Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2010, 22(10) 1491–1499
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Phytoplankton community from Lake Taihu, China, has dissimilar responses to inorganic and organic nutrients

Xiaodong Wang[1,2], Boqiang Qin[1,*], Guang Gao[1], Yongping Wang[1,2], Xiangming Tang[1], Timothy Otten[3]

1. State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China. E-mail: wangxd1201@163.com
2. Graduate School of the Chinese Academy of Sciences, Beijing 100039, China
3. Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC 27517, USA

Received 24 November 2009; revised 04 January 2010; accepted 25 February 2010

Abstract

To evaluate the response of phytoplankton from Lake Taihu to different types of nutrients, the phytoplankton responses were measured after adding inorganic nitrogen (N) and phosphorus (P) or decomposed algal scum (*Microcystis* spp.) into the lake water. Both types of nutrients promoted an increase in phytoplankton biomass as determined by chlorophyll *a* and algal wet weight. The addition of decomposed algal scum resulted in a significantly greater phytoplankton response than the addition of inorganic N and P alone. The dissolved inorganic N and P in the inorganic nutrient treatment were found not limit phytoplankton growth. The higher algal biomass obtained in the treatment with decomposed algal scum indicated the importance of other organic nutrients besides N and P such as trace elements, as well as the importance of the form of N since the levels of ammonia nitrogen (NH_4^+-N) from the decomposed algal treatment were actually higher than that of the inorganic N and P addition. *Microcystis* spp. (Cyanobacteria), *Scenedesmus* spp. (Chlorophyta) and *Synechocystis* spp. (Cyanobacteria) were the dominant taxa in the control, inorganic N and P treatment, and the decomposed algal scum treatment, respectively. *Microcystis* never bloomed in response to both types of nutrient additions indicating that the bloom propagation is not solely related to nutrient additions, but may be related to the absence of selective grazing from zooplankton.

Key words: phytoplankton; inorganic nutrients; decomposed algal scum
DOI: 10.1016/S1001-0742(09)60280-1

Introduction

Phytoplankton, especially harmful algal blooms, flourish in eutrophic waters (Anderson et al., 2002). The source and composition of nutrients driving phytoplankton production have been studied extensively, including those released from sediments (Søndergaard et al., 2001) and those deposited by external loads (Bootsma et al., 1999). The chemical speciation (inorganic vs. organic) has also been shown as an important factor (Gobler and Sañudo-Wilhelmy, 2001; Bronk et al., 2007).

Usually nitrogen (N) and phosphorus (P) concentrations in lake water are the main indices of eutrophication, and many previous investigations have focused on the response of phytoplankton to inorganic N and P additions, as well as the N versus P limitation of algal blooms (Pick and Lean, 1987; Hecky and Kilham, 1988). For phytoplankton in a shallow and hypertrophic lakes, Sommer (1989) showed that N was usually the most limiting nutrient, followed by P. However, Hecky and Kilham (1988) conducted a comprehensive review of nutrient enrichment studies (bioassays) and found that P was generally the limiting nutrient in freshwater systems. As such, Paerl (2009) recommended that both N and P should be given greater attention when considering the growth of phytoplankton in freshwater. In addition, there can be limitations of other trace elements such as iron (Goldman, 1972; Morel and Hudson, 1985; Zhang, 2000; North et al., 2007).

Since phytoplankton require not just N and P to grow, but other nutrients as well, the production of organic matter from phytoplankton decay may be critical to bloom sustainability because decay releases other nutrients into the water column which can in turn be sequestered again by phytoplankton leading to a positive feedback loop. Many studies have investigated the nutrients released from organic matter decomposition, especially phytoplankton decomposition. It has been shown that decomposition is a primary mechanism for the loss of organic carbon to bloom biomass (frequently constituted by blue-green algal dominance) (Fallon and Brock, 1980; Zohary et al., 1998). Phytoplankton growth in lakes is affected by organic nutrients, including those from decomposed algae.

* Corresponding author. E-mail: qinbq@niglas.ac.cn

Organic matter produced during phytoplankton blooms is thus crucial to nutrient cycling and ecosystem functioning (Fukami et al., 1985; Fujii et al., 2002). The release of different elements from decomposing phytoplankton debris has been quantified by many studies (Lee and Fisher, 1992; Gobler et al., 1997; Wang and Guo, 2001) and it is known to be an important nutrient source (Enoksson, 1993).

The studies highlighted above illustrate the significance of nutrient cycling on eutrophication and bloom propagation. Some studies have addressed this issue for phytoplankton growth (Antia et al., 1991; Berman, 1997). Wang et al. (2007) found that organic matter was related to higher phytoplankton biomass in Lake Taihu, China. However, no studies have addressed the differences between the effects of inorganic nutrients and the nutrients associated with decomposed biomass (phytoplankton) from Lake Taihu.

Lake Taihu, on the south side of the Changjiang (Yangtze) delta of China, is the third largest freshwater lake in China. The lake has become increasingly eutrophic since the 1980's due to abundant and diverse incoming pollutants (Qin et al., 2007). Consequently, it has been plagued by extensive, annual *Microcystis* blooms in recent decades (Chen et al., 2003; Guo, 2007). The large quantity of continuously growing, and then decomposing phytoplankton, especially during the summer blooms, has become a new type of internal pollutant. The subsequent release of organic matter and nutrients to the water column from the decomposition of algae leads to a positive feedback cycle providing increasingly more nutrients for the growth of new algae.

This article reported the effects of nutrients on the growth and community composition of phytoplankton from Lake Taihu when grown with the addition of inorganic N and P or the addition of decomposed algae from a bloom (mainly constituted by *Microcystis* spp.). The objectives of this study were: (1) to evaluate the role of inorganic N and P, and decomposed algae from a bloom to phytoplankton biomass; (2) to identify the phytoplankton community composition resulting from the two different types of nutrient addition; (3) to determine whether *Microcystis* dominance such as in Lake Taihu can be induced under experimental conditions.

1 Materials and methods

1.1 Experimental design

Nutrient addition bioassays were used as previously described (Paerl and Bowles, 1987). The experiment was conducted in plastic barrels on the shore of Meiliang Bay in the north of Lake Taihu during 15–27 July, 2008. The large-sized zooplankton and *Microcystis* spp. colonies of the initial lake water for the experiment were removed using a plankton net. Water was taken from Meiliang Bay, filtered by a no.13 plankton net (mesh size 0.112 mm), to remove zooplankton and large *Microcystis* spp. colonies, and was aliquoted into nine plastic barrels of 100 L each.

The experiment involved a control treatment without nutrient and two treatments consisting of different nutrients additions. Control had no nutrients added, and one treatment consisted of an addition of inorganic N (as KNO_3 and NH_4Cl, and NO_3^--N/NH_4^+-N weight ratio of 2:1) and P (as $K_2HPO_4{\cdot}3H_2O$) (+INORG), and the second treatment consisted of an addition of decomposed algal matter, mainly *Microcystis* spp. bloom (+ORG). Each condition was carried out in triplicates.

The *Microcystis* blooms in Meiliang Bay, Lake Taihu are very dense in recent years, and its nutrient concentrations are also high. According to the yearly report released by the Taihu Laboratory for Lake Ecosystem Research (TLLER) during 2006 to 2008, the highest TN and TP contents in Meiliang Bay were about 850 μmol/L and 20 μmol/L, respectively, although not concomitantly. The nutrient addition concentrations used in this experiment were on these levels.

The initial total nitrogen (TN) to total phosphorus (TP) ratio of the control was 40.1:1 (molar ratio). The +INORG treatment had a net increase of 714.3 μmol/L TN and 16.1 μmol/L TP in comparison with the control, and the initial TN/TP ratio (molar ratio) of +INORG treatment was 43.6:1. For the +ORG treatment, the decomposed algal scum consisting mainly of *Microcystis* spp., was harvested from the surface bloom in Meiliang Bay on 5 June 2008, and held in a clean glass jar without additional water at ambient temperature in the shade until the experiment began. Five hundred and ten milliliters of the decomposed algal scum was added to each of the 3 barrels to get a net increase of 714.3 μmol/L TN, and a net increase of 18.1 μmol/L TP in comparison with the control, and the initial TN/TP ratio (molar ratio) of +ORG treatment was 39.6:1.

1.2 Water chemistry and physical measurements

The weather, water temperature at 07:00 and 14:00, and the photosynthetically active radiation (PAR) at 14:00 were recorded daily. Water samples were taken from each barrel after gentle mixing every three days, and seven parameters were analyzed: total nitrogen (TN), total phosphorus (TP), dissolved total nitrogen (DTN), dissolved total phosphorus (DTP), soluble reactive phosphorus (SRP), nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N).

Water temperature was measured at the surface 3 cm of each barrel by mercury thermometer. The downwardly directed PAR (400–700 nm) was measured by using a LI-192SA underwater cosine corrected sensor connected to an Li-1400 data logger (LI-COR Biosciences, USA).

Water collected for DTN, DTP, SRP, NO_3^--N and NH_4^+-N analyses was filtered through GF/C filters (1.2 μm pore size, Whatman, UK). TN, TP, DTN and DTP analyses were measured according to Gross and Boyd (1998). NO_3^--N was measured by ultraviolet spectrophotometry and NH_4^+-N was measured by the Nessler's reagent method (Eaton et al., 1995).

1.3 Phytoplankton

The total phytoplankton biomass was expressed as both total wet weight biomass and chlorophyll *a* (Chl. *a*), and the biomass of different phytoplankton genera was

estimated by wet weight. To determine Chl-*a* concentration, water samples were filtered through GF/C filters, the residue extracted by 90% hot ethanol, and then Chl-*a* concentration was determined by colorimetry (Lorenzen, 1967; Jespersen and Christoffersen, 1987).

Samples were collected every three days following gentle mixing in the barrels. Phytoplankton samples (50 mL) had 100% Lugols solution added to them, giving a final concentration of 1%, and were stored in the dark until they could be identified microscopically. If the density of phytoplankton was too low for counting, the sample was concentrated by sedimentation. For enumeration, two replicate aliquots were placed in 0.1 mL plankton counting chambers according to Palmer and Maloney (1954).

Most cells were observed at 400× magnification by light microscopy, while large algal cells were observed at 100× magnification. Cells were identified to the genus level based on morphology (Hu and Wei, 2006). Algal volumes were calculated from cell density and cell size measurement. Cell volumes were estimated by approximation to the nearest simple geometric solid after measurement of at least 40 algal units (Zhang and Huang, 1991). Conversion to wet weight biomass assumed that 1 mm^3 of volume was equivalent to 1 mg of wet weight biomass (Chen et al., 2003).

1.4 Statistical analyses

The data comparison between control and two treatments was analyzed by two-way ANOVA (analysis of variance) with sampling time and treatment as the two factors. Before comparison, DTN, NH_4^+-N, NO_3^--N and (NO_3^--N + NH_4^+-N)/DTN were not transformed (as they all had homogeneity of variances), and water temperature at 14:00, TN, TP, DTP, SRP, TN/TP, DTN/DTP, SRP/DTP, PAR at 14:00, Chl-*a* and total wet weight biomass of phytoplankton were log-transformed, and SRP/DTP was transformed by arc-sin of the square-root to increase homogeneity of variances (Underwood, 1997). Analyses were made using the program SPSS 11.5 (Statistical Package for the Social Sciences). The differences are reported as significant if $P < 0.05$, and the LSD (Least Significant Difference) test was chosen as the post-hoc test for equal variances, whereas Tamhane's T2 test was chosen for unequal variances.

2 Results

2.1 Weather, water temperature and light intensity

The weather was clear and sunny on most of the experimental days, and the exceptions were 16 July when it was partly cloudy, and 19 July when there was a thunderstorm.

There was a negligible difference in the water temperature between control and two treatments at 07:00, and the mean water temperature of all at 07:00 was 30.5°C; and that of the control, +INORG, +ORG at 14:00 was 37.5, 37.3, 37.9°C, respectively (Fig. 1). There were no significant differences between control and two treatments of the water temperature at 14:00 ($P > 0.05$).

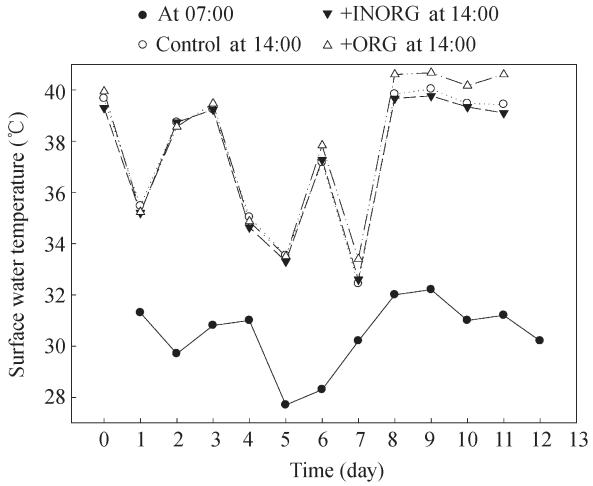

Fig. 1 Surface water temperature of control and two treatments taken daily at 07:00 and 14:00. Values at 07:00 were considered the same for both control and two treatments.

There was no significant difference between the control and two treatments of the surface photosynthetically active radiation (PAR) at 14:00 ($P > 0.05$), and the bottom PAR of +INORG was lower than that of the control and +ORG ($P < 0.05$), while there was no significant differences between the control and +ORG of the bottom PAR ($P > 0.05$) (Fig. 2).

2.2 Nutrients

Figure 3 shows the N and P concentrations and ratios. There were significant differences in SRP, NO_3^--N, TN, DTN and DTP concentrations between control and two treatments ($P < 0.05$) (Fig. 3). The NH_4^+-N concentrations in the +ORG treatment were higher than that of both +INORG and the control, while there was no significant difference between +INORG and the control. The rank order of differences in SRP, TN, DTN and DTP concentrations were: +INORG > +ORG > control ($P < 0.05$), and that of NO_3^--N was +INORG > control > +ORG ($P < 0.05$). The TP of +INORG and +ORG (which were not significantly different from each other) was significantly higher than that of the control ($P < 0.05$).

The TN/TP in +ORG treatment was significantly lower than that of the control ($P < 0.05$), while there was no significant difference between either the control and +INORG treatment or between the +INORG and +ORG treatments. There were no significant differences of DTN/DTP between the control and two treatments. (NH_4^+-N + NO_3^--N)/DTN was significantly higher in the control and the +INORG treatment (which were not significantly different from each other) compared with the +ORG treatment ($P < 0.05$). SRP/DTP was significantly lower in +ORG than that of +INORG ($P < 0.05$), and there was no significant difference either between the control and +ORG or between the control and +INORG.

2.3 Phytoplankton biomass

The peak Chl-*a* content of the +INORG treatment (102.76 μg/L) was on day 6, while that of the +ORG treatment (445.14 μg/L) was on day 9 (Fig. 4). The peak

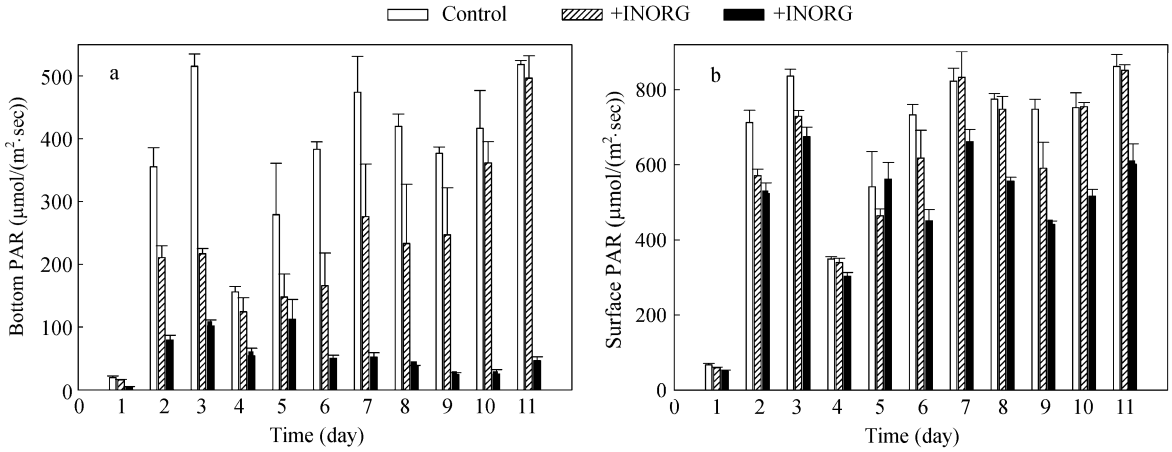

Fig. 2 Daily values (mean ± SD) of bottom photosynthetically active radiation (PAR) (a) and surface PAR (b) of control and two treatments at 14:00.

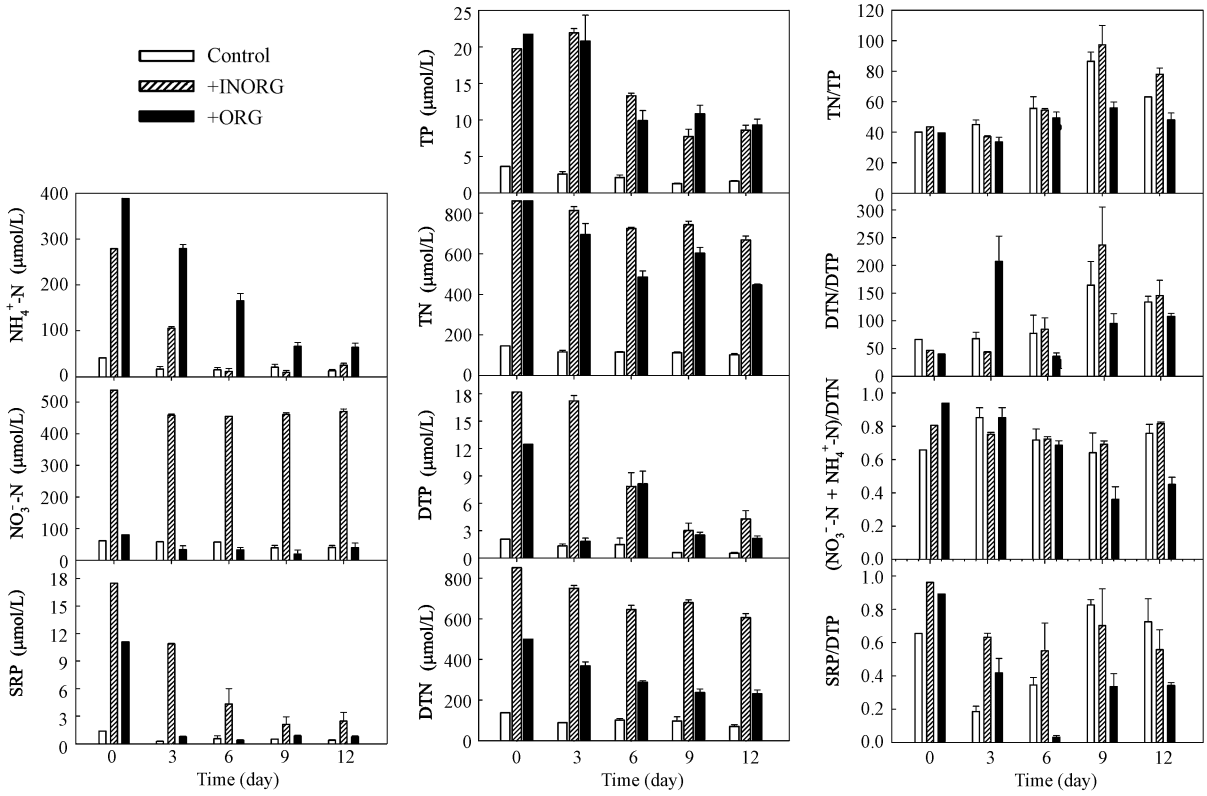

Fig. 3 Variation of mean (± SD) concentrations of N, P, and their molar ratios of control and two treatments on sampling days.

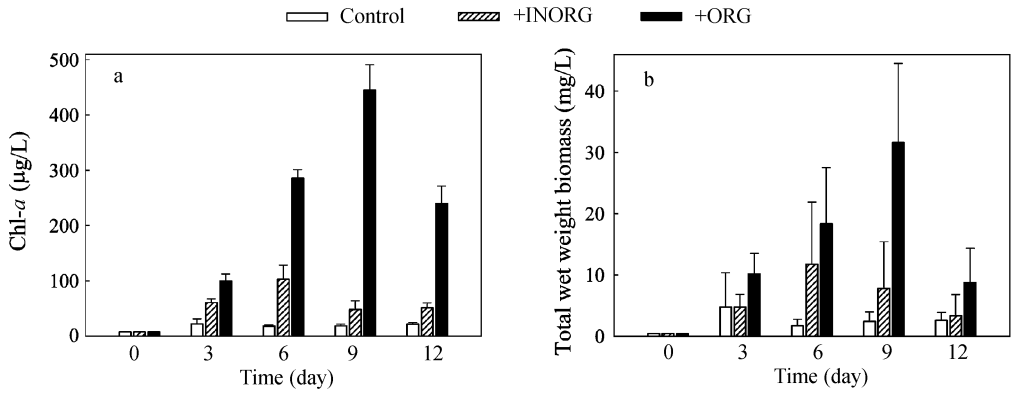

Fig. 4 Variations of mean (± SD) Chl-*a* (a) and total wet weight biomass (b) of the phytoplankton in the control and two treatments.

Chl-*a* contents in the +INORG and +ORG treatments were much higher than that of the control, which was 21.89 μg/L. The period of peak wet weigh biomass was the same as for the peaks in Chl-*a* content for +INORG and +ORG treatments. There were significant differences of both the Chl-*a* content and wet weight biomass between control and two treatments ($P < 0.05$), with an order: +ORG > +INORG > control.

2.4 Phytoplankton community composition

The phytoplankton community composition of the initial water filtered by 0.112 mm plankton net from Meiliang Bay was: Cyanobacteria: *Aphanocapsa* sp., *Chlorococcus* sp., *Microcystis* spp., *Planktothrix* sp. and *Synechocystis* spp.; Chlorophyta: *Ankistrodesmus* sp., *Chlamydomonas* spp., *Crucigenia* spp., *Oocystis* spp., *Quadrigula* sp., *Scenedesmus* spp. and *Selenastrum* spp.; Euglenophyta: *Trachelomonas* spp.; Crytophyta: *Cryptomonas* spp.; Bacillariophyta: *Fragilaria* spp.

Table 1 shows the wet weight biomass of the five dominant genera in the control and two treatments. *Microcystis* (Cyanobacteria) was the most dominant in the control on day 3, 9 and 12, while it was never the dominant genera in either of the +INORG or +ORG treatments. In contrast, *Scenedesmus* (Chlorophyta) was the dominant in +INORG on day 6 and 9, and *Synechocystis* (Cyanobacteria) and *Trachelomonas* (Euglenophyta) were the two dominant in +ORG on all sampling days. The predominant *Microcystis* species was *M. flos-aquae* in this experiment, which occurred in colonies with cell numbers ranging from dozens to hundreds.

During the experiment, 8 phyla representing 40 genera of phytoplankton were observed. In the control, +INORG and +ORG treatments, the number of Chlorophyta genera was 15, 15 and 16, respectively; the number of Cyanobacteria genera was 6, 7 and 8, respectively; the number of Bacillariophyta genera was 3, 4 and 1, respectively; and there were 2 genera of Euglenophyta in all 3 conditions.

Variations of wet weight biomass of different phytoplankton phyla (after deleting the phylum whose wet weight biomass were < 5% of the total biomass) in control and two treatments during the experiment (Fig. 5), showed that Cyanobacteria, Chlorophyta and Euglenophyta were dominant in the control, in which Cyanobacteria had the largest wet weight on all sampling days. Cyanobacteria and Chlorophyta were the dominant in +INORG, in which Chlorophyta had the largest wet weight on day 6, 9 and 12. Cyanobacteria, Chlorophyta and Euglenophyta were dominant in +ORG treatment, in which Cyanobacteria had the largest wet weight on days 3, 6 and 9.

3 Discussion

3.1 Effect of nutrients on phytoplankton biomass

The results of the water temperature (Fig. 1) and PAR (Fig. 2) indicated that the lower phytoplankton biomass in +INORG than in +ORG (Fig. 4) was not limited by water temperature or light condition; and the nutrient results (Fig. 3) suggested it was not due to inorganic N or P limitation. It is therefore likely that there are other reasons for the higher inorganic NO_3^--N and SRP contents in the +INORG treatment not being utilized efficiently by the phytoplankton for growth and proliferation. While the NH_4^+-N content of the +ORG treatment was higher than that of +INORG, it is probable that the higher Chl-*a* content of the +ORG

Table 1 Biomass of the five dominant genera in the control and two treatments (unit: mg/L wet weight)

Control (no nutrients addition)		+INORG treatment (addition of inorganic N and P)		+ORG treatment (addition of decomposed algal scum)	
Day 3					
Microcystis	2.669 ± 4.348	*Synechocystis*	1.503 ± 0.144	*Synechocystis*	3.930 ± 0.842
Trachelomonas	1.022 ± 0.432	*Trachelomonas*	1.334 ± 0.492	*Trachelomonas*	3.750 ± 1.447
Synechocystis	0.382 ± 0.186	*Cyclotella*	0.422 ± 0.278	*Selenastrum*	1.903 ± 0.255
Carteria	0.213 ± 0.239	*Pediastrum*	0.316 ± 0.273	*Microcystis*	0.273 ± 0.473
Pediastrum	0.154 ± 0.117	*Microcystis*	0.296 ± 0.263	*Chlamydomonas*	0.125 ± 0.114
Day 6					
Trachelomonas	0.424 ± 0.181	*Scenedesmus*	3.380 ± 2.516	*Synechocystis*	6.928 ± 1.327
Microcystis	0.422 ± 0.351	*Microcystis*	1.930 ± 2.731	*Trachelomonas*	4.531 ± 2.301
Cryptomonas	0.232 ± 0.218	*Trachelomonas*	1.870 ± 1.209	*Oocystis*	1.707 ± 0.721
Synechocystis	0.145 ± 0.011	*Micractinium*	0.804 ± 0.917	*Selenastrum*	1.536 ± 0.225
Scenedesmus	0.092 ± 0.007	*Tetrastrum*	0.675 ± 0.711	*Carteria*	1.333 ± 1.736
Day 9					
Microcystis	0.740 ± 0.182	*Scenedesmus*	4.033 ± 3.613	*Trachelomonas*	10.350 ± 1.922
Trachelomonas	0.647 ± 0.435	*Trachelomonas*	1.387 ± 1.178	*Synechocystis*	8.569 ± 1.263
Synechocystis	0.234 ± 0.025	*Micractinium*	0.511 ± 0.885	*Microcystis*	6.406 ± 5.397
Cryptomonas	0.144 ± 0.187	*Tetrastrum*	0.482 ± 0.197	*Selenastrum*	1.501 ± 0.262
Scenedesmus	0.139 ± 0.126	*Chodatella*	0.429 ± 0.516	*Scenedesmus*	1.018 ± 0.174
Day 12					
Microcystis	1.169 ± 0.521	*Tetrastrum*	1.301 ± 1.008	*Trachelomonas*	1.821 ± 0.762
Trachelomonas	0.481 ± 0.061	*Micractinium*	0.921 ± 1.061	*Synechocystis*	1.527 ± 0.667
Oocystis	0.199 ± 0.131	*Microcystis*	0.334 ± 0.512	*Oocystis*	1.444 ± 1.272
Cryptomonas	0.196 ± 0.132	*Trachelomonas*	0.228 ± 0.298	*Tetrastrum*	0.957 ± 0.157
Synechocystis	0.106 ± 0.022	*Golenkinia*	0.207 ± 0.270	*Crucigenia*	0.564 ± 0.468

The dominant genera by wet weight of the initial water were *Synechocystis*, *Trachelomonas*, *Microcystis*, *Chlroococcus* and *Cryptomonas*, and their biomass (mg/L) was 0.103, 0.092, 0.072, 0.051 and 0.022, respectively. Data are expressed as mean ± SD. Dominance was determined by wet weight biomass.

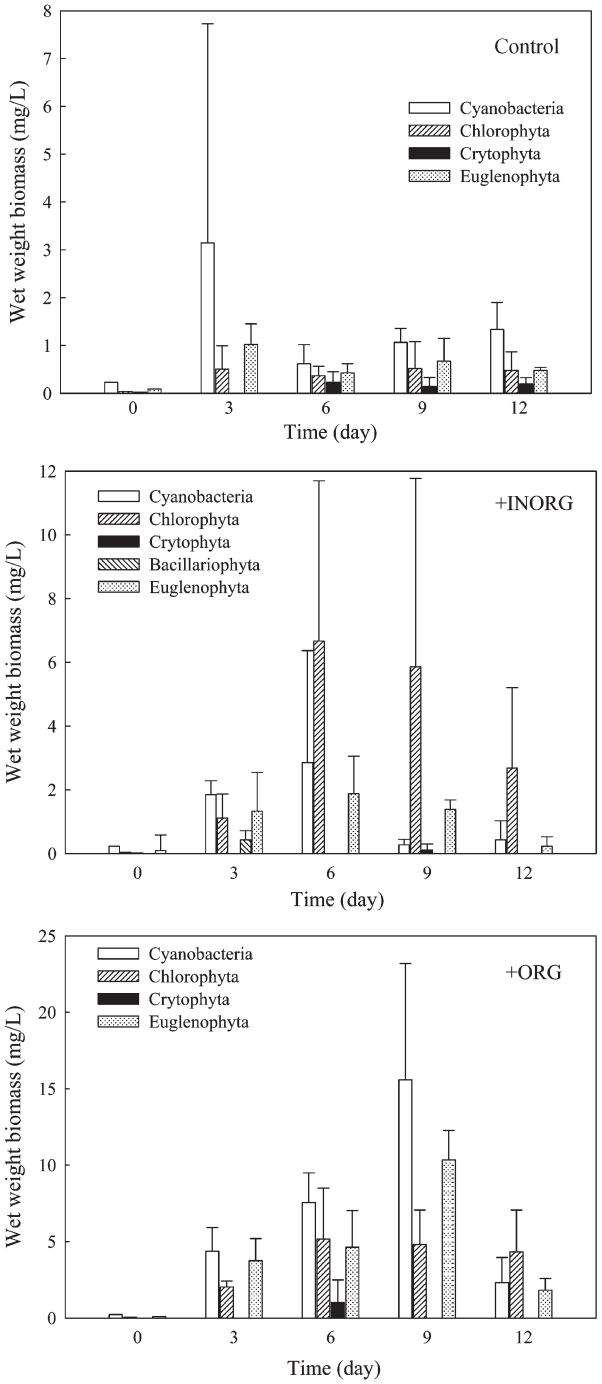

Fig. 5 Variation (mean ± SD) of wet weight biomass of different phytoplankton phylum in the control, +INORG and +ORG treatments, with deleting the phylum whose wet weight biomass were < 5% of the total biomass.

treatment was partially benefited by its higher NH_4^+-N content. Studies have shown that regenerated NH_4^+-N is an important source for phytoplankton growth (Smith and Kemp, 1995; Bronk et al., 1998).

Organic nutrients, as well as inorganic nutrients, play an important role in phytoplankton growth (Granéli et al., 1999; Lomas et al., 2001; Bronk et al., 2007), and many phytoplankton species with heterotrophic nature are able to utilize organic nutrients (Wheeler et al., 1977; Lewitus and Caron, 1991; Granéli et al., 1999). *Synechocystis* is a unicellular cyanobacterium with representative heterotrophic growth capacity (Anderson and McIntosh, 1991). It is therefore likely that the higher phytoplankton biomass of the +ORG treatment with *Synechocystis* dominance (Table 1) benefited from these organic nutrients.

The TN and TP contents of +ORG were not higher than those of +INORG (Fig. 3) during the experiment, however, the phytoplankton biomass of +ORG was higher than that of +INORG (Fig. 4), showing the higher phytoplankton biomass was not equal to the higher TN and TP contents, and it is not always that increased N and P concentrations lead to increased phytoplankton biomass. The reason for the concentration of dissolved inorganic N and P in the +INORG treatment remaining high without being used by the phytoplankton was probably that other elements were limiting instead of N or P.

Considering that for the growth and proliferation of phytoplankton additional nutrients besides N and P are required, algae must be efficient at sequestering other macronutrients and trace elements (Healey, 1973; Boyd and Tucker, 1998). Paerl (1997) demonstrated the importance of atmospheric deposition and groundwater recharge as "new" nitrogen and other nutrient sources, and these sources may also contain individually or synergistically acting nutrients, such as Fe, Se, and other trace metals. Thus, when N and P contents are low, other trace elements may be responsible for regulating phytoplankton growth. To date, growth limitation by trace metals in freshwaters has received less attention than the macronutrients N and P.

Elements besides N and P are released during the death and decomposition of phytoplankton, such as Se and Fe, which are available for subsequent generations of phytoplankton (Gobler et al., 1997). Sun et al. (2007) found that abundant colloidal, particulate, and dissolved nutrients could be released during death and decomposition of cyanobacteria bloom from Lake Taihu. Colloids have been shown to be supplementary sources of nutrition, including N, P, Fe and other trace elements promoting microalgae growth (Wang and Guo, 2001). Numerous field and laboratory studies focusing on the growth and species composition of phytoplankton illustrated the limiting role of trace nutrients such as iron (Zhang, 2000; North et al., 2007) and others (Goldman, 1972; Morel and Hudson, 1985) may play even in eutrophic waters. Thus, it is possible that the limiting agents in +INORG treatment were trace nutrients.

In Lake Taihu, a long duration of *Microcystis* spp. blooms has continuously occurred every year in recent decades (Chen et al., 2003; Guo, 2007). Once the accumulated algal blooms are decomposed, they provide many nutrients for subsequent algal blooms; in addition, blooms will be nourished by exogenous nutrients input and endogenous nutrients release from the sediment. Although the *Microcystis* spp. blooms of Lake Taihu are a consequence of eutrophication, the growing and decomposing bloom algae themselves provide further nutrients supporting their further growth; thus, nutrients are recycled.

Meanwhile, the exogenous nutrient inputs from point and non-point sources include not only the macronutrients

N and P, but usually a complex mixture of other elements with decomposing organic residues and high NH_4^+-N content (Smith and Kemp, 1995; Bronk et al., 1998). Such complex mixtures support phytoplankton growth, speed up eutrophication and increase the difficulty of controlling eutrophication and algal blooms in Lake Taihu. Thereby, it can be concluded that the development of phytoplankton biomass in Lake Taihu water was not just related to N and P contents.

3.2 Effect of nutrients on phytoplankton community composition

The different phytoplankton composition and dominant genera of the control and two treatments (Table 1, Fig. 5) showed the different roles of organic and inorganic nutrients. Phytoplankton composition is usually related to nutrient ratios, and Smith (1983) reported that low nitrogen to phosphorus ratios favor dominance by blue-green algae. However, in this experiment there were not differences in the ratios of TN/TP, DTN/DTP between the organic and inorganic nutrients addition treatments, and therefore these ratios are not likely the reason for differences in phytoplankton dominance.

Phytoplankton communities are affected by interspecific competition among algae for nutrients (Tilman, 1982) and dominant species competitively displace other species (Tilman, 1977; Sommer, 1985). The relative stability of the phytoplankton composition and *Microcystis* dominance in the control may be because the nutrient concentrations remained at relatively stable levels (Fig. 3). Reynolds (1984) singled out *Microcystis* as an example of a K-selected phytoplankter because it grows slowly in nature and is often the dominant taxa in a stable environment.

It has been shown that high nutrient loading in lakes leads to cyanobactera blooms (Gibson and Smith, 1982). In this experiment, Chlorophyta was always the most dominant phylum in +INORG treatment, particularly *Scenedesmus* spp. on day 6 and day 9, although cyanobacteria, particularly *Microcystis* spp., was dominant in the control, showing that cyanobacteria do not necessarily bloom when the nutrient load is high. There is a similar report of chlorophyte dominance in shallow hypertrophic lakes (Jensen et al., 1994), and *Microcystis* did not effectively out-compete *Scenedesmus* when grown with a high inorganic nutrients supply. This is likely due to the larger maximum uptake rates of ammonium per cellular carbon (V_{max}) of *Scenedesmus* than that of *Microcystis* (Watanabe and Miyazaki, 1996).

Cyanobacteria, particularly *Synechocystis* spp. was always dominant in +ORG treatment (Table 2), which was related to the organic nutrients added, and due to the fact that *Synechocystis* is a unicellular cyanobacterium with representative heterotrophic growth capacity (Anderson and McIntosh, 1991). Granéli et al. (1999) showed that the nutritional demands of a growing number of phytoplankton species can be met, at least partially, by heterotrophy, and that mixotrophic species (both phototrophic and heterotrophic) may out compete strict autotrophs.

Microcystis was not dominant in the two nutrient addition treatments, even though they were comparable with the nutrient conditions in Lake Taihu where *Microcystis* is always dominant. The reasons for this disparity may mainly be: (1) the water for this experiment was filtered through a no. 13 plankton net at the beginning, in which large zooplankton and large *Microcystis* particulates were removed; (2) differences between the food chain dynamics of Lake Taihu and the barrels in this experiment; (3) the physical environment of the experimental containers in this experiment is different from Lake Taihu.

The most important reason for it may be the absence of phytoplankton grazers. Laboratory studies suggest that green algae are a higher quality food source for zooplankton than are cyanobacteria (Arnold, 1971), and there are similar results that green algae such as *Scenedesmus* are thought to be preferentially grazed by zooplankton, whereas cyanobacteria, such as *Microcystis* are less preferentially grazed (de Bernardi and Giussani, 1990). And another study of ours finds that nutrient enrichment and selective predation by zooplankton promote *Microcystis* (Cyanobacteria) bloom formation (Wang et al., 2010). In this experiment, *Microcystis* blooms were not observed in either type of nutrient addition indicating that the occurrence of *Microcystis* bloom is not solely related to nutrient enrichment.

4 Conclusions

(1) Decomposed algal scum from Lake Taihu can more easily improve the phytoplankton biomass than inorganic N and P addition alone; even their initial TN concentrations are the same.

(2) Higher NH_4^+-N concentrations, organic nutrients and trace elements availability may all play roles in stimulating or restricting phytoplankton growth when inorganic N and P are not limiting for phytoplankton.

(3) *Microcystis* spp. can thrive in relatively low nutrient waters, and *Synechocystis* spp. and *Scenedesmus* spp. can out-compete *Microcystis* spp. under high decomposed algal scum addition and inorganic N and P addition, respectively, when grazing zooplankton are absent.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 40825004, 40730529). We thank Dr. Sarah L. Poynton and Dr. Steven W. Wilhelm for English editing and constructive comments.

References

Anderson D M, Glibert P M, Burkholder J M, 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries*, 25: 704–726.

Anderson S L, McIntosh L, 1991. Light-activated heterotrophic growth of the cyanobacterium *Synechocystis* sp. Strain PCC 6803: a blue-light-requiring process. *Journal of Bacteriology*, 173: 2761–2767.

Antia N J, Harrison P J, Oliveira L, 1991. Phycological reviews: the role of dissolved organic nitrogen in phytoplankton

nutrition, cell biology, and ecology. *Phycologia*, 30: 1–89.

Arnold D E, 1971. Ingestion, assimilation, survival, and reproduction by *Daphnia pulex* fed seven species of blue-green algae. *Limnology and Oceanography*, 16: 906–920.

Berman T, 1997. Dissolved organic nitrogen utilization by an *Aphanizomenon* bloom in Lake Kinneret. *Journal of Plankton Research*, 19: 577–586.

Bootsma M C, Barendregt A, van Alphen J C A, 1999. Effectiveness of reducing external nutrient load entering a eutrophicated shallow lake ecosystem in the Naardermeer nature reserve, The Netherlands. *Biological Conservation*, 90: 193–201.

Boyd C E, Tucker C S, 1998. Pond Aquaculture Water Quality Management. Kluwer Academic Publishers, Boston. 19.

Bronk D A, Glibert P M, Malone T C, Banahan S, Sahlsten E, 1998. Inorganic and organic nitrogen cycling in Chesapeake Bay: autotrophic versus heterotrophic processes and relationships to carbon flux. *Aquatic Microbial Ecology*, 15: 177–189.

Bronk D A, See J H, Bradley P, Killberg L, 2007. DON as a source of bioavailable nitrogen for phytoplankton. *Biogeosciences*, 4: 283–296.

Chen Y, Qin B, Teubner K, Dokulil M T, 2003. Long-term dynamics of phytoplankton assemblages: *Microcystis*-domination in Lake Taihu, a large shallow lake in China. *Journal of Plankton Research*, 25: 445–453.

de Bernardi R, Giussani G, 1990. Are blue-green algae a suitable food for zooplankton? An overview. *Hydrobiologia*, 200: 29–41.

Eaton A D, Clesceri L S, Greenberg A E, 1995. Standard Methods for the Examination of Water and Wastewater (19th ed.). American Public Health Association, Washington DC.

Enoksson V, 1993. Nutrient recycling by coastal sediments: effects of added algal material. *Marine Ecology Progress Series*, 92: 245–254.

Fallon R D, Brock T D, 1980. Planktonic blue-green algae: Production, sedimentation, and decomposition in Lake Mendota, Wisconsin. *Limnology and Oceanography*, 25: 72–88.

Fujii M, Murashige S, Ohnishi Y, Yuzawa A, Miyasaka H, Suzuki Y et al., 2002. Decomposition of phytoplankton in seawater. part I: Kinetic analysis of the effect of organic matter concentration. *Journal of Oceanography*, 58: 438–443.

Fukami K, Simidu U, Taga N, 1985. Microbial decomposition of phyto- and zooplankton in sea water. 1. Changes in organic matter. *Marine Ecology Progress Series*, 21: 1–5.

Gibson C E, Smith R V, 1982. Freshwater plankton. In: The Biology of Cyanobacteria (Carr N G, Whitton B A, eds.). Blackwell Scientific Publications, London. 463–490.

Gobler C J, Sañudo-Wilhelmy S A, 2001. Effects of organic carbon, organic nitrogen, inorganic nutrients, and iron additions on the growth of phytoplankton and bacteria during a brown tide bloom. *Marine Ecology Progress Series*, 209: 19–34.

Gobler C J, Hutchins D A, Fisher N S, Cosper E M, Sañudo-Wilhelmy S A, 1997. Release and bioavailability of C, N, P, Se, and Fe following viral lysis of a marine chrysophyte. *Limnology and Oceanography*, 42: 1492–1504.

Goldman C R, 1972. The role of minor nutrients in limiting the productivity of aquatic ecosystems. Symposium on nutrients and eutrophication. *Limnology and Oceanography*, 1: 21–23.

Granéli E, Carlsson P, Legrand C, 1999. The role of C, N and P in dissolved and particulate organic matter as a nutrient source for phytoplankton growth, including toxic species. *Aquatic Ecology*, 33: 17–27.

Gross A, Boyd C E, 1998. A digestion procedure for the simultaneous determination of total nitrogen and total phosphorus in pond water. *Journal of the World Aquaculture Society*, 29: 300–303.

Guo L, 2007. Doing battle with the green monster of Taihu Lake. *Science*, 317: 1166.

Healey F P, 1973. Inorganic nutrient uptake and deficiency in algae. *Critical Reviews in Microbiology*, 3: 69–113.

Hecky R E, Kilham P, 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnology and Oceanography*, 33: 796–822.

Hu H, Wei Y, 2006. The Freshwater Algae of China-systematics, Taxonomy and Ecology. Science Press, Beijing.

Jensen J P, Jeppesen E, Olrik K, Kristensen P, 1994. Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow Danish lakes. *Canadian Journal of Fisheries and Aquatic Science*, 51: 1692–1699.

Jespersen A M, Christoffersen K, 1987. Measurements of chlorophyll-*a* from phytoplankton using ethanol as extraction solvent. *Archiv für Hydrobiologie*, 109: 445–454.

Lee B G, Fisher N S, 1992. Degradation and elemental release rates from phytoplankton debris and their geochemical implications. *Limnology and Oceanography*, 37: 1345–1360.

Lewitus A J, Caron D A, 1991. Physiological responses of phytoflagellates to dissolved organic substrate additions. 1. Dominant role of heterotrophic nutrition in *Poterioochromonas malhamensis* (Chrysophyceae). *Plant and Cell Physiology*, 32: 671–680.

Lomas M W, Glibert P M, Clougherty D A, Huber D R, Jones J, Alexander J et al., 2001. Elevated organic nutrient ratios associated with brown tide algal blooms of *Aureococcus anophagefferens* (Pelagophyceae). *Journal of Plankton Research*, 23: 1339–1344.

Lorenzen C J, 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations. *Limnology and Oceanography*, 12: 343–346.

Morel F M M, Hudson R J M, 1985. The geobiological cycle of trace elements in aquatic systems: Redfield revisited. In: Chemical Processes in Lakes (Stumm W, ed.). New York, Wiley. 251–281.

North R L, Guildford S J, Smith R E H, Havens S M, Twiss M R, 2007. Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. *Limnology and Oceanography*, 52: 315–328.

Paerl H W, 1997. Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. *Limnology and Oceanography*, 42: 1154–1165.

Paerl H W, Bowles N D, 1987. Dilution bioassays: Their application to assessments of nutrient limitation in hypereutrophic waters. *Hydrobiologia*, 146: 265–273.

Paerl H W, 2009. Controlling eutrophication along the freshwater-marine continuum: Dual nutrient (N and P) reductions are essential. *Estuaries and Coasts*, 32: 593–601.

Palmer C M, Maloney T E, 1954. A new counting slide for nanoplankton. *Limnology and Oceanography*, (Special Publ. 21): 1–7.

Pick F R, Lean D R S, 1987. The role of macronutrients (C, N, P) in controlling cyanobacterial dominance in temperate lakes. *New Zealand Journal of Marine and Freshwater Research*,

21: 425–434.

Qin B Q, Xu P Z, Wu Q L, Luo L C, Zhang Y L, 2007. Environmental issues of Lake Taihu, China. *Hydrobiologia*, 581: 3–14.

Reynolds C S, 1984. The Ecology of Freshwater Phytoplankton. Cambridge University Press, Cambridge, UK.

Smith E M, Kemp W M, 1995. Seasonal and regional variations in plankton community production and respiration for Chesapeake Bay. *Marine Ecology Progress Series*, 116: 217–231.

Smith V H, 1983. Low nitrogen to phosphorus favors dominance by bluegreen algae in lake phytoplankton. *Science*, 221: 669–671.

Sommer U, 1985. Comparison between steady state and non-steady state competition: experiment with natural phytoplankton. *Limnology and Oceanography*, 30: 335–346.

Sommer U, 1989. Nutrient status and nutrient competition of phytoplankton in a shallow, hypertrophic lake. *Limnology and Oceanography*, 34: 1162–l173.

Søndergaard M, Jensen J P, Jeppesen E, 2001. Retention and internal loading of phosphorus in shallow, eutrophic lakes. *The Scientific World Journal*, 1: 427–442.

Sun X J, Qin B Q, Zhu G W, 2007. Release of colloidal phosphorus, nitrogen and organic carbon in the course of dying and decomposing of cyanobacteria. *China Environmental Science*, 27: 341–345.

Tilman D, 1977. Resource competition between algae: an experimental and theoretical approach. *Ecology*, 58: 338–348.

Tilman D, 1982. Resource Competition and Community Structure. Princeton University Press, Princeton. 296.

Underwood A J, 1997. Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge. 189–192.

Wang W X, Guo L D, 2001. Production of colloidal organic carbon and trace metals by phytoplankton decomposition. *Limnology and Oceanography*, 46: 278–286.

Wang X L, Lu Y L, He G Z, Han J Y, Wang T Y, 2007. Exploration of relationships between phytoplankton biomass and related environmental variables using multivariate statistic analysis in a eutrophic shallow lake: A 5-year study. *Journal of Environmental Sciences*, 19(8): 920–927.

Wang X D, Qin B Q, Gao G, Paerl H W, 2010. Nutrient enrichment and selective predation by zooplankton promote *Microcystis* (Cyanobacteria) bloom formation. *Journal of Plankton Research*, 32: 457–470.

Watanabe T, Miyazaki T, 1996. Maximun ammonium uptake rates of *Scenedemus quadricauda* (Chlorophyta) and *Microcystis novacekii* (Cyanobacteria) grown under nitrogen limitation and implications for competition. *Journal of Phycology*, 32: 243–249.

Wheeler P A, North B B, Stephens G C, 1977. Uptake of glycine by natural phytoplankton communities. *Limnology and Oceanography*, 22: 900–910.

Zhang J, 2000. Evidence of trace metal limited photosynthesis in eutrophic estuarine and coastal waters. *Limnology and Oceanography*, 45: 1871–1878.

Zhang Z, Huang X, 1991. The Research Methods for Freshwater Plankton. Science Press, Beijing. 342–344.

Zohary T, Pollingher U, Hadas O, Hambright K D, 1998. Bloom dynamics and sedimentation of *Peridinium gatunense* in Lake Kinneret. *Limnology and Oceanography*, 43: 175–186.