as ~1.4 mg/L were recorded on 26 May. TN in –N–P started to increase after 1 March and peaked at ~0.9 mg/L on 4 May.

A similar pattern was observed in TP concentrations, being significantly higher in the two treatments with P-addition (+N+P, 0.10 (0.01–0.26) mg/L; –N+P, 0.11 (0.01–0.38) mg/L) than in the two without (+N–P, 0.06 (0.01–0.31) mg/L; –N–P, 0.03 (0.01–0.09) mg/L) ( $n=10$ ;  $P<0.001$ ) (Fig.2b). The difference between the two P-addition treatments ( $n=10$ ;  $P=0.76$ ) was minor compared with that between the two treatments without ( $n=10$ ;  $P=0.13$ ), although none were significantly different. Prior to May 2011, TP was usually higher in the two P-addition treatments than in the two without, and the differences were both minor for the pairs of treatments with and without P-addition. After May, the treatments overlapped frequently. For the two treatments without P-addition, TP was usually <0.1 mg/L, with the exception of >0.15 mg/L on 4 July and August in +N–P.

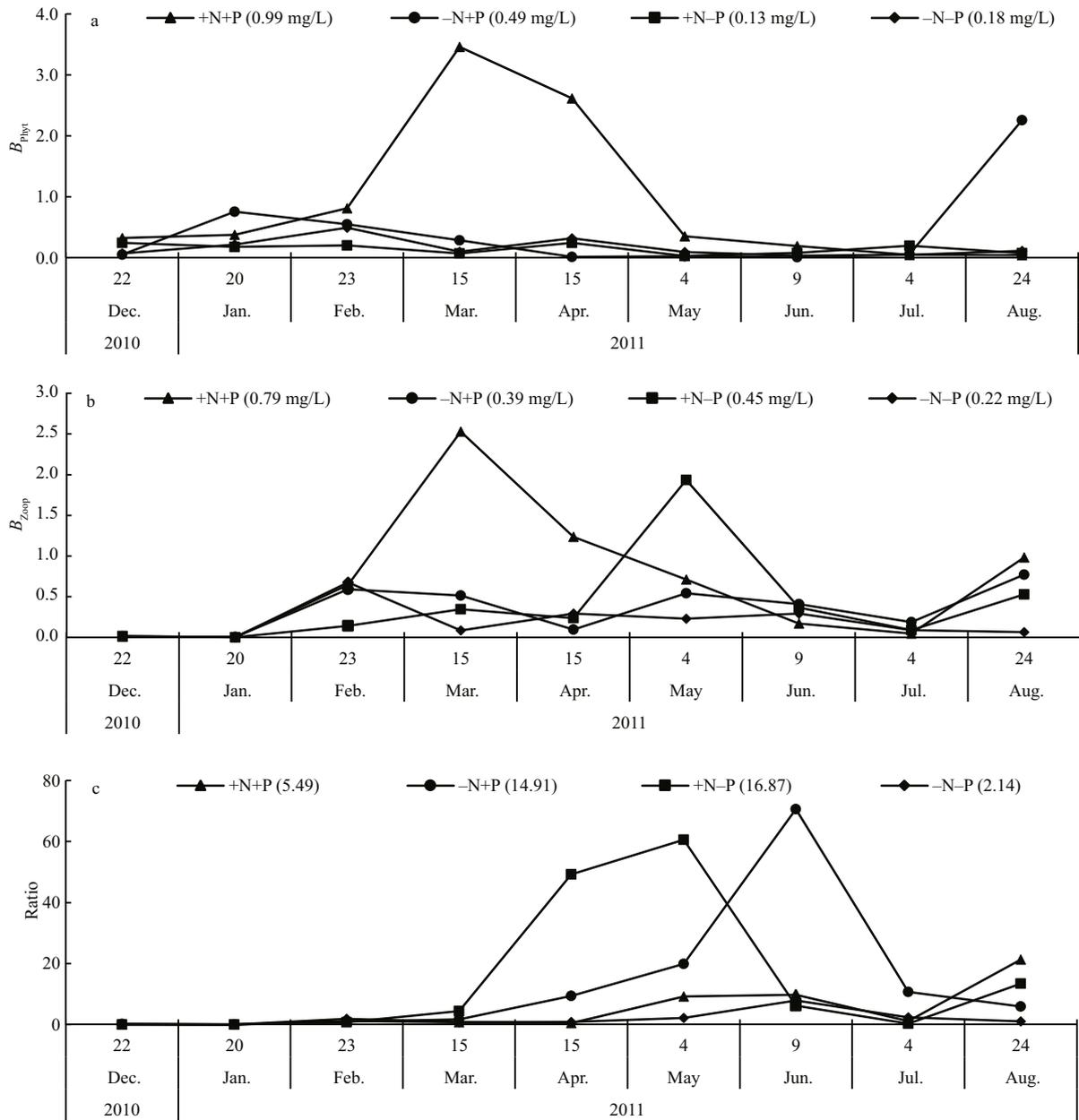
#### 3.2 Phytoplankton abundance

Regarding phytoplankton abundance as represented by Chl *a*, the P-addition treatments (+N+P, 16.33 (0.91–95.54)  $\mu\text{g/L}$ ; –N+P, 9.72 (0.10–33.49)  $\mu\text{g/L}$ ) were significantly higher than those without (+N–P, 4.17 (0.68–13.42)  $\mu\text{g/L}$ ; –N–P, 4.14 (0.50–8.02)  $\mu\text{g/L}$ ) ( $n=10$ ;  $P<0.04$ ) (Fig.2c). The difference was not significant between the two treatments without P-addition (+N–P versus –N–P;  $P=0.63$ ) or for those



**Fig.2** Total nitrogen (TN), total phosphorus (TP), ratio of TN to TP (TN/TP), and chlorophyll *a* concentration of phytoplankton (Chl *a*) in various treatments during the experiment

The values plotted for each treatment represent the mean of duplicate tanks.



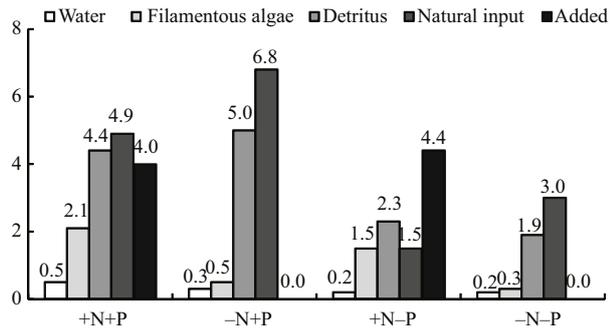
**Fig.3 Dried phytoplankton ( $B_{Phyt}$ ) and zooplankton ( $B_{Zoop}$ ) biomass and their ratio ( $B_{Phyt}/B_{Zoop}$ ) in various treatments during the experiment**

The values plotted for each treatment represent the mean of duplicate tanks.

with P-addition (+N+P versus -N+P;  $P=0.31$ ). The variation in differences among treatments over time differed from TN and TP. Prior to 22 February, Chl *a* were higher in the two P-addition treatments than in those without; however, this difference was not significant. -N+P began to decrease and overlap the treatments without P-addition from 22 February and peaked in most treatments after 10 August. +N+P began to decrease and overlap with the other treatments after 4 May. The two treatments without P-addition remained at low levels and frequently

overlapped during the experimental period.

Phytoplankton biomass ( $B_{Phyt}$ ) exhibited a similar pattern in variation to that of Chl *a*, being higher in the two P-addition treatments (+N+P, 0.99 (0.04–3.46) mg/L; -N+P, 0.49 (0.01–2.26) mg/L) than in the two treatments without (+N-P, 0.13 (0.03–0.24) mg/L; -N-P, 0.18 (0.03–0.49) mg/L), although the difference was not significant ( $n=9$ ;  $P>0.1$ ) (Fig.3a). No significant difference was found between the other paired treatments. The pattern of variation in  $B_{Phyt}$  over time was also similar to that of Chl *a*.



**Fig.4** Amount (g) of nitrogen (TN) contained in water, filamentous algae growing on the inter wall of the tanks, and detritus accumulated on the bottom measured at the end of the experiments and net nitrogen (TN) inputs (besides fertilization)

The values for each treatment represent the mean of duplicate tanks.

### 3.3 Zooplankton abundance

Zooplankton biomass ( $B_{Zoop}$ ) was higher, though not significantly, in treatment +N+P (0.79 (0.004–2.53) mg/L) than in the other treatments (–N+P, 0.39 (0.004–0.77) mg/L; +N–P, 0.45 (0.001–1.93) mg/L; –N–P, 0.22 (0.001–0.68) mg/L) ( $n=9$ ;  $P>0.19$ ) (Fig.3b). No significant difference was found between the other paired treatments. +N+P was higher in March and April and +N–P in May than in other months. Various treatments overlapped during the experimental period. –N+P and +N–P remained at relative low levels throughout the experiment.

When looking at zooplankton grazing stress on phytoplankton as reflected by their biomass ratio ( $B_{Zoop}/B_{Phyt}$ ), the two single nutrient treatments (–N+P, 14.91 (0.01–70.67) mg/L; +N–P, 16.87 (0.004–60.61) mg/L) were higher than the other two treatments (+N+P, 5.49 (0.02–21.34) mg/L; –N–P, 2.14 (0.005–7.92) mg/L), although the differences were not significant ( $n=9$ ;  $P>0.13$ ) (Fig.3c). No significant difference was found between the other paired treatments.  $B_{Zoop}/B_{Phyt}$  remained at low levels in all of the treatments prior to March. After March, +N–P and –N+P began to increase and decrease again in June and July, respectively. The other two treatments remained low for most of the experimental period.

### 3.4 N budget

The amounts of N contained in filamentous algae were clearly higher in the two N-addition treatments (+N+P, +N–P) than in those without (–N+P, –N–P) (Fig.4). Comparatively, the amounts of N in detritus were clearly higher in the two P-addition treatments (+N+P, –N+P) than in the other two (+N–P, –N–P).

For the net natural inputs, the results were similar to that of detritus, being higher in the two P-addition treatments than in the other two. Between the two P-addition treatments, the N-addition treatment (+N+P) was clearly lower than the one without (–N+P). The difference in net natural input between the two treatments without P-addition was the same, being clearly lower in treatment +N–P than in treatment –N–P.

## 4 DISCUSSION

### 4.1 Validity of N/P to indicate nutrient limitation

The results of our 9-month mesocosm experiment were in contrast to the predicted nutrient limitation according to N/P theory. In the treatments without P-addition (+N–P), no clear response in phytoplankton abundance was observed with the addition of N fertilizer, with Chl *a* and  $B_{Phyt}$  being as low as those in the treatments without fertilization. Actually, in one of the two N-addition treatments without P-addition, the initial ratio of TN to TP (TN/TP) of 5.6 (Fig.2c) clearly indicated N-limitation. In the treatments without N-addition, Chl *a* and  $B_{Phyt}$  increased rapidly in response to the addition of P fertilizer. In these two treatments, the initial TN/TP of 6.4 and 4.5 also indicated N-limitation. For both Chl *a* and  $B_{Phyt}$ : 1) both of the P-addition treatments (+N+P, –N+P) were higher than those without P-addition (+N–P, –N–P); 2) the difference between the two treatments with and without N-addition was significant with P-addition (+N+P, –N+P), but not significant without P-addition (+N–P, –N–P). Therefore, it can be concluded that N/P is not a reliable indicator of the type of nutrient limitation and hence controlling N is necessary.

The different cycling characteristics between N and P may explain the contrasting results between our experiment and the nutrient limitation predicted by the N/P theory. Because the P cycle is sedimentary, ecosystems are limited in their abilities to obtain P. However, the gaseous N cycle enables ecosystems to obtain atmospheric  $N_2$  through various natural processes such as biological fixation. Therefore, algal growth in lakes may not be limited by N deficiency. In the two treatments without N-addition in our experiment (–N+P, –N–P), N concentrations continuously increased although no N fertilizer was added. This suggests N compensation through natural loading. A similar phenomenon was observed in our 18-month whole-ecosystem experiment, where TN in the –N+P treatment was either similar to or even

higher than that in the +N+P treatment (Li et al., in revision). The N budget results further suggest the role of net natural inputs to compensate for the relative deficiency of N compared to P (Fig.4). The net natural N inputs were much higher in the two P-addition treatments (4.9 g and 6.8 g) than in those without P-addition (1.3 g and 3.0 g). Natural N inputs were higher in treatments without added N than in those with N-addition (6.8 g versus 4.9 g and 3.0 g versus 1.5 g), when P treatment was the same.

#### 4.2 Response rates of experimental systems to fertilization

In the present research, compensation for N deficiency did not begin immediately after the start of the experiment. In treatment -N+P, an obvious increase in TN was only observed after 3 weeks (13 January). TN increased further after approximately 2 months (3 March) and 4 months (4 May). In treatment -N-P, an obvious increase in TN was only observed after approximately 3 months (1 April) and continued until the end of April. In our whole-ecosystem experiments (Li et al., in revision), obvious increases in TN were only observed after approximately 8 months in treatment -N+P and approximately 10 months in treatment -N-P. The efficiency is even lower in the Arctic. For example, an obvious increase in TN was only observed after 3 years as a response to P fertilizer addition (Schindler et al., 2008).

Spivak et al. (2011), based on their results from a 7-day mesocosm experiment, concluded that results from small-scale experiments can be applied in larger, more natural aquatic systems. However, the duration of their experiment was limited making their conclusion questionable. Most previous experiments that support dual N and P control were less than 2 weeks long. Many of the experiments only lasted hours or days. In these short-term experiments, the natural process may not react fast enough to compensate for the lack of N, even though all other conditions may not be limiting. Actually, the short-term experiments were also quite space limited (Table 1). Limited space implies a lack of important N compensation factors, such as phytoplankton species pool and sufficient interaction with the atmosphere.

Zooplankton biomass remained at the initial level for 1 month, and only started to increase after 2 months. The response in the ratio of zooplankton to phytoplankton biomass was even slower, only starting to increase after 3 months. Zooplankton grazing stress

may have played a role in phytoplankton variation. However, such slow responses in either zooplankton biomass or its ratio to phytoplankton would not be observed in short-term experiments lasting either hours or days.

#### 4.3 Extrapolation of small to large experiments

The conclusion supporting the extrapolation of small-scale experiments to larger, more natural aquatic systems has been proved as misleading when whole-lake experiments are considered (Hecky and Kilham, 1988; Carpenter, 1996; Schindler, 2012). Various processes may influence the extrapolation of small to large experiments. The main causes underlying the weakness of small-to-large extrapolation have been analyzed by Schindler (2012). Species pool is usually a factor. Although there is no empirical evidence showing the increasing range of optimal N/P, it is reasonable to assume that a larger ecosystem is more abundant in species diversity and, hence, a wider N/P range and ability to adapt to N deficiency. In our whole-ecosystem experiments, a high abundance of N-fixers (*Anabaena* sp.) was responsible for the high TN concentration and total phytoplankton in treatment -N+P (Li et al., in revision). In our mesocosm experiment, only two N-fixers were found, one in treatment -N+P on 15 March and another in treatment -N-P on 4 July. In both cases, the appearance of N-fixers was not the result of N deficiency. TN/TP of both treatments was as high as 25.7 and 118, respectively, and TN was 0.84 mg/L and 0.59 mg/L, respectively. The scarcity of N-fixers in our experiment may be explained by the limited species pool because of the small volume and loss of anoxic conditions for N-fixation as a result of the shallowness and lack of sediment.

Although we cannot explain the underlying mechanism, the contrast between increased TN in treatments without N-addition and scarce planktonic N-fixers is an interesting phenomenon. It probably reflects a difference in N-fixation methods between mesocosms and natural ecosystems. In natural ecosystems, planktonic N-fixation is the norm, although filamentous algal growth may be favored in shallow littoral substrates. In mesocosms, dense filamentous algal mats may create the anoxic conditions ideal for biological N-fixation. In our mesocosm experiments, N-fixation supported by the abundant filaments may be the reason for the increased TN, although we did not identify the species or heterocyst.

## 5 CONCLUSION

Our 9-month mesocosm experiment demonstrates that N compensation through natural processes is slow as a response to N deficiency. Such processes, therefore, cannot be simulated in short-term fertilization experiments. The addition of P may accelerate the response rate as suggested by the earlier increases in TN in the P-addition treatments compared with those without.

Increased TN in treatments without N-addition cannot be explained by N-fixation if phytoplankton N-fixers were scarce, which may in turn be because of limited space to support a sufficient species pool.

Although there is no evidence, increased TN in treatments without N-addition may have been sourced from N-fixation supported by the dense filamentous algal mat, suggesting a different mode of N-fixation from large natural ecosystems.

Our results contradict those predicted by the nutrient limitation N/P theory, suggesting the weakness of N/P theory to support dual N and P control; They also highlight that extrapolating short and small experiments to large natural ecosystems is problematic.

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