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Effects of nitrogen and phosphorus on phytoplankton composition and biomass in 15 subtropical, urban shallow lakes in Wuhan, China

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ABSTRACT

This study aims at investigating the composition and biomass of the phytoplankton community in 15 urban shallow eutrophic lakes as well as the effects of main environmental factors, including nutrient concentrations and the ratio of nitrogen to phosphorus, temperature, COD, BOD, water depth, etc. on the phytoplankton community structure. Lake water samples were taken and analyzed on a bimonthly basis during the period from March 2004 to March 2006. The redundancy analysis (RDA) and regression analysis (RA) were performed to identify the effects of nutrients on the phytoplankton community and biomass in these typical urban lakes. The results indicate that most of these urban lakes were hypertrophic due to high concentrations of total phosphorus (TP) and total nitrogen (TN), with mean levels of 490 and 5380 mg m⁻³, respectively. The phytoplankton community was dominated by *Microcystis aeruginosa* and *Euglena caudate* in summer and *Cryptomonas ovata* and *Cyclotella meneghiniana* in winter. The mean biomass of the phytoplankton reached 456.87 mg L⁻¹ in summer months and the annual level was 189.24 mg L⁻¹. Temperature and TP content were found to be the principal limiting factors for phytoplankton growth on an annual basis. On the other hand, the results of RDA and RA demonstrate that the dominant phytoplankton species were not nutrient-limited during summer months. Low TN:TP ratios (<10) were detected accompanied with fewer occurrences of N-fixing cyanobacteria and other filamentous algae in most lakes in summer, which implies that low N:P ratio does not always shifts the dominance of phytoplankton community to the N-fixing cyanobacteria. Moreover, TP always had higher correlation with chlorophyll *a* (Chl-*a*) than TN, even when the TN:TP ratios of most samples were lower than 10. Therefore, it is concluded that the TN:TP ratio is not always a suitable index to determine whether nitrogen or phosphorus limits the phytoplankton biomass in urban shallow eutrophic lakes.

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Introduction

Nutrient enrichment typically stimulates phytoplankton growth in lakes. Some genera of phytoplankton, such as *Microcystis*, *Anabaena*, *Nostoc* and *Aphanizomenon*, usually break out and stand stably, leading to problems with hypoxia, toxins and changes in the structure of biological communities (Carmichael, 2001; Chen et al., 2008).

Understanding the links between nutrient concentration and algal biomass is important in the efforts for eutrophication management. Phosphorus (P) and Nitrogen (N) are often considered as the principal limiting nutrients for aquatic algal production due to their short supply compared to cellular growth requirements. Sakamoto (1966) proposed that the phytoplankton biomass was dependent on total phosphorus (TP) when TN:TP by weight was > 17, on total nitrogen (TN) when TN:TP was < 10

and both on TN and TP when the ratio of TN to TP was in the range of 10–17. The importance of TN:TP ratio for the relative proportion of cyanobacteria in phytoplankton where TN:TP by weight was < 29 has been emphasized by Smith (1982). Paerl et al. (2001) proposed, however, that the “N:P rule” was not suitable for highly eutrophic systems when the loadings of N and P exceed the assimilative capacity of the phytoplankton. Large-scale investigations in the Yangtze shallow lakes indicated that the N:P ratios varied greatly among eutrophic lakes (Wang et al., 2008). Therefore, they proposed that the TN:TP ratio was not an appropriate index to determine whether nitrogen or phosphorus is the limiting nutrient in a lake. In addition, the mesocosm experiment in Lake Donghu indicated that the low TN:TP ratio is rather a result than a cause of *Microcystis* blooms (Xie et al., 2003b). Therefore, further investigations in more lakes, especially in urban shallow eutrophic lakes, are urgently needed to identify the relationship between the phytoplankton population and the threshold of TN:TP ratios.

There are more than one hundred floodplain lakes in the urban area of Wuhan, characterized with low depth and increasingly

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severe eutrophication in recent decades. Most of these lakes were formed from geological subsidence, and were once connected with the Yangtze River before 1950s. Since then, a majority of these lakes were disconnected from the Yangtze River as a result of the construction of flood-control projects, except for a few of lakes being connected with the Yangtze River through man-made ditches and milldams. Since Wuhan City underwent rapid urbanization and industrialization in the 1980s, the remaining lakes have been increasingly exposed to nutrients and organic pollutants generated by untreated domestic and industrial wastewaters (Lu et al., 2007). Thus, these lakes have become more and more eutrophic, showing high N and P levels. Previous research has frequently been focused on the mesocosm experiments in the Lake Donghu, the largest urban shallow lake in Wuhan City (Qiu et al., 2001; Xie and Xie, 2002; Xie et al., 2003a); nevertheless, efforts for the *in situ* investigations into other typical urban eutrophic lakes are rarely made.

The objectives of the study are to investigate the succession of phytoplankton population in these typical urban shallow lakes of Wuhan at different seasons, and to identify the effects of phosphorus and nitrogen on phytoplankton composition and biomass at varying TN:TP ratios.

Methods and materials

Study site

Wuhan, situated in the middle of China (113° 41' E–115° 05' E, 29° 58' N–31° 22' N), is praised as “a city with hundreds of lakes” owing to its abundance in water resources. The Yangtze River joins the Hanjiang River at the centre of the city, dividing it into three sections. More than one hundred lakes were once scattered in the urban and suburban areas (Fig. 1). Wuhan is located in the humid subtropical monsoon climate zone at an approximate elevation of 23.3 m. The city's 30-year normal precipitation is 1280.9 mm, and rainfall is most frequent between April and October. The average atmospheric temperature is 16.9 °C with an extremum low of –18.1 °C and extremum high of 42.2 °C (Wuhan Water Authority, 2005). Most of the lakes have suffered from

overloading of nutrients and organic contaminants, resulting in toxic algal blooms, fish kills and water turbidity.

Samples from fifteen lakes, which belong to nine different drainage basins, were taken in the present study. The lakes of Moshui, Longyang, Sanjiao, Beitaizi and Nantaizi are of the same drainage basin and have been interconnected through artificial ditches since December 2005. Lake Nantaizi was a major receiving water body of wastes generated from such industries as food, automobile and grease manufacturing, which are situated in the Zhuankou Industrial Park. Water depth of the Lake Donghu averages 2.2 m, and displays little seasonal or year-to-year variation. This stability is maintained by pumping water into the lake from a river nearby during the dry season to ensure a continuous water supply (4 million L day⁻¹) for a large steel mill (Havens et al., 2001). Lake Shuiguo is a bay of Lake Donghu with high nutrient loadings. All of the rest studied lakes are seepage lakes and are disconnected with each other. A few kinds of aquatic macrophytes including *Alternanthera philoxeroides*, *Polygonum flaccidum* and *Eichhornia crassipes*, etc., which inhabited in the littoral zone of the studied lakes, have been reported. No macrophyte species has been determined in the lakes of Lianhua, Nantaizi and Hankoubei (Wuhan Water Authority, 2005).

Sampling methods

Field investigations were conducted every two months during the period from March 2004 to March 2006. Samples were collected at a depth about 0.50 m below the surface and mixed with water on the bottom at each location. Quantitative samples of the phytoplankton collected using a 5 L water sampler were divided into 3 portions, and preserved immediately with 1% of acidified *Lugol* iodine solution. Precisely 1 L sample was taken from each portion in the lab and concentrated to 30 mL after sedimentation for 48 h. After complete mixing, the concentrated samples were counted directly in a 0.1 mL counting chamber using a compound microscope (XSP-C202, Shanghai, China; at a magnification of 640×). The mean of all of the three portions was adopted as the final result. Colonial forms were counted by enumerating single cells after separation using an ultrasonic

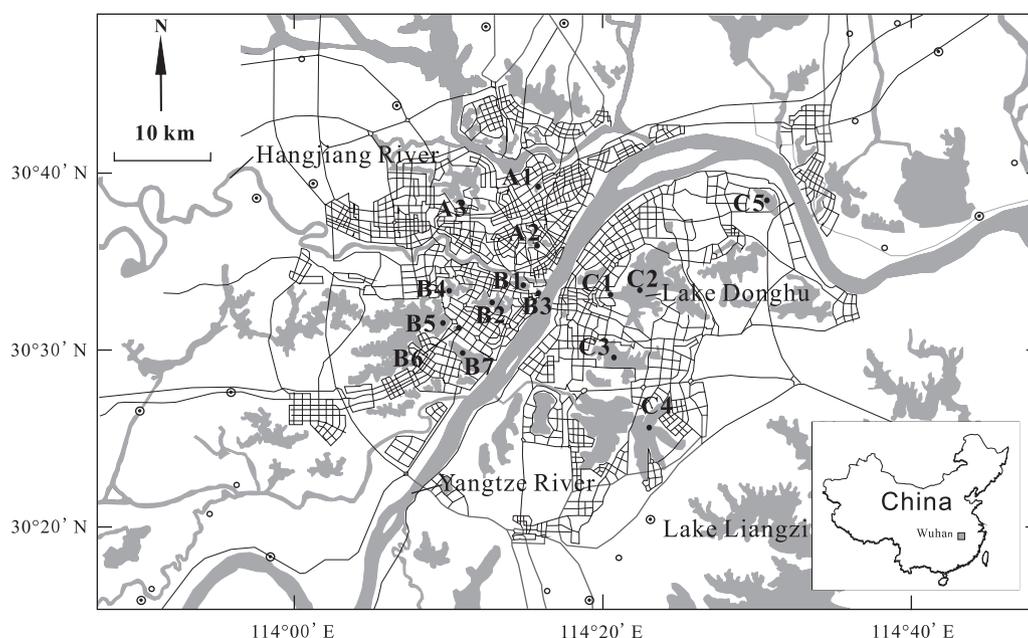


Fig. 1. Sampling stations at 15 lakes, Wuhan, China. A1: Lake Tazi; A2: Lake Hankoubei; A3: Lake Zhangbi; B1: Lake Yuehu; B2: Lake Moshui; B3: Lake Lianhua; B4: Lake Longyang; B5: Lake Sanjiao; B6: Lake Beitaizi; B7: Lake Nantaizi; C1: Lake Shuiguo; C2: Lake Donghu; C3: Lake Nanhu; C4: Lake Tangxun; C5: Lake Qingshanbei.

crusher, JY88-II (Scientiz, Ningbo, China). Phytoplankton species were identified according to Hu et al. (1979). Algal biomass was estimated from approximate geometric volumes of each taxon, assuming that $1 \mu\text{m}^3$ equals $10^{-6} \mu\text{g}$ fresh weights (Shei et al., 1993). The geometric dimensions were measured on 10–20 individuals for each species.

Analytical methods

Temperature (T) and pH were measured *in situ* using an Aanderaa probe (CTD sensor) and pH-206 pH meter, while both the surface and bottom temperatures were measured to determine whether the water column was thermally stratified. Transparency (SD) was measured with a *Secchi*-disk. Dissolved oxygen (DO) determination was made with an Orion 810 dissolved oxygen meter. Chemical oxygen demand (COD) was determined by acidic potassium permanganate method. Biochemical oxygen demand (BOD_5 at 20°C) was analyzed with the dilution and seeding method. TP was analyzed according to the ammonium molybdate method after being oxidized by potassium persulfate under pressure. TN was measured at an absorbance of 220 nm after being digested with alkaline potassium persulfate using an ultra-violet spectrophotometer (Unico UV-2000, Shanghai, China). Ammonium (NH_4^+) was analyzed by colorimetry with Nessler's reagent. Nitrate (NO_3^-) and nitrite (NO_2^-) were analyzed using the automated Korolev/cadmium reduction method and the α -naphthylamine method, respectively. Chlorophyll *a* (Chl-*a*) was measured in a 1 cm length cell at the absorbencies of 665 and 750 nm, respectively, using a spectrophotometer (Unico UV-2000, Shanghai, China) after ethanol extraction. All of the methods above were described in detail in Ref. (Huang, 1999).

Statistical analysis

The effects of environmental variables on the phytoplankton community were investigated with redundancy analysis (RDA). The measured environmental factors, including water area, water depth, T , SD, pH, DO, BOD, COD, TP, TN, NH_4^+ , nitrate plus nitrite (NPN) and TN:TP, were adopted as the explanatory variables. All of these environmental variables were \log_{10} -transformed before analysis except for pH. In the data matrix of species biomass, only those taxon that occurred greater than 3 times and accounted for greater than 1% of the total biomass at least in one sample were incorporated into the analysis. The biomass data of each genera were $\log_{10}(x+1)$ transformed before analysis to obtain consecutive distributions. Thus, two series of matrix were carried out from the measured results, one was the species abundance and the other was environmental factors. Detrended correspondence analysis (DCA) was performed to assess the length of the dominant gradient. The redundancy analysis (RDA) was appropriate for the analysis if the length of ordination axes in DCA was relatively low (< 3); otherwise the canonical correspondence analysis (CCA) would be the suitable method (Jan and Petrš, 2003). All ordinations were done in the Canoco program (Version 4.5, Plant research international, Wageningen, the Netherlands) (Ter-Braak and Šmilauer, 2002). The results of RDA were visualized in the form of ordination diagrams in the Canodraw for Windows program. Species scores are represented as symbol such as 'circle', 'square', etc. Environmental variables are represented by lines with arrows pointing in the direction of maximal variation. Variables with lines close to each other and headed in the same (opposite) direction are highly positively (negatively) correlated. Two lines at a 90° angle indicate that the corresponding variables are uncorrelated.

Pearson correlation analysis in the SPSS package (Version 13, SPSS Institute Inc) was used to evaluate the correlation between phytoplankton abundance and environmental factors. Linear regression and curve estimation were also performed with this software.

Results

Differences in physico-chemical composition

The surface areas of sampled lakes ranged from 3.01 to 5218.15 ha, with mean depth in the range of 0.82 and 2.5 m (Table 1). All lakes were not thermally stratified based on the temperature differences between the top and bottom water columns. The mean temperature was 28°C in summer and 5.23°C in winter. Most of the lakes were highly turbid with low transparency, since 65% of the SD was less than 0.5 m and 95% was less than 0.8 m. The concentration of TP ranged from 0.01 to 1.89 mg L^{-1} with the mean value of 0.49 mg L^{-1} , while the majority of the TP concentration was greater than 0.4 mg L^{-1} (87% in summer samples and 52% in all samples). Mean TN:TP ratio was only 12.69, since the ratio from 51.7% of the samples was less than 10 and 78.9% was less than 17. The mean ratios were 10.2 and 21.4 during the summer and winter periods, respectively. According to the fixed boundary classification system for lake trophic states accepted by the OECD (2008), most of the lakes investigated (100% assessed by *Secchi* depth, 91% by Chl-*a* and 100% by TP) belong to eutrophic terminology.

As a major receiving lake of industrial wastewaters, Lake Nantaizi showed extremely high TP and TN concentrations, representing almost all of the highest measured TP and TN levels among the lakes studied. Lakes Lianhua, Donghu, Nanhu and Lake Tangxun were relatively clear, with mean TP and TN concentrations of 0.42 and 3.61 mg L^{-1} , respectively.

Variations of phytoplankton community

A total of 88 genera, 156 species, which belong to *Cyanophyta* (12 genus, 24 species), *Chlorophyta* (53 genus, 97 species), *Euglenophyta* (4 genus, 13 species), *Bacillariophyta* (13 genus, 16 species), *Pyrrophyta* (2 genus, 2 species), *Chrysophyta* (2 genus, 2 species) and *Cryptophyta* (2 genus, 2 species), respectively, were determined from the lake samples. The phytoplankton biomass varied between 0.13 and $2542.98 \text{ mg L}^{-1}$ with an average of 189.24 mg L^{-1} . Fig. 2 shows seasonal changes of the phytoplankton community in all lakes. As a phylum with the maximal species, *Chlorophyta* algae were determined in all samples. Species of *Pyrrophyta* and *Chrysophyta*, belonging to occasional species, were rarely found. *Euglena caudate* peaked in summer and autumn, which accounted for at least 45% of the total biomass due to its size. On the other hand, it accounted for only 3.5% of the total densities. *Cryptomonas ovata*, peaking in the lakes of Zhangbi, Longyang and Nantaizi in winter and spring, was absolutely the dominant species of the community.

A total of 137 species were observed during summer months (from July to September), the mean biomass in all lakes reached 456.87 mg L^{-1} . *Microcystis aeruginosa* and *E. caudate* dominated the phytoplankton community in most lakes (Fig. 2b), with the mean biomass reaching 228.52 and 133.86 mg L^{-1} , respectively. The filamentous species, i.e. *Nostoc punctiforme*, *Anabaena circinalis*, *Spirulina maxima*, *Oscillatoria tenuis*, *Hormidium flaccidum* and *Ulothrix oscillarina*, had low abundance in all lakes. A total of 69 species were identified in winter with the biomass in the range of 0.13 – 17.34 mg L^{-1} , with a mean value of 3.28 mg L^{-1} . Several unicellular species, i.e. *C. ovata*, *Cyclotella*

Table 1

Physico-chemical variables and phytoplankton biomass of each phylum in the 15 lakes studied in Wuhan, China.

Parameters	n	Mean	Min.	Median	Max.	STD	CV (%)
Lake area (ha)	15	747.70	3.01	189.95	5218.55	1431.80	191
Lake volume (10 ⁴ m ³)	15	854.04	7.52	142.46	8349.68	2032.28	237
Mean depth (m)	15	1.62	0.82	1.60	2.50	0.44	27
Maximum depth (m)	15	3.07	1.78	2.80	5.50	1.27	41
Temperature (°C)	180	18.70	3.70	21.00	31.60	8.89	47
pH	180	7.85	6.8	7.81	9.1	0.52	7
Transparency (m)	180	0.44	0.16	0.39	1.07	0.18	41
DO (mg m ⁻³)	180	9570	5690	9240	16,550	2010	21
COD (mg m ⁻³)	180	8710	3010	9020	17,090	3190	37
BOD ₅ (mg m ⁻³)	180	6750	1170	6690	16,030	3140	49
NH ₄ ⁺ (mg m ⁻³)	180	2120	60	1180	16,500	2900	137
Nitrate plus nitrite (mg m ⁻³)	180	770	10	580	3580	690	90
Total phosphorus (mg m ⁻³)	180	490	10	420	1890	360	73
Total nitrogen (mg m ⁻³)	180	5380	350	3290	36,180	5790	108
TN:TP ratio	180	12.69	2	8	79	12.07	95
Chlorophyll <i>a</i> (µg L ⁻¹)	180	69.76	2.11	74.78	189.84	49.7	71
Total biomass (mg L ⁻¹)	180	189.24	0.13	29.16	2542.98	369.15	195
Cyanophyta (mg L ⁻¹)	180	88.61	0	3.21	1573.59	233.75	263
Chlorophyta (mg L ⁻¹)	180	24.91	0.01	5.35	236.56	43.37	174
Euglenophyta (mg L ⁻¹)	180	68.31	0	5.98	854.48	143.73	210
Bacillariophyta (mg L ⁻¹)	180	4.53	0	0.80	137.07	16.28	359
Pyrrophyta (mg L ⁻¹)	180	0.64	0	0	37.70	3.33	525
Chrysophyta (mg L ⁻¹)	180	0.04	0	0	2.49	0.22	521
Cryptophyta (mg L ⁻¹)	180	2.21	0	0.07	124.40	10.36	469

n, sample number; STD, standard deviation; CV, coefficient variation.

meneghiniana, *E. caudate*, *Chlamydomonas microspiraera*, *Navicula cuspidate*, *Surirella linearis* and *Ankistrodesmus acicularis* dominated the communities (Fig. 2d). The abundance of diatoms increased in winter but declined sharply in summer except for *C. meneghiniana*, which maintained higher abundance even during the period of cyanobacteria bloom.

The highest phytoplankton biomass in growing seasons was found in Lake Nantaizi, with means of 24.43, 1682.51 and 847.31 mg L⁻¹ in spring, summer and autumn, respectively. The phytoplankton communities in the lakes of Lianhua, Donghu, Nanhu and Tangxun were consistent with the nutrient concentrations, characterized by low biomass with means of 273.03, 123.44, 101.00 and 61.23 mg L⁻¹, respectively.

Effects of nutrients on phytoplankton communities

Similar to the biomass of cyanobacteria, *Chlorophyta* and *Euglenophyta*, phytoplankton chlorophyll was positively correlated to TP, BOD, COD, temperature, TN, NH₄⁺ but negatively correlated to SD, TN:TP ratios (Table 2). TP showed similar relations to phytoplankton chlorophyll with regard to the biomass of cyanobacteria, *Chlorophyta* and *Euglenophyta*. TP was found to be the most positively correlated factor with the biomass of *Bacillariophyta*. Linear regression for both the annual and summer data confirmed that TP plays a vital role in the Chl-*a* variations (Fig. 3). All of the TP–Chl-*a* regressions had higher coefficients of determination (*R*²) than the TN–Chl-*a* regressions. Moreover, most of the samples with low TN:TP ratios (< 10) had high TP and Chl-*a* concentrations but intermediate TN concentrations (Fig. 3a, b), which means high TP concentrations resulted in low TN:TP ratios.

Relationship between phytoplankton species and environmental factors

Annually, fifty-one species from all the samples were met with the data select criteria and consequently summed as the species data matrix, 180 × 51 (Fig. 4a). Besides, fifty species from the

summer samples were met and summed as 60 × 50 species matrix (Fig. 4b). Most of these species were not clustered by the two-dimension graph of RDA, although the physico-chemical variables explained 35.5% of the total variance of species distribution within annual periods, and 33.7% within summer periods (Table 3). As an index of seasonal succession, water temperature is the most effective factor influencing the community composition and distribution. All of the predominant species, including *M. aeruginosa*, *E. caudate*, *Chlorella vulgaris*, *Chroococcus minor*, *Phacus longicauda* and *A. acicularis*, were characterized with high TP, COD, BOD and TN concentrations but low SD (Fig. 4a). It seems that *C. ovata* preferred to colonize in large lakes but did not show special preference for eutrophic state and physical conditions. In summer, *M. aeruginosa* did not show preference to any physico-chemical factors adopted though it was the dominant species of the phytoplankton community in most lakes. Another dominant species, *E. caudate*, showed particular favor to high TP, pH, and to either high water depth or TN, but to low NPN. *Quadrigula chodatii* preferred clear water, peaking in those lakes with high SD. *C. vulgaris* and *C. ovata* showed high biomass in those large and deep lakes.

Discussion

The phytoplankton community was mainly composed of *Cyanophyta* (*Microcystis*, *Chroococcus* and *Chlamydomonas*), *Bacillariophyta* (*Cyclotella*), *Chlorophyta* (*Chlorella*), *Cryptophyta* (*C. ovata*) and *Euglenophyta* (*E. caudate*, *P. longicauda*). These genera have great significances in terms of occurrence or biomass in the studied lakes, and also are common in subtropical and eutrophic lakes (Alam et al., 2001; Abrantes et al., 2006; Alex and Linda, 2008). Overloading of phosphorus is usually considered as the primary causation, which leads to significant shifts in phytoplankton species composition towards bloom-forming cyanobacterial domination (Reynolds, 1987; Smith, 1983; Steinberg and Hartmann, 1988). The frequently reported bloom-forming cyanobacteria are *Microcystis*, *Anabaena*, *Nostoc* and *Aphanizomenon* (Carmichael, 2001; Abrantes et al., 2006; Chen et al., 2008). In the present study, the phytoplankton communities

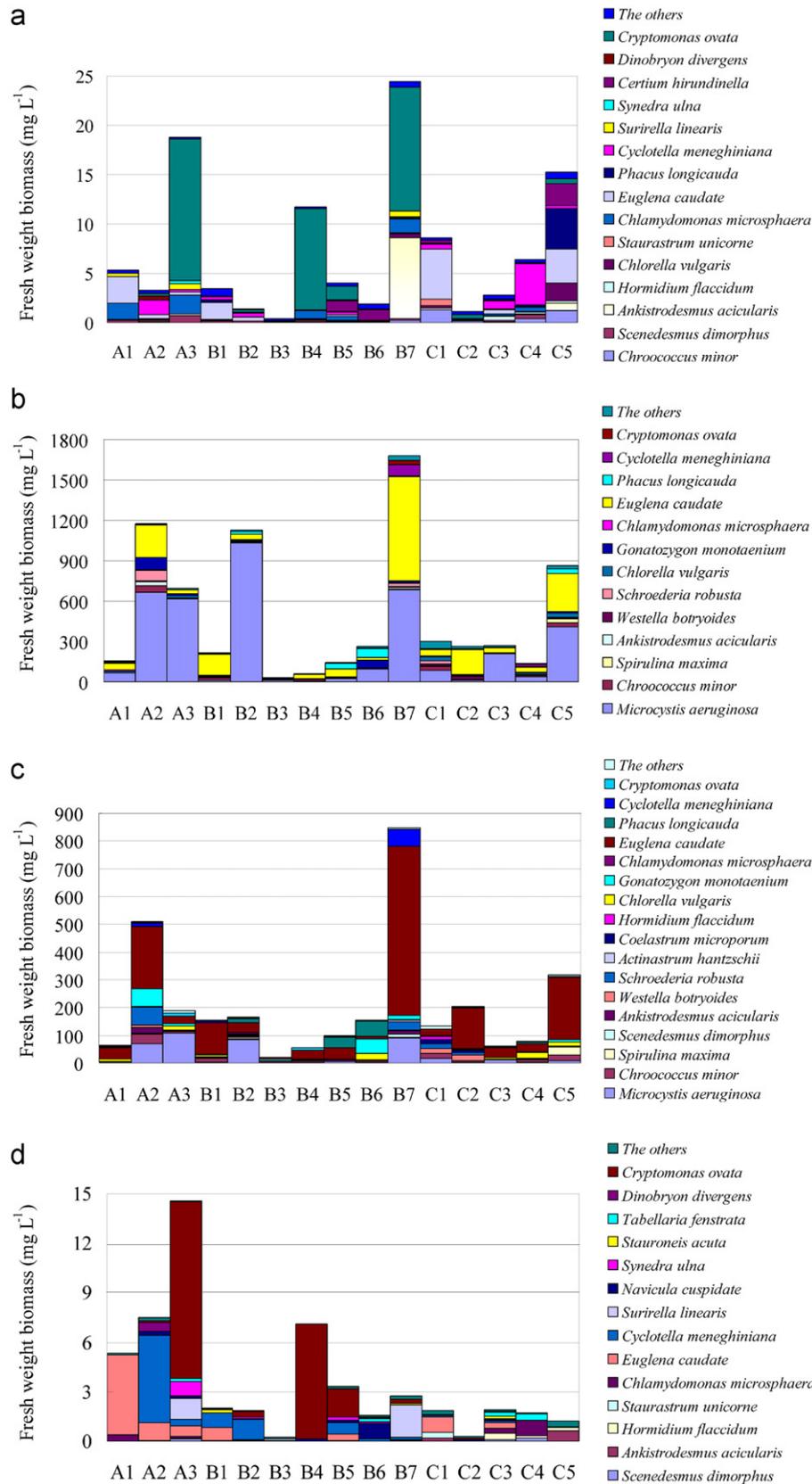


Fig. 2. Composition of phytoplankton during different seasons; at 15 lakes, Wuhan, China while the mean biomass of species was calculated with all the measurements in each season; (a) spring, from March to May; (b) summer, from July to September; (c) autumn, i.e., November and (d) winter, i.e., January.

were dominated by *M. aeruginosa* and *E. caudate* in growing seasons while the biomass of *Anabaena* and *Nostoc* never exceeded 0.5% of the total in all samples, and *Aphanizomenon*

was not found in all of the lakes investigated. In fact, filamentous species never dominated any phytoplankton community of the studied lakes. This is different than other shallow eutrophic lakes,

Table 2
Coefficients of the Pearson correlation between phytoplankton biomass and environmental indicators in the studied 15 lakes in Wuhan, China.

	Lake rea	Water depth	T	pH	SD	DO	COD	BOD ₅	TP	TN	TN:TP	NPN	NH ₄ ⁺	Chl-a
<i>B</i> _{Cyano}	-0.017	0.148*	0.717**	0.012	-0.641**	0.322**	0.537**	0.550**	0.697**	0.405**	-0.375**	-0.007	0.393**	0.688**
<i>B</i> _{Chlor}	-0.019	0.118	0.695**	0.069	-0.612**	0.321**	0.557**	0.568**	0.685**	0.427**	-0.333**	0.064	0.374**	0.756**
<i>B</i> _{Eugle}	0.045	0.165*	0.719**	0.086	-0.638**	0.256**	0.502**	0.528**	0.660**	0.383**	-0.359**	0.049	0.332**	0.739**
<i>B</i> _{Bacil}	0.193**	0.187*	0.241**	0.079	-0.351**	0.063	0.211**	0.270**	0.346**	0.295**	-0.069	0.106	0.284**	0.373**
<i>B</i> _{Pyrrr}	-0.047	-0.208**	0.203**	0.073	-0.040	0.024	0.157*	0.158*	0.160*	0.153*	0.002	-0.102	0.023	0.059
<i>B</i> _{Chrys}	-0.224**	0.041	-0.100	0.072	-0.001	-0.007	0.026	0.035	0.029	-0.066	-0.133	-0.12	0.04	-0.001
<i>B</i> _{Crypt}	0.098	0.011	-0.085	0.054	-0.052	0.232**	0.080	0.106	0.148*	0.177**	0.026	0.069	0.142	0.153*
<i>B</i> _{Total}	0.030	0.143	0.704**	0.065	-0.671**	0.346**	0.578**	0.606**	0.738**	0.453**	-0.370**	0.049	0.405**	0.808**
Chl-a	-0.003	0.145	0.665**	0.108	-0.755**	0.387**	0.687**	0.723**	0.817**	0.473**	-0.452**	0.099	0.414**	1

*B*_{Cyano}=biomass of *Cyanophyta*, *B*_{Chlor}=biomass of *Cyanophyta*, *B*_{Eugle}=biomass of *Cyanophyta*, *B*_{Bacil}=biomass of *Cyanophyta*, *B*_{Pyrrr}=biomass of *Cyanophyta*, *B*_{Chrys}=biomass of *Cyanophyta*, *B*_{Crypt}=biomass of *Cyanophyta*, *B*_{Total}=biomass of *Cyanophyta*, T=temperature, SD=Secchi-disk depth, NPN=Nitrate plus nitrite, Chl-a=chlorophyll a.

* Denotes *p* < 0.05.
** Denotes *p* < 0.01.

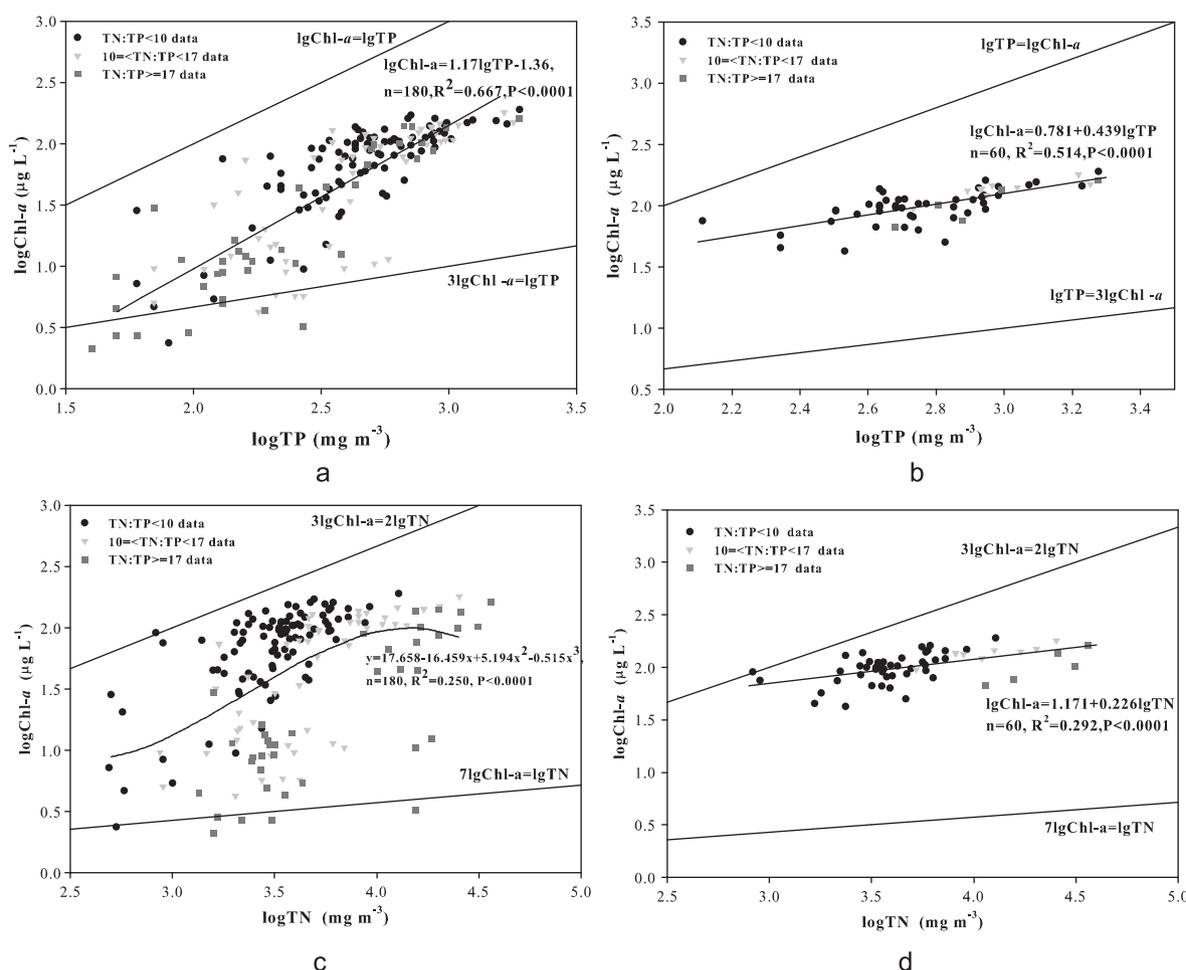


Fig. 3. Relationships between phytoplankton chlorophyll a to total phosphorus (TP) (a) overall and (b) in summer, and between chlorophyll a to total nitrogen (TN) (c) overall and (d) in summer in 15 lakes, Wuhan, China. Most of the data of log TP:log Chl-a located between the 1:1 and the 3:1 lines, as well as between the 3:2 and 7:1 of log TN:log Chl-a in (c) and (d).

in which the dominant species included *Cryptomonas* and some species of *Synechococcus* (Alam et al., 2001), or both the colonial algae *Microcystis* and *Chroococcus*, and the filamentous such as *Aphanizomenon* (Abrantes et al., 2006) and *Anabaena* (Alex and Linda, 2008).

It was proposed in a number of literatures (Peter and Kaj, 2006; Alex and Linda, 2008; David et al., 2008; Tobias et al., 2009) that the dominance of N-fixing cyanobacteria is strongly affected

by the changes in the N:P loading ratios, and low N:P ratio would promote the growth of N-fixing cyanobacteria. David et al. (2008) reported that the N-fixing cyanobacteria can provide sufficient N source, and allow phytoplankton biomass to continue to be produced in proportion to phosphorus content. Hence they suggested that nitrogen reduction cannot control the biomass of phytoplankton. In contrast to this hypothesis, most of the TN:TP ratios (85% in growing seasons) were lower than 10 in our lakes.

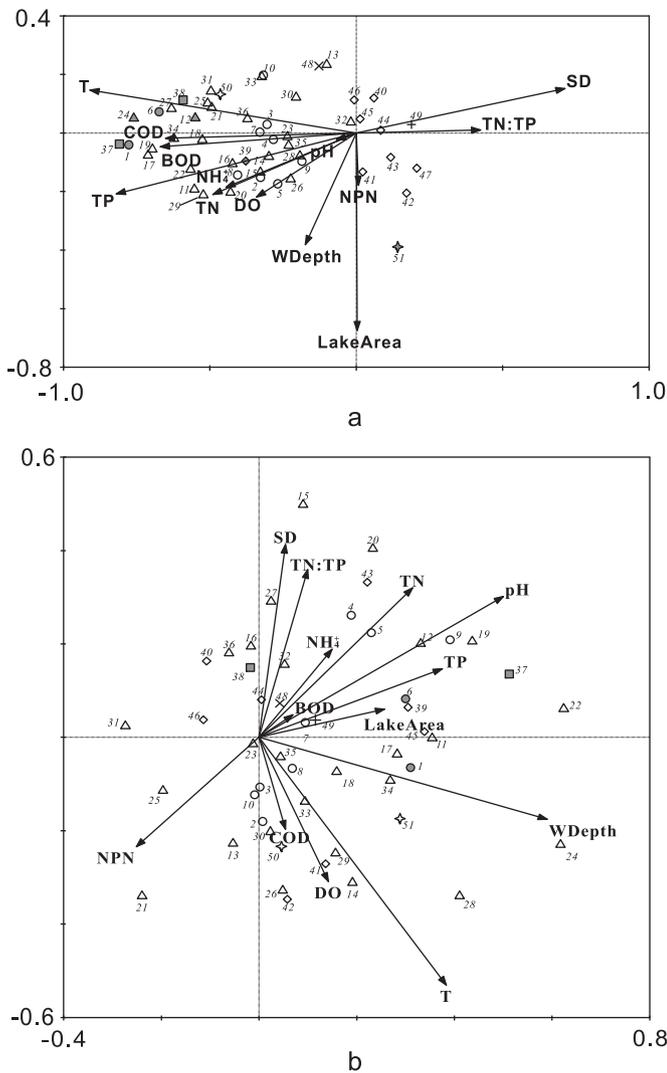


Fig. 4. The biplot of the first two axes of RDA analysis for environmental factors associated with the phytoplankton variation (a) annual; (b) in summer in 15 lakes, Wuhan, China. While ‘○’ denotes cyanophyta; ‘△’, chlorophyta; ‘□’, euglenophyta; ‘◇’, Bacillariophyta; ‘×’, pyrrophyta; ‘+’, chrysophyta and the ‘star’ denotes cryptophyta. Those symbols filled with gray represent predominant species. The “WDepth” means water depth in each sampling sites. The numbers represent the relevant species: 1-*Microcystis aeruginosa*, 2-*Synechocystis aquetilis*, 3-*Nostoc punctiforme*, 4-*Anabaena circinalis*, 5-*Merismopedia glauca*, 6-*Chroococcus minor*, 7-*Spirulina maxima*, 8-*Oscillatoria tenuis*, 9-*Dactylococcopsis acicularis*, 10-*Coelosphaerium dubium*, 11-*Scenedesmus dimorphus*, 12-*Ankistrodesmus acicularis*, 13-*Micractinium pusillum*, 14-*Closterium gracile*, 15-*Quadrigula chodatii*, 16-*Oocystis solitaria*, 17-*Westella botryoides*, 18-*Pediastrum simplex*, 19-*Schroederia robusta*, 20-*Actinastrum hantzschii*, 21-*Coelastrum microporum*, 22-*Hormidium flaccidum*, 23-*Westloppsis linearis*, 24-*Chlorella vulgaris*, 25-*Tetraedron minimum*, 26-*Staurastrum unicorn*, 27-*Gonatozygon monotaenium*, 28-*Chlamydomonas microsphaera*, 29-*Crucigenia quadrata*, 30-*Stichococcus bacillaris*, 31-*Ulothrix oscillarina*, 32-*Microspora floccose*, 33-*Tetrastrum hastiferum*, 34-*kirchneriella obesa*, 35-*Pandorina morum*, 36-*Selenastrum bibrarianum*, 37-*Euglena caudate*, 38-*Phacus longicauda*, 39-*Cyclotella meneghiniana*, 40-*Nitzschia microcephala*, 41-*Suriella linearis*, 42-*Navicula cuspidate*, 43-*Synedra ulna*, 44-*Stauroneis acuta*, 45-*Amphora ovalis*, 46-*Fragilaria capucina*, 47-*Tabellaria fenestrata*, 48-*Certium hirundinella*, 49-*Dinobryon divergens*, 50-*Chroomonas acuta*, 51-*Cryptomonas ovata*.

Low TN:TP was found in most lakes in summer but the N-fixing cyanobacteria were significantly inhibited. The depression of N-fixing cyanobacteria and other filamentous species perhaps was induced by the grazing stress of *Daphnia* (Paterson et al., 2002; Muylaert et al., 2006), or by the allelopathy effects from microcystin, which was yielded as a result of the decomposition of *Microcystis* (Chen et al., 2008).

Phosphorus was considered as the primary limiting factor to algal growth (Schindler, 1977; Smith, 2003; Jennifer et al., 2008), especially in lakes and streams with high N:P ratios (Dodds et al., 2002). In present study, the results of TP–Chl-*a* regression based on the annual data illustrated that all the TP–Chl-*a* regressions had higher coefficients of determination ($R^2=0.667$, $p < 0.0001$) than those of TN–Chl-*a* regressions ($R^2=0.250$, $p < 0.0001$). Although there was a positive correlation between TN and Chl-*a*, the great variations of Chl-*a* were observed irrespective of the TN concentration. Evidently, TN was not the limiting factor for phytoplankton chlorophyll. The regression results in summer demonstrated the effects of TP and TN on the phytoplankton biomass. However, the low slope of the regression equation implies that Chl-*a* increased smoothly with increase in TP and TN (Fig. 3c, d). Hence it is concluded that nutrients are not the limiting factors to phytoplankton biomass in summer. The mechanism was probably attributed to the excessive concentrations of nutrients, which was induced with the increase of rainstorm and domestic wastewaters in summer. Dokulil and Teubner (2003) and Dokulil et al. (2006) suggested that in lakes that are nutrient-limited, Chl-*a*:TP should be lower than or close to 1:1, Chl-*a* increasing with the increase of TP. While in lakes that are light-limited or have a high macrophyte community, Chl-*a* should be much lower than TP. Therefore, we believe it is the TP rather than TN that was the limiting factor to the phytoplankton biomass along the year, whereas neither TP nor TN was available in regulating the phytoplankton communities in summer. In these lakes, the dominant species, *M. aeruginosa* and *E. caudate*, did not show significantly negative correlation with SD in summer, which implied that the phytoplankton biomass was not light-limited either. Considering the complexity of ecosystem and interactions between nutrients and phytoplankton community in summer, it was difficult to find certain factor that alone limited the phytoplankton biomass in these urban eutrophic lakes.

Traditionally, N:P ratios were proposed as an index to classify lakes into N- or P-limited categories, while the assess criteria of nutrient limitation is proposed as 10–17 (Sakamoto, 1966). The C:N:P (106:16:1 by atoms or 41:7:1 by mass) proposed by Redfield (1958) and Redfield et al. (1963) was extensively used as the criterion to assess nutrient limitation. Nevertheless, some studies proposed that the Redfield ratio is not a universal optimum ratio, but an average of species-specific ratios due to great variation of the optimal N:P ratios among various eutrophic lakes (Klausmeier et al., 2004; Wang et al., 2008). Takamura et al. (1992) found that most of the TN:TP ratios were less than 10 during the *Microcystis* blooms, but exceeded 20 after the blooms disappeared in Kasumigaura Lake. In present study, the blooms of *M. aeruginosa* were found in the lakes of Lianhua and Nantaizi in May and July, respectively, in 2004, when all the TN:TP ratios were lower than 10. According to the assess criteria of nutrient limitation (Sakamoto, 1966), the phytoplankton biomass was limited by nitrogen. However, the results of RA and RDA revealed that the phytoplankton biomass was neither P-limited nor N-limited in summer. Xie et al. (2003b) proposed that the process of P-release from sediments was activated greatly by the outburst of *Microcystis* blooms, hence low TN:TP ratios was presumably the result instead of the cause of *Microcystis* bloom. The mechanism perhaps was that the organically bound P from freshly settled autochthonous particles induces alkaline phosphatase activity (APA) and leads to the release of bioavailable phosphates from the organic sediments (Zhou et al., 2008). These strengthened P-release resulted in increased TP concentration and decreased TN:TP ratios.

In conclusion, based on the annual investigation in urban shallow eutrophic lakes, we argue that low N:P ratio does not

Table 3

Summary of redundancy analysis (RDA) for the first two axes between environmental factors and phytoplankton biomass in annual periods and in summer in the studied 15 lakes in Wuhan, China.

Axes	Annual		Summer	
	1	2	1	2
Eigenvalues	0.275	0.018	0.070	0.059
Species–environment correlations	0.861	0.585	0.832	0.700
Cumulative percentage variance of species data	27.5	29.3	7.0	12.9
Cumulative percentage variance of species–environment relation	77.5	82.5	20.7	38.2
Sum of all eigenvalues	1.000		1.000	
Sum of all canonical eigenvalues	0.355		0.337	

always shifts the phytoplankton community to the dominance of N-fixing cyanobacteria. TP always had higher correlation with Chl-*a* than TN, even when the TN:TP ratio of most samples was less than 10. Thus, we believe that the TN:TP ratio is not a suitable index to be used as an index to distinguish N-limited lakes from P-limited ones in urban shallow eutrophic lakes.

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