



Iron operates as an important factor promoting year-round diazotrophic cyanobacteria blooms in eutrophic reservoirs in the tropics

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ABSTRACT

We hypothesize that iron availability plays an important role in driving phytoplankton structure and dynamics in tropical reservoir. This role has often been neglected in routine monitoring of water quality since the main focus has been addressed on macronutrients such phosphorus and nitrogen. To elucidate the potential regulation exerted by iron availability on phytoplankton, a limnological investigation was carried out in two neighboring eutrophic reservoirs in tropical China. Traditional physical and chemical variables were measured along with total and dissolved iron concentrations to explore how they could influence phytoplankton structure. Statistical analyses showed that dissolved iron (DFe) as well as dissolved nitrogen (DIN) and soluble reactive phosphorus (SRP) significantly explained the variance of phytoplankton assemblages in both reservoirs. We could also find that diazotrophic cyanobacteria blooms generally developed when DFe concentrations were above 0.025 mg/L. Moreover, the DFe availability in the water columns was modulated by the effects exerted by the stratification/circulation patterns on redox conditions and pH values. Anaerobic conditions favored the release of iron and phosphorus from sediments, and likely favored an enhanced nitrogen assimilation and a decrease of DIN in the epilimnion. Our results highlighted that iron functions as an important factor promoting diazotrophic cyanobacteria and driving seasonal phytoplankton succession in tropical eutrophic reservoirs, not only with a direct effect on phytoplankton growth but also indirect effect by modifying the availability of nitrogen and phosphorus.

1. Introduction

Eutrophication and cyanobacterial blooms are affecting freshwater ecosystem services worldwide, e.g., significantly increasing water treatment costs for drinking supply, promoting the decline of fishing and aquaculture, and impairing the recreational use of freshwaters (Paerl, 2018; Le Moal et al., 2019). In tropical latitudes, cyanobacterial blooms are more frequent and long-lasting when compared to those occurring at higher latitudes since the absence of seasonal fluctuations in light availability and water temperature can allow the year-round persistence of such events in eutrophic environments (Giani et al., 2020). However, the factors regulating cyanobacterial biomass and community composition during persistent blooms are less understood in tropical waters, because most of the available information is from temperate regions. To better understand the role exerted by iron on the dynamics of cyanobacteria, Molot et al. (2014) proposed a conceptual model which integrates macronutrient (N and P) availability, mixing and light regime, redox conditions and the dynamics of ferrous and ferric ions as drivers of

cyanobacterial blooms and of the alternance between cyanobacteria and eukaryotic phytoplankton.

Iron is an essential nutrient for phytoplankton growth since it is involved in several basic physiological processes of photosynthetic cells (e.g. oxygenic photosynthesis and respiration, synthesis of proteins and nucleic acids, nitrogen fixation) and have a role in enzymatic reactions and electron transport (Borowitzka et al., 2016). Differences in photosynthetic complexes and in nitrogen assimilation are considered important reasons for the different iron demand among species: as an example, diazotrophic cyanobacteria have iron requirements higher than eukaryotic algae because of their higher PS I:PS II ratio and N₂ fixation (Borowitzka et al., 2016). In spite of its biological importance and abundance on Earth's surface, iron is relatively unavailable to the aquatic biota (Emerson, 2016). In particular, at the pH values and in the oxidizing environment commonly found in the marine compartment, iron exists in the +3 valence state which reacts with water to form an extremely insoluble iron oxyhydroxide (Earhart and Lei, 2015). As a consequence, iron is considered one of the most important limiting

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factors to phytoplankton growth in open oceans (Martin & Gordon, 1988; Martin et al., 1990), especially in the so-called High-Nutrient, Low-Chlorophyll (HNLC) ocean regions (Hop et al., 2019). In tropical regions, diazotrophic cyanobacteria (e.g. *Raphidiopsis* spp. and *Dolichospermum* spp.) frequently dominate and form blooms in lakes and reservoirs (Padišák, 1997; Huszar et al., 2000; Barbosa & Padišák, 2002; Ndebele-Murisa et al., 2010). However, the effect of iron availability as a driver of phytoplankton succession in tropical freshwaters was seldom investigated.

Iron-rich red soil is abundant in tropical zones, and supplies a rich source of iron to freshwater ecosystems (Buol et al., 2011; Tang et al., 2019). However, the availability of iron strongly depends on redox conditions, and its release from sediments is influenced by several hydrodynamic, physical and chemical factors (Molot et al., 2014; Lis et al., 2015; Tang et al., 2019). Deep lakes and reservoirs are usually warm monomictic in the tropics, and they are characterized by a long stratification period followed by a few weeks of complete mixing (Lewis, 2000). During the stratification period, due to the photosynthetic activity of phytoplankton, high redox values and alkaline conditions generally occur in the euphotic layer which may cause a deficiency of soluble iron; simultaneously, anoxic hypolimnia, characterized by negative redox values and pH values lower than 7, may develop under eutrophic conditions; in these environments, insoluble Fe^{3+} is reduced to soluble Fe^{2+} and mobilized from the sediments (Millero et al., 1995; Lewis, 2000; Liu & Millero, 2002). Tropical and subtropical water bodies can be subjected to atelomixis, i.e. the effect of superficial heat-gain in the absence of vertical mixing. This thermal pattern causes a daily increase in the stability of stratification with the formation of a daily thermocline in the first centimeters of depth (Barbosa & Padišák, 2002), and promote an iron enrichment of the upper water layers by inflating Fe^{2+} -rich water from the anoxic deeper layers (Naselli-Flores, 2003). The specific thermal regime of tropical water body can contribute to the large variability of iron concentrations in the upper euphotic layers and exert a strong influence on phytoplankton dynamics in these environments.

Diazotrophic cyanobacteria commonly dominated in Chinese tropical reservoirs (Lei et al., 2014; Lin et al., 2020), although nitrogen and phosphorus were recognized as main factors of this succession pattern, the role of iron availability as a driver of the phytoplankton seasonal dynamics remains an open question. To test the hypothesis that iron availability can regulate the phytoplankton seasonal succession in tropical reservoirs and eventually promote the dominance of diazotrophic cyanobacteria, the environmental template and the structure of phytoplankton assemblages were investigated in two tropical moderately deep reservoirs (maximum depth <15 m), which showed similar trends in their physical and chemical variables, but different phytoplankton dynamics. Multivariate statistical analyses were used to detect the relationships existing among the physical (mixing regime) and chemical structure (redox conditions) of the water column, phosphorus and nitrogen dynamics and the effective availability of iron as a driver of the seasonal succession and structure of phytoplankton assemblages.

2. Materials and methods

2.1. Study site and local climate

A limnological investigation was carried out in two eutrophic reservoirs located in tropical southern China: Zhenhai reservoir (22°57' N, 112°57' E) and Dashaha reservoir (22°52' N, 112°43' E). The distance between the reservoirs is about 10 km, both are subjected to a tropical monsoon climate, and show a warm monomictic mixing regime. The normal storage volume is about $1.6 \times 10^8 \text{ m}^3$ of Dashaha and $1.2 \times 10^8 \text{ m}^3$ of Zhenhai, and the maximum depth is 14 m in Dashaha and 12 m in Zhenhai. The latter shows a more dendritic shape compared to Dashaha and also a lower average depth.

The sampling station in each reservoir was located in the lacustrine

zone near the dam and close to the deeper part of the water bodies (Fig. 1).

2.2. Sampling and environmental variables

Samples were collected at monthly intervals from January 2017 to December 2018. Water temperature (T), dissolved oxygen (DO) and pH values were measured with a Yellow Spring Instrument multiprobe (YSI 6600) at 1-m intervals from the surface to the bottom. Water transparency was measured using a Secchi disk (SD). Light availability in the water column was estimated by computing the ratio between euphotic depth and mixing depth ($Z_{\text{eu}}/Z_{\text{mix}}$). Z_{eu} was calculated as $2.7 \times \text{SD}$ whereas Z_{mix} was considered as the depth of the thermocline. Thermocline depth was estimated as the first water layer with a temperature decrease higher than 1 °C in 1 m interval. Relative water column stability (RWCS) was calculated following Padišák et al. (2003) according to the formula:

$$\text{RWCS} = (D_b - D_s)/(D_4 - D_5)$$

where D_s is the water density at surface D_b is the water density at bottom, D_4 and D_5 are water density at 4 °C and 5 °C.

Samples for nitrogen, phosphorus, chlorophyll-a and iron concentration were taken at three depths (0.5 m, 5 m, 10 m). Soluble reactive phosphorus (SRP), nitrate ($\text{NO}_3\text{-N}$), ammonium ($\text{NH}_4\text{-N}$), total nitrogen (TN) and phosphorus (TP) were measured according to the standards set by the Environmental Protection Agency of USA (APHA, 1989). Dissolved nitrogen (DIN) was considered as the sum of nitrate and ammonium. Chlorophyll-a (Chla) was extracted with acetone and measured spectrophotometrically after filtering 500-ml of lake water through a 0.45 μm pore size acetate filter (Lorenzen, 1967; Lin et al., 2005). The samples for total (TFe) and dissolved (DFe) iron were collected with special glass bottles treated with trace metal clean techniques. The samples for DFe were filtered in situ. Iron concentrations were measured with ICP-AES (OPTIMA, 2000DV). All measurements were completed within 24 h from sampling.

2.3. Phytoplankton identification and counting

Samples for phytoplankton were collected subsurface (0.5 m), and preserved in situ with 1% Lugol. Phytoplankton analysis was carried out under an inverted microscope (at 400 magnification) and taxa were identified according to the most updated taxonomic literature. Enumeration was performed according to Utermöhl (1958) using 10 ml sedimentation chambers. At least 400 settling units were counted, and the size of each counted algal cell was measured. Biomass was computed using the biovolume method (Hillebrand et al., 1999), assuming a specific gravity of 1 mg mm^{-3} . Species relative abundance was calculated as percentage of total biomass according to:

$$\text{Relative abundance(\%)} = (\text{Species biomass}/\text{total phytoplankton biomass}) \times 100$$

2.4. Statistical analysis

The seasonal dynamics of physical and chemical variables (T, DO, pH values) along vertical profiles were shown with isoline drawing using the software Surfer 8.0. Kriging was used as gridding method.

Twelve environmental variables (T, pH values, TN, DIN, TP, SRP, TFe and DFe concentrations at 0.5 m of depth, Z_{mix} , RWCS, SD, $Z_{\text{eu}}/Z_{\text{mix}}$) were used in the environmental data matrix. The environmental matrix was standardized (z-scores) before the analyses. To describe the structure of the assemblages, phytoplankton was grouped in the following groups: diazotrophic cyanobacteria, non-diazotrophic cyanobacteria, diatoms, chlorophytes, desmids and others. Biomass values of these groups were used in phytoplankton matrices, and Hellinger's transformation was used before statistical analyses (Borcard et al., 2011).

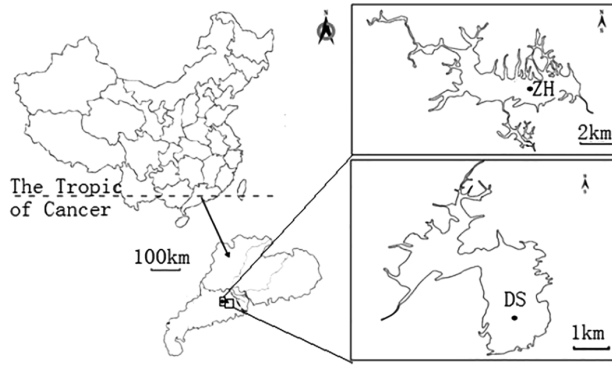


Fig. 1. Location of the studied reservoirs and of the sampling sites. DS indicates the sampling station in Dashahe reservoir and ZH that in Zhenhai.

Nonmetric multidimensional scaling analysis (NMDS) was used to identify the separation of environmental preferences among the selected genera. Redundancy analysis (RDA) was used to identify the relationships between the phytoplankton matrix and the matrix containing the most significant environmental variables. These variables were identified using a forward selection with 10,000 permutations. At the same time, the significance of each variable was tested with marginal effect. In redundancy analysis, datasets were set by two scales: a dataset for each reservoir was used to identify the main factors for phytoplankton seasonal dynamics, and a combined dataset including both the reservoirs was used to identify the main factors which contributed to the differences in the structure of their phytoplankton assemblage. Local regression fitting (Loess) and Linear Model (LM) were used to detect the

regression relationships between the significant environmental variables and the variation of the biomass and of the relative abundance of phytoplankton. All calculations were carried out in R platform version 3.0.0 (Borcard et al., 2011; R Core Team, 2014).

3. Results

3.1. Physical conditions

Water temperature ranged between 13 °C and 32 °C with an average annual value of 24 °C in both reservoirs. In Zhenhai, the high values of water temperature in spring and summer promoted the stratification of the water column with the establishment of a thermocline that

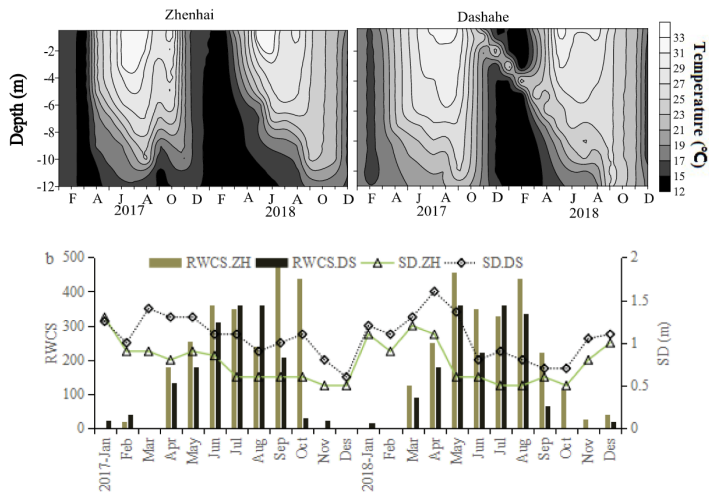


Fig. 2. Isotherms of water temperature in the water column (a) and relative water column stability (RWCS, bar chart) and Secchi disk (SD, line graph) dynamics (b) in the studied reservoirs. (ZH: Zhenhai reservoir; DS: Dashahe reservoir).

progressively moved upward from a depth of about 6 m in April to about 3 m in July 2017 and June 2018; in addition, a thermocline located at about 1 m of depth was recorded in October 2017 (Fig. 2a). This reservoir was also characterized by high RWCS values (318 ± 112) which denoted a well-developed summer stratification with a quite steep temperature gradient between the surface and the bottom (Fig. 2b). In addition, according to the trends of RWCS, the reservoir showed a transition from stratification to circulation more abrupt in 2017 than in 2018. Thermocline started developing in April also in Dashahe. However, it was located at about 7 m of depth until June and progressively moved upward reaching a depth of 2 m in August 2017. In 2018, the thermocline fluctuated between 1 and 2 m from May to August 2018. In both the investigated years, this reservoir started circulating earlier than Zhenhai and showed lower RWCS values (222 ± 130). Both reservoirs were characterized by a shallow euphotic zone (<3 m) in most of the study period, especially during stratification; SD showed an average value of 0.7 ± 0.2 m in Zhenhai and of 1.0 ± 0.3 m in Dashahe, during the period April–October.

3.2. Chemical conditions and Fe dynamics

Both reservoirs showed alkaline ($\text{pH} > 7.5$) and well oxygenated ($\text{DO} > 8$ mg/L) subsurface waters, but pH and DO values rapidly

tended to decrease below 3 m of depth, showing a steep gradient along the water column, especially close to the thermocline (Fig. 3a). As regard the hypolimnion, both reservoirs showed anaerobic and neutral or acidic conditions around and/or immediately below the thermocline, and these were more pronounced in Zhenhai reservoir.

According to the redox regime of the reservoirs, dissolved iron (DFe) showed a pronounced spatial gradient along the water column. DFe concentration in the upper layers (0.5 m) ranged from below 0.001 mg/L to 0.1 mg/L with a peak at the end of summer in both reservoirs. Another peak (≈ 0.06 mg/L) occurred in Zhenhai at circulation, but not in Dashahe (Fig. 3b). The subsurface values significantly correlated ($R^2 > 0.56$, $p < 0.001$) with DFe concentrations in the middle layer (5 m). DFe in the middle layer (5 m) was influenced not only by pH and DO but also by the DFe concentrations in the hypolimnetic zones. A significant negative correlation ($R^2 = 0.53$, $p < 0.01$) was found between DFe at 5 m and pH. During the stratification period, DFe concentrations in the anoxic hypolimnion (10 m) were above 4 mg/L in both reservoirs. Conversely, very low values were detected in the deeper layers at circulation.

3.3. The dynamics of phytoplankton

Phytoplankton biomass had similar trends (with a maximum during

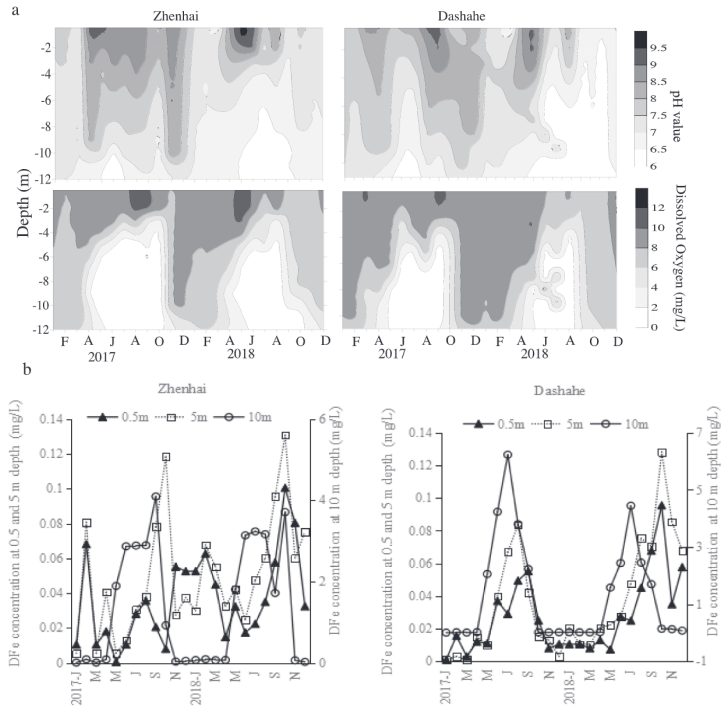


Fig. 3. (a) Vertical profiles of pH values and dissolved oxygen concentrations and (b) DFe concentrations at 0.5 m, 5 m and 10 m recorded in the study period.

stratification and a minimum at circulation) in the studied reservoirs and its average value was 7.2 ± 6.6 mg/L in Zhenhai, and 4.0 ± 3.8 mg/L in Dashahe. Diazotrophic cyanobacteria, including *Dolichospermum* spp. and *Raphidiopsis raciborskii*, represented the most important phytoplankton group in Zhenhai reservoir, with a relative abundance constantly higher than 75% of total biomass, except at circulation in 2017 (Fig. 4a). In Dashahe, this group was also dominant during the stratification period, whereas diatoms, chlorophytes and desmids dominated during circulation. The alternance of diazotrophic cyanobacteria and eukaryotic phytoplankton strongly marked the seasonal pattern observed in the phytoplankton dynamics of Dashahe reservoir. In total, 91 phytoplankton genera were identified in the two reservoirs; however, only 11 showed annual average relative abundances higher than 1%. They were *Raphidiopsis*, *Dolichospermum*, *Microcystis*, *Limnotherix*, *Cyclotella*, *Aulacoseira*, *Fragilaria*, *Chlorella*, *Scenedesmus*, *Closterium*, *Staurastrum*. These genera were well separated in the NMDS plot, as a result of their different ecological requirements in the environmental gradient (Fig. 4b). Most of them occurred in Dashahe at circulation with relative abundance >10%. Conversely they were rare in Zhenhai where *Raphidiopsis* was found to be strongly dominant during most of the investigation period.

3.4. Relationships between iron, nitrogen and phosphorus

The two studied reservoirs showed similar concentration values as regard nitrogen and phosphorus. In particular, in surface waters total nitrogen (TN) and total phosphorus (TP) were 1.1 ± 0.15 mg/L and 0.025 ± 0.005 mg/L in Zhenhai and 0.97 ± 0.28 mg/L and 0.028 ± 0.006 mg/L in Dashahe. In both reservoirs, dissolved nitrogen (DIN) was above 0.3 mg/L and soluble phosphorus (SRP) below 0.01 mg/L in most of the samples (Fig. 5). TN concentrations showed quite constant values, especially in Zhenhai, with no clear relationships with TFe. Conversely, a slight increase in TP concentrations was recorded with increasing TFe concentrations (Fig. 5a). A positive correlation between DFe and SRP concentrations and a negative correlation between DFe and DIN

concentrations were detected in both the reservoirs. A significant regression line ($R_{adj} > 0.11$, $p < 0.05$) was found between DFe concentrations and both DIN and SRP concentrations in Zhenhai reservoir (Fig. 5b).

3.5. The importance of iron for phytoplankton assemblage

Linear Model (LM) showed positive and significant relationships ($p < 0.01$, $R_{adj} = 0.2$) between DFe and Chla concentrations, and significant, negative relationships ($p < 0.01$, $R_{adj} = 0.42$) between DIN and Chla concentrations. A higher Chla concentration per unit of potentially available phosphorus (Chla/TP) was detected in the samples with a higher DFe concentration (Fig. 6).

In the redundancy analysis (RDA) carried out with the dataset of each reservoir, the importance of DFe was ignored in forward selection because of the collinearity detected between DFe and RWCS and other variables, even though DFe was a significant variable ($p < 0.01$) in explaining the variance of phytoplankton assemblages. In the matrix combining the datasets from the two reservoirs, DFe was the most important variable explaining the difference of phytoplankton assemblages between the two reservoirs, and it alone explained more than 20% of the variance in the phytoplankton assemblage (Table 1).

In the tri-plot of the RDA, diazotrophic cyanobacteria (c1) were separated from other groups, and positively correlated with DFe ($r > 0.63$, $p < 0.01$, $n = 48$, Spearman), and negatively with DIN ($r > 0.68$, $p < 0.01$, $n = 48$, Spearman). As regard the other groups (c2 to c6), they positively depended on DIN and negatively correlated with DFe. In the Linear Model (LM), all nine variables (TN, TP, TFe, DIN, SRP, DFe, pH, T and Z_{eq}/Z_m) could explain 48% of the variance in the relative abundance of diazotrophic cyanobacteria, and DFe concentration was the most important (Table 1). A positively significant linear regression relationship ($R_{adj}^2 = 0.28$, $p < 0.01$) was found between DFe concentrations and the relative abundance of diazotrophic cyanobacteria. The local regression fitting (Loess) revealed that the relative abundance of these genera was above 50% when DFe concentrations were above 0.025 mg/

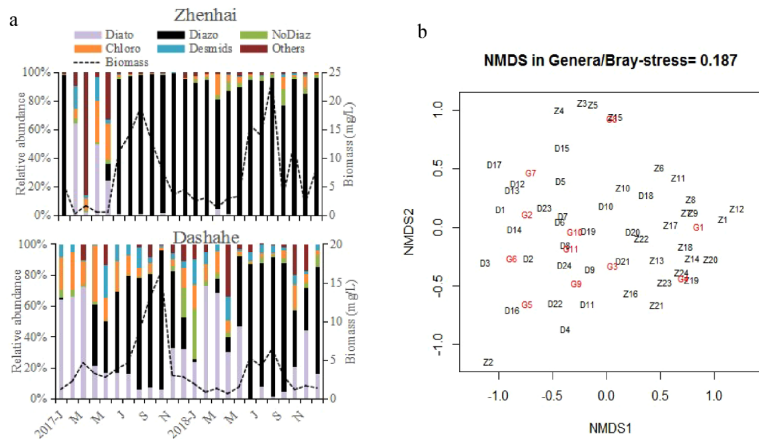


Fig. 4. (a) The structure of phytoplankton assemblages in the studied reservoirs (Diatom: Diatoms; Diazotrophic cyanobacteria; NoDiazotrophic cyanobacteria; Chloro: Chlorophytes; Biomass: standing phytoplankton biomass, dashed line), and (b) the results of Nonmetric Multidimensional Scaling (NMDS) analysis carried out on the 11 most important genera recorded in the studied reservoirs (G1: *Raphidiopsis*, G2: *Microcystis*, G3: *Dolichospermum*, G4: *Limnotherix*, G5: *Cyclotella*, G6: *Aulacoseira*, G7: *Fragilaria*, G8: *Chlorella*, G9: *Scenedesmus*, G10: *Closterium*, G11: *Staurastrum*; D1 to D24 are the samples from Dashahe, and Z1 to Z24 are the samples from Zhenhai).

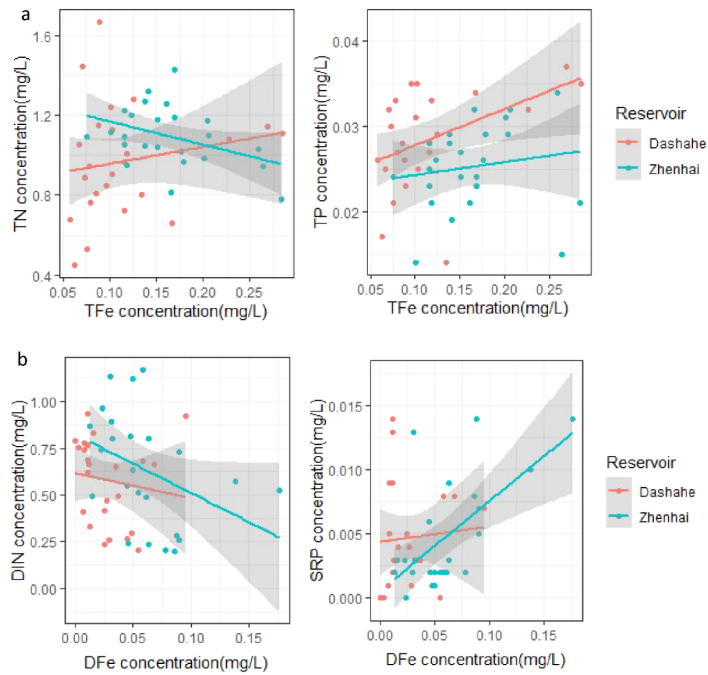


Fig. 5. Response of TN and TP concentrations to increasing TFe concentrations (a), and response of DIN and SRP concentrations to increasing of DFe concentrations (b) (the blue solid point were the samples from Zhenhai reservoir, and red solid points were the samples from Dashahahe reservoir, solid line was the fitting line, and the grey area is the double error range of fitting line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

L in both reservoirs (Fig. 7).

4. Discussion

4.1. Iron availability and phytoplankton dynamics in the studied reservoirs

Our results allowed to identify DFe availability in the studied reservoirs as a significant variable to explain the observed variance of phytoplankton assemblages. During our limnological investigation, the water temperature ranged between 13 °C and 32 °C; these values are high enough to allow cyanobacteria growth throughout the year (Giani et al., 2020). Moreover, it is important to consider that the higher is the temperature, the higher is the RWCS resulting from 1 °C of difference between two adjacent water layers. Therefore, at high water temperatures, as those recorded in the studied water bodies, water bodies stratify more easily, often in the first centimeters of depth, and tend to form a more stable stratification than their temperate counterparts.

DIN and SRP concentrations (0.3 mg/L and 0.003 mg/L respectively) were above the limiting threshold for phytoplankton growth (Reynolds, 2006) and the only detectable difference between the reservoirs was related to DFe availability. In Dashahahe reservoir, where epilimnetic DFe availability was lower, a higher diversity of phytoplankton groups and genera was recorded, especially during circulation periods. Conversely,

in Zhenhai reservoir, diazotrophic cyanobacteria outcompeted the other members of the phytoplankton assemblage and reached percentage of dominance close to 100% for long periods. Morton & Lee (1974), were the first who showed that iron concentrations in the range 0.1–1.0 mg/L, similar to those recorded in Zhenhai reservoir at circulation, caused a shift from green algae to cyanobacteria. In fact, green algae are known to be able to outcompete cyanobacteria under low iron availability (e.g. Jin et al., 2009; Molot et al., 2010; Wang et al., 2015). Diazotrophic cyanobacteria have higher iron requirements compared to eukaryotic algae because enzymes involved in N₂ fixation need iron to work (Molot et al., 2010) and many cyanobacteria species can overcome iron starvation by increasing the production of extracellular iron chelators (siderophores) and/or by down-regulating their physiological rates (González et al., 2018). The latter mechanism however strongly decreases their growing rates. When iron limitation occurs, green algae and diatoms can rely on a more efficient uptake system similar to that involved in the “strategy I” of plants (Hutchins et al., 1999; McQuaid et al., 2018; Coale et al., 2019; Jiang et al., 2020). In particular, they can increase the production of membrane enzymes (ferric chelate reductases) which promote the reduction of Fe³⁺ to Fe²⁺ and, coupled with a specific transporter, facilitate the uptake of Fe²⁺ across the plasma membrane (Weger et al., 2002; Jeong et al., 2008). This mechanism allows green algae and diatoms to store iron and to use it to support growth for several generations under conditions of low iron availability (Marchetti et al., 2009;

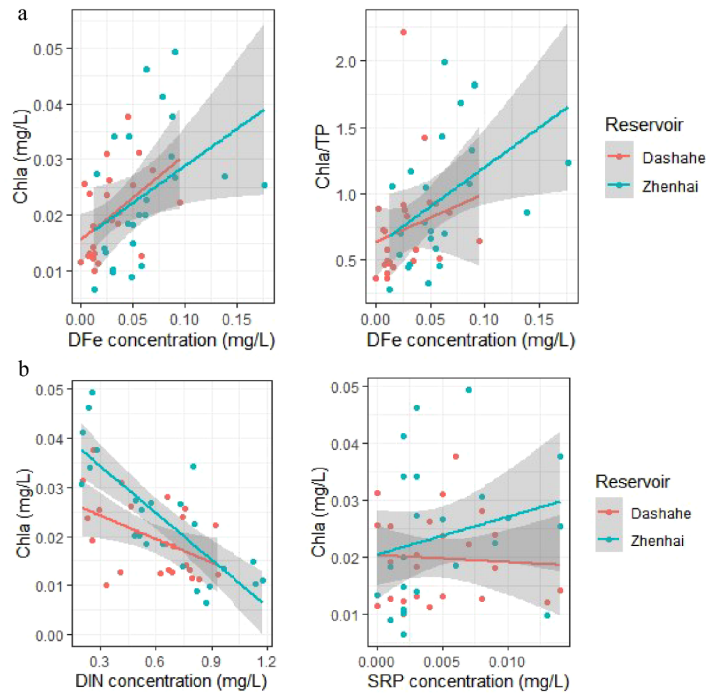


Fig. 6. Linear Model (LM) showing the regression fitting computed on Chla and Chla/TP with DFe concentrations (a) and on Chla with DIN and SRP concentration (b) (the blue solid circles were the samples from Zhenhai and red solid circles were the samples from Dashahae reservoir, solid line was the fitting line, and the grey area is the double error range of fitting line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Significant environmental variables explaining the variance in phytoplankton assemblage structure determined by forward selection and testing of marginal effects, and their R_{adj} determined by redundancy analysis. (n.s.: no significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

| Dataset | Significant variables selected by forward selection | Significant variables from the test of marginal effects (R_{adj}) |
|------------------|---|---|
| Dashahae | T, RWCS, SRP, ($R_{adj} = 0.50$) | DFe** (0.22), DIN*** (0.35), T*** (0.4), SD* (0.16), Z_m ** (0.27), RWCS** (0.23) |
| Zhenhai | n. s. | DIN* (0.14), DFe* (0.05) |
| Combinet dataset | DFe, DIN, ($R_{adj} = 0.28$) | TFe** (0.1), DFe*** (0.22), DIN*** (0.13), T** (0.13), SD*** (0.2), Z_m * (0.11), RWCS** (0.12) |

Briat et al., 2010) as those observed in Dashahae reservoir at circulation.

The two studied reservoirs, although similar in the physical and chemical characteristics of their waters, were characterized by quite different phytoplankton dynamics. The RDA carried out on combined dataset showed that the variance of phytoplankton assemblages in the two reservoirs could significantly be explained by the different concentrations of DFe, whose explaining effect in the analysis was more

pronounced than those of DIN and SRP. The higher DFe likely allowed the diazotrophic cyanobacteria to be dominant for most of the investigated period in Zhenhai reservoir, and during stratification in Dashahae reservoir. In Zhenhai, the strengthening of the stratification in June 2017 led diazotrophic cyanobacteria to be dominant for the rest of the investigated period. In Dashahae reservoir, a seasonal alternance between diazotrophic cyanobacteria (mostly *Raphidiopsis*) and eukaryotic algae (desmids, chlorophytes and diatom) was observed. As shown in several studies, a spatial or a temporal alternance of these phytoplankton groups commonly occurs in tropical lakes; for example, *Staurastrum* spp. and/or *Cosmarium* spp. were found to be dominant in the epilimnion of many lakes in the middle Rio Doce area of Brazil, whereas *Raphidiopsis* dominated in the metalimnion (Barbosa & Padisák, 2002), where the access to a higher concentration of available iron was more likely. The alternate dominance of desmids and *Raphidiopsis* was also recorded in the African tropical lakes Ogelube and Kariba (Nweze, 2006; Ndebele-Murisa et al., 2010). These studies analyzed the patterns of phytoplankton dynamics and structure considering both the physical properties of water in tropical climate (Barbosa & Padisák, 2002) and the availability of N and P (Huszar et al., 2000; Nweze, 2006; Ndebele-Murisa et al., 2010) but not the iron availability. A temporal segregation of these phytoplankton groups was also detected in our study and, as shown by NMDS, it can be related to the different resource requirements

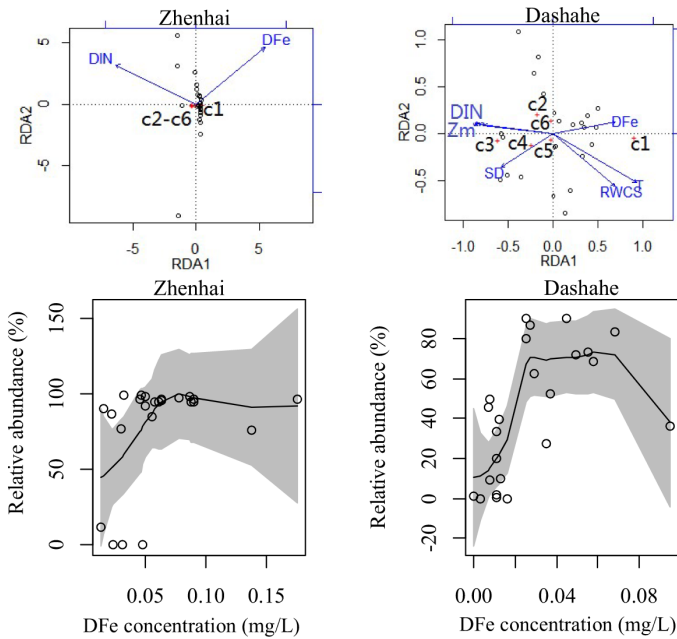


Fig. 7. Above, tri-plots of the RDA results showing the relationships among significant environmental variables and phytoplankton biomass of six group (c1: diazotrophic cyanobacteria, c2: non-diazotrophic cyanobacteria, c3: diatoms, c4: chlorophytes, c5: desmids, c6: others); open circles represent the samples. Below, local regression fitting computed on relative abundance of diazotrophic cyanobacteria (the open circles are the samples, solid line is the fitting line, and the grey area is the double error range).

of the functional groups to which they belong (Reynolds et al., 2002).

4.2. Thermal stability and nutrients dynamics in the studied reservoirs

No differences in climate and geological features of the catchment characterized the two studied reservoirs. It was therefore not surprising that they showed similar seasonal trends of temperature and of their stratification/mixing patterns. However, the studied water bodies showed a different strength in the thermal stability of their water columns. In particular, Zhenhai showed RWCS values much higher than Dashahe. This higher stability, along with the high summer values of water temperature, as already observed in some tropical and subtropical reservoirs (Barbosa & Padišák, 2002; Naselli-Flores, 2003), have likely favored the establishment of daily thermoclines (atelmixis) in the upper layer of the water column which may have caused a downward reduction of oxygen availability and pH values. Such decrease, as shown in the vertical profiles of the two variables, could explain the different availability of nutrients, and especially of DFe, in the upper layers of the studied reservoirs. In lakes and reservoirs, the nutrients availability often depends on the physical, chemical and hydrodynamical conditions along the water column (Kalf, 2002; Tang et al., 2019; Liang et al., 2020). As already observed in other aquatic ecosystems (e.g. Millero et al., 1995; Shaked et al., 2005; Earhart and Lei, 2015). DFe concentrations showed a marked spatial gradient along the water columns of the study reservoirs: the availability of dissolved iron was quite low (<0.05 mg/L) in the oxygen-rich surface waters and increased below the thermocline up to values higher than 4 mg/L. The vertical profiles of dissolved iron were against the concentration gradient of dissolved oxygen and negatively related to pH value in both the studied reservoirs.

Nitrogen and phosphorus availability are considered the major drivers of phytoplankton growth and seasonal succession (Sommer et al., 2012). Our results showed that their availability was linked to that of iron which ultimately was regulated by the thermal patterns occurring in the reservoirs. Phosphate and Fe^{3+} are actually bound to form insoluble ferric phosphate in well oxygenated lacustrine sediments. Under anaerobic conditions, Fe^{3+} is reduced to Fe^{2+} and ferric phosphate is transformed in the soluble ferrous phosphate (Millero et al., 1995). As a consequence, both phosphorus and iron are released from the sediments to hypolimnetic waters and eventually made available to phytoplankton in the upper layers of a water body (Kalf, 2002; Molot et al., 2014). According to the results shown by Gautreau et al. (2020), anaerobic conditions can promote the mobilization of high amount of nitrogen and phosphorus from reservoirs sediments. Anaerobic conditions and low pH values, as shown in subtropical reservoirs under a Mediterranean climate (Naselli-Flores, 2003), can be determined by the onset of atelmixis which contribute to the release of bioavailable iron and phosphorus from the sediments. DFe and SRP values in the upper layers, which in the studied reservoirs were positively related, were higher in Zhenhai than those recorded in Dashahe reservoir, likely because of the more stable stratification, more pronounced anaerobic conditions in its hypolimnion, and shallower anaerobic hypolimnetic zone. According to the data, it is possible to speculate that, especially in the shallower, coastal zone of these reservoir, atelmixis may have occurred under calm conditions, promoting oxygen consumption, favoring the release of DFe and SRP from the sediments and promoting the growth of diazotrophic cyanobacteria. Moreover, the lower oxygen concentrations recorded along the water column of Zhenhai reservoir even during circulation can explain the higher DFe concentrations

measured in that period.

The increase in DFe was also linked to the decrease of DIN in epilimnion of the studied reservoirs. This effect was already observed in Lake Erie (Havens, et al., 2012), and two hypotheses were proposed to explain it: (i) the increase of Fe availability stimulates the growth of Fe-limited phytoplankton and enhances inorganic nitrogen assimilation; (ii) the increase of Fe availability promotes an increased intracellular Fe content of phytoplankton which enhances the ability of cells to assimilate inorganic nitrogen. Since DFe is an important factor for the dynamics of high-iron-demanding diazotrophic cyanobacteria, the first hypothesis seems more reliable in our case.

Our results suggest that iron availability in the epilimnion of the two reservoirs mostly depended on the redox and acid conditions of their deeper layers, which in turn depended on the strength of their thermal stratification. Thus, it is not surprising that temperature and RWCS, which determined the stratifying regime and the strength of mixing, resulted as significant explaining variables in RDA forward selection, but not DFe.

In addition, the significant relationships found between DFe concentration and both Chla and Chla/TP indicate a positive effect of iron availability on phytoplankton biomass. The highest values of phytoplankton biomass were recorded in summer when diazotrophic cyanobacteria (*Raphidiopsis* and *Dolichospermum*) were dominant. *Raphidiopsis* and *Dolichospermum* both can fix atmospheric nitrogen and show a quite high uptake affinity for phosphorus; moreover, they both have the capacity to increase alkaline phosphatase activity under P-limiting conditions (Istvánovics et al., 2010; Willis et al., 2019; Wan et al., 2019). Both nitrogen fixation and alkaline phosphatase activity need a continuous iron supply (Fu et al., 2019; Yong et al., 2014), which in the studied reservoirs was likely conveyed from the iron-rich, anaerobic hypolimnion. This mechanism supported their growth even in the decreasing DIN and low SRP condition, allowing these species to outcompete eukaryotic phytoplankton. However, spatial replications are needed to better describe the relationships among diazotrophic cyanobacteria and DFe, DIN and SRP concentration in the reservoirs.

5. Conclusion

The results of an investigation on the seasonal succession of phytoplankton in two tropical, eutrophic reservoirs, and the differences observed in the dynamics of its assemblages, strongly indicated iron availability as an important factor in promoting permanent year-round cyanobacteria blooms. The effect of iron was mediated by the complex interactions between the physical (establishment and strength of thermal stratification) and chemical (redox conditions, pH) features of the water column. Such interactions contributed to regulate iron availability and, in cascade, that of other nutrients such as nitrogen and phosphorus. This regulating effect can have masked the role of iron in modulating phytoplankton dynamics in lakes and reservoirs. The environmental scenario resulting from these interactions can contribute to allow the persistence of permanent year-round blooms of diazotrophic cyanobacteria. In particular, the strengthening of water stratification as a result of global warming can favor the establishment of anoxic hypolimnia in eutrophic water bodies, and the release of available DFe which in turn can enhance cyanobacteria blooms. Our results suggest that the analysis of iron dynamics should be routinely included in the monitoring programs addressed at elucidating phytoplankton dynamics and eutrophication patterns.

CRedit authorship contribution statement

Li-Juan Xiao: Data curation, Writing - original draft. **La-Mei Lei:** Supervision. **Liang Peng:** Methodology, Software. **Qiu-Qi Lin:** Visualization, Investigation. **Luigi Naselli-Flores:** Writing - review & editing.

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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