PRIMARY RESEARCH PAPER

# Growth response of *Microcystis* spp. to iron enrichment in different regions of Lake Taihu, China

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**Abstract** Iron (Fe) is an essential micronutrient for algal growth and can be a potential limiting nutrient in aquatic system, especially regions that exhibits nitrogen (N) limitation. Using short-term nutrient addition bioassays, we evaluated the potential role that iron might play in modifying the response of *Microcystis* spp. to the anthropogenic phosphorus (P) and N enrichment in hypereutrophic Lake Taihu, the third largest freshwater lake in China. Three nutrient enrichment experiments involving additions of N (as $NO_3^-$) and P (as $PO_4^{3-}$) with and without Fe were conducted during 2009–2010 in Meiliang Bay, a region characterized by summer cyanobacterial (*Microcystis* spp.) blooms, and East Taihu, a region largely free of cyanobacterial blooms and dominated by macrophytes. In Meiliang Bay, Fe addition alone did not significantly increase *Microcystis* spp. biomass. However, Fe addition occasionally increased the stimulatory effect of N and P additions on *Microcystis* spp., indicating that Fe was not a primary limiting nutrient for *Microcystis* spp. growth. Occasionally Fe was co-limiting with N and P in this region. In East Taihu, the addition of Fe alone significantly stimulated *Microcystis* spp. growth, while addition of N and/or P had no effects on growth, indicating that Fe was a primary limiting nutrient in East Taihu. The combined addition of Fe and N resulted in a growth response similar to Fe alone, while combined addition of Fe and P yielded greater biomass increases than the addition of Fe alone. This indicated that in East Taihu, N was not limiting and Fe and P supplies facilitated *Microcystis* spp. growth. These results reflect differential availabilities and limitations of N, P, and Fe in distinct regions of Taihu. The potential role of Fe in eutrophication dynamics of large, regionally complex lakes like Taihu requires further attention.

**Keywords** Lake Taihu · CyanoHABs · Iron · Nitrogen · Phosphorus · *Microcystis* spp.

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## Introduction

Harmful cyanobacterial blooms (CyanoHABs) frequently occur in freshwater ecosystems worldwide. The colony-forming genus *Microcystis* is one of the most ubiquitous CyanoHABs in eutrophic freshwater eosystems (Huisman et al., 2005). *Microcystis* blooms often form thick surface scums that cause various

environmental problems, including reduced transparency, decreased biodiversity, potential occurrence of oxygen depletion, production of odor and taste compounds and toxins hazardous to animals, including humans (Dokulil & Teubner, 2000; Carmichael, 2001). Consequently, CyanoHABs represent a serious threat to drinking water supplies, and the ecological and economic sustainability of freshwater ecosystems (Carmichael, 2001; Huisman et al., 2005). Anthropogenic nutrient over-enrichment of lakes and reservoirs has been linked to CyanoHAB proliferation (Paerl et al., 2001; Huisman et al., 2005). Identifying the nutrients controlling the growth of CyanoHABs is an essential step for reducing this negative effect of eutrophication.

Phosphorus has been implicated traditionally as playing a central role in the control of freshwater primary production (Likens, 1972; Schindler, 1977; Schindler et al., 2008) and CyanoHAB bloom formation (Paerl, 1988, 2008). Moreover, based on recent research in a variety of lakes and reservoirs, primary production and bloom formation may be controlled by both N and P inputs (Havens et al., 2001; Kronvang et al., 2005; Elser et al., 2007; Jeppesen et al., 2007; Lewis & Wurtsbaugh, 2008; Özkan et al., 2010; Lewis et al., 2011). However, since Martin & Gordon (1988) showed that iron deficiency limited phytoplankton growth in the subarctic North Pacific Ocean, a series of more recent studies have focused on the potential for Fe limitation of phytoplankton growth in diverse aquatic ecosystems. Iron (Fe) is an essential micronutrient for algal growth and plays an important role in many biochemical processes such as photosynthesis, chlorophyll synthesis, respiration and nitrogen assimilation, including nitrate reduction and $N_2$ fixation (Murphy et al., 1976; Hyenstrand et al., 2000). Fe limitation of phytoplankton growth in oceanic high-nutrient, low-chlorophyll (HNLC) regions has been well documented, including the equatorial Pacific (Martin et al., 1994; Coale et al., 1996), the subarctic Pacific (Tsuda et al., 2003) and regions of the Southern Ocean (Boyd et al., 2000). Iron limitation is not expected to occur in lakes because of their proximity to terrestrial sources of this metal, but prior studies have shown that Fe can in fact limit phytoplankton growth in hardwater calcareous lakes (Schelske, 1962; Schelske et al., 1962; Wetzel, 1966). In addition, Fe limitation of phytoplankton growth has been demonstrated in two of the largest freshwater lakes of the world, Lake Superior and Lake Erie (Twiss et al., 2000; Sterner et al., 2004; North et al., 2007).

Cyanobacteria have relatively high cellular iron requirements when compared to eukaryotic algae (Brand, 1991). The addition of Fe can stimulate primary production in eutrophic lakes exhibiting $N_2$-fixing cyanobacterial dominance, because Fe is an essential constituent of the enzyme complex mediating the $N_2$ fixing process (nitrogenase) (Wurtsbaugh & Horne, 1983; Wurtsbaugh, 1988; Hyenstrand et al., 2001). The availability of Fe also controlled the growth of bloom-forming non-$N_2$ fixing cyanobacteria in shallow eutrophic lakes (Aizaki & Aoyama, 1995; Imai et al., 1999; Nagai et al., 2004). In laboratory competition experiments, Morton & Lee (1974) found that Fe additions caused a shift in phytoplankton community composition from green algal to cyanobacterial genera such as *Anabaena*, *Gloeotrichia*, and *Microcystis*, which implied that increases in available Fe supply may favor cyanobacterial dominance.

Lake Taihu is the third largest freshwater lake in China, and it has experienced accelerating eutrophication over the past three decades (Qin et al., 2007, 2011). 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). Recently, the *Microcystis* spp. blooms have expanded throughout the bays and into the north, western and central regions of the lake, where thick scums are now a regular feature from May through October (Guo, 2007). In May 2007, a very large ''cyanobacterial mat'' overwhelmed and incapacitated the drinking water plant in nearby Wuxi, a large city with approximately 4 million inhabitants; leading to a highly publicized drinking water crisis (Guo, 2007; Qin et al., 2011). Surprisingly, the southeastern part of the lake has remained relatively free of CyanoHABs, and the floating macrophytes *Trapa maximowiczii* and *Nymphoides peltata* dominate the water column (Gu et al., 2005). Recently, Xu et al. (2010) and Paerl et al. (2011) investigated the potential for N and P limitation of phytoplankton using seasonal short-term (<1 week) nutrient addition bioassays. They found spring P limitation, followed by summer and autumn N and P co-limitation of *Microcystis* spp. blooms in Meiliang Bay. However, so far no studies have evaluated the potential role that iron limitation might play in Taihu.

In the present study, three nutrient enrichment experiments were conducted over a 2-year period (2009–2010) to investigate the response of Taihu to additions of N and P with or without addition of Fe in

both bloom-impacted and bloom-free regions. The objective was to examine the roles Fe might play in modifying the response of *Microcystis* spp. to the anthropogenic P and N enrichment that the lake is experiencing. Short-term (up to 2 days) nutrient addition bioassays were incubated in the lake under natural light and temperature conditions. These bio-assays provide a rapid assessment of nutrient limitation characteristics, i.e., immediate growth responses, rather than predicting long-term phytoplankton succession patterns (Paerl & Bowles, 1987; Piehler et al., 2009).

## Materials and methods

### Study area and sampling sites

Lake Taihu is located in the Yangtze River delta; the most rapidly developing region of China (Fig. 1). Taihu is a large, shallow (mean depth <2 m) poly-mictic lake, with an area of 2,338 km$^2$, a catchment area of 36,500 km$^2$ and a volume of 4.4 billion m$^3$ (Qin et al., 2007, 2011). The Taihu basin is characterized by a complex set of river networks, with 117

rivers and tributaries draining into the lake. The annual freshwater input to the lake is about 57 × 10$^8$ m$^3$, and its water retention time is approximately 284 days (Qin et al., 2007). Generally, freshwater inputs enter the lake from the southwest or western mountainous watershed and the dominant discharge points are Eastern Taihu Bay and River Taipu in the southeast of basin. The Taihu Basin accounts for only 0.4% of China's land area, but the gross domestic product (GDP) of this region accounts for ~11% of the national GDP (Qin et al., 2007). Approximately 40 million people live within the Taihu watershed. The lake is a key drinking water, fishing and tourism resource for the region. However, it also serves as a repository of waste from urban, agricultural, and industrial sources in the watershed (Guo, 2007; Qin et al., 2007).

Taihu exhibits two ecologically distinct regions. The north-western region of the lake is characterized by the absence of macrophytes, high water turbidity and the occurrence of dense cyanobacterial blooms from late spring through early fall (Fig. 1).The eastern region of the lake is characterized by floating and submersed macrophytes, relatively clear water, and diverse communities of invertebrates and fishes (Fig. 1).



**Fig. 1** Location of sampling sites in Lake Taihu, showing Meiliang Bay, the extent of the *Microcystis* spp. bloom in summer and location of macrophyte dominance
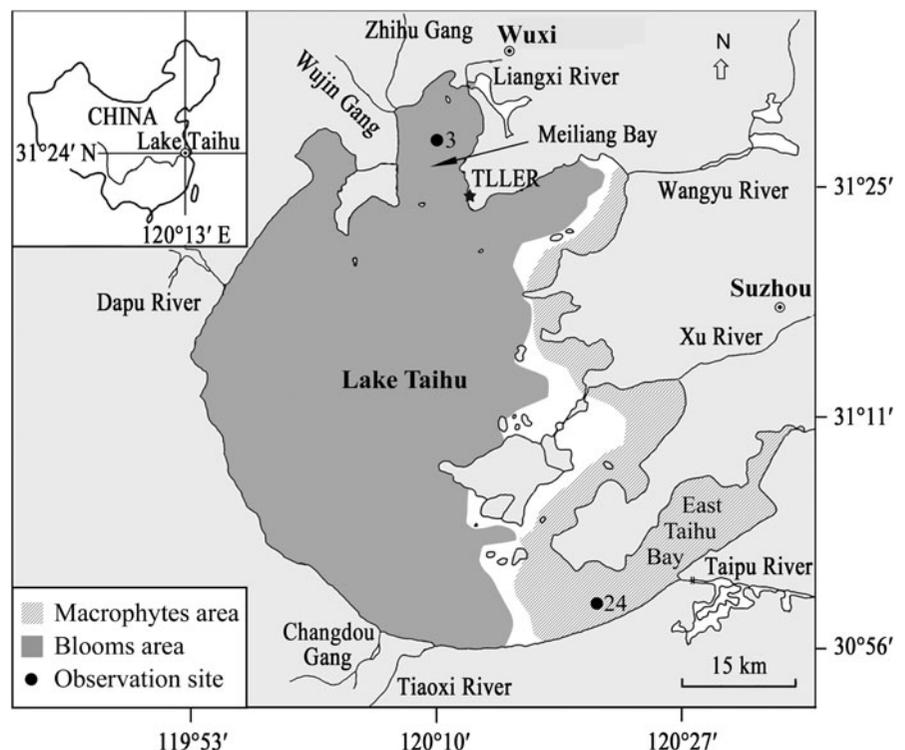
One of our study sites was located in Meiliang Bay, northern Taihu (Fig. 1), which is one of the lake's most eutrophic bays. The surface area of the bay is 123 km$^2$, and the average depth is 1.8 m (Chen et al., 2003). Two main rivers empty into the bay; the Liangxi and Zhihugang. Large amounts of untreated wastewater from factories and urban areas are discharged to these rivers. This bay experiences *Microcystis* spp. blooms every summer. Another site was located in East Taihu Bay, which is a macrophyte-dominated area in the southeastern part of the lake with a surface area of 131 km$^2$ and mean water depth of 1.2 m (Qin et al., 2007). Cyanobacterial blooms are absent from this bay. It is dominated by floating macrophytes, including *Trapa maximowiczii* and *Nymphoides peltata* (Gu et al., 2005).

Sample collection

Water samples for bioassay experiments were collected from 0.2 m below the water surface from each location with acid-clean level 20-l polyethylene carboys. Parallel water samples were collected for analysis of total nitrogen (TN), total dissolved nitrogen (TDN), ammonium ($NH_4$-N), nitrate ($NO_3$-N), nitrite ($NO_2$-N), total phosphorus (TP), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), suspended solids (SS), dissolved carbon(DOC), total dissolved iron (TDFe) chlorophyll *a* (Chl *a*), and phytoplankton community.

Natural populations of *Microcystis* spp. used for bioassay experiments were collected from Meiliang Bay using 46-μm mesh plankton net and rinsed with deionized water.

Physical–chemical parameters, including surface water temperature (WT), dissolved oxygen (DO), and pH were measured in the field using a YSI 6600 multi-sensor sonde (Yellow Springs Instruments Inc.). Secchi disc depths were determined in parallel with field measurements.

Nutrient enrichment bioassay experiment

A total of three nutrient limitation bioassay experiments were carried out during summers of 2009 and 2010. The experimental design is summarized in Table 1.

In August 2009, we specifically examined difference in response of bloom-forming *Microcystis* spp. to N, P additions between Meiliang Bay and East Taihu. The experimental treatments were designed as Control (no nutrient additions), N addition (+N), P addition (+P), and combined N and P addition (+NP). In September 2009, we included additional Fe amended

**Table 1** Experimental conditions for the three enrichment experiments conducted in Lake Taihu, 2009–2010

| Date | Locations | Depth of sample (m) | Experimental treatment |
|---|---|---|---|
| August 2009 | Meiliang Bay, East Taihu | 0.2 | Control |
| | | | 2.0 mg/l N |
| | | | 0.2 mg/l P |
| | | | 2.0 mg/l N and 0.2 mg/l P |
| September 2009 | Meiliang Bay, East Taihu | 0.2 | Control |
| | | | 0.1 mg/l Fe |
| | | | 0.1 mg/l Fe, 2.0 mg/l N and 0.2 mg/l P |
| August 2010 | Meiliang Bay, East Taihu | 0.2 | Control |
| | | | 2.0 mg/l N |
| | | | 0.2 mg/l P |
| | | | 0.1 mg/l Fe |
| | | | 2.0 mg/l N and 0.2 mg/l P |
| | | | 0.1 mg/l Fe and 2.0 mg/l N |
| | | | 0.1 mg/l Fe and 0.2 mg/l P |
| | | | 0.1 mg/l Fe, 2.0 mg/l N and 0.2 mg/l P |

treatments to determine if Fe might stimulate growth of *Microcystis*, and enrichment bioassay included Control, Fe addition (+Fe) and combined Fe, P and N addition (Fe + NP). In August 2010, the experimental treatments were modified as Control (no nutrient additions), N addition (+N), P addition (+P), Fe addition (+Fe), N and P addition (+NP), Fe and N addition (Fe + N), Fe and P addition (Fe + P), and combination of Fe, P and N (Fe + NP).

Natural phytoplankton assemblages in Taihu are characterized by significant spatial variation in both species composition and abundance. Thus, differences in growth rate and cell yields in nutrient bioassays from different locations may depend to a large extent on spatial variation in initial phytoplankton biomass and community structure. To avoid this problem, water samples for bioassays were screened through 0.7-μm pore-size Whatman GF/F glass fiber filters to remove ambient phytoplankton and inoculated with field *Microcystis* spp. collected from Meiliang Bay. This enabled us to assess the responses of a representative *Microcystis* spp. assemblage to nutrient supplies present at different sites. Water samples containing inoculated field *Microcystis* spp. were distributed among acid (0.001 N HCl) and deionized water washed 1-l polyethylene Cubitainers (Hedwin Co.) in triplicate for each treatment. Cubitainers are chemically inert, unbreakable, and transparent (80% PAR transmittance) incubation vessels (c.f., Paerl et al., 2011).

Nutrients were added into Cubitainers as shown in Table 1. N was added as $KNO_3$ because nitrate is the dominant form of inorganic N in the lake, P was added as $K_2HPO_4 \cdot 3H_2O$ and Fe was added as $FeCl_3 \cdot 6H_2O$ in September 2009 and EDTA-Fe in August 2010. The final concentrations of N, P, and Fe were 2.00, 0.20, and 0.1 mg/l, respectively. These concentrations were designed to reflect the relatively high values periodically observed in the lake, and to saturate initial growth rates of *Microcystis* spp.

After nutrient additions, the Cubitainers were incubated in lake surface water (below 0.2 m water surface) near the TLLER for 2 days by placing them in a floating frame. This ensured natural light, temperature, and surface turbulence conditions as outlined in Xu et al. (2010). One layer of neutral density screening was placed over the frame, which reduced incident irradiance by 30% and prevented photoinhibition during the course of incubations. The containers were sampled after 2 days incubation for Chl *a* analyses. This allowed for measurements of short-term responses in phytoplankton growth to provide a snapshot of the nutrient limitation state of *Microcystis* spp., while minimizing "bottle effects" such as prolonged nutrient stress, which can occur in longer-term incubations.

Water sample analysis

Water samples were analyzed for dissolved nutrients by filtering freshly collected samples through pre-cleaned Whatman GF/F glass fiber filters. SRP was determined using the molybdenum blue method (APHA, 1995). $NH_4$-N was measured by the indophenol blue method, and $NO_3$-N and $NO_2$-N with the cadmium reduction method (APHA, 1995). TP, TDP, TN, and TDN were analyzed using a combined persulphate digestion (Ebina et al., 1983), followed by spectrophotometric analysis as for SRP and $NO_3$-N. TN and TP recovery efficiencies were 98.4 and 99.7%, respectively.

Water samples for Chl *a* determination were filtered onto Whatman GF/F glass fiber filters. The filters were frozen at $-20$°C for no more than 2 days prior to extraction. Chl*a* concentration was determined spectrophotometrically after extraction in 90% hot ethanol (Papista et al., 2002).

Water samples for DOC measurement were filtered through pre-combusted Whatman GF/F glass fiber filters and analyzed by a Rosemount Dohrmann DC-190 High-Temperature TOC analyzer. Suspended solids (SS) in lake water were quantified directly by filtration of freshly collected water through a pre-combusted and pre-weighed 0.7 μm pore-size Whatman GF/F glass fiber filter (Gray et al., 2000).

Water samples for trace element analysis were filtered through pre-cleaned Whatman GF/F filters, and acidified with trace metal-clean HCL (Baseline; Seastar) to pH 2. The total dissolved Fe (TDFe), total dissolved manganese (TDMn), total dissolved copper (TDCu), and total dissolved zinc (TDZn) concentrations in filtrate was measured using inductively coupled plasma/mass spectrometry (ICP/MS) with detection limits of 2 μg/l for Fe, 0.02 μg/l for Mn, 0.01 μg/l for Cu and 0.1 μg/l for Zn.

Phytoplankton samples were preserved with Lugol's iodine solution and sedimented for 48 h prior to count with a Sedgwick-Rafter counting chamber

under microscopic magnifications of 200–400×. Phytoplankton species were identified according to Hu et al. (1980). Total algal biovolumes were calculated from cell numbers and cell size measurements. Conversion to biomass assumed that 1 mm$^3$ of volume is equivalent to 1 mg of fresh-weight biomass. Cyanobacterial species for bioassays were also identified at the start of experiments.

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 using the SPSS 13.0 statistical package (SPSS Inc., Chicago, IL, USA), and the level of significance used was $P < 0.05$ for all tests.

**Results**

In situ conditions at the initiation of bioassay experiments

The properties of the lake water collected from two sites for bioassays are shown in Table 2.

The surface WT was around 30°C during experiments. The SS values were lower and Secchi depths were higher in East Taihu than in Meiliang Bay, indicating a relatively clear water column in East Taihu during summer. The DO concentrations and pH in the surface water (below 0.5 m water surface) were generally lower in East Taihu than in Meiliang Bay.

The TDFe concentrations in lake water collected from East Taihu were undetectable in experiments during 2009 and lower than that in Meiliang Bay in experiment during 2010. Generally, TDMn and TDCu concentrations were low in water column, sometime below detect limit at both stations. TDZn were detected during three sampling data and concentrations were higher in Meiliang Bay than in East Taihu. The differences in nutrient concentrations between the two sites were apparent when measurements from all sampling dates were compared (Table 2). In Meiliang Bay, $NH_4$-N was the predominant inorganic nitrogen

form and concentrations ranged from 0.13 to 0.18 mg/l. In East Taihu, $NO_3$-N was the predominant inorganic nitrogen form (ranging from 0.21 to 0.79 mg/l) and concentrations were relatively higher compared with Meiliang Bay during the three sampling dates. Phosphorus concentrations, including TP and TDP, were higher in Meiliang Bay than in East Taihu Bay.

The phytoplankton assemblage of Taihu was mainly composed of four algal groups, including cyanobacteria, diatoms, green algae, and cryptophytes (Chen et al., 2003). During summer, ambient Chl $a$ concentrations in Meiliang Bay ranged from 40.0 to 60.3 µg/l at all sampling dates (Table 2). Cyanobacteria dominated phytoplankton biomass (Fig. 2), forming periodic blooms in summer. Generally, *Microcystis* dominated the cyanobacterial community, accounting for 85.7% of the total cyanobacterial biomass in Meiliang Bay (Liu et al., 2011). However, phytoplankton biomass in East Taihu was much lower, with Chl $a$ concentrations ranging from 2 to 5 µg/l (Table 2). The phytoplankton assemblage there tended to be dominated by cryptophytes and chlorophytes (Fig. 2).

Nutrient addition bioassays

The *Microcystis* species for three bioassay experiments were composed of *Microcystis aeruginosa*, *M. flos-aquae*, and *M. wesenbergii*, which jointly contributed 95% of total cyanobacterial biomass (data not shown).

In August 2009, *Microcystis* spp. biomass (as Chl $a$) showed no response to N or P alone addition, while the combined N and P additions led to the strongest positive response of *Microcystis* spp. in surface water collected from Meiliang Bay (Fig. 3). However, in lake water obtained from East Taihu, *Microcystis* spp. showed no response to enrichment when N and P were added alone or combined (Fig. 3).

In September 2009, Fe was included in the bioassay experiment. The addition of Fe alone had no effect on the Chl $a$ concentration in Meiliang Bay. In contrast, enrichment with N and P led to a significant ($P < 0.05$) increase in Chl $a$ concentrations relative to the control treatment. *Microcystis* spp. growth showed the strongest positive response to Fe addition combined with N and P (Fig. 4). This was not the case for East Taihu water, in which the Fe alone addition

**Table 2** Properties of lake water used for bioassay experiment collected from both sites

|  | Parameter | August 2009 | | September 2009 | | August 2010 | |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Meiliang Bay | East Taihi | Meiliang Bay | East Taihi | Meiliang Bay | East Taihi |
| Physical | WT (°C) | 29.1 | 28.9 | 29.1 | 28.9 | 33.3 | 32.6 |
|  | Secchi (m) | 0.38 | 2.4 | 0.4 | 2.3 | 0.45 | 0.8 |
|  | SS (mg/l) | 27.07 | 4.04 | 31.55 | 5.00 | 25.96 | 6.24 |
|  | pH | 9.13 | 8.13 | 8.17 | 7.78 | 8.22 | 7.87 |
| Chemical | TDFe (mg/l) | 0.012 | – | 0.01 | – | 0.106 | 0.003 |
|  | TDMn (mg/l) | – | – | 0.002 | – | 0.007 | 0.004 |
|  | TDCu (mg/l) | – | – | – | – | 0.003 | 0.002 |
|  | TDZn (mg/l) | 0.009 | 0.005 | 0.015 | 0.006 | 0.005 | 0.002 |
|  | TN (mg/l) | 2.12 | 0.76 | 1.89 | 0.85 | 0.99 | 2.36 |
|  | TDN (mg/l) | 0.82 | 0.62 | 0.80 | 0.75 | 0.46 | 2.21 |
|  | $NH_4$-N (mg/l) | 0.13 | 0.06 | 0.18 | 0.08 | 0.16 | 0.03 |
|  | $NO_3$-N (mg/l) | 0.10 | 0.31 | 0.09 | 0.5 | 0.11 | 0.79 |
|  | $NO_2$-N (mg/l) | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 |
|  | DIN (mg/l) | 0.25 | 0.38 | 0.28 | 0.59 | 0.28 | 0.84 |
|  | TP (mg/l) | 0.126 | 0.012 | 0.20 | 0.014 | 0.046 | 0.013 |
|  | TDP (mg/l) | 0.024 | 0.005 | 0.050 | 0.007 | 0.017 | 0.006 |
|  | $PO_4$-P (mg/l) | 0.003 | 0.003 | 0.035 | 0.004 | 0.005 | 0.002 |
|  | DOC(mg/l) | 4.55 | 2.09 | 3.98 | 2.5 | 4.34 | 3.3 |
|  | DO (mg/l) | 10.01 | 7.27 | 7.18 | 6.75 | 15.03 | 6.82 |
| Biological | Chl $a$ (µg/l) | 50.2 | 2.0 | 60.3 | 3.0 | 40.0 | 5.0 |

– Below detection limit (0.002 mg/l)

**Fig. 2** Relative contribution of major phytoplankton groups to total phytoplankton fresh biomass
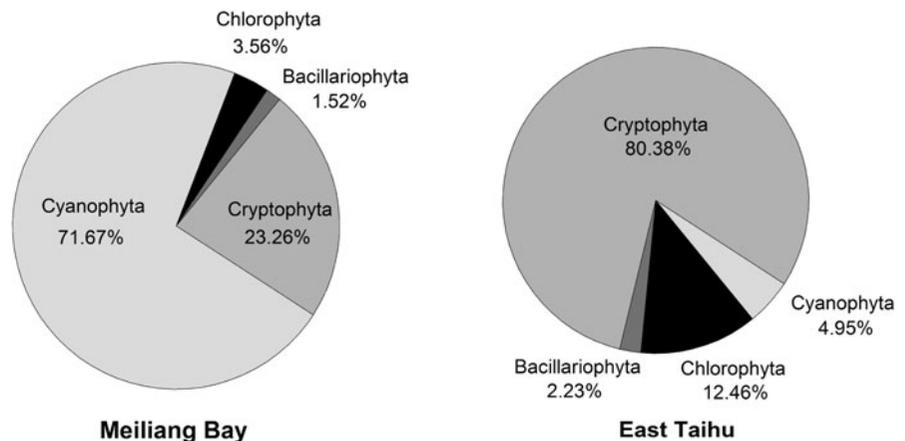


significantly ($P < 0.05$) stimulated Chl $a$ concentration, while the combined N and P addition had no effect on Chl $a$ concentration relative to the control treatment. Fe enrichment with N and P produced the strongest positive responses (Fig. 4).

In order to examine the interaction of Fe with N or P, a bioassay experiment which included Fe additions

combined with N or P was conducted in August 2010. The results showed enrichment with Fe and/or P in Meiliang Bay water did not result in a significant increase of *Microcystis* spp. biomass, whereas N addition alone led to a significant increase in Chl $a$ concentrations. The combined addition of Fe, P, and N yielded the same Chl $a$ increases as the combined N

**Fig. 3** Phytoplankton biomass (chlorophyll *a*) responses in bioassays conducted in August 2009. Water samples for bioassay were collected from the surface at the location in Meiliang Bay and East Taihu. Initial chlorophyll *a* content is shown. Responses were for 2-day incubations. Mean values are shown. *Error bars* represent ±1SD of triplicate samples. Differences between treatments are shown based on ANOVA post hoc tests (*a* > *b* > *c*)
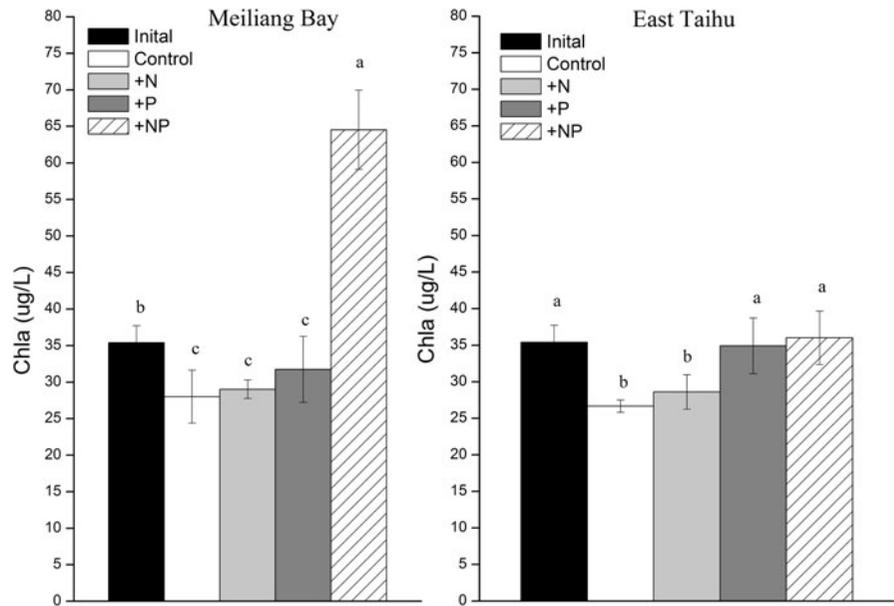


**Fig. 4** Phytoplankton biomass (chlorophyll *a*) responses in bioassay conducted in September 2009. Water samples for bioassay were collected from the surface at location in Meiliang Bay and East Taihu. Initial chlorophyll *a* content is shown. Responses were for 2-day incubations. Mean values are shown. *Error bars* represent ±1SD of triplicate samples. Differences between treatments are shown based on ANOVA post hoc tests (*a* > *b* > *c* > *d*)
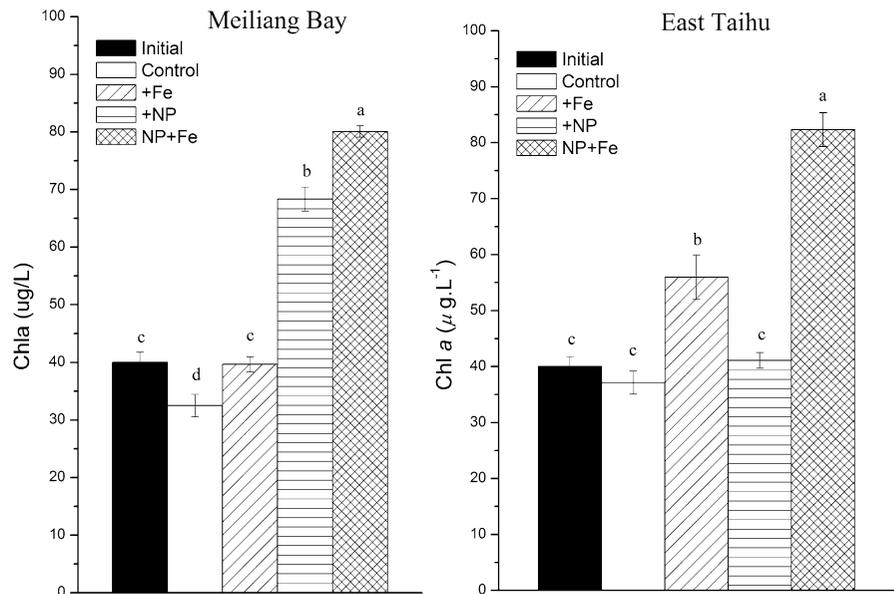
and P addition (Fig. 5). In East Taihu, enrichment with N or P alone or combined did not produce a significant increase of *Microcystis* spp. biomass. However, Fe addition alone significantly increased Chl *a* concentrations. Combined addition of Fe and P yielded larger biomass increases than the addition of Fe alone, while the combined addition of Fe and $NO_3^-$ resulted in a similar growth response as addition of Fe alone (Fig. 5).

## Discussion

Spatial variability of nutrient limitation for growth of *Microcystis* spp.

It is generally accepted that P is the primary limiting nutrient in freshwater ecosystems (Likens, 1972; Schindler, 1977; Schindler et al., 2008; Paerl & Piehler, 2008). However, numerous bioassays and

**Fig. 5** Phytoplankton biomass (chlorophyll *a*) responses in bioassay conducted in August 2010. Water samples for bioassay were collected from the surface at location in Meiliang Bay and East Taihu. Initial chlorophyll *a* content is shown. Responses were for 2-day incubations. Mean values are shown. *Error bars* represent ±1SD of triplicate samples. Differences between treatments are shown based on ANOVA post hoc tests (a > b > c > d)
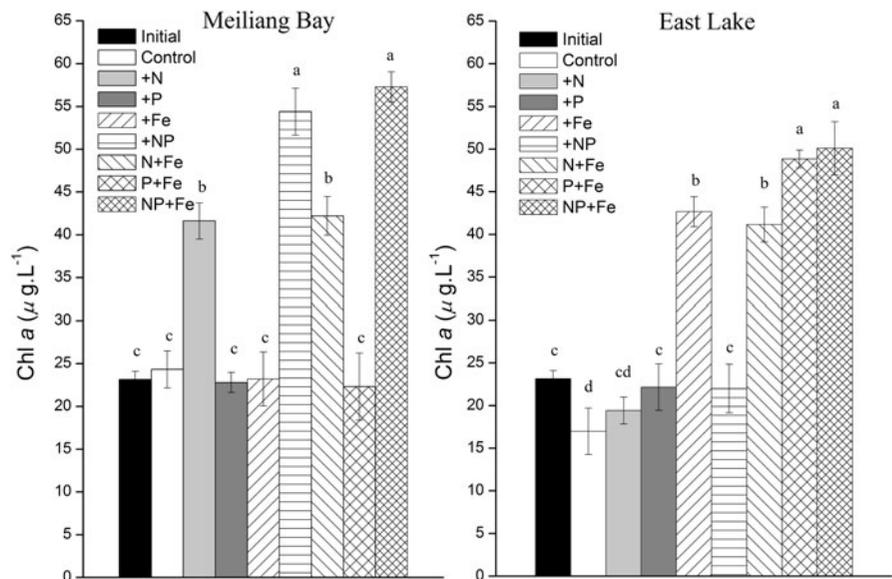


whole lake experiments have shown N to be limiting in lakes and streams as frequently as they are limited by P (Fee, [1979]; Elser et al., [1990]; Francoeur, [2001]; Lewis & Wurtsbaugh, [2008]; Conley et al., [2009]). *Microcystis* spp. blooms were extensive during summer in Meiliang Bay of Taihu. N concentrations showed seasonal low values during the period of severe bloom activity (Xu et al., [2010]). This suggests that the high biomass may deplete DIN, causing N to be the limiting nutrient for *Microcystis* spp. growth. Using short-term bioassays, Xu et al. ([2010]) and Paerl et al. ([2011]) showed that N and P were the co-limiting nutrients for the cyanobacterial bloom in summer and fall in Meiliang Bay. However, the results from the enrichment experiment conducted in August 2009 showed that the growth responses of *Microcystis* spp. to N and P additions were different between Meiliang Bay and East Taihu (Fig. [3]). In Meiliang Bay, the combined N and P additions led to the strongest positive response of *Microcystis* spp., which further confirmed previous conclusion that N and P were the co-limiting nutrients. However, in East Taihu, *Microcystis* spp. did not respond positively in lake water supplied with excessive N and/or P, indicating factors other than N and P were limiting. We checked trace element and found TDFe concentration was relatively higher in Meiliang Bay than in East Taihu. The availability of Fe may have played an additional role in controlling phytoplankton production. We tested the role of Fe may have played in modifying the growth response of

*Microcystis* spp. to N and P additions in September 2009. When Fe was added alone to lake water collected from Meiliang Bay, no significant stimulatory effects of Fe were observed, suggesting Fe was not the primary limiting nutrient. However, Fe addition increased the positive effect of N and P on *Microcystis* spp. growth, indicating moderate Fe co-limitation at the sampling date in Meiliang Bay. In contrast, only treatments containing Fe were able to stimulate *Microcystis* spp. growth in East Taihu, indicating that Fe was a primary limiting nutrient there (Fig. [4]). An additional experiment was conducted in August 2010 to further examine the interactions of Fe with N or P. Fe was not a limiting nutrient for *Microcystis* spp. growth at this sampling date in Meiliang Bay because combined addition of Fe, P, and N yielded the same Chl *a* increases as combined addition of P and N. However, in East Taihu Fe addition alone significantly increased Chl *a* concentrations and combined addition of Fe and P yielded larger biomass increases than the addition of Fe alone, showing Fe and P were co-limiting nutrients and Fe was the primary limiting nutrient. Studies in Lake Tahoe (USA) and Lake Erie (USA/Canada) also demonstrated co-limitation of Fe and P on phytoplankton growth (Chang et al., [1992]; Twiss et al., [2000], [2005]).

$NO_3^-$ concentration was relatively high, while TDFe and $NH_4^+$ were relatively low in East Taihu (Table [2]). Bioassay experiments showed that enrichment with $NO_3^-$ alone or combined with P did not

significantly increase *Microcystis* biomass in East Taihu water (Figs. 3, 4, 5). Because Fe is a constituent of the nitrate reductase enzyme, which is required for $NO_3^-$ assimilation, phytoplankton were able to access the ambient $NO_3^-$ only when exogenous Fe was supplied. Therefore, phytoplankton $NO_3^-$ uptake and growth can potentially be restricted by Fe limitation (Milligan & Harrison, 2000; Timmermans et al., 2004). The combined addition of Fe and $NO_3^-$ resulted in a similar growth response as addition of Fe alone in East Taihu (Fig. 5), suggesting adequate N supplies were available for supporting *Microcystis* spp. growth when Fe was supplied. Fe is the primary limiting nutrient for both phytoplankton growth rates and export production in approximately 30% of the global ocean where high concentrations of $NO_3^-$ are present in surface waters throughout the year (de Baar et al., 2005; Boyd et al., 2007).

Prior work has shown that the dominant cyanobacteria tend to prefer $NH_4^+$ over $NO_3^-$ in Taihu (Paerl et al., 2011). In our bioassay, $NO_3^-$ was employed as the sole nitrogen source. Therefore, *Microcystis* spp. growth response to N addition might have been greater if the nitrogen additions had included $NH_4^+$, which does not require Fe for N assimilation.

Factors affecting iron availability

Iron limitation may occur because of low total amounts of iron and/or by low iron bioavailability in the aquatic ecosystem. Iron limitation in the ocean usually occurs only at very low dissolved iron concentrations (<0.2 nM, Johnson et al., 1997; Achterberg et al., 2001). Fe limitation is usually not expected to occur in most freshwater lakes because of their proximity to land-based iron sources. However, many studies showed Fe limitation in lakes may occur over a much wider range of concentrations (<2–140 nM) (Chang et al., 1992; Auclair, 1995; Imai et al., 1999; Twiss et al., 2000; Sterner et al., 2004; Nagai et al., 2004). In large eutrophic, shallow Taihu, Fe was shown to be limiting even when TDFe concentration was 0.003 mg/l in East Taihu and 0.01 mg/l in Meiliang Bay. These concentrations are much higher than those found in the world's oceans. For example, Fe has been shown to be limiting when concentrations are near 2 ng/l in the Ross Sea (Bertrand et al., 2011). The large Fe concentration differences between Taihu and the oceans must be

reconciled with its bioavailability. Fe bioavailability is dependent on Fe chemical speciation, and not necessarily on concentration. Only a small fraction of total ambient Fe occurs in forms available to phytoplankton (Wells et al., 1995). Fe(II) is considered to be a biologically available form and its formation via reduction of Fe(III) is a common phenomenon in aquatic systems (Wells et al., 1995; Croot et al., 2001; Weger et al., 2002). In seawater, high concentrations of $Cl^-$ and $SO_4^-$ complex Fe(II) and retard its oxidation (Millero et al., 1987). As a result, transient accumulation of Fe(II) must be considered in marine systems. In contrast, rapid re-oxidation of Fe(II) is expected in most lakes since $[Cl^-]$ and $[SO_4^-]$ are much lower and hydroxide contributes to the oxidation of Fe(II) at rates proportional to $[OH^-]^2$. Fe(III) is the main form of iron in aerobic systems. Fe(III) exhibits very low solubility in circumneutral solutions and is rapidly hydrolyzed to various colloidal or precipitated Fe(III)-oxyhydroxides, which exhibit varying degrees of biological availability (Yoshida et al., 2006).

Most dissolved Fe is present as organically bound Fe(III) in aquatic system (van den Berg, 1995). Marine humic substances exist at rather low concentrations, rarely exceeding 0.25 mgC/l (Harvey & Boran, 1985). In contrast, dissolved humic substances (fulvic acid, and to a lesser extent, humic acid) in freshwaters are generally more abundant (>1 mgC/l) and comprise up to 80% of total dissolved organic C (Kemp & Johnston, 1979; Steinberg & Muenster, 1985). Nagai et al. (2004) determined the speciation of dissolved iron by cathodic stripping voltammetry (CSV) and showed that >99.9% of the dissolved iron is present as organic species in Lake Kasumigaura, Japan. A chemical speciation model (WHAM) has been used to estimate that >99% of the dissolved Fe species in Lake Erie, are complexed to organic ligands (Twiss et al., 2000).

Availability of organically bound Fe to phytoplankton likely depends on the quality of the DOC (Wang & Dei, 2003), which in turn will affect dissociation and photo-reduction rates. Imai et al. (1999) reported that the ambient level of fulvic acid in Lake Kasumigaura significantly inhibited the growth of *Microcystis aeruginosa* due to complexation of Fe(III) with fulvic acid. They concluded that organic complexation of Fe likely plays a role in regulating the formation of blooms of this species. Nagai et al. (2006) reported that the growth of *M. aeruginosa* and *P. agardhii* in water from Lake Kasumigaura was

constrained by iron limitation as a consequence of iron complexation with ambient dissolved organic matter. These studies suggest that the iron limitation may occur even in eutrophic lakes that contains a high concentration of TDFe due to low bioavailability.

The present study showed that the extent of Fe limitation was greatest in the eastern part of Taihu. Unfortunately, measurements of free $Fe^{3+}$ and $Fe^{2+}$ were not available at the time of the bioassays reported here. This made it difficult to estimate the bioavailability of iron and impossible to determine the limiting Fe concentration from our results. However, our results may at least partially be explained by spatial differences of total dissolved iron between East Taihu and Meiliang Bay when the bioassays were conducted (Table 2).

It is known that Fe distribution in lakes is strongly affected by the chemical conditions of the lake water (e.g., pH, DO, $E_h$), Fe inputs and removal processes, as well as internal recycling (Davison, 1993; Hongve, 1997). High pH and redox potential may reduce Fe availability by promoting high rates of oxidation of bioavailable ferrous $Fe^{2+}$ to ferric $Fe^{3+}$, followed by the formation of the highly insoluble ferric hydroxide (Stumm & Morgan, 1996), while the presence of organic substances enhances organic complexation of $Fe^{3+}$ (Imai et al., 1999; Sun et al., 2005). The water column at both sites in Taihu is fully oxygenated, which would restrict soluble Fe ($Fe^{2+}$) availability. In East Taihu, pH ranged from 7.78 to 8.13, which was lower than that in Meiliang Bay (Table 2), suggesting pH was not a major factor controlling Fe availability in East Taihu. However, the dissolved organic carbon concentrations were lower in East Taihu than in Meiliang Bay (Table 2), which indicated that lower concentrations of organic substances in East Taihu may lead to low amounts of complexed Fe in water column.

Fluvial inputs may be an important source for elevated concentrations of Fe in lakes. Meiliang Bay is near Wuxi city, where two main rivers, the Liangxi and Zhihu Gang, discharge to the bay. Large amounts of wastewater from factories and residential areas are discharged to these rivers, which in turn discharge large amounts of anthropogenic nutrients including Fe into the northern lake (Huang et al., 2001; Qu et al., 2001). In contrast, East Taihu is more removed from waste pollution sources, and the external Fe load is low. This also can be shown by heavy metal pollution in the sediments. A distinct spatial distribution of

heavy metals in sediments has been observed (Qu et al., 2001; Wang et al., 2002). The sediments in northern Lake Taihu, such as Meiliang Bay are polluted with heavy metals. However, heavy metal pollution in the southern lake is much lower. The Fe content in surface sediment ranged from 23,400 to 47,200 mg/kg (dry weight), with a mean value of 42,000 mg/kg (dry weight) in Meiliang bay and 28,233 mg/kg (dry weight) in East Taihu (Qu et al., 2001).

The shallow and well mixed regions of lakes are not expected to exhibit signs of Fe limitation because of the reductive dissolution of Fe oxides in the sediments that become remobilized and diffuse into the water column (Schoemann et al., 1998). The formation of an anoxic microlayer at the sediment surface is an important factor in releasing Fe to the water column (Penn et al., 2000), and thus it is possible that some internal Fe loading from epilimnetic sediments occurs in shallow eutrophic lakes. Taihu is a very large shallow ($\sim 1.9$ mean depth) lake, in which the sediments are frequently resuspended by wind mixing, a phenomenon particularly evident in exposed bays like in Meiliang Bay (Qin et al., 2004). This may introduce relatively more Fe into water column in Meiliang Bay. Internal P loading is known to occur in shallow eutrophic lakes via desorption of soluble P and wind induced resuspension of particulate P and soluble porewater P (Kleeberg & Kozerski, 1997; Spears et al., 2007); this suggests that shallow sediments in eutrophic waters can contribute to internal loading of soluble P and possibly $Fe^{2+}$, even when the overlying water is oxygenated. However, in East Taihu, aquatic macrophytes are dominant in summer period. Macrophytes can reduce sediment resuspension by attenuating wave and current energy (Madsen et al., 2001). This proved to be the case in the summer, when higher transparency and lower SS were observed (Table 2). When lake water remains relatively calm and poorly mixed, sediment resuspension will be minimal and losses of water column Fe by sedimentation will be highest, with resultant total dissolved iron concentrations remaining quite low.

Effects of iron supply on cyanobacterial growth and dominance

As a phytoplankton group, cyanobacteria have higher Fe requirements than eukaryotic algae (Morton & Lee,

1974; Parr & Smith, 1976; Brand, 1991), with $N_2$ fixation imposing a particularly high demand (Murphy et al., 1976; Glass, et al., 2009). Parparova & Yacobi (1998) suggested that cyanobacteria were particularly sensitive to a reduction in Fe bioavailability due to their relatively high Fe requirements. It has been suggested that coastal pico-cyanobacteria were unable to lower their Fe requirements in a low-Fe environment (Maldonado & Price, 1999). Production of siderophores by cyanobacteria is therefore considered to be a mechanism to access more bioavailable iron from aquatic ecosystems. A laboratory study showed that removal of siderophores decreased iron-uptake rates by $\sim 60\%$ compared to siderophore-replete conditions (Wirtz et al., 2010). In contrast, coastal eukaryotic species appear to adapt to a low-Fe environment by lowering their Fe requirements (Brand, 1991; Sunda & Huntsman, 1995). Iron limitation has been observed for picocyanobacteria, but not for chlorophytes and diatoms in Lake Tanganyika (de Wever et al., 2008). In East Taihu, Fe concentrations were relatively lower than those in Meiliang Bay (Table 2). Cyanobacterial blooms were largely absent and the dominant phytoplankton species were eukaryotic cryptophytes and chlorophytes during summer. However, the bioassays demonstrated that *Microcystis* spp. grew well when Fe was added to filtered water collected from East Taihu. Therefore, Fe availability may be an important factor regulating phytoplankton production and community structure, although dominant macrophytes may also have a negative effect on phytoplankton by the competing for macronutrients, living space, light and exhibiting allelopathy (Gopal & Goel, 1993; Inderjit & Dakshini, 1994; Gross, 2003; van Donk & van de Bund, 2002).

The results presented here underscore the importance of Fe in controlling lake phytoplankton production and possibly a role in promoting the growth of Fe-demanding bloom-forming cyanobacteria (Guildford et al., 2003; Sterner et al., 2004; North et al., 2007, 2008). It follows that increases in Fe fluxes from the watershed, point sources of Fe, or increased Fe liberation from sediments may enhance cyanobacterial dominance over eukaryotic phytoplankton species as suggested by Molot et al. (2010). Therefore, management and manipulation of Fe availability in lakes and their catchments may present an opportunity to reduce the severity of CyanoHABs. The potential contribution of Fe and possibly micronutrient enrichment to the eutrophication of large, regionally complex lakes like Taihu needs further attention.

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