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Quantifying the dependence of cyanobacterial growth to nutrient for the eutrophication management of temperate-subtropical shallow lakes

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ABSTRACT

The increasing global occurrence of cyanobacterial blooms, attributed primarily to human-induced nutrient enrichment, significantly degrades freshwater ecosystems and poses serious risks to human health. The current study examined environmental variables and cyanobacterial biovolume (B_{Cyano}) of 28 shallow lakes in the eastern China plains during the spring and summer of 2018. We used a 95% quantile regression model to explore season-specific response of B_{Cyano} to total nitrogen (TN), or total phosphorus (TP), and robust linear relationships were observed between $\log(B_{Cyano}+0.001)$ and log(TN), or log(TP) in both spring and summer periods. Based on these regressions, regional-scale and season-specific TN and TP thresholds are proposed for these lakes to ensure the safety for recreational waters and drinking water source. However, actual B_{Cyano} for a given concentration of TN (or TP) for many observations were considerably lower than the results of the 95% regression model predict, indicating that other factors significantly modulated nutrient limitation of B_{Cyano}. Generalized additive model and quantile regression model were used together to explore potentially significant modulating factors, of which lake retention time, macrophytes cover and N: P ratio were identified as most important. Thus, it is necessary to develop type-specific nutrient thresholds with the consideration of these significant modulating factors. Furthermore, nutrient-B_{Cyano} relationships of our studied lakes with lake retention time>100 days and no macrophyte were further explored and nutrient thresholds of this lake type were proposed. Nutrient thresholds proposed in this study may play an essential role in achieving a cost-effective eutrophication management for shallow lakes both in the eastern China plains and elsewhere with similar climatic background. On a broader scale, the approaches and findings of this study may provide valuable reference to formulate reasonable nutrient reduction targets for other ecoregions with different climatic conditions.

1. Introduction

Cyanobacteria are photosynthetic bacteria that play key roles in primary production and the material cycling of aquatic ecosystems. However, where cyanobacterial growth is excessive, so-called “blooms” increase turbidity and their decomposition may deplete oxygen, alter biogeochemical cycling and suppress communities of aquatic plants, oxygen-sensitive invertebrates and fishes (Huisman et al., 2018; Le Moal et al., 2019). Toxins associated with cyanobacterial blooms also pose a range of risks to human health, from mild skin irritations to digestive stress, liver disease, impairment of neurological function and even death (Codd et al., 2005; Giani et al., 2005). There is strong evidence that cyanobacterial blooms are increasing in frequency and intensity in lakes and reservoirs worldwide (Hallegraeff, 1993; Qin et al., 2010; Ho et al., 2019), resulting in a pressing need for knowledge as to when and where such events are likely to occur and to what extent, management can mitigate the problems they cause.

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It is widely believed that cyanobacterial blooms are driven mainly by human-induced nutrient (nitrogen and phosphorus) over-enrichment (Beaulieu et al., 2013; Carvalho et al., 2013; Paerl and Otten, 2013). Studies exploring the quantitative responses of cyanobacteria to nutrient enrichment have therefore been a key aspect of eutrophication management in recent decades. Most of these studies focus on the relationship between cyanobacterial dominance (abundance %) and nutrient levels (Smith, 1986; Ptacnik et al., 2008; Wagner and Adrian, 2009), while relatively few studies consider the response of actual cyanobacterial biovolume (B_{Cyano}) to nutrient enrichment, despite this being more relevant to the health and functioning of aquatic ecosystems (Dolman et al., 2012; Carvalho et al., 2013). Of these few studies, majority have been based on a statistical approach of data averaging followed by ordinary least-squares regression (OLSR) model (Smith, 1986; Downing et al., 2001; Lars et al., 2007). However, OLSR-derived models are unlikely to yield meaningful results if datasets exhibit a wedge-shaped scatter of points, which indicates that cause-effect relationships are confounded by stressors other than nutrient levels (Cade and Noon, 2003; Philips et al., 2019). The relationship between Chlorophyll *a* (Chl*a*) and total phosphorus (TP) is one example. Ideally, a robust linear relationship between log(Chl*a*) and log(TP) would be observed if algal growth was primarily limited by TP (Fig. 1 A). However, in winter observations, Chl*a* exhibited insensitive response to TP due to the considerably modulating effect of temperature. As a result, OLSR-derived model tend to exhibit large uncertainty where the datasets include winter observations (Fig. 1B). Therefore, to explore the essential relationship between Chl*a* and TP, it might be more appropriate to regard nutrients as an upper limit for Chl*a*, and to apply instead a 95% quantile regression model (Cade and Noon, 2003; Xu et al., 2015a,b).

In shallow lakes, there are number of regional-scale factors (e.g, flushing) in addition to temperature that may interact significantly with nutrient limitation to exert a modulating effect on cyanobacterial growth during growing seasons (Liu et al., 2016; Qin et al., 2020). This suggests that more specific information regarding ecological background of individual lakes may be necessary in order to contextualize monitoring data and establish cost-effective nutrient reduction targets. However, for developing world (e.g., China), the vast majority of current lake nutrient management strategies are based on very general criteria (China State Environmental Protection Administration, 2002), a practice may easily lead to over or under-protection of individual lakes (Xu et al., 2015a). Identification and quantification of significant modulating factors will help identify which lakes are more susceptible to cyanobacterial blooms under nutrient enrichment process, and provide important information for lake subdivision and development of type-specific nutrient reduction target. In this study, environmental variables and phytoplankton samples were collected from 28 subtropical-temperate shallow lakes in the eastern China plains during spring and summer of 2018 (Fig. 2). Our objectives were; 1) to explore the season-specific maximal (95% quantiles) and average responses of B_{Cyano} to total nitrogen (TN) and total phosphorus (TP), using 95% quantile regression and OLSR-derived models; 2) to quantify the effect of hydromorphological and biogeochemical background on nutrient limitation of cyanobacteria during the growing periods and identify the significant modulating factors, and 3) to provide useful information for the development of cost-effective nutrient reduction targets for lakes both in our studied ecoregion and elsewhere. We hypothesized that while nutrient levels may define the growth potential of B_{Cyano} in growing seasons. In most cases, this potential would not be reached because of other modulating and controlling factors.

2. Materials and methods

2.1. Study area

We conducted our study in the eastern China plains, which is across the subtropical and warm temperature zone (Wang and Dou, 1998). This ecoregion comprises a vast area of floodplain along the middle and lower reaches of the Yangtze River plain and downstream of the Huai River Plain, which are the most densely populated and agricultural productive region of China (Ma et al., 2010). The total area of natural lakes larger than 1 km^2 in the ecoregion is 21,053 km^2, accounting for 25.9% of total freshwater lake area in China (Ma et al., 2010), and lake area varied greatly in this ecoregion. For lake depth, these lakes are very shallow (generally 1—5 m) and exhibit intensive wind-induced resuspension of sediment (Hamilton and Mitchell, 1996; Huo et al., 2013). In addition, Yangtze River-connected lakes and some lakes in the Huai River Basin were observed with rapid flushing, while the rest of them (e.g., Yangtze River-isolated lakes) tend to have longer residence time (Wang and Dou, 1998). In the past several decades, the anthropogenic activities, including urbanization, agriculture, aquaculture and industrial development, have led to declines in water quality (Ma et al., 2010). Human-induced nutrient enrichment and its associated cyanobacterial blooms are key environmental concerns and challenges facing local governments (Le et al., 2010; Shi et al., 2015; Duan et al., 2017).

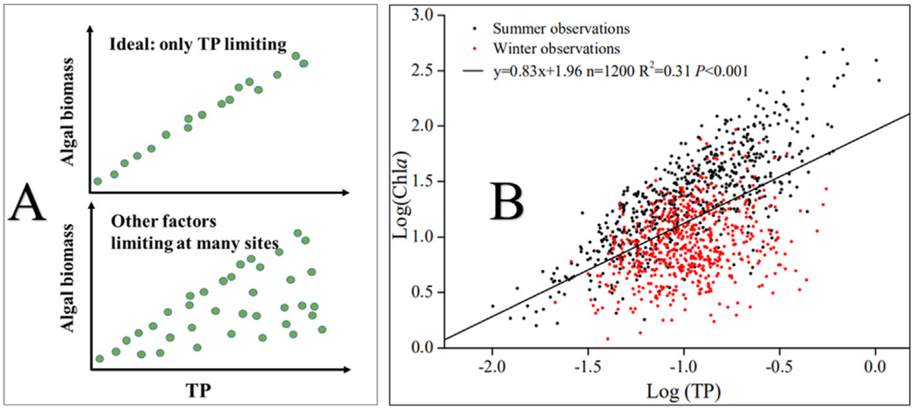

Fig. 1. Conceptual graph of the response of algal biomass to total phosphorus (Fig. 1A, modified from Cade and Noon, 2003). The response of Chlorophyll *a* (Chl*a*, $\mu g.L^{-1}$) to total phosphorus (TP, $mg.L^{-1}$) for summer and winter observations in a large shallow lake (Lake Taihu) from 2005 to 2015 (Fig. 1B, unpublished data). The solid black line was derived from ordinary least-squares regression.

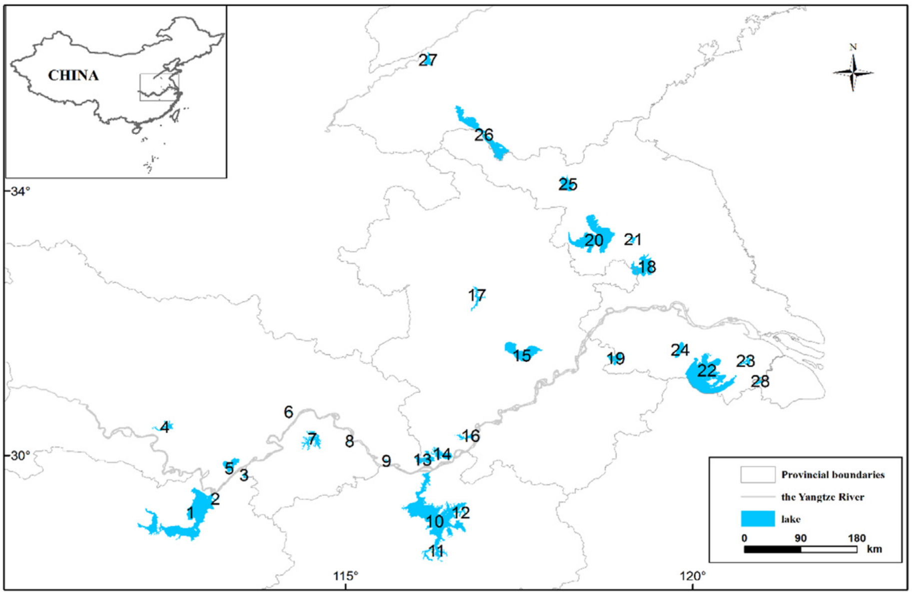

Fig. 2. The spatial distribution of our study lakes. Detailed information of each lake can be found in Table S1.

2.2. Data acquisition

Environmental variables and phytoplankton samples were collected from 28 shallow lakes (184 sites) in the eastern China plains during summer months 2018 (Fig. 2). Moreover, 27 shallow lakes (169 sites) were investigated in spring months 2018, and Shijiu Lake (number 19 in Fig. 2) was not sampled due to very low water depth. On each sampling site, water depth (WD) and Secchi depth (SD) were measured using a Speedtech SM-5 Portable Depth Sounder and Secchi disk, and macrophytes cover (MC) was estimated *in situ*. Water temperature (WT), pH and electric conductivity (EC) of the upper 0.5 m of the water column were measured with an YSI 6600 V2 multi-sensor sonde. Additional water samples were collected within 50 cm of the surface for chemical analysis in the laboratory. Total nitrogen (TN), total phosphorus (TP), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP) and Chl*a* were determined according to standard methods (APHA, 2012). The suspended solids in quantified water samples were collected on 0.70 μm porosity membrane filters (Whatman GF/F) with tared weight 1 (W1), and combusted in a muffle furnace (550 °C for 4 h) to determine ash-free weight. Inorganic suspended solids (ISS) were derived by subtraction from values for pre and post-filtered samples. Phytoplankton were sampled within 50 cm of the surface and fixed with Lugol's iodine solution (1%, V/V) followed by 48 h settling (APHA, 2012). Cell density was counted directly in a 0.1 ml counting chamber, using an Olympus BX53 microscope at × 400 magnification. The taxonomic identification of algae and the calculation of B_{Cyano} were conducted according to Hu and Wei (2007).

Lake retention times (LRT) were acquired from previously published data. For some lakes, LRT are readily available in the literatures (Wang and Dou, 1998; Cai et al., 2012; Liu et al., 2016). While for others, the LRT were calculated. Previous studies demonstrated that the ratio of average annual precipitation in the basin to lake volume was significantly correlated with LRT calculated the traditional way (i.e. by dividing lake volume by flow in or out of the lake). The equation was expressed as $y = 0.25x+25.38$ ($R^2 = 0.99$, $P < 0.001$, $n = 27$). Where y is the ratio of average annual precipitation in the basin to lake volume, and x represents LRT calculated by the traditional method, ranging from 18 to 13977 days (Shi et al., 2018). In most cases, average annual precipitation in the basin and lake volume could be obtained from the literature (Wang and Dou, 1998), but there remained four lakes in this study, including Wuchang East Lake (number 6 in Fig. 2), Wushan Lake (number 9 in Fig. 2), Zhu Lake (number 12 in Fig. 2), and Huangda Lake (number 14 in Fig. 2), for which the LRT values are not available. Hence, the four lakes were removed in the later difference analysis and generalized additive model analysis.

2.3. Statistical analysis

2.3.1. Difference analysis and regression models

The temporal differences between environmental variables and B_{Cyano} were tested using pared-sample T test. Both OLSR-derived and quantile regression models were used to explore relationships between B_{Cyano} and nutrient levels (TN or TP). The quantile regression model can be viewed as an extension of classical least squares estimation of conditional mean models to estimate an ensemble of models for several conditional quantile functions (Cade and Noon, 2003). Recognizing that certain undetermined factors may limit the accuracy of B_{Cyano} values estimated from nutrient levels (Cade and Noon, 2003; Carvalho et al., 2013), 95% quantile regression model was used to explore the response of cyanobacteria to nutrient enrichment under ideal conditions where cyanobacterial growth was limited primarily by TN (or TP). The difference analysis and OLSR-derived models were performed using SPSS 19.0, and the 95% quantile regression model were conducted with the *quantreg* package of R software.

2.3.2. Generalized additive model

The generalized additive model (GAM) is an analysis which assumes that the response variable for covariates depends on smoothing splines, rather than linear coefficients (Hastie and Tibshirani, 1987). In this study, GAM was used to identify the factors that significantly modulated TN (or TP) limitation of cyanobacterial growth in spring and summer. We assumed that the response of B_{Cyano} to TN (or TP) under ideal conditions in which cyanobacterial growth is limited primarily by TN (or TP) could be quantified using the 95% quantile regression model. Meanwhile, on a regional scale, other factors (e.g., LRT) may have a significantly modulating effect on the nutrient limitation of cyanobacterial growth, even during the growing season (Liu et al., 2016). As a result, the actual values of B_{Cyano} at a given TN (or TP) concentration may exhibit considerable difference compared to those derived from the 95% quantile regression model. This difference, deemed to reflect the total modulating effect of the other factors on the nutrient limitation of cyanobacteria (Fig. 3), was used as a response variable (y) in the GAM analysis.

In this study, we explored season-specific (spring and summer) responses of B_{Cyano} to nutrient levels (TN and TP). Theoretically, there are four distinctive response (y1-y4) variables for the analysis of GAM. y1 is the difference between the expected B_{Cyano} derived from 95% quantile regression at a given TN concentration and actual B_{Cyano} in spring; y2 is the difference between expected B_{Cyano} derived from 95% quantile regression at a given TP concentration and actual B_{Cyano} in spring; y3 is the difference between expected B_{Cyano} from 95% quantile regression at a given TN and actual B_{Cyano} in summer and y4 is the difference between expected B_{Cyano} from 95% quantile regression at a given TP and actual B_{Cyano} in summer.

To our knowledge, WD is closely related with sediment resuspension (Hamilton and Mitchell, 1996), thus it may interfere the nutrient-cyanobacteria relationships by indirectly influencing underwater light conditions, bioavailability of nutrient to phytoplankton (Lind, 1986; Havens et al., 2001). For similar reason, ISS content is likely to affect cyanobacterial response to nutrient (Dzialowski et al., 2011). As to LRT, previous studies showed that rapid flushing can directly inhibit the algal response to nutrient input (Reynolds, 2006; Liu et al., 2016). In addition, previous study showed that, in lakes with high N: P ratio, insignificant relationships between Chl*a* and TN have been observed, thus we hypothesize that nutrient limitation of cyanobacteria may also be modulated by LRT and N:P ratio (Nõges et al., 2008; Carvalho et al., 2011). As to biological variables, macrophytes is most likely to affect nutrient-cyanobacteria relationships (Nakai et al., 2000). Therefore, these five variables were considered as explanatory variables in GAM analysis to identify those which exerted significant influence on the nutrient-cyanobacteria relationships (i.e., variations in the total modulating effect). Limited multiple collinearity was observed between the five explanatory variables indicated by very low value of variance inflation factor (<2).

The analysis of GAM using all explanatory variables mentioned above were performed four times, namely y1~(WD + LRT + ISS + N:P + MC); y2~(WD + LRT + ISS + N:P + MC); y3~(WD + LRT + ISS + N:P + MC) and y4~(WD + LRT + ISS + N:P + MC), and $P < 0.05$ of an explanatory variable (e.g., LRT) indicated the flushing have significantly modulated the nutrient limitation of cyanobacteria. Moreover, the R^2-adjusted were also used to identify significant modulating factors in our study, the analysis of GAM was thus performed using each single explanatory variable. The estimated degrees of freedom (edf) used to determine the amount of smoothing for each explanatory variable, were automatically selected by the model fitting procedure, using restricted maximum likelihood (Hastie and Tibshirani, 1990). Before analysis of regression and GAM, values for B_{Cyano} and MC were $\log_{10}$-transformed with an additional small arbitrary constant of 0.001 to eliminate zeros before transformation and the remaining variables were $\log_{10}$ transformed straightforwardly. GAM was performed using the *mgcv* package of R software.

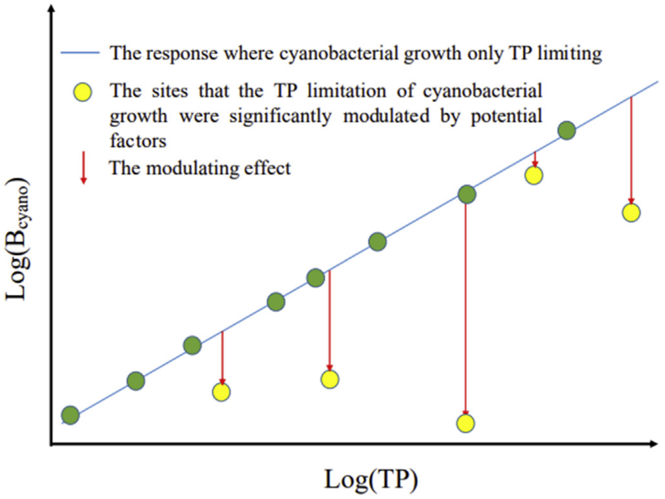

Fig. 3. Conceptual graph, showing how total phosphorus (TP) limitation of cyanobacterial biovolume (B_{Cyano}) was modulated by potential factors.

2.4. B_{Cyano} thresholds of recreational waters and drinking water source

In our study, the nutrient thresholds of shallow lakes were calculated based on cyanobacterial abundance targets established for recreational waters and drinking water source in the eastern China plains. In recreational waters, cyanobacterial cell densities of 20,000 and 100,000 cells.mL^{-1} are considered to pose a ‘low’ and ‘moderate’ risk to health, respectively, associated with symptoms such as skin irritations and gastrointestinal illness (Bartram et al., 1999; WHO, 2003). We adopted these thresholds and multiplied the cell densities by a typical cyanobacterial cell volume, assuming a spherical cell with a diameter of 5.7 μm, to achieve equivalent biovolumes of 2 mm^3.L^{-1} and 10 mm^3.L^{-1} (Bartram et al., 1999). As to drinking water source, thresholds of cyanobacterial cell densities were proposed by Burch (1993), Bartram et al. (1999) and again by House et al. (2004). In this most recent revision, thresholds of cell densities of 2000 and 5000 cells.mL^{-1} (the equivalent biovolumes of 0.2 mm^3.L^{-1} and 0.5 mm^3.L^{-1}) amount to ‘low’ and ‘moderate’ health risk for drinking water source and were used in the current study.

3. Results

3.1. Descriptive statistics of nutrient and B_{Cyano}

In spring, the WT of our study lakes ranged from 13.80 to 25.24 °C, with an average value of 20.63 ± 2.57 °C. Average values of TN, TDN, TP, and TDP in this season were 1.73 ± 0.91 mg.L^{-1}, 1.38 ± 0.83 mg.L^{-1}, 94.59 ± 68.70 μg.L^{-1} and 44.03 ± 32.29 μg.L^{-1}, respectively. In summer, significantly higher ($P < 0.001$) WT were observed, with average values of 30.31 ± 1.54 °C. TDN values were significantly ($P < 0.001$) lower than in spring, averaging 0.97 ± 0.65 mg.L^{-1}, while TP increased by about 30% from spring to summer ($P = 0.03$). No significant seasonal differences were observed in TN and TDP ($P > 0.05$), but the average B_{Cyano} increased considerably ($P < 0.001$), from 0.51 ± 2.16 mm^3.L^{-1} in spring to 3.14 ± 11.62 mm^3.L^{-1} in summer (Table 1). More details of the season-specific nutrient levels and B_{Cyano}, as well as other important variables (i.e., lake area, WD, LRT, SD, EC, ISS and Chl*a*) of each lake are provided in Table S1.

3.2. Regression analysis between B_{Cyano} and nutrient concentrations

A significant positive linear relationship was observed between $\log(B_{Cyano}+0.001)$ and log(TN), or log(TP), based on the 95% quantile regression model for both spring and summer datasets

Table 1
Statistical descriptions of water temperature (WT), total nitrogen (TN), total dissolved nitrogen (TDN), total phosphorus (TP), total dissolved phosphorus (TDP) and cyanobacterial bivolume (B_{Cyano}) in our study lakes. Seasonal differences in physicochemical variables and B_{Cyano} were tested using independent-samples T test or Mann-Whitney *U* test depending on the normality and homoscedasticity of the data.

Variables	Spring (n = 169)			Summer (n = 184)			*P*
	Max	Min	Mean ± SD	Max	Min	Mean ± SD	
WT (°C)	25.24	13.80	20.63 ± 2.57	33.98	25.64	30.31 ± 1.54	<0.001
TN ($mg.L^{-1}$)	5.71	0.41	1.73 ± 0.91	15.28	0.26	1.70 ± 1.44	0.49
TDN ($mg.L^{-1}$)	5.10	0.23	1.38 ± 0.83	3.65	0.18	0.97 ± 0.65	<0.001
TP ($\mu g.L^{-1}$)	397.0	23.0	94.6 ± 68.7	1417.5	14.3	120.3 ± 147.4	0.03
TDP ($\mu g.L^{-1}$)	233.4	6.7	44.0 ± 32.3	330.2	4.9	41.1 ± 48.6	0.70
B_{Cyano} ($mm^3.L^{-1}$)	20.00	0	0.51 ± 2.16	116.66	0	3.14 ± 11.62	<0.001

($P < 0.001$). These regression lines exhibited a relatively high determination coefficient (R^2 range from 0.49 to 0.52), implying that both TN and TP limit the maximum capacity of B_{Cyano} for the shallow lakes in spring and summer. As expected, a wedge-shaped scatter of points between log(B_{Cyano}+0.001) and log(TN), or log(TP) was observed in both two growing seasons. Meanwhile, OLSR-derived regression lines exhibited with low R^2. In addition, for most sites in both growing seasons, considerable differences were observed between actual B_{Cyano} values at a given TN (or TP) and those derived from the 95% quantile regression model (Fig. 4).

3.3. Identification of the modulating factors

For spring observations, the GAM using all selected explanatory variables explained 51.3% of deviance in y1 (R^2-adjusted = 0.45, n = 169) and 58.1% of deviance in y2 (R^2-adjusted = 0.52, n = 169),

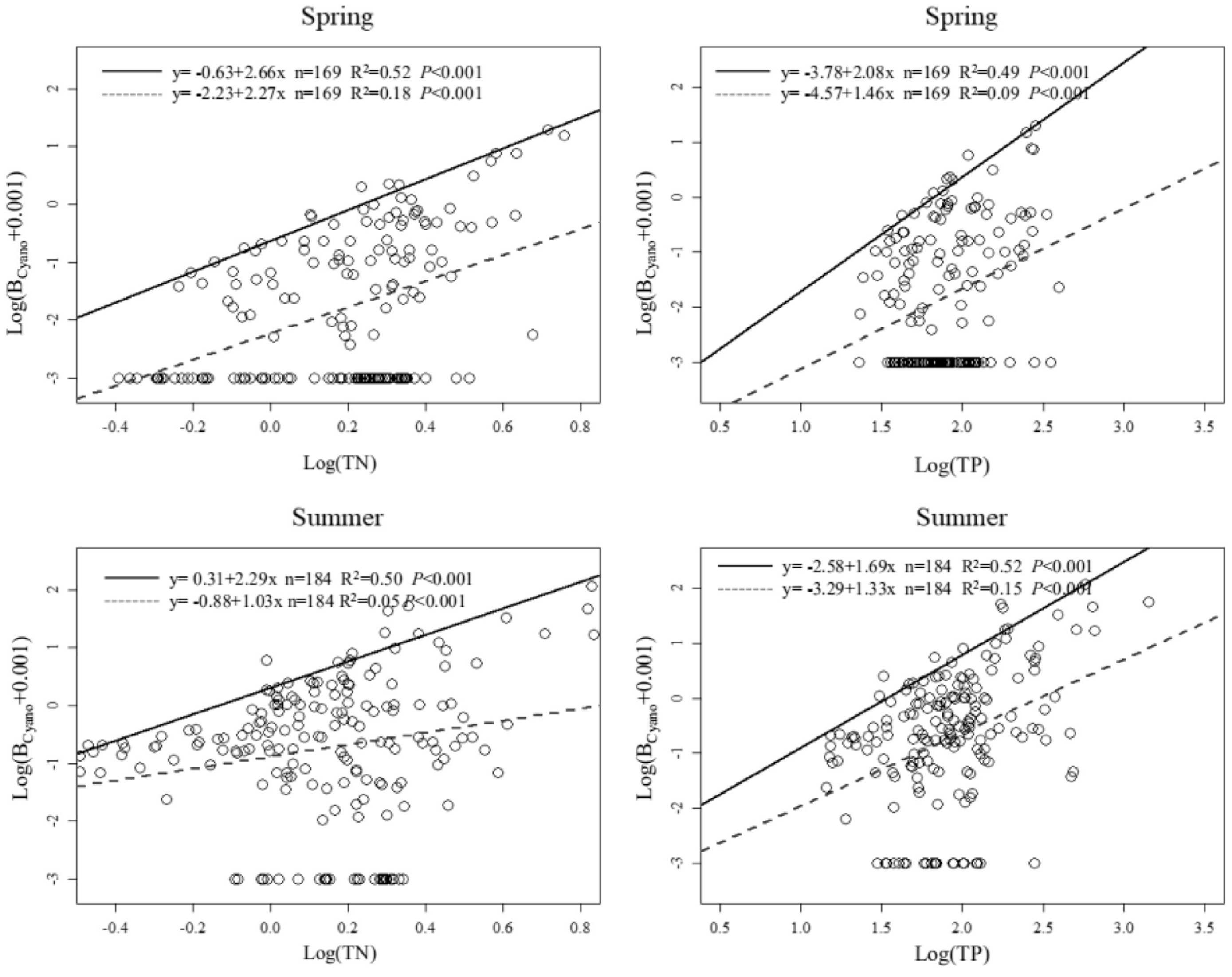

Fig. 4. The relationships between cyanobacterial biovolume (B_{Cyano}, $mm^3.L^{-1}$) and nutrient concentrations (total nitrogen, TN, $mg.L^{-1}$; total phosphorus, TP, $\mu g.L^{-1}$) derived from 95% quantile regression model (dark solid lines) and ordinary least-squares regression-derived model (blue dashed lines) for spring and summer observations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

respectively. Moreover, LRT, ISS, N: P ratio, and MC were identified as significant explanatory variables in both models ($P < 0.01$). In terms of summer observations, the GAM using all possible explanatory variables explained 68.7% of deviance in y3 (R^2-adjusted = 0.66, n = 184) and 58.1% of deviance in y4 (R^2-adjusted = 0.55, n = 184), respectively. Correspondingly, the results of GAM analysis indicated that LRT and N:P ratio were the significant modulating factors ($P < 0.05$) (Table 2).

The results of GAM analysis incorporating single explanatory variable for spring observations showed that the LRT accounted for the largest variations in y1 (R^2-adjusted = 0.30), followed by MC (R^2-adjusted = 0.06). For y2, LRT also exhibited largest accountability for its variations (R^2-adjusted = 0.28), N: P ratio and MC accounted for relatively low variations in y2, with the R^2-adjusted of 0.13 and 0.09, respectively. As to summer datasets, The results of GAM analysis incorporating single explanatory variable showed that more than half variations of y3 was accounted by LRT (R^2-adjusted = 0.64), and N:P ratio also accounted for relatively high variations in y2, with the R^2-adjusted of 0.25. For y4, LRT also exhibited largest accountability for its variations (R^2-adjusted = 0.33), followed by N: P ratio, with R^2-adjusted of 0.09 (Table 3). On the other hand, the sites with short LRT were generally associated with high values of y1, y2, y3, and y4. In contrast, higher values of y1 and y2 were observed in the sites with abundant MC. As to N: P ratio, on the whole, this variable exhibited positive relationships with y1 (or y3); in contrast, negative relationships were observed between N: P ratio and y2 (or y4). In addition, our results also showed linear negative correlation between ISS content and y1 (or y2) (Fig. 5).

4. Discussion

4.1. The nutrient limitation of B_{Cyano} and potential modulating factors

Studies exploring the responses of actual B_{Cyano} to nutrient enrichment mostly conducted on small number of lakes. As a result, the nutrient abundance was generally considered to be the single dominant limiting factor for cyanobacterial growth for these lakes, and robust nutrient-B_{Cyano} relationships (R^2: 0.50–0.70) derived from OLSR model were observed (Smith, 1985, 1990; Trimbee and Prepas, 1987; Dzialowski et al., 2011). In current region-scale study, however, OLSR-derived models between log(B_{Cyano}+0.001) and log(TN), or log(TP) exhibited very low R^2. In contrast, robust linear responses of log(B_{Cyano}+0.001) to log(TN), or log(TP) were observed using 95% quantile regression model for both spring and summer observations ($P < 0.001$). These results indicate that nutrient levels (TN and TP) set the maximal capacity of B_{Cyano} in our study lakes. On the other hand, actual B_{Cyano} at a given TN (or TP) were considerably lower than those derived from the 95% quantile regression model in both spring and summer, pointing to a significant modulation of cyanobacterial growth by other factors.

Table 2
The R^2-adjusted of generalized additive model incorporating all explanatory variables and the P values of each explanatory variable. $P < 0.05$ indicated that the explanatory variable exerted significant effect on the variations of response variable (y1-y4). y1 is the difference between expected cyanobacterial biovolume (B_{Cyano}) derived from 95% quantile regression at a given TN and actual B_{Cyano} for spring datasets; y2 is the difference between the expected B_{Cyano} derived from 95% quantile regression at a given TP and actual B_{Cyano} for spring datasets; y3 is the difference between the expected B_{Cyano} derived from 95% quantile regression at a given TN and actual B_{Cyano} for summer datasets and y4 is the difference between the expected B_{Cyano} derived from 95% quantile regression at a given TP and actual B_{Cyano} for summer datasets.

Response variable	R^2-adjusted	$P_{(WD)}$	$P_{(LRT)}$	$P_{(ISS)}$	$P_{(N:P)}$	$P_{(MC)}$
y1	0.45	0.553	<0.001	0.002	0.012	<0.001
y2	0.52	0.779	<0.001	<0.001	0.001	<0.001
y3	0.66	0.553	<0.001	0.56	0.029	0.568
y4	0.55	0.612	<0.001	0.27	<0.001	0.578

Table 3
The R^2-adjusted of generalized additive models incorporating each single explanatory variable.

Response variable	Log(WD)	Log(LRT)	Log(ISS)	Log(N:P)	Log(MC)
y1	0.03	0.30	0.02	0.03	0.06
y2	0.01	0.28	0.05	0.13	0.09
y3	0.03	0.64	0.06	0.25	0.01
y4	0.03	0.33	0.04	0.09	0.01

Based on the results of GAM analysis, we verified that LRT is the most significant factor modulating TN (or TP) limitations of cyanobacteria in our study lakes (Tables 2 and 3). In fact, it is well known that short LRT can suppress algal blooms by flushing biomass before the standing crop reaches the maximum permitted by the available nutrient (Dillon, 1975). However, a total absence of B_{Cyano} was recorded in the most study sites with LRT less than 35 days, such as Yangtze River-connected lakes (upper quartile = 0, Fig. 6), indicating that rapid flushing may especially suppresses cyanobacterial growth. Previous studies have demonstrated that small phytoplankton cells tend to dominate the community in rapid flushing lakes (Dickman, 1969; Bailey-Watts et al., 2010), providing a possible explanation for much greater controls of cyanobacteria, compared to fast-growing diatoms, small chlorophytes and flagellates. Overall, bloom-forming cyanobacteria have relatively slow reproductive rates, which in such cases are not sufficient to compensate for removal by flushing (Reynolds, 2006). In contrast to LRT, WD was not identified as significant explanatory variable in any of the GAM analysis (Table 2). This may be because the range of WD in the studied shallow lakes is small (Table S1).

Our results also indicated that N: P ratio has significantly modulated the nutrient limitation of cyanobacteria (Tables 2 and 3) both in spring and summer. Specifically, B_{Cyano} response to TN (or TP) would be closer to the lines derived from 95% quantile regressions with decrease of N: P ratio (or with increase of N: P ratio) (Fig. 5). These results are reasonable because both TN and TP co-limited cyanobacterial growth in our study lakes. Take the response of B_{Cyano} to TP as an example, increasing N: P ratio indicates that cyanobacterial growth is more likely to be limited by TP. Therefore, the B_{Cyano} theoretically exhibit higher utilization efficiency of TP, and the difference between actual B_{Cyano} and those derived from the 95% quantile regression models at a given TP would decrease with the increase of N: P ratio.

Furthermore, the GAM analysis (Tables 2 and 3) indicated that, in spring, MC exerted significant ($P < 0.05$) effect on the nutrient limitation of cyanobacteria, and boxplot analysis (upper quartile = 0, Fig. 6) revealed that cyanobacterial response to nutrient were considerably suppressed in the sites with abundant macrophytes. The mechanisms for this inhibitive effect may include shading, allelopathy and increasing grazing pressure as abundant macrophytes beds can provide shelter for large zooplankton (Nakai et al., 2000; Pakdel et al., 2013). By contrast, no macrophytes were observed in the majority of our sampling sites during summer due to the massive mortality of the spring predominating macrophytes species (i.e., *Potamogeton crispus*) (Cao et al., 2018). As a result, insignificant ($P > 0.05$) effect of MC on nutrient-cyanobacteria relationships were observed as the small range of MC.

In terms of non-algal turbidity, analysis of GAM also indicated that, in spring, cyanobacteria exhibited more sensitive response to TN (or TP) when higher ISS content were recorded. To our

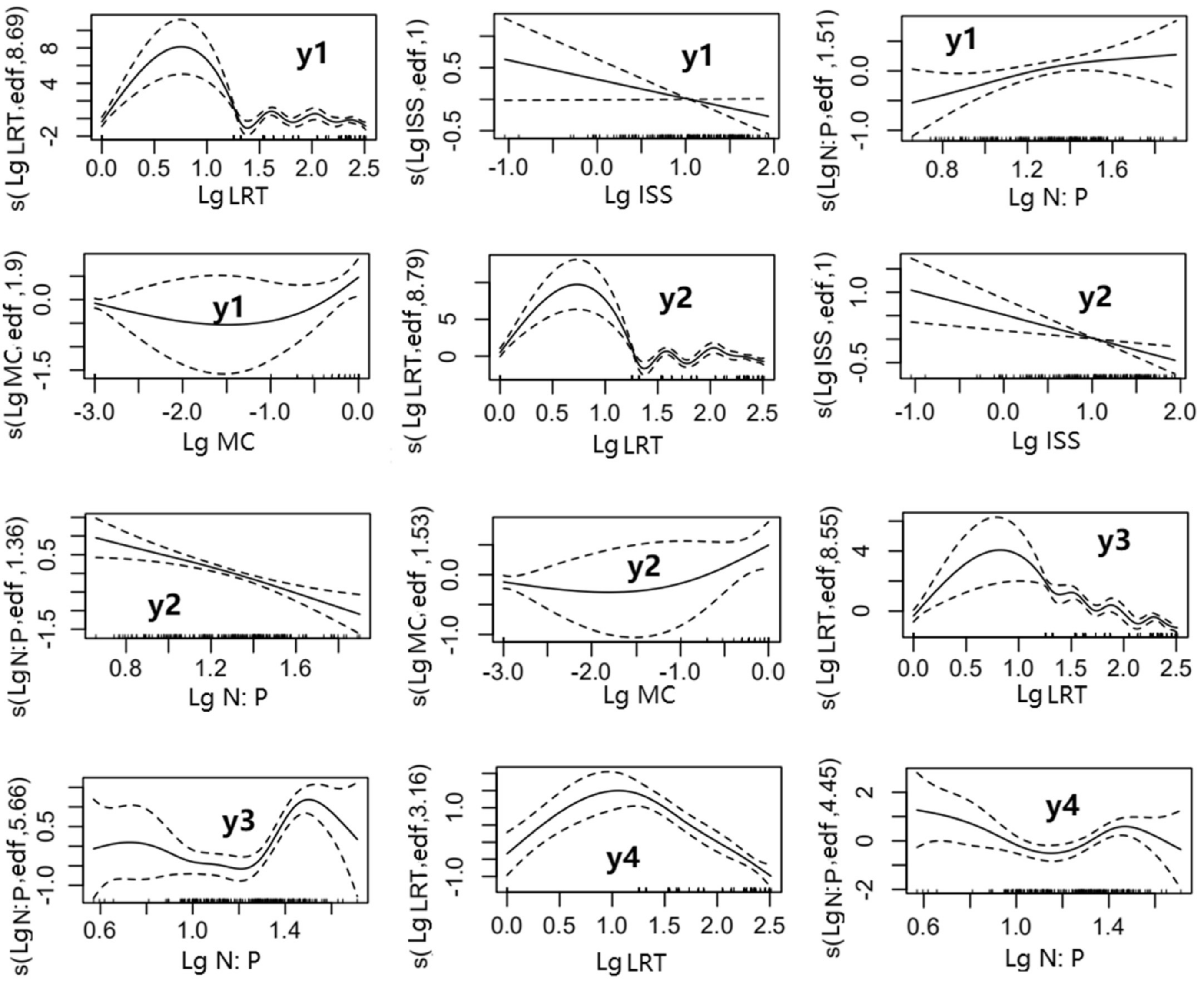

Fig. 5. Fitted plot of generalized additive models between response variables (y1-y4) and each significant explanatory variable. Each plot displays the response of y1-y4 to the individual explanatory variable with dashed lines indicate the standard errors, and edf represents the estimated degrees of freedom used to fit each variable. y1 is the difference between expected cyanobacterial biovolume (B_{Cyano}) derived from 95% quantile regression at a given TN and actual B_{Cyano} for spring datasets; y2 is the difference between the expected B_{Cyano} derived from 95% quantile regression at a given TP and actual B_{Cyano} for spring datasets; y3 is the difference between the expected B_{Cyano} derived from 95% quantile regression at a given TN and actual B_{Cyano} for summer datasets and y4 is the difference between the expected B_{Cyano} derived from 95% quantile regression at a given TP and actual B_{Cyano} for summer datasets.

knowledge, however, high ISS content can reduce water transparency (Brezonik et al., 2019), causing photoinhibition in cyanobacterial growth (Lind, 1986). Moreover, increased ISS often represents a high proportion of particulate nutrient with relatively low bioavailability (Havens et al., 2001; Chung et al., 2009). Correspondingly, previous studies have demonstrated that cyanobacterial biomass in sites with high ISS contents are less responsive to nutrient enrichment (Smith, 1990; Dzialowski et al., 2011). We suggest that the contrasting current results may be because that the sites with low ISS content were typically associated with abundant macrophytes. The influence of macrophytes may be sufficient to account for the larger difference between actual B_{Cyano} and that predicted for a given a TN (or TP) by the 95% quantile regression model in sites with lower ISS content in spring. Thus, ISS may, in essence, exert limited effect on nutrient-cyanobacteria relationships in shallow lakes. In contrast, as the decline of macrophytes (*P. crispus*) in summer, ISS alone was not identified as significant modulating factor because its values remained generally high and exhibited relatively small gradients in shallow lakes (Hamilton and Mitchell, 1996).

4.2. Implications for nutrient management

The fundamental question in nutrient (nitrogen and phosphorus) management is the extent to which variations will promote "good" ecological status or reduce the potential threat to human health (Poikane et al., 2019). Thus, the thresholds of B_{Cyano} for safe recreational waters and drinking water source were used to derive nutrient reduction targets. However, the wedge-shaped scatter points on a plot of B_{Cyano} and nutrient levels (i.e., TN and TP) indicate that the traditional binary regression lines based on OLSR-derived model are of limited relevance for nutrient management. Our results support that the 95% quantile regression model may be more suitable for exploring the essential response of

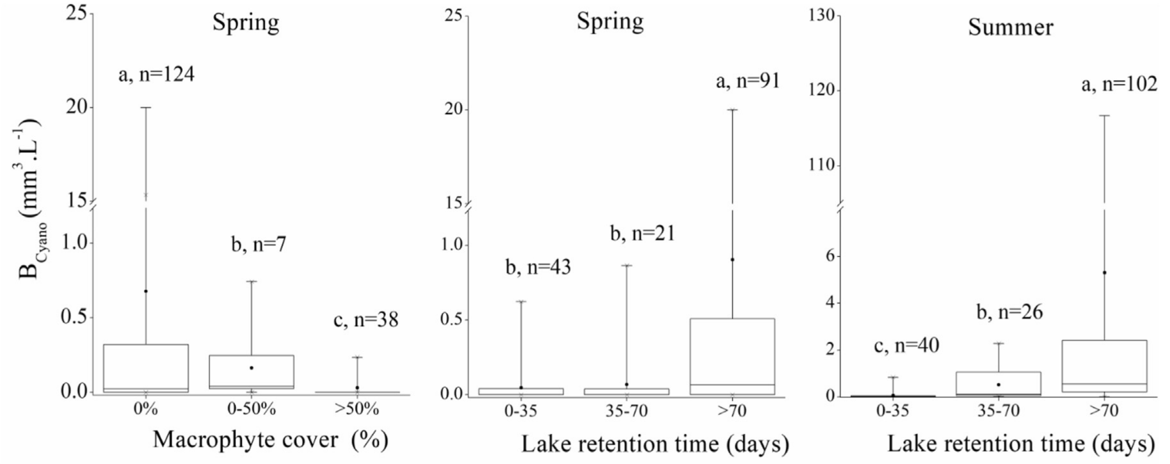

Fig. 6. Boxplot of between cyanobacterial biovolume (B_{Cyano}) in sites with different macrophytes cover and lake retention time.

B_{Cyano} to nutrient levels (Cade and Noon, 2003; Philips et al., 2019). In addition, the results using 95% quantile regression model demonstrated robust linear relationships between log(B_{Cyano}+1) and log(TN), or log(TP) in both spring and summer, suggesting that reductions in both N and P are needed to control cyanobacterial blooms in shallow lakes (Xu et al., 2014; Paerl et al., 2016). Thus, to ensure the safety for recreational waters and drinking water source of our study lakes, regional-specific and season-specific thresholds for TN and TP thresholds were established based these 95% quantile regression models (Table 4) (Bartram et al., 1999; WHO, 2003).

However, it should be noted that the generalized regional nutrient thresholds proposed above may be considered overly restrictive for some lakes. For individual lakes, the significant modulating factors may should also be taken into the consideration and establish type-specific nutrient thresholds (Carvalho et al., 2013; Zhang et al., 2015). It seems that we need to make subdivision of our lakes based on LRT, MC and N: P ratio because GAM analysis indicated all three factors have significantly modulated nutrient-cyanobacteria relationships on a regional scale, but the truth may be more nuanced. In our study, the sites with rapid flushing and abundant macrophytes were generally observed with the total absence of cyanobacteria (Fig. 6), indicating that the two factors can cause considerable difference between actual B_{Cyano} and those derived from the 95% quantile regression model for individual sites. However, if the sites with relatively short LRT (<100 days) and with macrophytes were removed (in this case, the effect of LRT and macrophytes on the nutrient-cyanobacteria relationships is theoretically limited), small difference between the actual B_{Cyano} and those derived from the 95% quantile regression model were observed (Fig. 7). These results indicate that, for individual sites, the modulating effect of N: P on the nutrient limitation of cyanobacteria seems not very eye-catching. In this condition, there is no need to subdivide lakes with the consideration of N: P ratio. Previous study indicate that in lakes with a LRT higher than 100 days, flushing is likely to have limited effect on cyanobacterial abundance (Reynolds, 2006). Thus, OLSR-derived models were further performed to estimated nutrient—B_{Cyano} relationships using the datasets with LRT>100 days and no macrophytes. As a result, the significant nutrient—B_{Cyano} relationships with relatively high R^2 were observed, especially for summer datasets (Fig. 7), and season-specific thresholds of TN and TP were further proposed for this lake type (Table 5). In contrast, in lakes with rapid flushing (most for the Yangtze-connected lakes in our study) or abundant macrophytes, where cyanobacteria are generally absent, or present in very low abundance, blooms control measures should focus more on significant these non-nutrient modulating factors. For the rest lakes, the current study may not go so far as to estimate nutrient-cyanobacteria relationship due to the relatively small sampling size after lake subdivision and further research may be needed in order to obtain corresponding nutrient reduction targets. In addition, we emphasize that the summer thresholds proposed in current study have more practical significance because, for a large lake, it is difficult to significantly reduce the nutrient levels in short time.

5. Conclusion

In current study, the response of B_{Cyano} to nutrient of shallow lakes in the eastern China plains were examined. We demonstrated that, at regional scale, nutrient limitation of cyanobacteria was seriously modulated by other factors. In such condition, classical

Table 4
Regional-specific thresholds of total nitrogen (TN), total phosphorus (TP) concentrations for shallow lakes in the eastern China plains to maintain acceptable cyanobacterial biovolume (B_{Cyano}). B_{Cyano} of 2 mm^3.L^{-1} and 10 mm^3.L^{-1} (0.2 mm^3.L^{-1} and 0.5 mm^3.L^{-1}), considered to represent ‘low’ and ‘moderate’ health risk, respectively, in lakes used for recreational waters (drinking water source). TN and TP thresholds are obtained from season-specific regression lines based on 95% quantile regression model between B_{Cyano} and nutrient (TN and TP) for our study lakes.

Season	Usage	% exceeded	Health risk	TN (mg.L^{-1})	TP (μg.L^{-1})
Spring	Recreational waters	5%	Low	2.24	91.6
Spring	Recreational waters	5%	Medium	4.10	198.6
Summer	Recreational waters	5%	Low	0.99	50.7
Summer	Recreational waters	5%	Medium	2.00	131.3
Spring	Drinking water source	5%	Low	0.94	30.3
Spring	Drinking water source	5%	Medium	1.33	47.1
Summer	Drinking water source	5%	Low	0.36	13.0
Summer	Drinking water source	5%	Medium	0.54	22.3

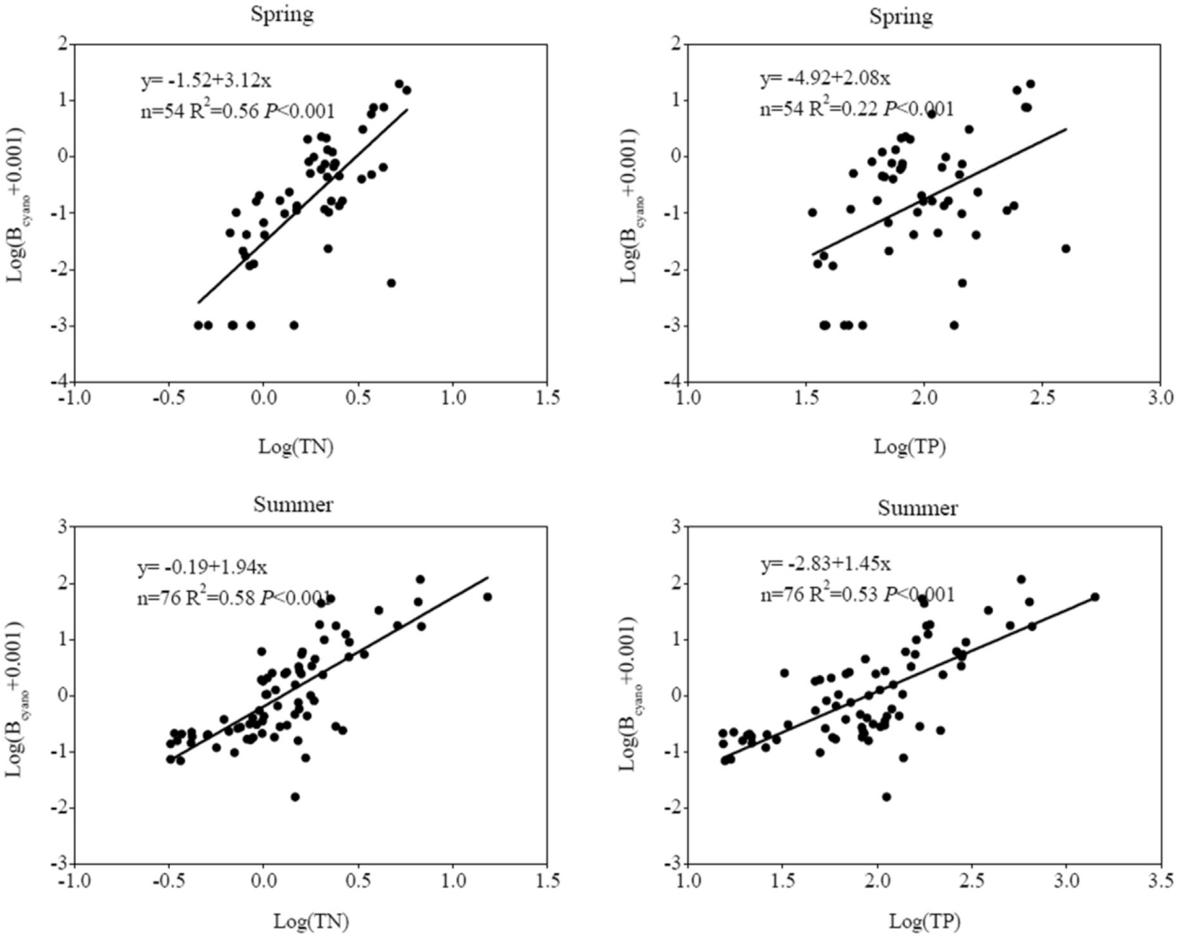

Fig. 7. The relationships between cyanobacterial biovolume (B_{Cyano}, $mm^3.L^{-1}$) and nutrient concentrations (total nitrogen, TN, $mg.L^{-1}$; total phosphorus, TP, $\mu g.L^{-1}$) for shallow lakes with lake retention time longer than 100 days and no macrophyte derived from the ordinary least-squares regression model for spring and summer observations.

Table 5

Season-specific thresholds of total nitrogen (TN), total phosphorus (TP) concentrations for shallow lakes with lake retention time longer than 100 days and no macrophyte in the eastern China plains to maintain acceptable cyanobacterial biovolume (B_{Cyano}). B_{Cyano} of 2 $mm^3.L^{-1}$ and 10 $mm^3.L^{-1}$ (0.2 $mm^3.L^{-1}$ and 0.5 $mm^3.L^{-1}$), considered to represent ‘low’ and ‘moderate’ health risk, respectively, in lakes used for recreational waters (drinking water source). TN and TP concentrations are obtained from season-specific regression lines based on ordinary least-squares regression-derived model between B_{Cyano} and nutrient (TN and TP) for our study lakes.

Season	Usage	Health risk	TN ($mg.L^{-1}$)	TP ($\mu g.L^{-1}$)
Spring	Recreational waters	Low	3.83	323.7
Spring	Recreational waters	Medium	6.42	701.7
Summer	Recreational waters	Low	1.79	144.3
Summer	Recreational waters	Medium	4.11	437.9
Spring	Drinking water source	Low	1.83	107.0
Spring	Drinking water source	Medium	2.46	166.2
Summer	Drinking water source	Low	0.55	29.5
Summer	Drinking water source	Medium	0.88	55.5

OLSR-derived models are ill-suited to explore the essential nutrient-B_{Cyano} relationships, and it might be more appropriate to regard nutrient as an upper limit for B_{Cyano}. For our studies lakes, robust nutrient-B_{Cyano} relationships were observed using 95% quantile regression model, we therefore used these relationships to establish regional-specific TN and TP reduction targets of the shallow lakes. On the other hand, regional-specific nutrient thresholds may be overly restrictive for some lakes. For example, Yangtze River-connected lakes are not susceptible to cyanobacterial blooms under nutrient enrichment process due to rapid flushing. Thus, for individual lake management, it is advisable that nutrient reduction targets should be considered in relation to other important modulating factors (e.g., lake retention time). In this study, quantile regression model and generalized additive model were used together to explore potentially significant modulating factors, of which lake retention time, macrophytes cover and N:P ratio were identified as most important. The subdivision of our studied lakes was performed based on the most significant non-nutrient modulating factors. As expected, robust nutrient—B_{Cyano} relationship were observed in a specific lake type (shallow lakes with retention time>100 days and no macrophyte). Given the fact that the nutrient management in many countries, including China, are based on “one size fits all” policy, a practice which may easily lead to over or under-protection in individual lakes. Thus, nutrient thresholds proposed in our study may provide essential information for cost-effective eutrophication management of shallow lakes both in the eastern China plains and in elsewhere with similar climatic background. On a broader scale, it may be not rare to see that regional scale nutrient-cyanobacteria relationships were affected by other modulating factor, thus the approaches and findings of this study can also serve as a valuable reference to develop reasonable nutrient reduction targets for lakes in other ecoregions with different climatic conditions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2020.115806.

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