

# Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China

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## Abstract

Lake Taihu (Taihu) is the third largest freshwater lake in China and an important drinking water, fishing, and tourism resource for Jiangsu Province. Recent toxic cyanobacterial blooms caused by excessive human nutrient loading have focused attention on arresting blooms and restoring the lake to acceptable water quality conditions by reducing nutrient inputs. Field sampling and in situ nutrient enrichment bioassays were conducted to determine seasonal patterns of nutrient limitation and nutrient thresholds for phytoplankton growth. The TN:TP and TDN:TDP mass ratios in the ambient water showed high seasonal variation and changed from 33–80:1 and 52–212:1, respectively, in winter and spring, and both declined to below 20:1 in summer. In spring and winter, total phytoplankton biomass and growth rates increased significantly with additions of P, with no primary effects from N, suggesting P limitation of phytoplankton growth. During the summer and fall bloom periods, however, N additions alone revealed a significant positive effect on phytoplankton growth, and P additions only stimulated phytoplankton growth once N had been added, suggesting that N was the primary limiting nutrient, with P being a secondarily limiting nutrient. When P enrichment was  $\geq 0.20 \text{ mg P L}^{-1}$  and N enrichment  $\geq 0.80 \text{ mg N L}^{-1}$ , growth of the toxin-producing, dominant bloom-forming cyanobacteria *Microcystis* spp. was not nutrient limited. This study suggests that availability of N during the summer is a key growth-limiting factor for the proliferation and maintenance of toxic *Microcystis* spp. blooms. Therefore, although P load reduction is important, N load reduction is essential for controlling the magnitude and duration of algal booms in Taihu.

Aquatic ecosystems worldwide have been negatively affected by eutrophication, many of them driven by increasing nutrient inputs from untreated domestic sewage and industrial and agricultural wastewater. Systems affected by accelerating eutrophication frequently exhibit harmful algal blooms, which foul waterways and water intakes, disrupt food webs, fuel hypoxia, and produce secondary metabolites that are toxic to water consumers and users, including zooplankton, fish, shellfish, cattle, domestic pets, and humans (Paerl 1988). The need to reduce anthropogenic nutrient inputs to aquatic ecosystems has been widely recognized as essential for reducing these negative effects of eutrophication (Nixon 1995; Smith 2003).

Diverse studies have shown that nitrogen (N), phosphorus (P), or availability of both controls phytoplankton growth (Elmgren and Larsson 2001; Smith 2003), biomass (Cloern 2001; Bledsoe et al. 2004), and species composition (Duarte et al. 2000; Smayda and Reynolds 2001). With regard to the control of algal production and bloom formation, it is generally accepted that nitrogen is the prime limiting nutrient in marine systems, whereas phosphorus is the prime limiting nutrient in freshwater systems (Hecky and Kilham et al. 1988; Nixon 1995). This paradigm has led to widespread reductions in inputs of phosphorus to control eutrophication in freshwater lakes (National Research Council 1992). Recently, Schindler et al. (2008) suggested that only P reductions were needed to protect freshwater and coastal marine ecosystems and that N reductions might be ineffective and therefore not necessary.

However, this could be an oversimplification, in that there are numerous freshwater and marine exceptions to this conclusion (Conley et al. 2009; Paerl 2009). For example, in some freshwater ecosystems, particularly in the tropics, the subtropics, high-altitude environments, and diverse large lake ecosystems, N can be the primary limiting nutrient for phytoplankton production (Lewis and Wurtsbaugh 2008). Conversely, some marine environments can exhibit P-limited conditions (Phlips et al. 1999; Smith 2003; Sylvan et al. 2006).

Located in the Changjiang (Yangtze) River delta in eastern China, Lake Taihu (Taihu, meaning “Great Lake” in Chinese) is the third largest freshwater lake in China. It is shallow (mean depth  $\approx 2 \text{ m}$ ), polymictic, and has become increasingly eutrophic over the past three decades (Qin et al. 2007). About 40 million people live in cities (including Shanghai) and towns within the Taihu watershed. The lake is a key drinking water source for the human population, and tourism, fisheries, and shipping are also important economic activities. Ironically, it is also a repository of waste from urban centers and nearby agricultural and industrial segments of the local economy (Guo 2007; Qin et al. 2007). The Taihu basin accounts for 0.4% of China’s land area, but the gross domestic product (GDP) in this region accounts for 11% of the Chinese economy (Qin et al. 2007). With recent economic growth and urbanization and continuing population increases, nutrient loadings and eutrophication of Taihu have rapidly accelerated to the point that harmful cyanobacterial blooms are now a common feature (Guo 2007; Qin et al. 2007). Since the mid-1980s, blooms of the toxin-producing cyanobacteria of

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*Microcystis* spp. have occurred every summer in the northern part of the lake (Qin et al. 2007). These blooms have led to serious environmental, economic, and societal problems, including a lack of safe water resources for the local population (Guo 2007).

During the summer of 1990, a cyanobacterial bloom dominated by *Microcystis* spp. covered one of Taihu's main bays, Meiliang Bay. This bloom caused 116 factories to halt work and deprived local towns and the regional city of Wuxi (~ 3 million inhabitants) of safe drinking water. More recently, the *Microcystis* blooms have expanded from the bays to the center of Taihu (Guo 2007). The northern and western regions of the lake are now regularly covered by thick blooms from late spring into autumn. In May 2007, a very large "cyanobacteria mat" caused the drinking water plant to cease functioning, leading to a highly publicized drinking water crisis in Wuxi, which forced residents to resort to bottled drinking water (Qin et al. in press). This crisis led to a public outcry, which has increased pressure on local, provincial, and central governments to identify causative factors and initiate solutions. Although substantial efforts have gone toward restoration of aquatic vegetation and related fish habitat since 1991 (Pu et al. 1998), efforts to restore water quality to the pre-cyanobacterial bloom era have yet to be implemented (Chen et al. 2003).

To restore water quality of Taihu to an acceptable (i.e., no harmful blooms) level, it is necessary to first identify those growth-limiting nutrients responsible for bloom development and proliferation. The potential for nutrient limitation of phytoplankton growth can be assessed in many ways on the basis of geochemical, ecological, or physiological processes (Hecky and Kilham 1988; Beardall et al. 2001). The effect of altered nutrient regimes on phytoplankton biomass can be quantified by measuring the community growth response to a controlled nutrient environment over short time intervals (Paerl 1982; Paerl and Bowles 1987). In this regard, short-term (< 1 week) nutrient manipulation bioassays provide a management tool for addressing the issue of immediate phytoplankton response to enhanced nutrient concentrations. In Taihu, it has been suggested that nutrient concentrations are so high (particularly in the northern lake) that algal growth is mainly controlled by light availability, with P being the nutrient closest to "limiting" (Dokulil et al. 2000). Long-term field investigations at Taihu have shown that the key indicator of algal biomass, chlorophyll *a* (Chl *a*) is positively and significantly correlated with total P, whereas NO<sub>3</sub> concentrations were inversely related (Chen et al. 2003). On the basis of these findings, Yang (2004) suggested that P, and not N, was the nutrient limiting *Microcystis* spp. growth. However, field verification of nutrient limitation and its potential for control of Taihu's blooms is needed. Our study focused on Meiliang Bay, a main recipient of anthropogenic nutrients, an important drinking water source, and the site of repeated blooms.

Water quality monitoring and in situ nutrient enrichment bioassays were conducted to better characterize nutrient limitation in Taihu. The objective was to test: (1) whether phytoplankton is nutrient limited, (2) whether the

degree to which either P or N are limiting is seasonally shifted, and (3) how much nutrient concentrations must be decreased to prevent bloom formation. This information is essential for developing best management practices to reduce N or P transport from land to surface waters and for calibrating and verifying models for predicting future trends in eutrophication of the lake.

## Methods

**Study site**—Taihu is located in China's coastal plain, which is dominated by a subtropical monsoon climate. The lake has a surface area of 2338 km<sup>2</sup> and a catchment area of 36,500 km<sup>2</sup>. The Taihu basin has a complex, high-density set of river networks, with 117 rivers and tributaries draining into the lake. The annual runoff into the lake is about  $57 \times 10^8$  m<sup>3</sup>, and the water retention time of the lake is approximately 284 d (Qin et al. 2007). Generally, water enters the lake from the western side and exits from the eastern side.

Our study sites were situated in Meiliang Bay, northern Taihu (Fig. 1), one of the lake's most eutrophic bays. The surface area of the bay is ~ 123 km<sup>2</sup>, and the average depth is 1.8 m (Chen et al. 2003). Two main rivers empty into the bay, the Liangxi and Zhihu Gang Rivers. Large amounts of untreated wastewater from factories and residential areas are discharged to these rivers. Two sampling stations were selected for monthly sampling; one inside Meiliang Bay (Sta. 1) and another near the central lake (Sta. 2) to observe differences between the bay and the open lake. An intermediate location near the Taihu Laboratory for Lake Ecosystem Research (TLLER), Nanjing Institute of Geography and Limnology, Chinese Academy of Science, Sta. 3, was chosen as the incubation site for the nutrient addition bioassays (Fig. 1).

**Ambient physical, chemical, and biological conditions**—Monthly physical, chemical, and biological data were available for the calendar years 2006, 2007, and 2008 from TLLER. Data from precipitation collectors at seven weather stations around lake, water levels at five hydrology stations, and monthly total riverine discharge to the lake were made available by the Taihu Basin Authority, Ministry of Water Resources.

Physical parameters, including surface water temperature (WT), dissolved oxygen (DO), pH, and electrical conductivity (EC), were measured in the field using a Yellow Springs Instruments (YSI) 6600 multisensor sonde. Photosynthetically active radiation (PAR; 400–700 nm) at the water surface was measured continuously with a spherical quantum sensor (LI-COR 192SA). A continuously recording multiparameter underwater sensor (YSI 6600, Yellow Springs Instruments) was also deployed at Sta. 3 in 2008. At this location, temperature, DO, and pH were monitored continuously in the upper water column (0.2 m below water surface) and near-bottom waters (0.5 m above the bottom sediments). Before deployment, DO and pH sensors were calibrated and accuracy was checked by measuring standards with a average error of 2%. Data were collected at 10-min intervals from January to December

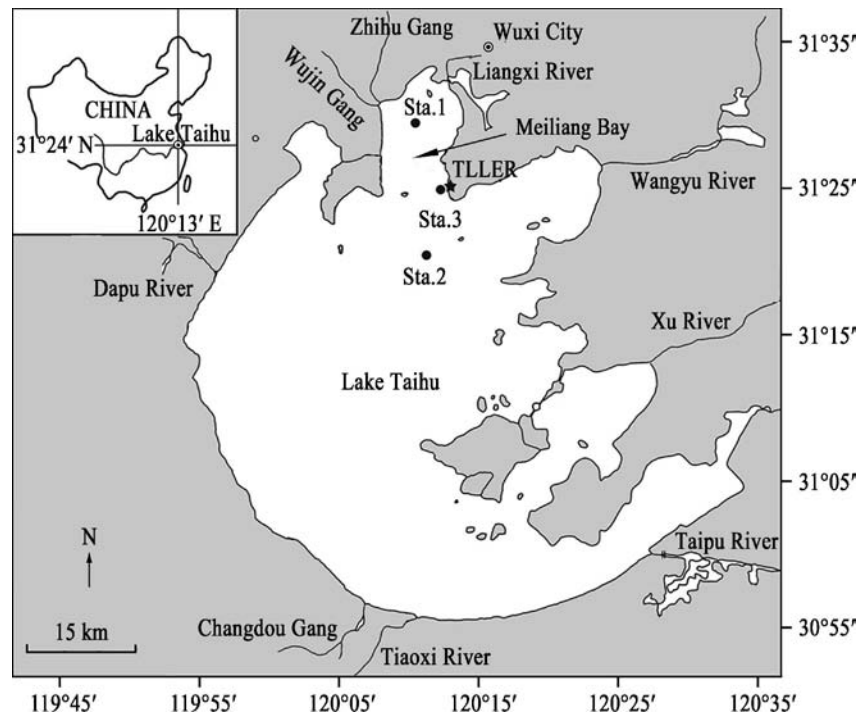


Fig. 1. Lake Taihu: Sta.1 and Sta. 2 are locations from which water samples were routinely collected, and Sta. 3 shows the location at which in situ nutrient addition bioassays were incubated.

2008. Hourly datasets were analyzed to show the influences of diurnal photosynthesis and water temperatures on DO. The data were downloaded biweekly. Integrated water samples were taken with a 2-m-long, 0.1-m-wide handmade plastic tube with a one-way valve at the upper part of the tube. Chemical analyses of water samples included total nitrogen (TN), total dissolved nitrogen (TDN), ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP). SRP concentrations were determined spectrophotometrically, according to the molybdenum blue method (APHA 1995).  $\text{NH}_4^+$  concentrations were measured by the indophenol blue method, and  $\text{NO}_3^-$  and  $\text{NO}_2^-$  concentrations were analyzed by the cadmium reduction method (APHA 1995). TP, TDP, TN, and TDN concentrations were determined after thawing with the use of a combined persulfate digestion (Ebina et al. 1983), followed by spectrophotometric analysis as for soluble reactive phosphorus and nitrate. TN and TP recovery efficiencies were 98.4% and 99.7%, respectively. Particulate nitrogen (PN) was obtained from the difference between TN and TDN, and particulate phosphorus (PP) was obtained from difference between TP and TDP. Error estimates were determined as the average percent coefficient of variation (CV) of triplicates. Average errors are 6.3% for PP and 5% for PN. Phytoplankton samples were fixed with Lugol's iodine solution (2% final conc.) and sedimented for 48 h. Cell density was measured with a Sedgwick–Rafter counting chamber under microscopic magnification of  $\times 200$ – $400$ . Phytoplankton species were identified according to Hu et al. (1980). Algal biovolumes

were calculated from cell numbers and cell size measurements. Conversion to biomass assumes that  $1 \text{ mm}^3$  of volume is equivalent to 1 mg of fresh weight biomass. Chl *a* concentrations were determined spectrophotometrically after extraction in 90% hot ethanol (Papista et al. 2002).

*Nutrient limitation bioassay experiments*—To address whether N or P limited phytoplankton growth, four in situ nutrient addition experiments were performed in May, July, October, and December 2008 with Sta. 3 as the incubation site (Fig. 1). Water samples containing natural phytoplankton assemblages were collected from 0.2 m below the surface with precleaned (0.01 N HCl-washed and then lake water-washed) 20-L polyethylene carboys at Sta. 1. Water samples were screened through 200- $\mu\text{m}$  mesh to remove large zooplankton grazers and distributed into acid-washed 1-L polyethylene Cubitainers (Hedwin Co.) which are chemically inert, unbreakable and transparent (80% PAR transmittance). The methodology and deployment procedures for deployment of in situ Cubitainer bioassays is detailed in Paerl and Bowles (1987) and Paerl et al. (2005). Additional water samples were collected for analyses of Chl *a* and nutrients at the initiation of the bioassays.

Three treatments in addition to a control (no nutrient additions) were administered: N addition (+N), P addition (+P), and N and P addition (+NP). N was added as  $\text{KNO}_3$  because nitrate is the dominant form of inorganic N in the lake. P was added as  $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ . The final concentration of N was  $2.00 \text{ mg N L}^{-1}$ , and the final concentrations of P were  $2.00 \text{ mg P L}^{-1}$  in spring and  $0.50 \text{ mg P L}^{-1}$  in



summer, fall, and winter. These concentrations were designed to reflect the relatively high values periodically observed in the lake and to saturate initial growth rates of phytoplankton.

To detail the effects of varying N and P concentrations on phytoplankton growth, treatments with various P concentrations (i.e., 0, 0.02, 0.20, 0.50, 1.00, 1.50, and 2.00 mg P L<sup>-1</sup>) and a fixed N level (2.00 mg N L<sup>-1</sup>) and with various N concentrations (i.e., 0, 0.50, 1.00, 1.50, 2.00, 3.00 mg N L<sup>-1</sup>) and a fixed P level (2.00 mg P L<sup>-1</sup>) were also run in July 2008. In October and December 2008, we ran a series of variable P concentrations (i.e., 0, 0.50, 1.00, 2.00 mg P L<sup>-1</sup>) with and without N enrichment (2.00 mg N L<sup>-1</sup>) and variable N concentrations (i.e., 0, 0.50, 1.00, 2.00 mg N L<sup>-1</sup>) with and without P enrichment (0.50 mg P L<sup>-1</sup>).

All treatments were conducted in triplicate. After nutrient additions, the Cubitainers were incubated in situ near the surface for 4 or 6 d by placing them in a floating steel frame. This allowed for natural light, temperature, and surface turbulence conditions. One layer of neutral-density screening was placed over the frame to prevent photoinhibition during the course of incubations. The containers were sampled at intervals of 2 or 3 d for Chl *a*, nutrient, and pH analyses. The growth rate ( $\mu$ ) under each set of treatment conditions was calculated according to the modified exponential growth equation,

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

where  $X_1$  is the concentration of Chl *a* at the initial incubation stage ( $T_1$ ), and  $X_2$  is the concentration of Chl *a* at the peak incubation stage ( $T_2$ ).

The maximum growth rate ( $\mu_{\max}$ ) and half-saturation constant ( $K_u$ ) were calculated according to the Monod kinetic equation (Monod 1950).

**Statistical analyses**—The differences in the growth responses between the various treatments were analyzed by one-way ANOVA. Post hoc multiple comparisons of treatment means were performed by Tukey's least significant difference procedure. Untransformed data in all cases satisfied assumptions of normality and homoscedasticity. Statistical analysis was performed with the SPSS 13.0 statistical package for personal computers, and the level of significance used was  $p < 0.05$  for all tests.

## Results

**Seasonal dynamics of ambient physical, chemical and biological conditions**—During sampling in 2006, 2007, and 2008, daily photosynthetically available radiation (PAR) changed from a minimum of 45.2 mol m<sup>-2</sup> d<sup>-1</sup> to a maximum of 179.4 mol m<sup>-2</sup> d<sup>-1</sup> (Fig. 2A). From winter to summer, PAR gradually increased and showed one regular summer peak each year. The water temperature variations followed PAR and changed from a minimum of 2.9°C in January to a maximum of 31.9°C in August (Fig. 2A).

Rainfall is the main source of surface water in the Taihu basin. Rainfall patterns are strongly affected by the

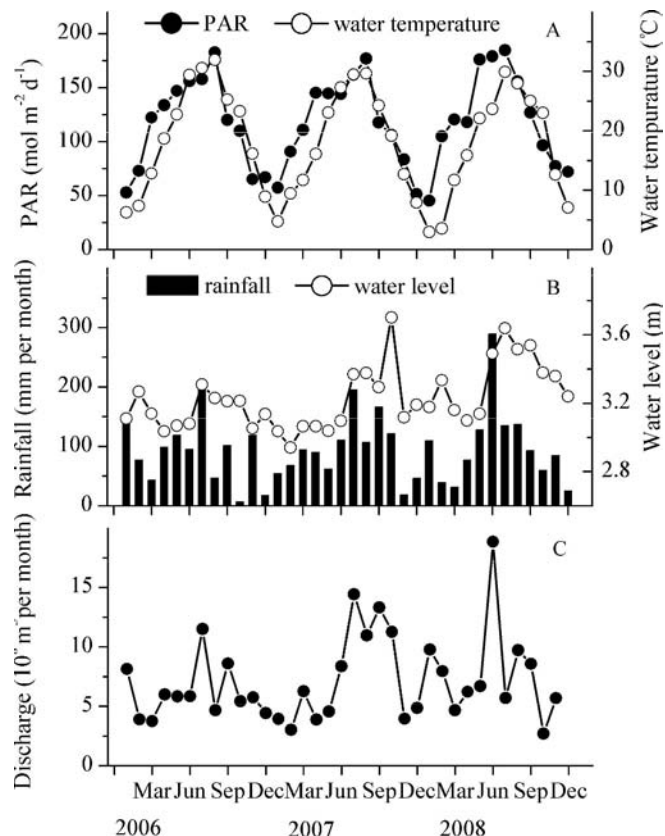


Fig. 2. Seasonal variation in Taihu Basin from 2006 to 2008 of (A) monthly mean water temperature and monthly mean daily accumulative photosynthetically available radiation (PAR), (B) monthly total rainfall and monthly mean water level, and (C) monthly total riverine discharge. Water temperature and PAR data were obtained from Taihu Laboratory for Lake Ecosystem Research (TLER). Rainfall, water level, and total riverine discharge data were obtained from the Taihu Basin Authority, Ministry of Water Resources.

southeast monsoon. As a result, local rainfall showed strong seasonal variation, with the highest amounts occurring in June and July (Fig. 2B). The variation of average water level in Taihu followed rainfall patterns, with a slight time lag, with lowest values from January to May and highest values from July to September (Fig. 2B). River discharge was strongly influenced by rainfall, with maxima occurring in summer (Fig. 2C).

Water chemistry also showed large variations in Taihu. The EC showed high seasonal variation and increased in April to 815  $\mu\text{S cm}^{-1}$  in Meiliang Bay and 695  $\mu\text{S cm}^{-1}$  in the central lake and then declined from May to November at both locations (Fig. 3A). The DO of the lake varied seasonally, with the peaks ( $> 10$  mg O<sub>2</sub> L<sup>-1</sup>) during winter (Fig. 3B). Commonly, the lowest DO concentrations coincided with periods of maximum water temperature in summer. However, relatively high DO concentrations ( $> 10$  mg O<sub>2</sub> L<sup>-1</sup>) were also observed in Meiliang Bay in July and August 2008, most likely because of high phytoplankton photosynthetic rates. The pH of the lake ranged from 7.73 in February to 9.53 in July during 3 yr and revealed a

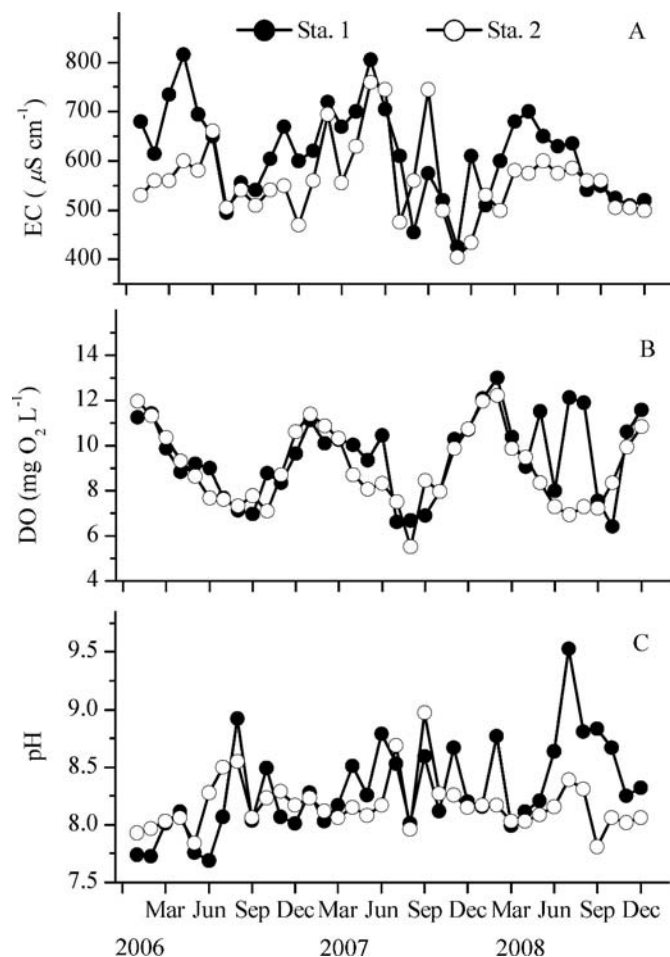


Fig. 3. Seasonal variation at the two sampling stations in Taihu from 2006 to 2008 of (A) EC, (B) DO, and (C) pH. Data were obtained from TLLER. EC and DO represent electrical conductivity and dissolved oxygen, respectively.

seasonal pattern approximately inverse to that of DO (Fig. 3C).

Continuous investigation in July at Sta.3 revealed that the surface was only slightly warmer (up to 0.3°C) than the bottom on most days (19 d), regardless of daytime or nighttime. Surface waters were cooler (up to 1.0°C) than the bottom on some days (9 d; Fig. 4A), most likely because of rapid cooling associated with poor weather. This suggested that the lake was not strongly thermally stratified in summer. The DO concentrations ranged from 5.9 to 17.7 mg O<sub>2</sub> L<sup>-1</sup> in surface water and from 5.0 to 15.2 mg O<sub>2</sub> L<sup>-1</sup> in bottom water, and hypoxic events were not observed during the study period (Fig. 4B). Diel fluctuation in DO was strong during the observation period. Daily peaks of DO normally occurred between 14:00 h and 18:00 h—most frequently around 16:00 h. The lowest values usually occurred between 04:00 h and 07:00 h—most commonly around 06:00 h. During 28 d of observation, DO exceeded 12 mg O<sub>2</sub> L<sup>-1</sup> in surface water 10 d, and the maximum value was 17.7 mg O<sub>2</sub> L<sup>-1</sup> (Fig. 4B). Overall, relatively vigorous photosynthetic oxygen production in

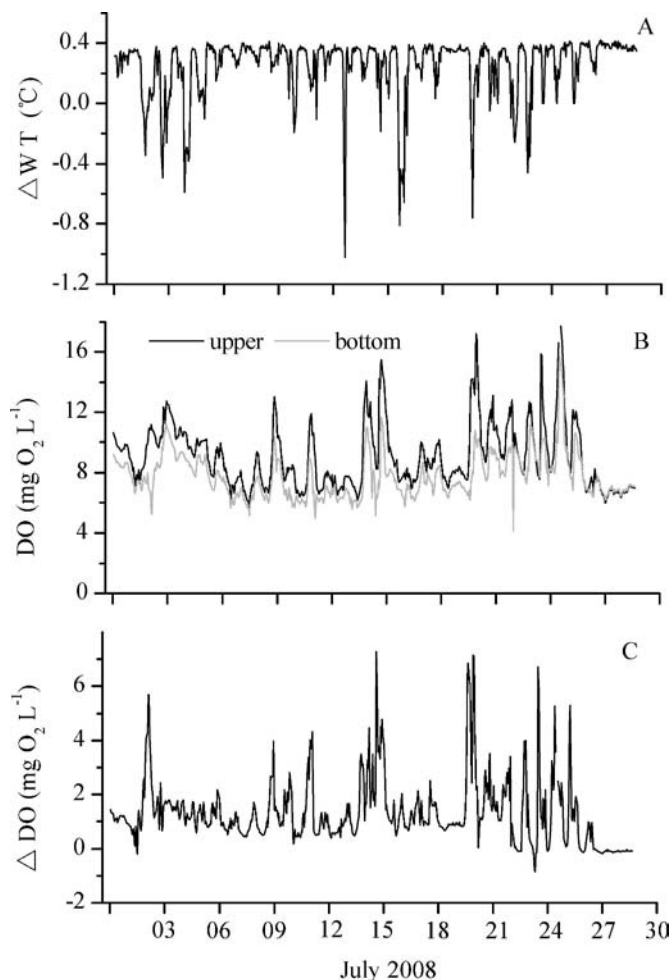


Fig. 4. Fluctuations in (A) water temperature differences between the upper water column (0.2 m below the water surface) and near-bottom waters (0.5 m above the bottom sediments), (B) dissolved oxygen (DO), and (C) DO differences between the upper water column (0.2 m below the water surface) and near-bottom waters (0.5 m above the bottom sediments) over the period of observation from 01 July to 28 July 2008.

well-illuminated surface water resulted in relatively higher DO concentrations than that in bottom waters (Fig. 4C).

Various forms of nitrogen in Taihu showed strong seasonal variation. The TN concentrations ranged from 1.24 mg N L<sup>-1</sup> in the central lake to 9.48 mg N L<sup>-1</sup> in Meiliang Bay, with an average of 3.54 mg N L<sup>-1</sup> (Fig. 5A). Maximum values normally occurred in winter and spring, whereas minimum values were normally confined to summer and autumn during the 3-yr period. The PN concentrations varied from 0.13 to 2.17 mg N L<sup>-1</sup> in the central lake and 0.13 to 3.24 mg N L<sup>-1</sup> in Meiliang Bay (Fig. 5B). Maximum values were normally recorded in summer (from July to September) during the most severe algal blooms, which contributed 55–80% of TN in Meiliang Bay. The TDN concentrations exhibited a similar seasonal pattern to TN concentrations, with peaks occurring in early spring (March–April) and then abruptly decreasing during May–August at both stations (Fig. 5C). NO<sub>3</sub><sup>-</sup> concentra-

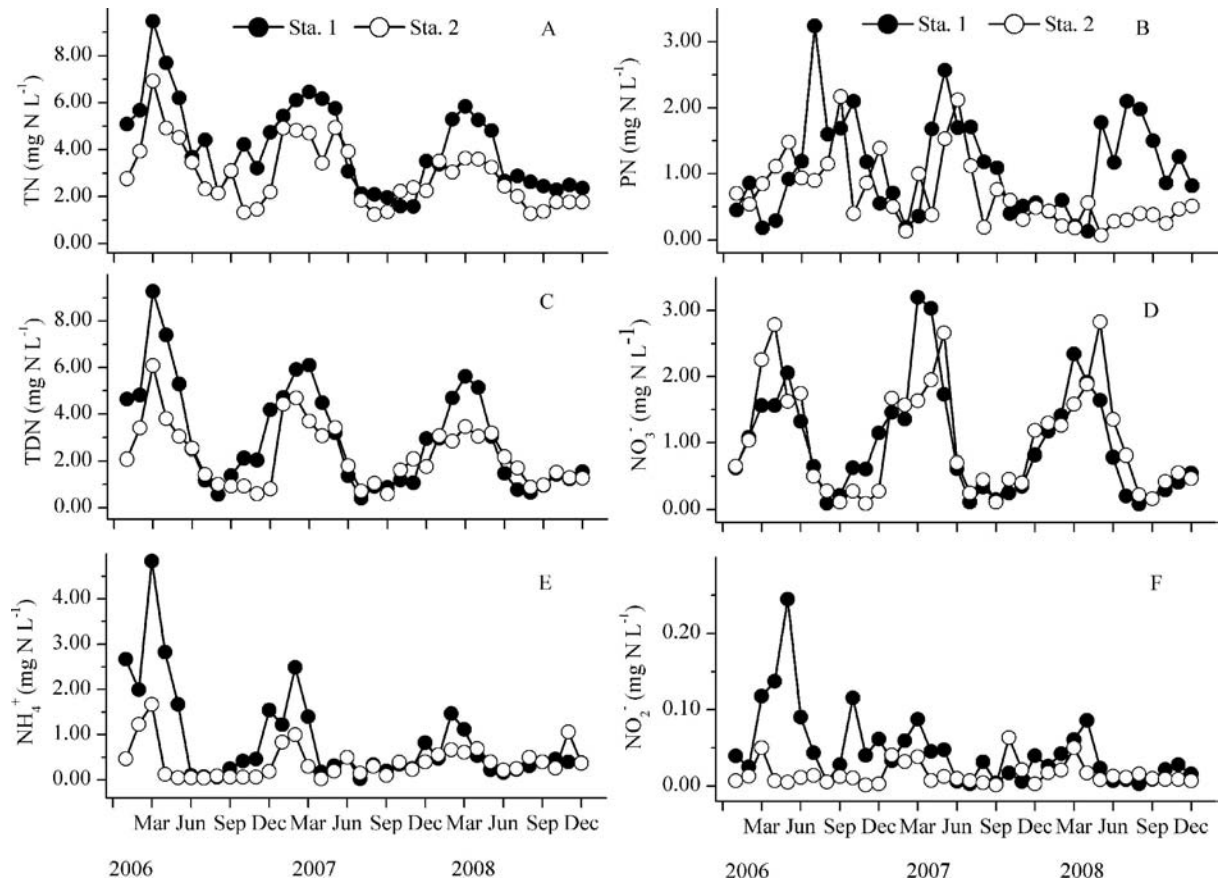


Fig. 5. Seasonal variation at the two sampling stations in Taihu from 2006 to 2008 of (A) TN, (B) PN, (C) TDN, (D)  $\text{NO}_3^-$ , (E)  $\text{NH}_4^+$ , and (F)  $\text{NO}_2^-$ . Data were obtained from TLLER. TN, PN, TDN,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and  $\text{NO}_2^-$  represent total nitrogen, particulate nitrogen, total dissolved nitrogen, nitrate, ammonium, and nitrite, respectively.

tions at two locations varied between 0.09 and 3.20 mg N  $\text{L}^{-1}$ , with peaks in early spring and declines ( $< 0.65$  mg N  $\text{L}^{-1}$ ) in summer and autumn (Fig. 5D).  $\text{NH}_4^+$  concentrations ranged from 0.05 to 2.67 mg N  $\text{L}^{-1}$  at the two stations, with an average of 0.63 mg N  $\text{L}^{-1}$ . The concentrations in Meiliang Bay were higher than those in the central lake during the peak winter and spring periods (Fig. 5E).  $\text{NO}_2^-$  concentrations were  $< 0.25$  mg N  $\text{L}^{-1}$  in Meiliang Bay and  $< 0.07$  mg N  $\text{L}^{-1}$  in the central lake during the 3 yr (Fig. 5F).

Similar to TN, TP concentrations were higher in Meiliang Bay than that in the central lake. The TP concentrations in Meiliang Bay changed from 0.08 to 0.32 mg P  $\text{L}^{-1}$ , with an average of 0.15 mg P  $\text{L}^{-1}$ , and values in the central lake varied between 0.06 and 0.25 mg P  $\text{L}^{-1}$ , with an average of 0.10 mg P  $\text{L}^{-1}$  (Fig. 6A). However, TP revealed an inverse seasonal pattern to TN, with maxima occurring from July to September and minimal values occurring from winter to early spring. PP concentrations at the two stations ranged from 0.035 to 0.273 mg P  $\text{L}^{-1}$  and showed a similar seasonal pattern to TP, with peaks in summer (Fig. 6B). PP was the primary form of P in summer, which contributed 60–84% of TP in Meiliang Bay. The TDP concentrations ranged from a minimum of 0.01 mg P  $\text{L}^{-1}$  in the central lake to a maximum of 0.10 mg P  $\text{L}^{-1}$  in Meiliang Bay, with a mean

value of 0.04 mg P  $\text{L}^{-1}$  during the 2006–2008 period (Fig. 6C). SRP concentrations at both stations changed from 0.002 mg P  $\text{L}^{-1}$  in April to 0.046 mg P  $\text{L}^{-1}$  in September throughout this period (Fig. 6D).

The TN:TP mass ratios in Taihu displayed a seasonal pattern driven by asynchronous dynamics of TN and TP (Fig. 7A). During winter and spring, TN:TP ratios ranged from 33 to 80:1 in Meiliang Bay, but in summer, this ratio dropped below 20:1. The ratios in the central lake varied between 30:1 and 64:1 during winter and spring and then declined in late summer to early fall below 20:1. TDN:TDP mass ratios revealed a seasonal dynamic similar to TN:TP ratios (Fig. 7B). During January–May, TDN:TDP ratios in Meiliang Bay changed from 52 to 212:1, and then declined to below 20:1 in late summer. The TDN:TDP ratios in the central lake were higher than those in Meiliang Bay during a 3-yr period (2006–2008). The PN:PP mass ratios ranged from 4 to 18:1, with an average value of 9:1 (Fig. 7C), which was similar to the cellular element mass ratio (7:1) of algae (Redfield 1958).

Chl *a* concentrations indicated recurring seasonal phytoplankton blooms (Fig. 8A). Blooms were most profound from spring to autumn, with Chl *a* concentrations showing maxima in summer. Generally, Chl *a* values were higher in Meiliang Bay compared with those in the central lake, especially in the summer of 2008. A majority



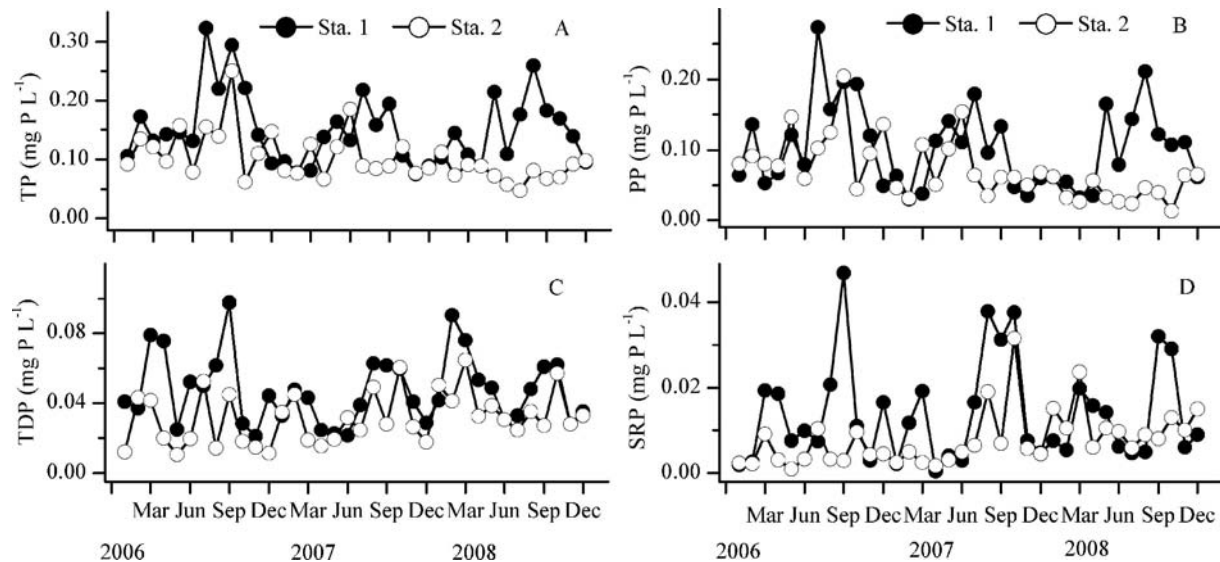


Fig. 6. Seasonal variation at the two sampling stations in Taihu from 2006 to 2008 of (A) TP, (B) PP, (C) TDP, and (D) SRP. Data were obtained from TLLER. TP, PP, TDP, and SRP represent total phosphorus, particulate phosphorus, total dissolved phosphorus, and soluble reactive phosphorus, respectively.

of blooms were dominated by *Microcystis* spp., which occupied 58–98% of the total algal biomass from July to December (Fig. 8B).

**Phytoplankton growth and limiting nutrients**—The physical and chemical properties of the lake water used for bioassays are shown in Table 1. The phytoplankton responses to various nutrient additions are shown in Figs. 9 and 10. In spring, Chl *a* in unamended and N-alone addition treatments did not increase significantly ( $p < 0.05$ ) compared with initial Chl *a*, but P addition treatments (P and N + P) led to significantly ( $p < 0.05$ ) higher Chl *a* concentrations than the control (Fig. 9). The combined N and P additions led to the strongest positive response. This was also true for phytoplankton community growth rates (Fig. 10). In contrast to spring results, the P-alone addition had no effect on Chl *a* and growth rate compared with control during summer and fall, whereas N showed low levels of Chl *a* stimulation above controls. The N + P addition had the strongest effect on phytoplankton biomass during this period, with Chl *a* concentrations and growth rates being significantly higher than either the P, N addition treatments, and control in both summer and fall. In winter, P and P + N enrichments caused an increase of Chl *a* that was significantly ( $p < 0.05$ ) higher than controls and N additions. The difference between P and N + P addition treatments during winter were not significantly different. Overall, phytoplankton showed no response to N-alone additions in winter and spring.

**Phytoplankton growth under various N and P concentrations**—To examine the relationships between the range of bioassay-amended nutrient concentration and phytoplankton growth in Taihu specifically, growth rate responses to different N and P concentrations were examined during summer when the *Microcystis* blooms were most devel-

oped. The final concentrations of available N or P in the various treatments were obtained by summing ambient available N and P concentrations with added N and P concentrations. The results are shown in Fig. 11. During the summer bloom, the growth rate consistently increased until the P concentration reached approximately  $0.20 \text{ mg P L}^{-1}$  (Fig. 11A), whenever enough N was available. When sufficient P was supplied, the increase in growth rates tended to be very small after N concentration reached approximately  $0.80 \text{ mg N L}^{-1}$  (Fig. 11B). The results plotted with the Monod equation indicated a maximum growth rate of  $0.41 \text{ d}^{-1}$ , with a half-saturation concentration of  $0.031 \text{ mg P L}^{-1}$  for P. For N, maximum growth rate and half-saturation concentration were  $0.53 \text{ d}^{-1}$  and  $0.45 \text{ mg N L}^{-1}$ , respectively.

To complement summer results, the responses of phytoplankton growth to various concentrations of N and P added were also examined in fall and winter. Natural lake water and lake water with sufficient N or P additions were used for these bioassays. The results are shown in Fig. 12. In fall, an addition of  $0.50 \text{ mg N L}^{-1}$  was enough to enhance phytoplankton growth, independent of whether P was supplied. Unlike results obtained with N, a range of P additions without N supplied to lake water had no effect on phytoplankton growth. When sufficient N was supplied to lake water, phytoplankton growth over a range of P additions was stimulated. However, a P addition of  $0.20 \text{ mg P L}^{-1}$  was sufficient to maximize phytoplankton growth. During winter, phytoplankton could not grow at various N concentrations without sufficient P addition. When sufficient P was supplied to lake water, phytoplankton growth was stimulated, and growth rates showed no significant differences ( $p < 0.05$ ) among the range of N addition treatments. However, a range of P additions had the same stimulatory effect on phytoplankton growth, which was independent of the level of N supplied.

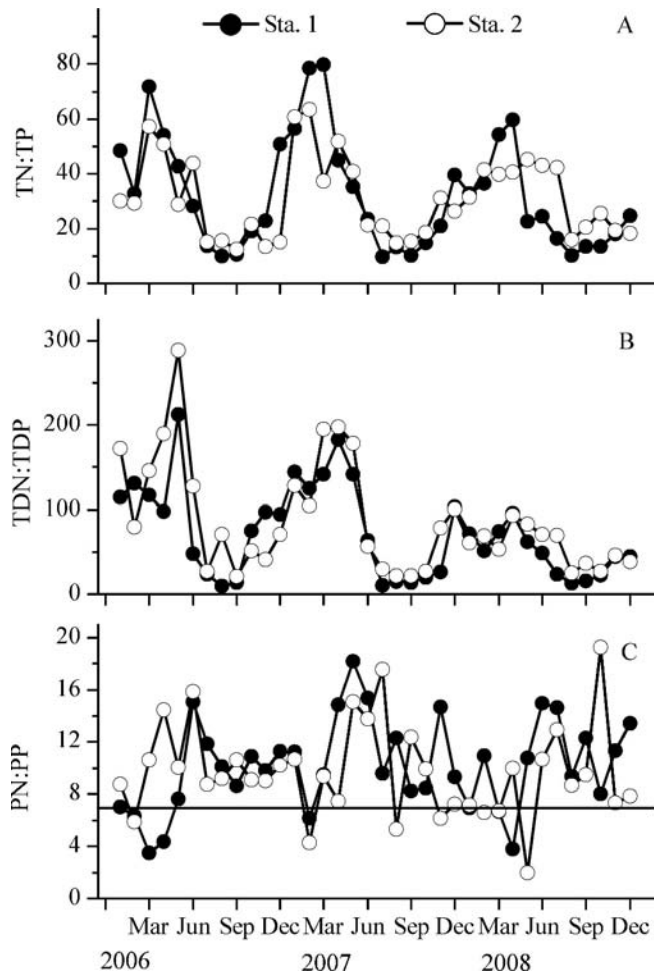


Fig. 7. Seasonal variation at the two sampling stations in Taihu from 2006 to 2008 of mass ratios of (A) TN:TP, (B) PN:PP, and (C) TDN:TDP. Data were obtained from TLLER. TN, TP, PN, PP, TDN, and TDP represent total nitrogen, total phosphorus, particulate nitrogen, particulate phosphorus, total dissolved nitrogen, and total dissolved phosphorus, respectively. The line in panel C represents the Redfield ratio (7 : 1).

## Discussion

**Nutrient limitation of phytoplankton growth**—In situ nutrient enrichment bioassays have been used extensively to identify potential nutrient limitation in phytoplankton communities (Elser et al. 1990). Results from these bioassays show short-term responses of the native phytoplankton to identifiable changes in nutrient conditions, essentially by “interrogating” them as to their nutrient preferences and immediate growth responses. It has been pointed out that such a direct bioassay approach has advantages over nutrient limitation inferred from stoichiometric nutrient ratios and fluxes, which often do not account for nutrient recycling, sediment–water interactions, and cellular storage (Paerl 1982; Fisher et al. 1992). However, generalizations from in situ bioassay experiments must be drawn with caution (Hecky and Kilham 1988). Bioassays are often conducted on a relatively small scale, and it has been argued that the responses observed in them

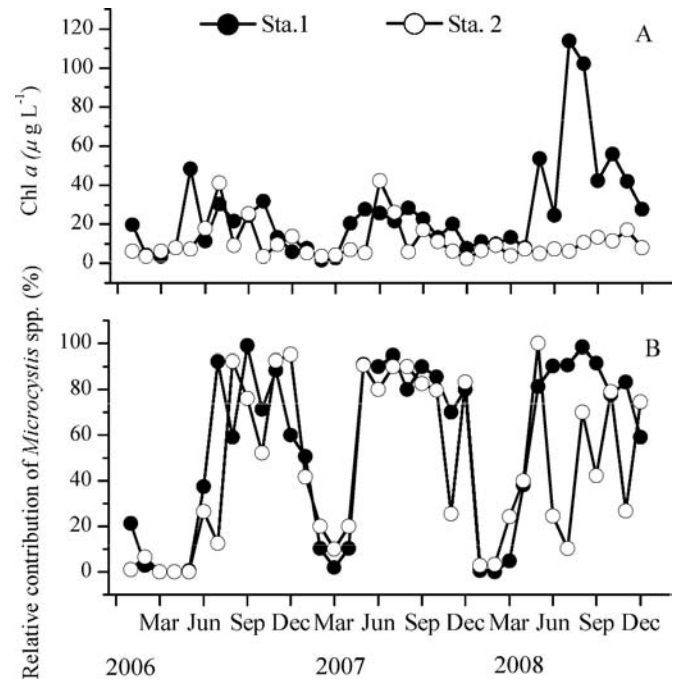


Fig. 8. (A) Seasonal variation of chlorophyll *a* and (B) relative contribution of *Microcystis* spp. fresh biomass to total phytoplankton fresh biomass at the two sampling stations in Taihu. Data were obtained from TLLER.

might not necessarily reflect all the ecological interactions of the system in which they are conducted (Schindler et al. 2008). In bioassays, water samples are incubated in closed containers with a finite supply of nutrients (dependent on initial concentrations and enrichment treatments). Thus, concentrations of nutrients will tend to decline as a consequence of cellular uptake and division to the point at which a particular nutrient can become limiting to further growth. In contrast, in the field, the nutrient flux from autochthonous and allochthonous sources is continual, which could potentially balance or exceed rates of uptake by phytoplankton. Therefore, exhaustion of nutrient supplies in bioassay batch cultures might not necessarily reflect limitation by the same nutrient in the field because net growth rates, and hence nutrient demand, are maximized under the confined conditions. An additional issue is whether stimulation of primary productivity or biomass accumulation in response to the addition of a nutrient is sufficient evidence that the nutrient limits primary productivity or biomass accumulation. To circumvent and minimize many of these concerns, relatively short incubation periods were used in our bioassays. In addition, we used 3 yr of background field data to help interpret our results within the context of the nutrient dynamics of the Taihu ecosystem.

The results of these enrichment experiments showed a strong seasonal pattern in the response of phytoplankton growth to nutrient additions (Figs. 9, 10). In the spring bioassay, P addition and combined P and N additions had a statistically significant positive influence on Chl *a* concentrations and growth rates. However, N-alone



Table 1. Physical and chemical properties of the lake water for bioassays collected from surface water at Sta.1. WT, EC, DO, TN, TDN,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , TP, TDP, SRP represent water temperature, electrical conductivity, dissolved oxygen, total nitrogen, total dissolved nitrogen, nitrate, ammonium, nitrite, total phosphorus, total dissolved phosphorus, and soluble reactive phosphorus, respectively.

	Spring	Summer	Fall	Winter
WT ( $^{\circ}\text{C}$ )	21.8	31.5	22	8.8
pH	8.10	8.81	8.67	8.32
EC ( $\mu\text{S cm}^{-1}$ )	660	540	525	520
DO ( $\text{mg O}_2 \text{ L}^{-1}$ )	11.51	11.9	6.43	11.59
TN ( $\text{mg N L}^{-1}$ )	3.42	1.63	2.29	2.37
TDN ( $\text{mg N L}^{-1}$ )	2.51	0.98	1.43	1.55
$\text{NO}_3^-$ ( $\text{mg N L}^{-1}$ )	1.90	0.13	0.30	1.22
$\text{NH}_4^+$ ( $\text{mg N L}^{-1}$ )	0.037	0.166	0.039	0.030
$\text{NO}_2^-$ ( $\text{mg N L}^{-1}$ )	0.005	0.008	0.000	0.023
TP ( $\text{mg P L}^{-1}$ )	0.133	0.103	0.169	0.096
TDP ( $\text{mg P L}^{-1}$ )	0.044	0.035	0.062	0.035
SRP ( $\text{mg P L}^{-1}$ )	0.003	0.015	0.023	0.003

additions had no significant influence on phytoplankton growth. This can be explained by ambient DIP concentrations that were quite low ( $\sim 0.003 \text{ mg P L}^{-1}$ ; Table 1), providing further evidence that P limitation characterized spring conditions. In summer and fall, however, more exclusive and consistently strong N limitation was observed on the basis of P additions alone having no effect on phytoplankton biomass and growth rate, whereas N additions alone revealed a significant positive effect on phytoplankton growth. The combination of P and N had the strongest influence at these times, indicating that P was a secondary potential limiting nutrient. Alleviating N limitation, while stimulating phytoplankton biomass, quickly led to depletion of bioavailable P in lake water.

Generally in freshwater, N is believed to function as a secondary nutrient capable of producing a synergistic effect

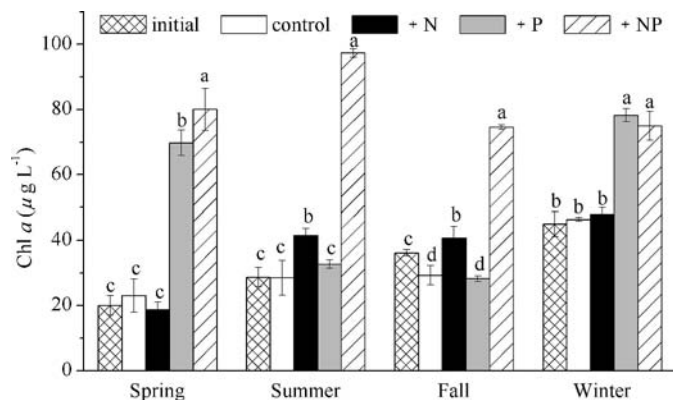


Fig. 9. Initial and maximum phytoplankton biomass (Chl *a*) responses in bioassays conducted in May, July, October, and December 2008. Water samples for bioassays were collected from the surface at Sta.1. Response is 3-d Chl *a* average in spring, summer, and fall and 6-d Chl *a* average in winter. Error bars represent  $\pm 1$  SD of triplicate samples. Differences between treatments are shown on the basis of ANOVA post hoc tests ( $a > b > c$ ;  $p < 0.05$ ).

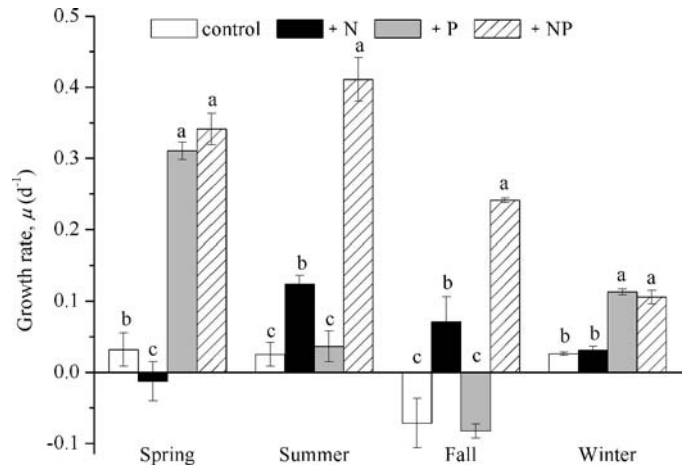


Fig. 10. Growth rates of natural phytoplankton assemblages in bioassays conducted in May, July, October, and December 2008. Water samples for bioassays were collected from the surface at Sta.1. Error bars represent  $\pm 1$  SD of triplicate samples. Differences between treatments are shown on the basis of ANOVA post hoc tests ( $a > b > c$ ;  $p < 0.05$ ).

in the presence of P (Elser et al. 1990). This contrasts with our observation that N was the primary limiting nutrient in Taihu during the critical summer period when water quality is most adversely affected by algal blooms. In winter, there was no response to N additions, whereas P and P + N additions had similar effects on phytoplankton, suggesting that the limiting nutrient shifted from N to P. Fisher et al. (1992) concluded from Chesapeake Bay field studies that the seasonal shift from nitrogen to phosphorus limitation took place when the ratio of N and P in allochthonous nutrient loads changed. Taihu's nutrient concentrations varied widely between seasons (Figs. 5, 6). The TN concentrations were lowest in summer and fall and highest in spring and winter. Conversely, TP concentrations were highest in summer and fall and lowest in spring and winter. As a result, TN:TP ratios in Lake Taihu showed strong seasonal variations, with highest values (52 to 212:1) in non-growth season and lowest values ( $< 20:1$ ) in growth season (Fig. 7A), which supported our seasonal bioassay-based variations in nutrient limitation.

Bioavailability of both N and P during the summer season plays a role in controlling bloom formation and magnitude. Sources of N and P to the lake during summertime include external runoff (dissolved inorganic and organic N), atmospheric deposition, and internal sediment release. The external N and P (including point and non-point sources) supplied to Taihu is dominated by riverine discharge. On the basis of the spatially distributed and mechanistic SWAT Model, Lai et al. (2006) suggested that sewage released to the rivers was the most important nutrient source, and the industrial point sources were the second highest contribution, each contributing 31% and 30% to TN and 47% and 16% to TP, respectively. Agricultural fertilizer application and livestock were also important nutrient sources, which contributed 15% and 10% to TN, and 14% and 11% to TP, respectively. Furthermore, annual nutrient loading from river runoff is

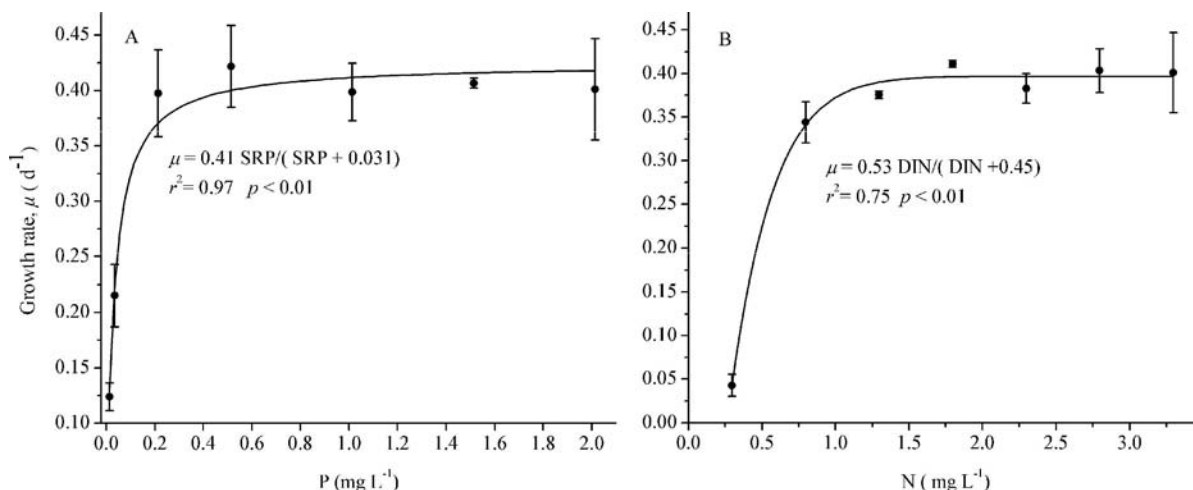


Fig. 11. Growth kinetics of natural phytoplankton assemblages in response to a range of (A) P concentrations and (B) N concentrations during summer 2008. Curves were fitted by nonlinear regression. Water samples for bioassays were collected from the surface at Sta.1. The final concentrations of dissolved inorganic N or soluble reactive P in the various treatments were obtained by summing ambient dissolved inorganic N or soluble reactive P concentrations in the lake water with added N or P levels. Error bars represent  $\pm 1$  SD of triplicate samples.

highly variable, with TN and TP loading showing peaks during summer (June–August). This variability largely reflects the pulsed nature of rainfall and river discharge events (Fig. 2B,C). Therefore, summer minimal N concentrations in the lake are largely the result of rainfall dilution and phytoplankton uptake, as evidenced by high levels of Chl *a* through the summer season, especially in embayments like Meiliang Bay (Fig. 8). Sediments provide sites for denitrification, a potentially important N loss mechanism that can drive aquatic systems toward N limitation and regulate nutrient supply ratios (Seitzinger 1988). In Meiliang Bay, denitrification might exacerbate N limitation by removing excess N in summer (McCarthy et al. 2007). As with TN, DIN and  $\text{NH}_4^+$  peaked in early spring in Meiliang Bay, perhaps because the low water level coincided with the lowest rainfall and freshwater inflows at that time (Fig. 2B,C) and the rate of biogeochemical cycling was low because of minimal temperatures at that time (Fig. 2A). Furthermore, during this time of the year, transplanting of rice into paddies is accompanied by maximal applications (and losses via runoff) of chemical and organic N fertilizers (Gao et al. 2004).

In addition to external nutrient loading, sediment release and remineralization of ammonium, nitrate, and phosphate is an important source of nutrients. Static release of P from sediment mainly depends on chemical diffusion induced by concentration gradient, which is controlled by temperature, dissolved oxygen concentration at the sediment–water interface, oxidation–reduction potential, and pH. For example, increases in temperature stimulate microbially mediated mineralization, which liberates organically bound P in the sediment pore water. Increases in microbial activity also lowers the redox potential in the surface sediments, which might induce the release of Fe-bound P (Kamp-Nielsen 1975). High pH in the overlying water triggers  $\text{PO}_4^{3-}$  release from aerobic sediments by  $\text{OH}^-$  ion exchange

with  $\text{PO}_4^{3-}$  on surfaces of metal oxides–hydroxides (Andersen 1975).

Wind-driven mixing and shallow depth prevent stable thermal stratification in Taihu. For example, the difference in water temperature between surface and bottom layers in July was approximately  $0.3^\circ\text{C}$  or smaller (Fig. 4A), and as a result, bottom waters remained oxic in summer (Fig. 4B). Elevated pH levels observed during photosynthetically active summer algal blooms (Fig. 3C) will induce massive P release from sediment, which might be an important factor driving the seasonal changes in the internal loading of phosphorus in this shallow lake (Xie et al. 2003; Jin et al. 2006). Investigation in Taihu suggest that sediments are frequently disturbed and resuspended by wind waves, which results in large short-term pulses of nutrients released to the overlying water. Such pulsed releases tend to be greater sources of nutrients than more chronic static releases (Qin et al. 2004). In addition, alkaline phosphatase produced by algae and bacteria also plays an important role in P cycling in Taihu. This process can hydrolyze about 58% of the total phosphorus to inorganic phosphate and compensate for phosphorus deficiency of algal and bacterial growth (Gao et al. 2006). In winter, sediment P release will be reduced because of relatively lower temperatures and pH (Figs. 2A, 3B), and the rates of P cycling are also reduced because of relatively low rates of microbial activity. Together, these factors can account for the observed low dissolved P concentrations and strong P limitation of phytoplankton in winter.

In our bioassay,  $\text{NO}_3^-$  was employed as the sole nitrogen source, largely because  $\text{NO}_3^-$  is the primary form of inorganic N in Taihu, especially in winter and spring. Phytoplankton biomass (Chl *a*) and growth rates responded to nitrogen additions in summer and fall, whereas phosphorus played a secondary role at this time of year. Prior work has shown that the dominant cyanobacteria

tend to prefer  $\text{NH}_4^+$  over  $\text{NO}_3^-$  (Dokulil and Teubner 2000). Therefore, phytoplankton growth response might have been even stronger if the nitrogen additions had included  $\text{NH}_4^+$ . Thus, if anything, the potential for N limitation, and responses to N enrichment, might have been underestimated in our bioassays.

**Nutrient threshold of phytoplankton growth**—Bioassay results showed phytoplankton growth responded strongly and proportionately to P additions over a range from 0.014 to 0.214 mg P L<sup>-1</sup>. Approximately 0.20 mg P L<sup>-1</sup> could be regarded as an upper limit for a P effect on the growth rate of a cyanobacterial bloom. Through luxury consumption, cyanobacteria are likely to store enough cellular P for several rounds of cell division; hence, their growth potential would not necessarily reflect ambient phosphate concentration (Goldman et al. 1987). In our experiments, because ambient P concentration (0.014 mg P L<sup>-1</sup>) promoted cyanobacterial growth, a lower limit for P could not be clearly defined. Phytoplankton growth was no longer N limited when N concentrations reached approximately 0.80 mg N L<sup>-1</sup> (Fig. 11). However 0.30 mg N L<sup>-1</sup> did not stimulate cyanobacteria growth (Fig. 11), indicating that higher N levels were needed to promote the bloom. A concentration of 0.30 mg N L<sup>-1</sup> is well above what is considered limiting in most estuarine and coastal systems. It is likely that the colony-forming *Microcystis* is larger than most phytoplankton in estuarine and coastal waters; hence, the utilization efficiency on  $\text{NO}_3^-$  would be expected to be low, with a high half-saturation constant (0.45 mg N L<sup>-1</sup>). The results from fall and winter bioassays further indicate that concentrations of 0.20 mg P L<sup>-1</sup> and 0.80 mg N L<sup>-1</sup> are enough to sustain the cyanobacterial bloom (Fig. 12).

The eutrophication thresholds of P for freshwaters (rivers and lakes) are from 0.02 to 0.10 mg P L<sup>-1</sup> and of N are from 0.50 to 1.00 mg N L<sup>-1</sup> (Lin et al. 2008). In 1960, Taihu was categorized as oligotrophic because total inorganic nitrogen (TIN) in the lake was only 0.05 mg N L<sup>-1</sup>, SRP was 0.02 mg P L<sup>-1</sup>. By 1981, TIN had increased to 0.89 mg N L<sup>-1</sup> and SRP remained stable (Sun and Huang 1993). In 1988, TIN and TN concentrations were 1.12 and 1.84 mg N L<sup>-1</sup>, respectively, and total phosphorus (TP) was 0.032 mg P L<sup>-1</sup> (Sun and Huang 1993). However, by the year 1998, TIN and TN concentrations had increased to 1.58 and 2.34 mg N L<sup>-1</sup>, whereas TP was 0.085 mg P L<sup>-1</sup> (Qin et al. 2007). Reacting to increases in the nutrient levels since the mid-1980s, blooms of the toxin-producing cyanobacteria *Microcystis* spp. have occurred every summer in the northern part of the lake (Qin et al. 2007). Therefore, the 0.89 mg L<sup>-1</sup> TIN in 1981 can be regarded as a threshold, above which large-scale cyanobacterial blooms regularly occur. This agrees with our results, which indicate that approximately 0.80 mg TIN L<sup>-1</sup> is enough to sustain the cyanobacterial bloom. During the summer cyanobacterial blooms in Taihu, available N levels are below the saturating N concentration (0.80 mg N L<sup>-1</sup>; Fig. 5). These results underscore the conclusion that further increases in available N load to the ecosystem can be expected to greatly enhance algal bloom potential in

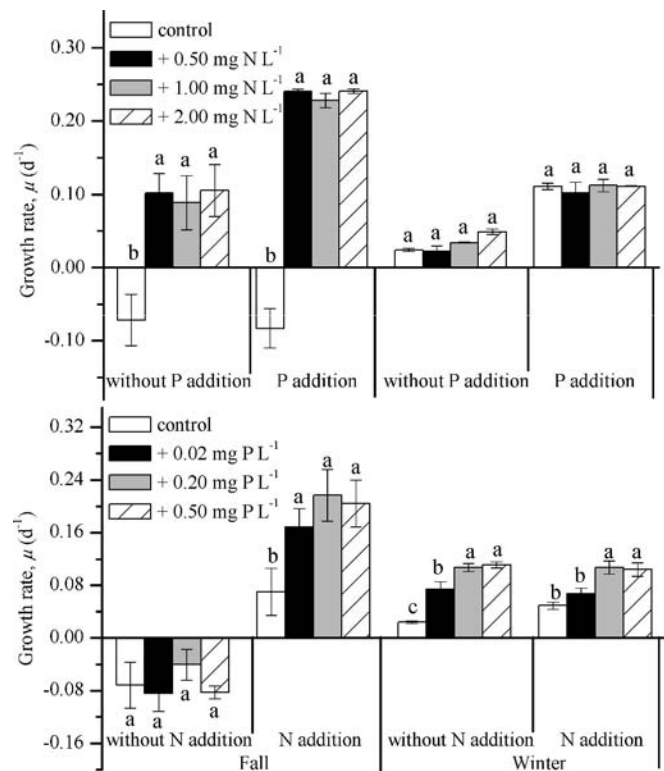


Fig. 12. Growth rates of natural phytoplankton assemblages in response to various concentrations of N and P additions during fall and winter 2008. Water samples for bioassays were collected from the surface at Sta.1. Error bars represent  $\pm 1$  SD of triplicate samples. Differences between treatments are shown on the basis of ANOVA post hoc tests ( $a > b > c$ ;  $p < 0.05$ ).

Taihu, given the continued presence of surplus P. Furthermore, it is stressed that the main bloom organisms, *Microcystis* spp. belong to a non-nitrogen-fixing cyanobacterial genus (Paerl et al. 2001), making them highly dependent on exogenous combined N sources (DIN) to support growth. Hence, controlling N inputs should be effective in reducing the bloom potential for this organism. Emphasis continues to focus on the reduction of P loads into freshwater bodies as a means of controlling eutrophication (Schindler et al. 2008). However, results from this study indicate that, although P load reduction is important, N load reduction is probably more critical for controlling the severity, geographic extent, and duration of Taihu's cyanobacterial blooms. Therefore, a long-term nutrient management strategy for the Taihu watershed should include reductions in both N and P inputs.

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