Storm and floods increase the duration and extent of phosphorus limitation on algal blooms in a tributary of the Three Gorges Reservoir, China

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ABSTRACT

Excessive anthropogenic nutrient input has resulted in eutrophication and algal blooms which have severely impacted the function and sustainability of aquatic ecosystems, underscoring the need to implement nutrient management strategies. It was assumed that the increasing rainfall during flood season would affect the stoichiometric ratio of total nitrogen (TN): total phosphorus (TP), driving the nutrient limitation of algal growth. In order to test this concept and explore corresponding nutrient management strategies, nutrient addition bioassays were carried out in Xiangxi Bay, one of the largest tributaries of the Three Gorges Reservoir (TGR), China. Results indicated that nutrient limitation on algal growth fluctuated from nitrogen (N) to phosphorus (P) limitation. N limitation dominated in the early flood season. However, the reduction of dissolved P, accompanied with an increase of TN: TP caused by an increase in extreme rainfall events intensified P limitation throughout the bay. Then P limitation was alleviated due to the reduction of rainfall and the process of impoundment after the flood season. The variation of TN: TP caused by the increasing of rainfall and flooding could be the main driving factor of the nutrient limitation shift in aquatic ecosystems mainly affected by external nutrient inputs. Nutrient dilution and enrichment bioassays showed that TN and TP concentration thresholds should be targeted at below 0.55 mg/L and 0.057 ~ 0.064 mg/L respectively, to limit the growth of algae and maintain chlorophyll *a* below 30 μg/L. Dual nutrient (N & P) reductions were required for long-term bloom mitigation in the entire basin. This study provided a scientific basis for a nutrient management strategy to combat eutrophication and reduce algal bloom potentials in the tributaries of the TGR. We recommend that long-term determinations of nutrient limitation and nutrient threshold will be needed to control algal growth, considering future anticipated changes in land use, population density and the impacts of climate change.

1. Introduction

It is well known that excessive anthropogenic nutrient loading has promoted eutrophication and the expansion of algal blooms on a global scale (Smith 2003; Glibert and Burford 2017; Huisman et al., 2018; Ho et al., 2019), seriously threatening the water quality security and sustainable development. Nitrogen (N) and phosphorus (P) are important nutrients mediating algal growth and bloom proliferation; therefore, controlling the input of N and P is considered to be an essential and effective way to mitigate these symptoms of water quality degradation (Lewis et al., 2011; Xu et al., 2015a; Shan et al, 2020). Accurate assessment of nutrient limitation of algal growth in aquatic ecosystems is of fundamental importance in formulating nutrient input controls (Liang et al., 2018; Liu et al., 2021). Determining nutrient limitation of algal growth in the aquatic ecosystem is challenging because of the highly dynamic interactions of varying nutrient inputs, rates of internal nutrient cycling and seasonal shifts in algal community composition and bloom potentials. Furthermore, we are now aware that there is a great deal of variability in the long-held paradigm that P limitation prevails in freshwater while N limitation characterizes marine systems (Hecky and

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Kilham 1998; Kagami et al., 2013; Ly et al., 2014; O'Donnell et al., 2017), with co-limitation (such as N&P, N&Fe, P&Si) increasingly reported along the freshwater to marine continuum (Guildford and Hecky 2000; Pilkaityte and Razinkovas 2007; Paerl et al., 2011, 2016; Browning et al., 2017). In addition, nutrient limitation of aquatic ecosystem reflects seasonality in weather, hydrology, and algal community successional patterns (Xu et al., 2015b; Wang et al., 2019; Zhang et al., 2021), which translate into dynamic changes in nutrient concentration and stoichiometric ratio, especially when considering climatic changes taking place (warming, increasing rainfall, increasing water temperature etc.), particularly the increasing extreme rainfall and flooding which has been reported to play an important role in the dynamic change of nutrient (Chaffin et al., 2014; Woolway and Merchant 2019; Radbourne et al., 2020; Díaz-Torres et al., 2021). It is therefore necessary to clarify the impacts of variations in nutrient inputs and utilization, as well complex hydrologic and biogeochemical interactions on a seasonal scale (such as flood season) in order to assess nutrient limitation conditions on appropriate temporal and spatial scales.

Effective nutrient management based on nutrient limitation knowledge has been implemented in some ecosystems, for example, P control was initially deemed successful in reducing the eutrophication in some freshwater systems (Schindler 2012), while more recent analyses indicate that dual N and P input controls will be needed to controlling eutrophication and harmful algal blooms along the freshwater to marine continuum (Paerl et al., 2016, 2018; Wurtsbaugh et al., 2019). In addition, a season-specific nutrient reduction strategy has been proposed, based on seasonal nutrient limitation characteristics (Ma et al., 2015; Tong et al., 2021). However, a long-term scientific and sustainable nutrient management strategy requires clear control targets, which means establishing a nutrient threshold standard to protect the ecosystems from excessive nutrient enrichment and algal blooms (Chambers et al., 2012; Shan et al., 2020; Kim et al., 2021). The nutrient threshold can be determined by the response of algal growth to a nutrient gradient, with the assumption that algal growth can be controlled when the nutrient concentration is below the threshold (Xu et al., 2015a; Cao et al., 2016), thereby providing managers with more specific management objectives. However, developing thresholds is a challenge in aquatic ecosystems experiencing climatic (temperature, hydrology) changes simultaneously with changes in nutrient inputs.

The Three Gorges Reservoir (TGR) (Fig. 1) is the largest reservoir in China, algal blooms have proliferated in its tributaries due to the increasing nutrient loads upon its impoundment in 2003 (Zeng et al., 2006; Yang et al., 2018b; Zhang et al., 2020a). Cyanobacterial blooms have become more prevalent especially in the flood season with relatively higher temperature, more stable stratification and higher light intensity (Wang et al., 2011; Xu et al., 2021). Previous studies have reported nutrient limitation of algal growth in the TGR. For instance, these studies reported P limitation on the mainstream and N limitation on Xiangxi Bay (Zhou et al., 2009), while more recent studies showed P limitation of algal blooms in the Pengxi River throughout the four seasons and in Xiangxi Bay in autumn (Li et al., 2019; Huang et al., 2020). These studies also showed temporal and spatial variation in nutrient limitation in the tributary bays. However, these studies will not work in reflecting dynamic varying process of nutrient limitation of algal growth, nor did these studies establish nutrient-growth thresholds needed to control eutrophication algal blooms under the current pressure of human nutrient enrichment and climate change. We hypothesized that the increasing rainfall during flood season would affect the

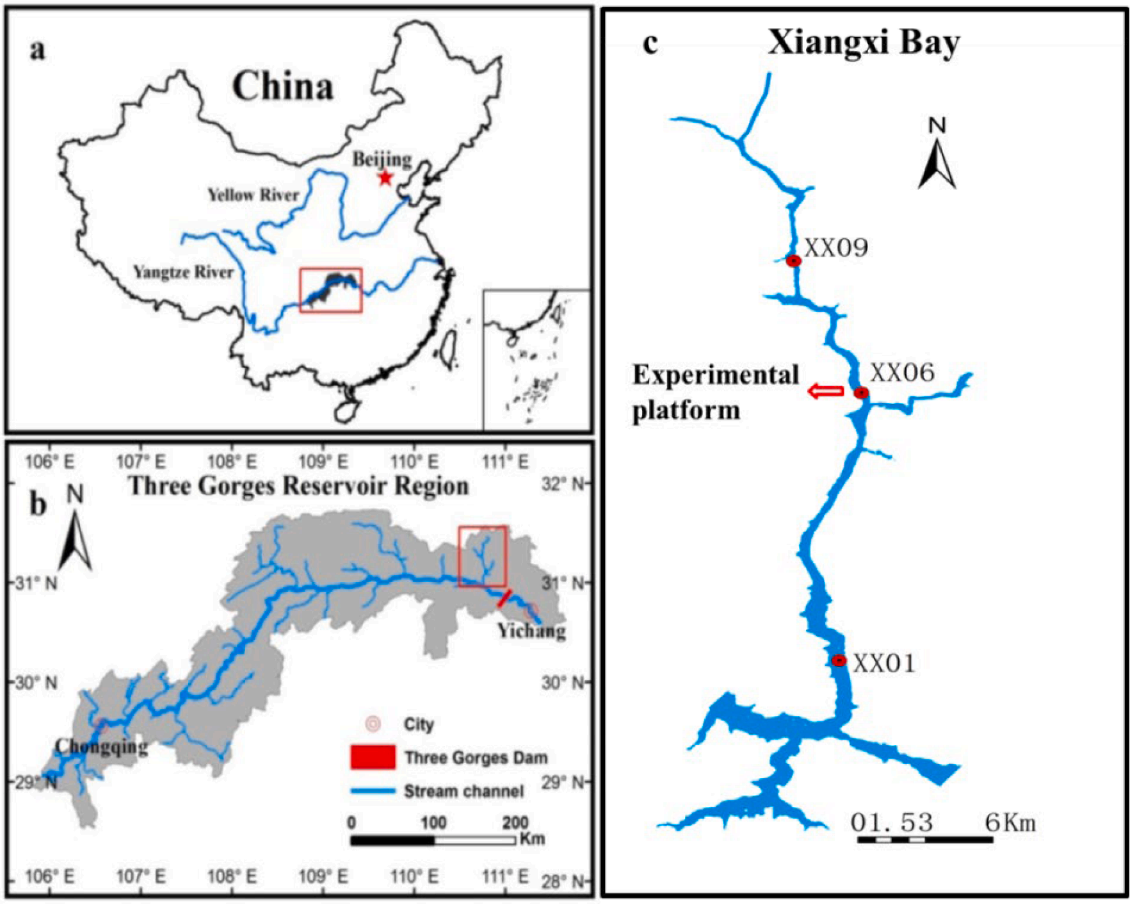

Fig. 1. Study area and sampling sites. a, the location of the TGR Region in China; b, the location of Xiangxi River in the TGR Region; c, distribution of sampling sites in Xiangxi Bay, an experimental platform has been built in XX06 and the *in situ* algal bioassays were incubated in this site.

TN: TP and driving the shift of nutrient limitation of algal growth. Therefore, we chose a representative tributary on the TGR for deploying nutrient manipulation bioassays, nutrient dilution and enrichment bioassays during flood season, the objectives were to: 1) explore the spatio-temporal variation of nutrient limitation on algal blooms during the flood season. 2) clarify the impact of increasing extreme rainfall and flood on the nutrient limitation characteristic of algal growth. 3) determine the response of algal growth to various nutrient regimes thus determine the nutrient thresholds needed to control algal growth on the increasing rainfall condition. The study could help to provide the technical basis for a nutrient management strategy.

2. Materials and methods

2.1. Study area and sampling sites

The Xiangxi River is located in the upstream of the Three Gorges Dam (TGD) (Fig. 1) and its mouth is about 32 km away from the TGD. The length of its mainstream is 94 km, and the basin area is 3183 km^2, located in a subtropical continental monsoonal climate. Rainfall varies widely with an annual average rainfall of about 1100 mm (Song et al., 2017), the 'flood season' is mainly concentrated from June to mid-September (Xiong et al., 2018; Wu et al., 2021), accounting for about 60% of the annual rainfall. The average annual upstream inflow is 40.18 m^3/s. A permanent backwater area was formed within a range of about 27 km from the estuary in Xiangxi Bay due to the impoundment of the TGR at the water level of 145 m, and the countercurrent area was about 40 km at 175 m, forming stratified density currents intruding into the bay from the mainstream. This has an important influence on water temperature and other hydrodynamic conditions of the Xiangxi Bay (Ji et al., 2017).

According to the characteristics of Xiangxi Bay, three sampling sites (XX01, XX06, XX09) were established from the river mouth to the upstream, XX01 in the downstream and close to the river mouth (about 3 km), XX06 in the middle reach and XX09 in the upstream and near the terminal of the permanent backwater area of the Xiangxi Bay respectively (Fig. 1). Our experimental site where the *in situ* bioassays were deployed was located in the middle reach of Xiangxi Bay (XX06), which is not only affected by mainstream invasion but is also a high-risk area for algal blooms (Yang et al., 2018b).

2.2. Experimental methods

(1) Nutrient addition bioassays method for nutrient limitation of temporal and spatial variation

Nutrient addition bioassays were implemented every half a month to one month from June to September in 2020 to determine nutrient limitation of phytoplankton growth during the flood season. Water samples were collected from XX06 site (0.2 m below the water surface) and dispensed in a large plastic container (about 280 L), thoroughly mixed and 4 L was dispensed into 5 L acid-cleaned polyethylene, chemically inert, 85% PAR transparent Cubitainers which were rinsed with river water before dispensing. Six treatments were set up and 10 mg/L sodium bicarbonate ($NaHCO_3$) was supplied for each treatment including the control treatment to avoid inorganic carbon limitation. Three parallel samples were set up for each treatment (three replicates), and the methodology and procedures for deployment of the *in situ* bioassays conducted following Paerl and Bowles (1987), Xu et al. (2010), and Huang et al. (2020).

Six different treatments were administered (Table 1): no nutrient addition (Control); N1 addition (+NO_3-N); N2 addition (+NH_3-N); P addition (+P); N1 and P addition (+N1 + P); N2 and P addition (+N2 + P). N1 was added as KNO_3, N2 was added as NH_4Cl, with the concentration of N at 1.00 mg/L. P was added as K_2HPO_4, at a P concentration of 0.10 mg/L. The concentrations added were determined according to the nutrient concentration in the field (Huang et al., 2020). After nutrient additions, the Cubitainers were incubated *in situ* to maintain the natural light and water temperature of the natural river, while covered with one neutral density screen to minimize photoinhibition. The day of sampling in the field, nutrient additions and cultivation started was marked as Day0. The days of incubation (marked as Day + number) were determined according to the growth of algae including the initial Chl-*a* at the beginning of the bioassays. The entire bioassay was held to at most 3–5 days to reduce the potential container effects (Xu et al., 2015a). Subsamples were collected for analysis of chlorophyll *a* (Chl-*a*) at 1–2 day intervals. Initial water samples in bioassays were also collected for analysis of Chl-*a* and initial condition of algal species compositions.

Table 1
Experimental treatments administered for nutrient limitation variation.

sodium bicarbonate	Nutrient addition	Sampling site
+10 mg/L $NaHCO_3$ each	①Control (no nutrient additions) ②1.0 mg N/L + KNO_3 (N1) ③1.0 mg N/L + NH_4 Cl (N2) ④0.1 mg P/L + K_2HPO_4 (P) ⑤+N1 + P ⑥+N2 + P	XX06

Water samples were collected from XX01, XX06 and XX09 respectively for nutrient limitation bioassays at different regions on August 2nd, 2020, and four different treatments were administered showed in Table 2. The procedures for deployment were the same as the nutrient addition bioassays described above.

(2) Nutrient dilution and enrichment bioassays method

Nutrient dilution bioassays during flood season were carried out during August 2020. Water samples were collected on XX06 and diluted 30% to 70%, respectively with the main ion solution (MIS) containing Si, Ca, Mg, Na, etc. (Table S1 and S2) which represented the water of Xiangxi Bay without N and P. Then the N and P were added separately or simultaneously to different treatments (Table S2), the no dilution (0%) treatments were set as control. The remaining methodological procedures were the same as nutrient addition bioassays to determine the algal growth limiting factors and the threshold for eutrophication abatement based on the response of algae to different nutrient concentrations (Xu et al., 2015a). Cubitainers were incubated from August 7th (Day0) – August 10th (Day3). Water samples were collected for analysis of Chl-*a*, TN and TP at 1–2 day intervals according to the growth of algae including the initial conditions at the beginning of the bioassay.

Another enrichment bioassay during flood season was carried out in August 2020 to determine the response of algae growth to different N and P gradients. Water samples were collected at XX06, three replicates for each treatment and the gradient concentrations of N (added as KNO_3) were set as 0, 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 mg/L with enough P (0.2 mg/L); the gradient concentrations of P (added as K_2HPO_4) were set as 0, 0.005, 0.01, 0.02, 0.04, 0.06 and 0.08 mg/L with enough N (2.0 mg/L). The nutrients added daily over the first six days, and the culture cycle was ten days, water samples were collected every day for analysis of Chl-*a* to calculate growth rates, especially the maximum growth rate for each nutrient gradient during the experiment. Then establish the response curves of phytoplankton growth kinetics using the Monod equation to determine the nutrient threshold (Xu et al., 2015a). The algal growth could be controlled when the nutrient concentration is below the

Table 2
Experimental treatments administered for nutrient limitation at different regions.

sodium bicarbonate	Nutrient addition	Sampling site
+10 mg/L $NaHCO_3$ each	①Control (no nutrient additions) ②1.0 mg N/L + KNO_3 (N1) ③0.1 mg P/L + K_2HPO_4(P) ④+N1 + P	XX01, XX06, XX09

threshold.

The growth rate (μ) of each treatment was calculated according to the modified exponential growth equation (Xu et al., 2015a).

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

Where X_1 is the concentration of Chl-*a* at the beginning of a certain interval (T_1), and X_2 is the concentration of Chl-*a* at the end of a certain interval (T_2), T_2 and T_1 represented the time interval.

2.3. *Water sample collection and analysis*

The samples from the nutrient addition bioassays were collected at 1–2 day intervals according to the experimental method. Daily surface samples in the field were collected at XX06 site from June to September in 2020 to determine the ambient TN, TP and Chl-*a* for chemical and biological conditions analysis during the flood season. Daily surface samples collection at XX06 site for TN, TP measurements were continued until October to track the variation of TN, TP after the flood season. TP, TN and Chl-*a* were measured using spectrophotometric measurements following China's national standard method (Wang et al., 2002).

Data on daily rainfall in Xingshan Site were sourced from Meteorological Data Network of China, the inflow of Xiangxi Bay were sourced from the official website of Bureau of Water Resources and Lakes of Yichang (http://shj.yichang.gov.cn), and the data of daily water level (Wusong datum) and inflow in the mainstream were sourced from the official website of China Three Gorges Corporation.

2.4. *Statistical analysis*

Each set of data in the experiments was conducted in triplicate and the analytical result was the mean ± standard deviation (SD) of three measurements. Statistical significance (5 % level of significance) between different experimental treatments was analyzed using one-way analysis of variance (ANOVA). Post hoc multiple comparisons were performed by Least Significant Difference (LSD) ($p < 0.05$) after normal distribution test (Shapiro-Wilk) and homogeneity of variance test (Levene). Post hoc multiple comparisons were performed by Tamhane's T2 if not satisfied the homogeneity of variance. The response curves of phytoplankton growth kinetics were established by nonlinear regression fitting on the basis of Monod equation. Statistical analysis was implemented using Origin 2021 and IBM SPSS Statistics 22.

3. Results

3.1. *Characteristics of hydrological, chemical, and biological conditions during flood season*

The main sources of water in Xiangxi Bay are intrusions from the mainstream, upstream inflow and rainfall confluence. Average value of monthly rainfall over ten years (2007–2016) and monthly rainfall in 2020 are showed in Fig. 2. The rainfall during the flood season accounted for about 60% of the annual rainfall. The daily inflow, water level in the mainstream and inflow, rainfall in the upper reaches of Xiangxi Bay during the flood season in 2020 are showed in Fig. 3. Five serial floods were entering the TGR during the flood season and the inflow on the TGR of the fifth flood reached the maximum (about 75000 m^3/s) on August 20th. The variation of daily water level was closely related to the inflow in the mainstream, the lowest water level was 145.27 m before the formal flood season, due to the increase of rainfall and inflow, the water level in the mainstream began to rise at the end of June. Subsequently, it showed a relatively lagged fluctuation with the inflow with a maximum of 167.48 m and a minimum of 153.26 m. Local rainfall during the flood season is mainly concentrated in June and July, and the inflow was strongly affected by the rainfall in the upper reaches of Xiangxi Bay, varying from 11 to 370 m^3/s.

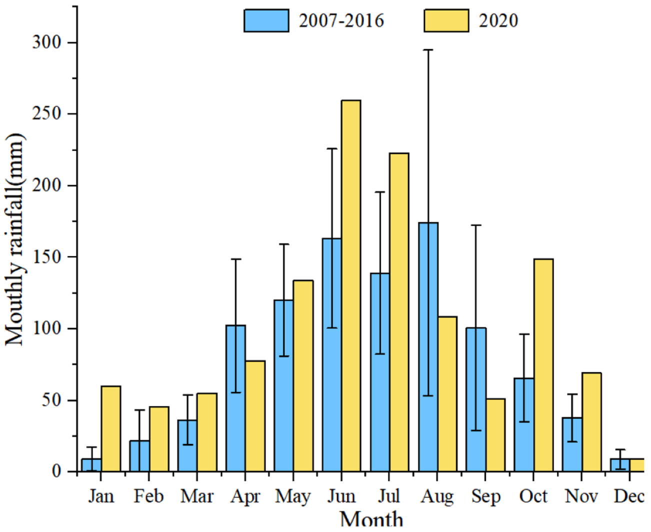

Fig. 2. Average value of monthly rainfall over ten years (2007–2016) and monthly rainfall in 2020. Error bars represent ± SD of monthly rainfall from 2007 to 2016.

The daily concentration of TN and TP (Fig. 4a) in the field varied greatly during flood season while relatively stable in October in 2020. The TN ranged from 0.645 to 3.441 mg/L with an average value of 1.760 mg/L. The TP fluctuated greatly from an oligotrophic concentration of 0.013 mg/L to hypereutrophic concentration of 0.142 mg/L with an average value of 0.060 mg/L. The TN increased in early July, higher than the average value, and fluctuated steadily on other periods, while the TP showed a decreasing trend from June to September as a whole and increased in October (Fig. 4c). The TN: TP increased from June to July, remained at a relatively high level from July to September, and decreased in October (Fig. 4d). The concentration of Chl-*a* (Fig. 4b) represented phytoplankton biomass, and 30 μg/L was used as the threshold for an algal bloom in the tributaries of the TGR (Yao, 2013). The daily Chl-*a* varied from 3.60 to 115.26 μg/L with a large fluctuation above and below the bloom threshold in June and July. The Chl-*a* varied from 20.23 to 112.68 μg/L during the August to mid-September, basically in a state of persistent algal bloom, and then the Chl-*a* eventually began to decrease from the mid-September.

3.2. *Characteristic nutrient limitation of phytoplankton growth in flood season*

3.2.1. *Variation in nutrient limitation on phytoplankton growth in flood season*

The phytoplankton biomass (Chl-*a*) in nutrient addition bioassays during flood season are shown in Fig. 5. A total of six bioassays were conducted from June to September in 2020. Treatments with N2 addition (N2 and N2 + P) led to higher Chl-*a* in the bioassay began on June 18th (Fig. 5A). Treatments with P addition (P, N1 + P and N2 + P) showed a significantly ($p < 0.05$) higher Chl-*a* than others, and Chl-*a* in N2 + P combined addition led to highest production in early July (Fig. 5B). Responses of Chl-*a* to P additions were both significantly ($p < 0.05$) higher than treatments without P addition in the bioassays from July 19th to September 7th (Fig. 5C, D, E). In late September, treatments with N2 addition resulted in significantly ($p < 0.05$) higher Chl-*a* in prophase of cultivation. P-alone/N2-alone addition treatments resulted in significantly ($p < 0.05$) higher Chl-*a* than N1-alone addition and control at the end of cultivation, and N2 + P combined addition treatment led to highest Chl-*a* (Fig. 5F).

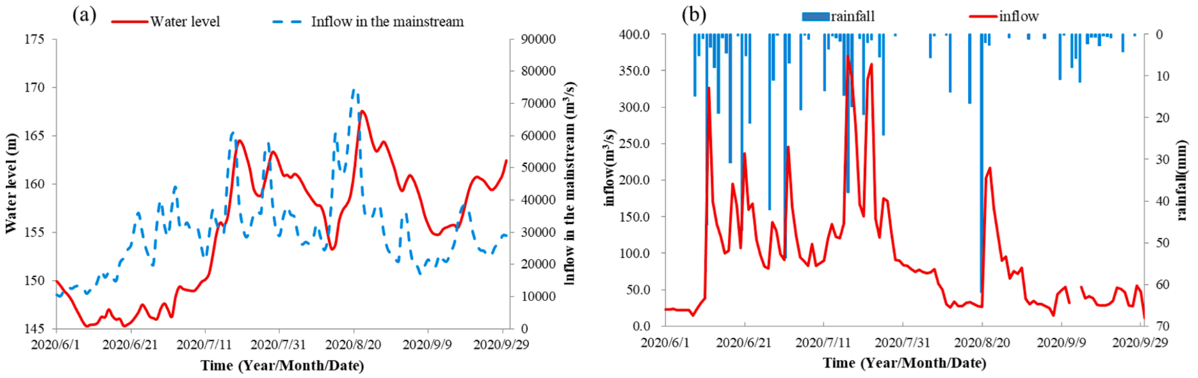

Fig. 3. Daily inflow, water level in the mainstream (a) and inflow, rainfall in the upper reaches of Xiangxi Bay (b) during flood season, 2020.

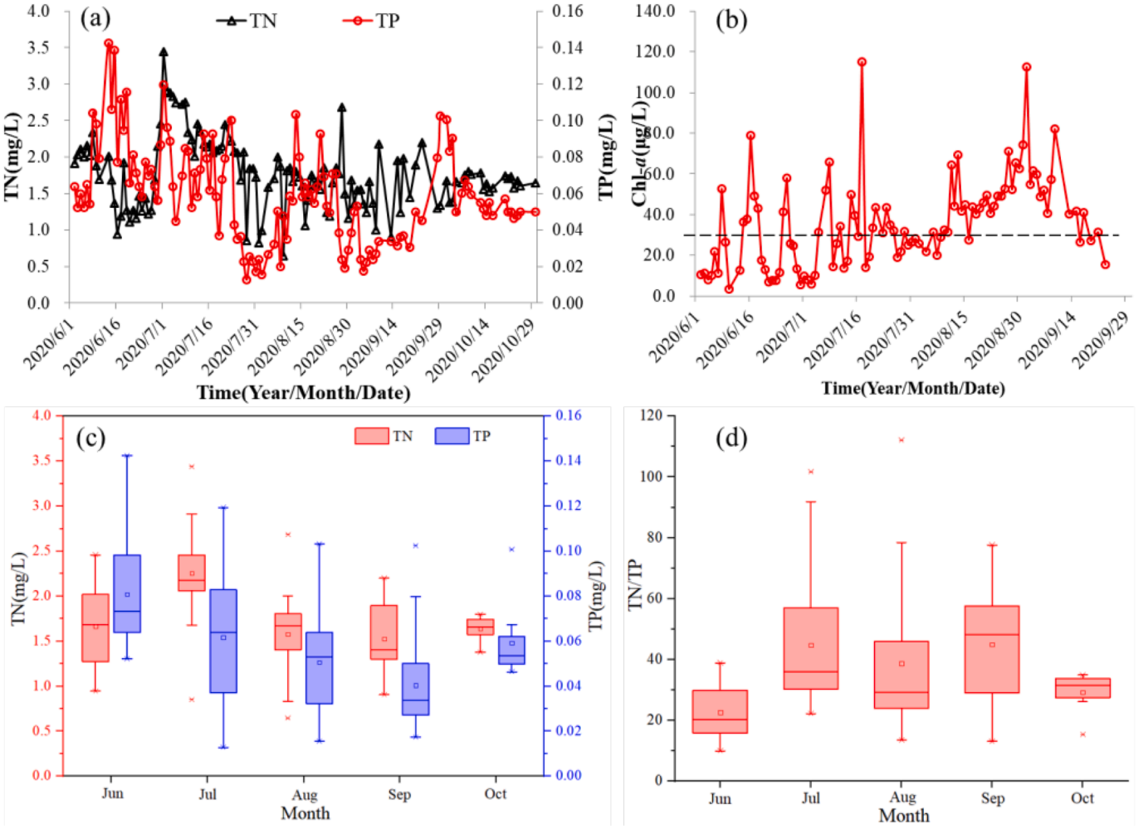

Fig. 4. Variation of the TN, TP and Chl-*a* at the surface water in XX06; a, daily variation of TN, TP from June to October in 2020; b, daily variation of the Chl-*a* from June to September in 2020, the dotted line represent the threshold for algal bloom of 30 μg/L; c, boxplots of monthly variation of TN and TP from June to October in 2020; d, boxplots of monthly variation of TN: TP (by mass) from June to October in 2020.

3.2.2. *Nutrient limitation of phytoplankton growth at different regions during flood season*

The phytoplankton biomass (Chl-*a*) on the bioassays of different regions from August 2nd (Day0) – August 5th (Day3) are shown in Fig. 6. The initial concentration of XX01, XX06 and XX09 were 24.83 ± 0.63 μg/L, 26.85 ± 1.38 μg/L, and 63.32 ± 4.70 μg/L, respectively. Treatments of P additions in different regions all yielded significantly ($p < 0.05$) higher Chl-*a* production than without P addition on day1, similar responses appeared at the end of the incubation (day3), though the Chl-*a* decreased in XX09, indicating P limitation at different regions and more P was needed on the upstream (XX09) to maintain relatively high phytoplankton biomass.

3.3. *The responses of phytoplankton growth to nutrient dilutions and daily additions during flood season*

The Chl-*a* and growth rates on the dilution bioassay from August 7th (Day0) – 10th (Day3) are shown in Fig. 7, the concentrations of TN and TP for the different treatments on the dilution bioassay are shown in Fig. 8. The initial Chl-*a* of four dilution levels (0%, 30%, 50%, and 70%) varied from 24.03, 18.48, 12.92 and 6.54 μg/L, the corresponding TN and TP concentrations ranged from 1.94, 1.44, 1.11, 0.55 mg/L and 0.091, 0.057, 0.043, 0.033 mg/L respectively.

N1-alone additions had no acceleration on the increase of the Chl-*a* compared to the control among all the dilutions, the corresponding Chl-*a* in those treatments for all the dilutions were lower than the bloom

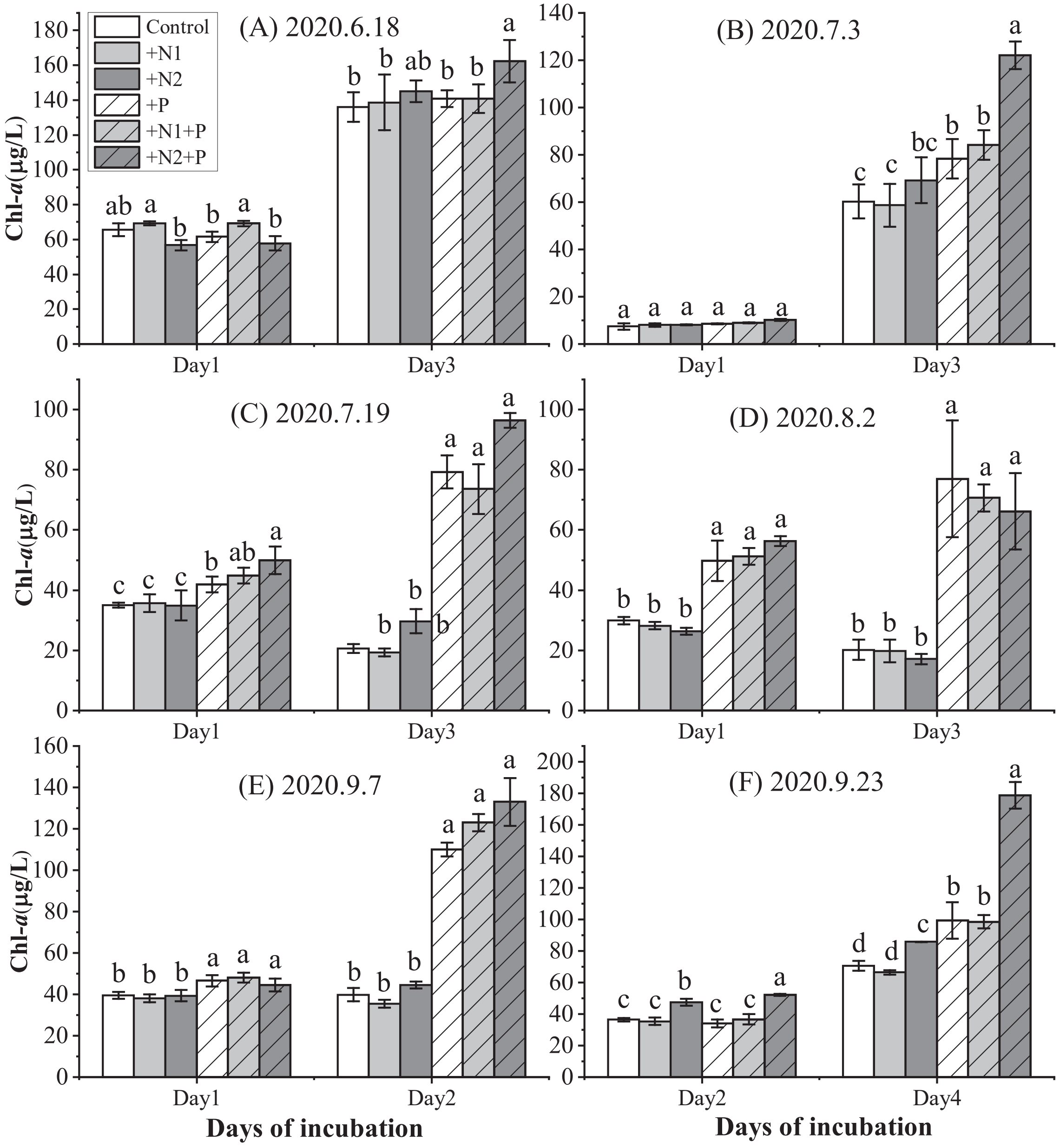

Fig. 5. Phytoplankton biomass (Chl-*a*) in nutrient addition bioassays in XX06 during flood season. The date represents the day when cultivation started of each bioassay (Day0). Error bars represent ± SD of the triplicate sample. Differences between treatments are shown based on ANOVA post hoc tests (a > b > c > d; $p < 0.05$).

threshold of 30 μg/L. The P additions (P and N1 + P) significantly ($p <$ 0.05) promoted the growth of the algal growth among all the dilutions. Furthermore, Chl-*a* was lower than 30 μg/L until 70% dilution with enough P. As for the growth rates, all treatments with P additions showed a high growth rate at the beginning (Day1), among which the 70% dilution group was lower than the other three diluted groups. With the consumption of nutrients, the growth rates of all treatments decreased significantly as the nutrients in the Cubitainers are not replenished as in the field, the growth rates of the P additions were reduced to lower than 0.4 d^{-1}, the treatments without P additions has adequate N while P was almost exhausted, and showed negative growth rates. The algal growth was controlled under the TN concentration (0.55 mg/L) of the treatments diluted with 70%, and the concentration of TP (0.057 mg/L) in the 30% dilution group, meanwhile, the total biomass of algae did not exceed the bloom threshold.

Growth rates in responses to daily addition N (a) and P (b) of varying concentrations during flood season in 2020 were showed in Fig. 9. The initial concentrations of TN and TP were 1.81 ± 0.09 mg/L and 0.037 ± 0.014, respectively. The growth curve fitted by nonlinear regression showed that the growth rate of natural phytoplankton in the N-gradient controlled (with enough P) group reached to highest rapidly at a relatively low N concentration (N was about 0.1 mg/L), due to the obvious P limitation during the experiment, the growth rate of natural phytoplankton in the P-gradient controlled group increased to the maximum until the P concentration reached about 0.03 ~ 0.04 mg/L.

4. Discussion

4.1. *P limitation (intensity, duration and range) of tributaries during flood season*

The response of phytoplankton growth to nutrient additions in the

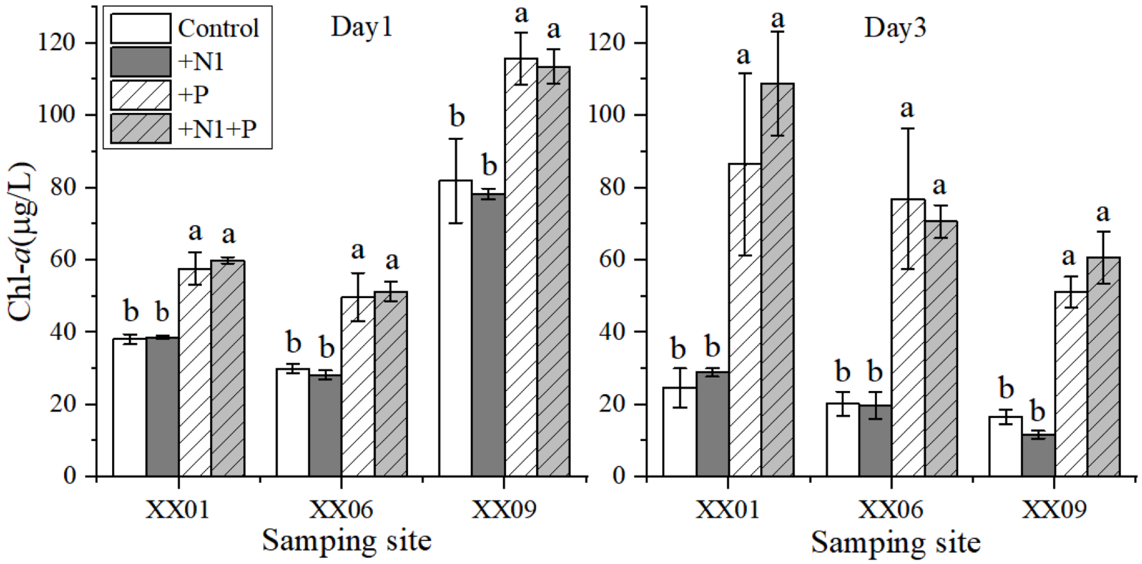

Fig. 6. Phytoplankton biomass (Chl-*a*) in bioassays at different regions from August 2nd (Day0) - August 5th (Day3) in 2020. Error bars represent ± SD of the triplicate sample. Differences between treatments are shown based on ANOVA post hoc tests (a > b; $p < 0.05$).

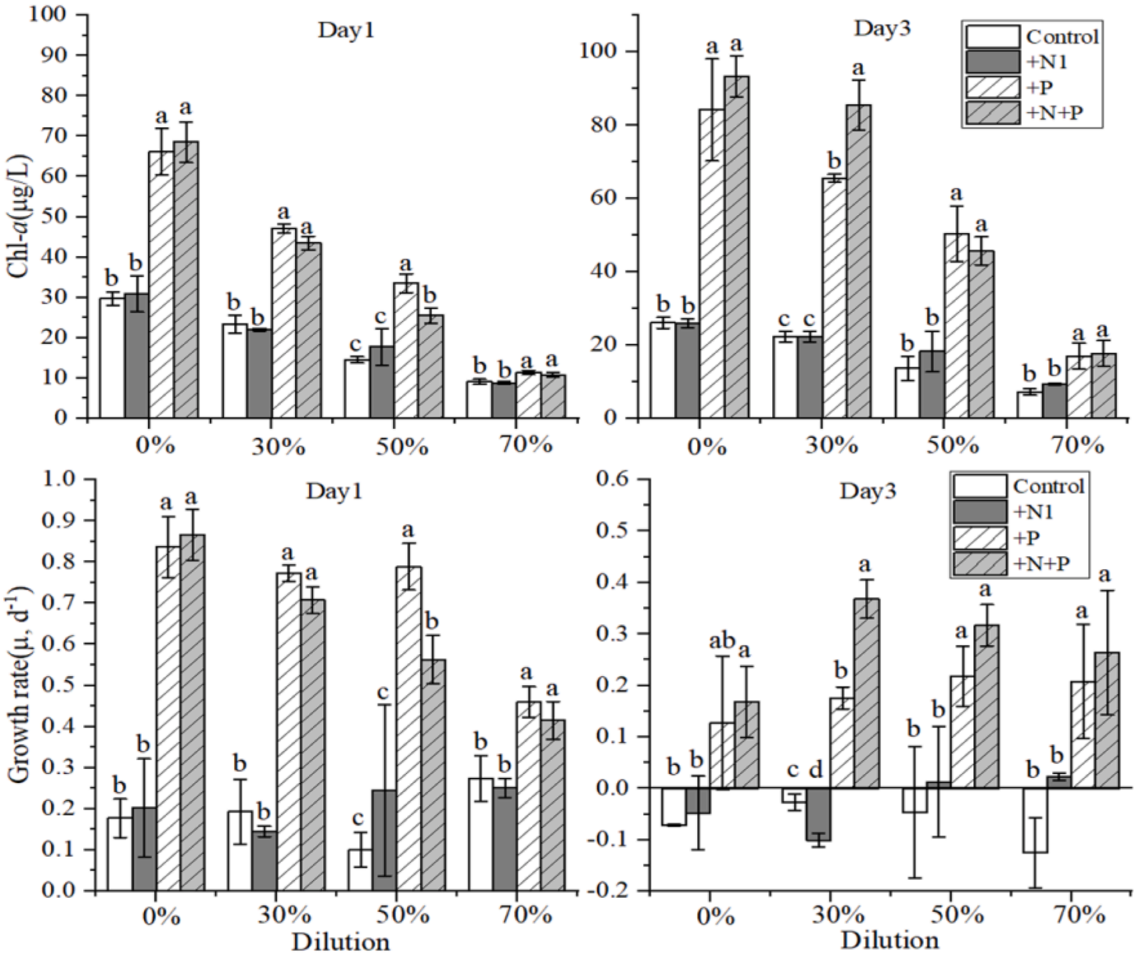

Fig. 7. Phytoplankton biomass (Chl-*a*) and growth rates in the dilution bioassay in XX06 from August 7th (Day0) – 10th (Day3) during flood season, 2020. Error bars represent ± SD of the triplicate sample. Differences between treatments are shown based on ANOVA post hoc tests (a > b > c > d; $p < 0.05$).

bioassays (Fig. 5) shows a distinct nutrient limitation variation of phytoplankton growth during flood season in 2020, the nutrient limitation variation was well corresponding to the characteristics of ambient TN, TP and TN: TP. The nutrient addition bioassay in mid-June showed a weak serial N2 limitation, and P limitation started to show up in early July with a weak co-limitation of N2 (Harpole et al., 2011). This can be

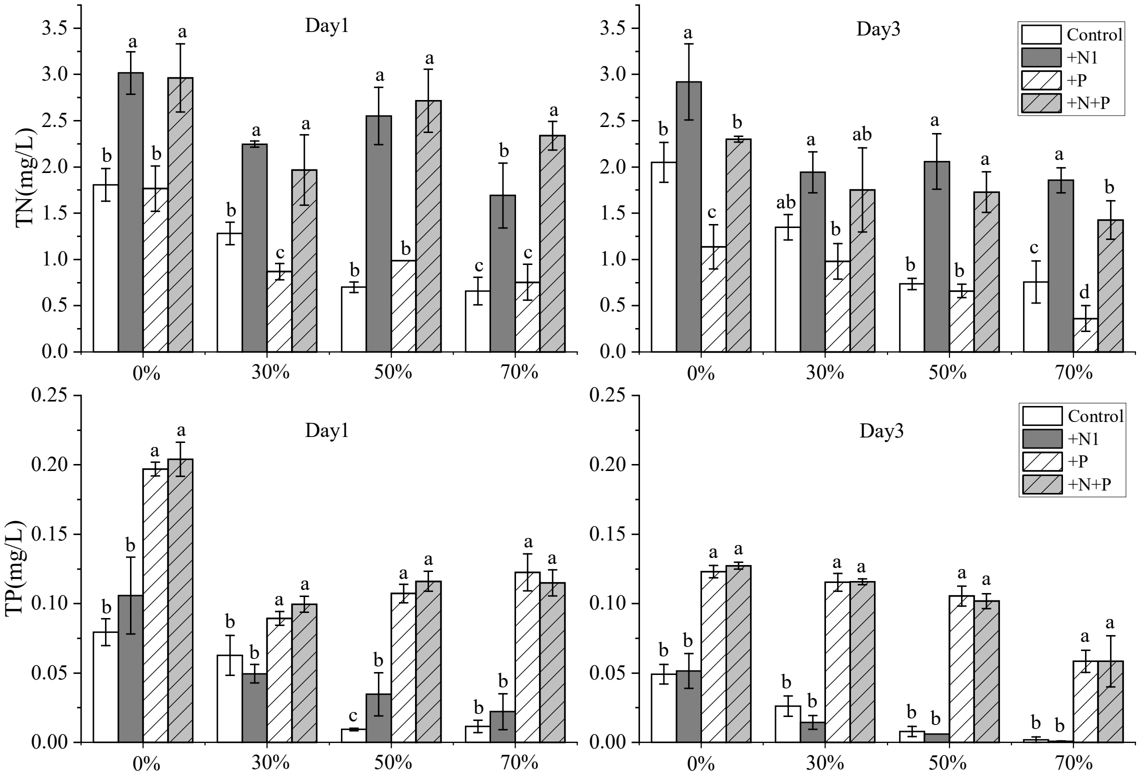

Fig. 8. The concentration of TN and TP on different treatments in the dilution bioassay in XX06 from August 7th (Day0) – 10th (Day3) during flood season, 2020. Error bars represent ± SD of the triplicate sample. Differences between treatments are shown based on ANOVA post hoc tests (a > b > c > d; $p < 0.05$).

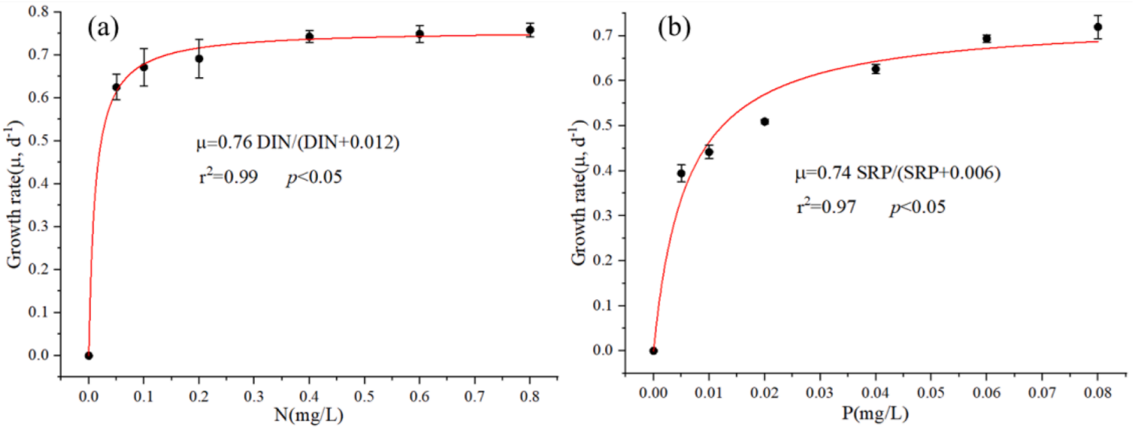

Fig. 9. Growth kinetics of phytoplankton in response to daily addition N (a) and P (b) of varying concentrations during flood season in 2020. The bioassay was carried out from August 12th - August 22th. Error bars represent ± SD of the triplicate sample. DIN is directly available N, SRP represents soluble reactive phosphorus.

well explained with the increasing of TN, decreasing of TP and increasing of TN: TP from June to July (Fig. 4c, d). A prominent P limitation from late July and lasted until the beginning of September due to the continuously decreasing TP during the flood season. We observed the Chl-*a* in treatments without P addition decreased at the end of cultivation because water samples are incubated in closed containers with limited supply of nutrients. However, the addition of P greatly increased the intensity of the algal bloom. The bioassay in late September showed a serial N2 limitation on the previous stage of cultivation as P addition has no effect on promoting the increasing of Chl-*a*. This indicated that N2 was the main limiting factor. An independent co-limitation of N2 and P appeared at the end of the incubation period (Elser et al., 2009; Harpole et al., 2011), while this may be reflected nutrient depletion due to the longer cultivation (Xu et al., 2010). Thus P was the secondary potential limiting factor. Actually TP has an increasing trend and TN: TP has a decreasing trend from the late September to October (Fig. 4c, d) explained the P limitation alleviation.

Studies have shown that seasonal shifts in nutrient limitation of phytoplankton growth are common in many aquatic ecosystems (Xu et al., 2010; Rogers et al., 2020). Most nutrient limitation succession is caused by seasonal variation of internal nutrient dynamic changes or external input, such as seasonal P release from sediments leading to the seasonal variation from significant N limitation in summer and autumn to P limitation in winter and spring in Lake Taihu (Paerl et al., 2011; Ding et al., 2018), seasonal variation of hydrologic condition, such as inflow, storm and snowmelt, could affect shifts in limitation characteristics in downstream lakes and reservoirs between N or P, and even the limitation succession in a shorter period (Bukaveckas and Crain 2002; Andersen et al., 2020). The present study showed a similar phenomenon that nutrient limitation of algal growth shift from N2 to P limitation, then back to the main N2 limitation during flood season in 2020. Our previous nutrient addition bioassay showed a potential serial N limitation of phytoplankton growth in Xiangxi Bay in early August in 2019 (Fig. S1). However, significant P limitation occurred from the early July in 2020 indicating an earlier P limitation or longer duration.

On the other hand, the spatial distribution of nutrient limitation on algal growth is equally important (Xu et al., 2013; Gikuma-Njuru et al., 2013; Zhang et al., 2021), for instance, research in the North Sea verified the offshore regional change from P to N limitation (Burson et al., 2016), nutrient limitation of N, P or even Fe and other metal elements in Lake Taihu showed obvious regional characteristics (Xu et al., 2015b; Zhang et al., 2019). Stratified density currents have been existed in Xiangxi Bay for a long time, and the intrusions from the TGR could supply nutrients to the tributaries especially N. As a result, N decreased longitudinally from estuary towards the end of tributary bays. Abundant phosphate rock resources are deposited in the upstream basin of the Xiangxi Bay, resulting higher P concentration in the upstream and lower in the middle reaches (Huang et al., 2014). Our previous nutrient addition bioassay of different regions conducted in late July 2019 (Fig. S2) showed a significant N limitation in the upstream and potential P limitation in the middle reach. The distribution of N and P could well explain the distinct regional distribution of nutrient limitation in Xiangxi Bay. Although N2 addition (N2 and N2 + P) was not conducted in the bioassays at different regions, the concurrent nutrient addition bioassay in XX06 (Fig. 5D) indicated that N2 addition has no effect on the algal growth. The regional distribution of nutrient limitation during the flood season in 2020 was in contrast to the previous studies as P limitation was proven to exist in the whole Xiangxi Bay in early August 2020 (Fig. 6) which showed a more extensive area of P limitation.

4.2. The influence of extreme rainfall during flood season on P limitation in a tributary of the TGR

The intensity and occurrence of rainfall events have increased significantly under the influence of climatic changes taking place (Lehmann et al., 2015; Giorgi et al., 2019). It was reported that extreme rainfall events are occurring more frequently and the annual rainfall showed an increasing tendency in the TGR (Lü et al., 2018; Zhu et al., 2021). Typically, the precipitation in the whole Yangtze River Basin was very abundant during flood season in 2020 and the peak discharge of the flood in the TGR has broken historical records (Fig. 3), second only to 1954. The monthly rainfall of June and July in Xiangxi Bay was about 1.6 times than the average monthly rainfall over ten years (2007–2016) (Fig. 2). Characteristics of rainfall and its frequency distribution in a river basin play an important role in the nutrient input to the ecosystem. It could significantly affect the nutrient concentration and stoichiometric ratios (TN: TP) through direct atmospheric deposition and runoff nonpoint source pollution (Elser et al., 2009; Camarero and Catalan, 2012; Xiong and Hoyer, 2018), thus driving the variation of nutrient limitation of phytoplankton growth.

As show previously, the nutrient limitation of algal growth shift from N2 to P limitation in the early flood season and the P limitation persist until late September in the tributary of the TGR. The nutrient limitation variation during the flood season was well supported by the variation of TN, TP concentration and TN: TP which could be affected by increasing the extreme rainfall. First, the increasing precipitation and runoff may bring more N load input to the water ecosystem and could exacerbate eutrophication (Sinha et al., 2017; Huang et al., 2020). In particular, agricultural watersheds are vulnerable to the impact of precipitation. Wet deposition under the influence of human activities (e.g., N fertilizer applications) increase the N concentration thus may lead to a shift from N limitation to P limitation (Williams et al., 2017), which has also been verified in Lake Dianchi (Zhan et al., 2017). Wet deposition of the TGR indicated that the concentration of NH_3-N was much higher than NO_3-N, which may be related to ammonia emission from farmland in the reservoir basin (Zhang et al., 2020b). We measured the nutrient concentrations in the rainfall several times in July and verified this finding (Table S3). This can be more conducive to the rapid alleviation of N2 limitation and shift to P limitation in the early flood season, which supported the results of our bioassays.

Secondly, we observed a dramatic decrease of the proportion of total dissolved phosphorus to TP (TDP/TP) in flood season in 2020 (Fig. S3), and the decreasing trend of TP during the flood season (Fig. 4c). This could further explain the observed intensive P limitation in 2020, among them, that the suspended particles might play an important potential role. Many researches demonstrated that particle phosphorus (PP) could reach more than 90% of TP loss from farmland to the surrounding water carried by rainfall-runoff (Bah et al., 2020). The concentration of SRP could not increase parallel with the corresponding rainfall (Davis et al., 2015). Higher concentration of suspended particles in the mainstream of the TGR with higher ability to adsorb dissolved P and particle settling (Tang et al., 2018; Wu et al., 2021) may present a dilution effect for P concentration of the tributaries especially the dissolved P through density currents (Han et al., 2018; Yang et al., 2018a), leading to the reduction of P supply (especially SRP) of the tributary. In general, the increase of rainfall (intensity and frequency) will increase the TN: TP of the whole ecosystem and thus intensify of P limitation on the whole bay.

Finally, our bioassay in late September showed a recovery of N2 limitation and an alleviation of P limitation (Fig. 5F). This can be attributed to a decrease in rainfall from August to September, followed by an increase in TP and a decrease in TN: TP from the end of September to October (Fig. 2 and Fig. 4). In addition, the P limitation alleviation could be attributed to the initial impoundment of the TGR in mid-September, the variation of stratified density currents in the process of impoundment (Fig. S5) will increase water exchange between mainstream and the tributaries (Zhao et al., 2016), weaken the water temperature stratification in tributaries and destroy the appropriate environment for algae growth (Liu et al., 2012; Chuo et al., 2019), which will help to reduce the algal blooms and accelerate the accumulation of nutrients comprehensively to ease nutrient limitation. However, nutrients input accompanied by the increasing extreme rainfall and flooding are not always leading to the variation of nutrient limitation. The variation of TN: TP and shift of nutrient limitation mainly driven by internal nutrient cycling (e.g., seasonal P release from sediments) in shallow eutrophication lakes such as Lake Taihu and Chaohu (Paerl et al., 2011; Ding et al., 2018; Tong et al., 2021). In contrast, our study in the tributaries of the TGR supported that the variation of TN: TP caused by the increasing of extreme rainfall and flooding is the main driving factor of the nutrient limitation succession in external nutrient inputs dominated systems. This will be helpful to better identify the nutrient limitation and develop more scientific nutrient management under the climatic change.

4.3. Nutrient threshold needed to control for phytoplankton growth under extreme rainfall in flood season

The flood season is a high occurrence season of algal blooms in the tributary bays of the TGR, which is dominanted by Cyanophyta and Chlorophyta, with Cryptophyta or Bacillariophyta as sub-dominants (Wang et al., 2011; Chuo et al., 2019; Zhou et al., 2019). Similar dominant species of algal blooms occurred throughout our study during the flood season, 2020. *Microcystis* spp. belong to Cyanophyta as the dominant species with the highest initial frequency of occurrence, species such as *Chlorella* sp. belong to Chlorophyta and *Chroomonas acuta*, *Cryptomonas erosa* belonging to the Cryptophyta more appeared from late July to early August (Fig. S6). To alleviate eutrophication and reduce the risk of algal bloom, determining the nutrient threshold is the important foundation to clear reduction target in nutrient management strategies (Huo et al., 2015; Cao et al., 2017).

Short-term *in situ* dilution bioassays can identify the response of phytoplankton growth under nutrient reductions, and thus can provide a reference for the nutrient threshold needed to control eutrophication (Paerl et al., 1978; Xu et al., 2015a). In our dilution bioassay, the concentration of TN and TP that can limit algal growth and algal bloom are approximately 0.55 mg/L and 0.057 mg/L, respectively. The bioassays also indicated a significant P limitation. In addition, growth curve (Fig. 9) in responses to daily N and P of varied concentrations additions indicating that the growth of algae could not be controlled until the concentration of SPR below 0.03 ~ 0.04 mg/L (Xu et al., 2015a; Zeng et al., 2016), in consideration of the average proportion of SPR to TP during the flood season, 2020 was about 47%, estimated the threshold of TP needed to control eutrophication is about 0.064 mg/L. Furthermore, the maximum growth rate of algae can be restricted only when the N pulse nutrient addition concentration was controlled lower than 0.1 mg/L due to obvious P limitation during the bioassay and original relatively abundant N, thus the growth curve of N-gradient controlled may not be representative.

Many relevant studies have been carried out to determine the nutrient threshold to protect the ecosystems from excessive nutrient enrichment and algal blooms (Carleton et al., 2009; Chambers et al., 2012; Chen et al., 2018). The concentration of TN and TP was recommended to be controlled under 0.8 mg/L and 0.061 mg/L respectively, to prevent excessive cyanobacteria bloom in deep-water (greater than3.1 m) lakes and reservoirs in the U.S. (Yuan and Pollard 2015), and below 0.80 mg/L and 0.05 mg/L respectively to limit the growth of *Microcystis* in Lake Taihu (Xu et al., 2015a). Our bioassays showed that the control threshold of TN is about 0.55 mg/L, and TP is 0.057 ~ 0.064 mg/L to alleviate eutrophication and reduce algal bloom (Chl-$a < 30$ μg/L) on the typical tributaries of the TGR. The control threshold of TP concentration is similar to those freshwater systems, while TN is relatively stricter, possibly due to the intensified P limitation as the increasing TN: TP and the originally severe overloaded N on the reservoir during the flood season (Xiang et al., 2021).

How to alleviate water eutrophication and reduce algal blooms is a global concern raising important scientific issue (Ho et al., 2019; Gobler 2020). The reduction of the nutrient load input is the most fundamental method to control algal bloom though it requires a long time to achieve which also applies to the tributaries of the TGR (Yang et al., 2018a). Our results of the nutrient threshold of TN and TP can provide a reference for managers to set scientific nutrient management goals for alleviating eutrophication in the whole Basin, and formulate corresponding pollution load reduction measures. In addition, we recommend that P could be priority control relative to N especially in the context of increasing rainfall and flood as the incidence and intensity of the P limitation have increased in tributaries of the TGR (Li et al., 2019; Huang et al., 2020) and the relatively economical way to remove from the water. Nevertheless, the process of nutrient limitation succession also emphasizes the important role of N in algal growth (N limitation or N and P co-limitation), indicating the need for N control, which also can reduce the adverse effects of the reduced imbalance of N and P on the downstream ecosystems (Paerl et al., 2018). Besides, high N concentration has been reported to lead to higher microcystin concentrations in water ecosystems (Davis et al., 2015). Thus we confirmed that implementing dual nutrient input controls over the long-term will be more effective. Finally, we recommend that long-term determinations of nutrient limitation and nutrient threshold will be needed to control algal growth, as a series of complex reactions of the aquatic ecosystems will respond to nutrient structure variation caused by the increasing impact of human activities. Further to take hydraulic retention time, transparency, phytoplankton composition, etc. comprehensively to determine the nutrient threshold for algal blooms control (Vuorio et al., 2020; Zou et al., 2020), as well as the impact of future climate change (increasing extreme rainfall, global warming, and rising atmospheric CO_2 etc.) on the standard of nutrient threshold and regularly improve the nutrient management solution will be more advantageous (O'Neil et al., 2012; Huo et al., 2019; Wang et al., 2020).

5. Conclusion

The present study reports the characteristics and causes of the temporal and spatial distribution of nutrient limitations in a tributary bay of the TGR during flood season in 2020, as well as the nutrient thresholds needed to control eutrophication and reduce algal blooms. Nutrient limitation shift from N2 to P limitation, then back to the main N2 limitation during the flood season. The reduction of dissolved P, accompanied by an increase of TN: TP caused by the increasing rainfall and flooding intensified P limitation of algal growth of the whole bay. The reduction of rainfall and the process of impoundment after the flood season combined to alleviate the P limitation. The variation of TN: TP caused by the increasing of rainfall and flooding could be the main driving factor of the nutrient limitation shift in external nutrient inputs dominated systems. Thus short-term variation of nutrient limitation need be considered to develop more scientific nutrient management under the influence of climate change particularly in external nutrient inputs dominated systems. Nutrient dilution and enrichment bioassays indicated that algal blooms could be controlled (Chl-$a < 30$ μg/L) when the concentrations of TN and TP were lower than 0.55 mg/L and 0.057 ~ 0.064 mg/L, respectively. The control threshold of TN is relatively stricter, possibly due to the intensified P limitation as the increasing TN: TP and the severe overloaded N on the reservoir during the flood season, thus more relates research is needed to verify this threshold. P could be priority control short-term while a long-term nutrient management of the whole basin needs a dual control of N and P. Further, we recommend sustained attention on nutrient limitation and long-term determinations of nutrient threshold needed to control algal blooms, as well as more comprehensive factors taken into consideration. The present study is helpful to further understand the responses of algal growth to nutrient dynamics and can provide a reference for scientific nutrient management strategy.

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.jhydrol.2022.127562.

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