

Research papers

Relationships between biomass of phytoplankton and submerged macrophytes and physicochemical variables of water in Lake Caohai, China: Implication for mitigation of cyanobacteria blooms by CO₂ fertilization

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ABSTRACT

Reducing nutrient input in aquatic ecosystems has been widely recognized as a way to mitigate eutrophication. The paradigm of nutrient limitation by N and P is pervasively accepted, without considering the possibility of C limitation. However, our assumption that C might affect the aquatic biological composition, lacks fieldwork support. We tested the co-limitation of C/N/P on the growth of submerged macrophytes and phytoplankton in the Caohai Lake, a typical shallow karst lake in southwestern China. This study documented the seasonal variations in the concentration and comparative availability of nutrients C/N/P, in addition to physicochemical parameters, submerged macrophytes biomass, and phytoplankton composition in the lake. Our results show that dissolved inorganic carbon plays a substantial role in enhancing the primary production of phytoplankton and macrophytes, which suggests that primary production can be suppressed not only by P and/or N (as generally believed) but also by C, owing to the unique physicochemical characteristics that prevail in typical karst regions. Additionally, submerged macrophytes have been found to inhibit phytoplankton growth. Further analysis showed that the proportion of Cyanobacteria over Chlorophyta and Bacillariophyta throughout the year was reduced at dissolved carbon dioxide/total nitrogen (CO₂/TN) ratios (>0.2) and dissolved carbon dioxide/total phosphorus (CO₂/TP) ratios (>10) in this study. Isotopic evidence further indicated that the abundance of Cyanobacteria was reduced when CO₂ >30 μmol/L. In summary, the combined results suggest that when controlling N and P, CO₂ fertilization may significantly affect both the growth and composition of phytoplankton communities/submerged macrophytes and carbon sequestration in karst surface water ecosystems. This implies a new “paradigm” in the mitigation of cyanobacteria blooms by adding more CO₂ to aquatic ecosystems with low pCO₂, during which carbon sequestration may also be enhanced.

1. Introduction

The concentration of atmospheric CO₂ which has been dramatically increasing, on average, to 408 ppm (IPCC, 2018; Myers et al., 2014; Schimel et al., 2000) is exacerbated by human activities, resulting in an increase in eutrophication (Anderson et al., 2002; Smith, 2003; Tang et al., 2012). Since the 1980s, evidence of consistent relationships

between P and phytoplankton biomass has supported “the phosphorus (P) limitation paradigm” (Edmondson, 1970; Hecky and Kilham, 1988; Schindler and Fee, 1973). It is generally believed that a natural mechanism compensates for nitrogen (N) and carbon (C) deficiencies in eutrophic lakes (Schindler, 1971). However, without an external source, P does not participate in the atmospheric gas-phase cycle, which forms the theoretical basis of this paradigm (Schindler, 1977; Smith, 1983). In

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recent decades, there has been growing evidence that contradicts this, with an emphasis on N limitation or N–P co-limitation, particularly in marine ecosystems (Conley et al., 2009; Lewis Jr and Wurtsbaugh, 2008; Liang et al., 2020; Smith et al., 2016). Many lakes are supersaturated with CO₂, leading to the misconception that the CO₂ in freshwater systems is sufficient to support the carbon needs of aquatic plants through photosynthesis, causing C limitation to be overlooked (Cole et al., 1994; Schindler, 1971; Sobek et al., 2005).

However, inorganic carbon potentially limits photosynthesis in aquatic systems. In fact, previous understanding of carbon limits was based on investigations of oligo-mesotrophic waters. Cyanobacterial blooms can easily transform a supersaturated lake with CO₂ into an undersaturated one (Balmer and Downing, 2017). The diffusion of CO₂ is 10⁴-fold lower in water than in air (Stumm and Morgan, 1981; Zagarese et al., 2021). The atmospheric CO₂ invasion rate calculated by Visser et al. (2016) was approximately 7 mmol m⁻² d⁻¹ during a dense cyanobacterial bloom. Dissolved inorganic carbon (DIC) in karst water exists in three interchangeable forms: HCO₃⁻, CO₂, and CO₃²⁻, with a CO₂ concentration of <1 % DIC when pH > 8 in alkaline waters (Bao et al., 2020, 2022; Riebesell et al., 1993; Zeng et al., 2019). Thus, C limitation is worthy of attention (Hamdan et al., 2018; Riebesell et al., 1993; Zeng et al., 2019), particularly in the karst aquatic ecosystems, where alkalinity is high and CO₂ concentrations is low. Furthermore, CO₂ can deplete locally and seasonally when aquatic plants grow rapidly (Zeng et al., 2019) or in highly eutrophic lakes where C supply cannot meet demand (Flynn and Raven, 2016; Talling, 2010; Low-Décarie et al., 2014). Previous studies have divided the factors limiting production into rate and yield-limiting factors (King, 1970; Schindler, 1971; Schindler and Fee, 1973; Smith, 1983; Sterner, 2008). Whereas C was defined as the rate-limiting resource owing to its concentration affected growth rates; N and P were defined as yield-limiting resources because their concentrations limit the potential maximum biomass. The absolute concentrations of these nutrients and their comparative availability (C/N, C/P, and N/P) have important implications for limiting aquatic plant growth (Bao et al., 2022; Bhavya et al., 2017; Elser et al., 2009; Gulis et al., 2017) and water quality regulation (Dodds and Oakes, 2008; Smith, 1983). In addition, increases in N and P in many modern ecosystems may reduce their restrictions on productivity and correspondingly increase the importance of limited CO₂ (Low-Décarie et al., 2015).

The concept of a biological carbon pump (BCP) was first proposed in the 1980s by marine scientists who studied the ocean carbon cycle. It refers to the transfer of C from the water surface to depth via a series of biological processes, including organic matter production, consumption, transmission, deposition, and decomposition (Longhurst and Harrison, 1989). Liu et al. (2010, 2015, 2018) showed that a strong BCP effect also exists in karst surface water ecosystems through photosynthesis of aquatic plants, which fixes part of the carbonate-weathering DIC and forms stable carbon sinks as sedimentary organic carbon. The terrestrial BCP effect describes the impact of DIC fertilization on the processes and intensity of biomass accumulation; the higher the DIC concentration, the stronger the C stabilization and formation of C sinks (Liu et al., 2010, 2018). Furthermore, it has been proven that macrophytes, the principal BCP effect product, can improve water quality and alleviate eutrophication (Dhote and Dixit, 2009; Song et al., 2019). The Redfield ratio (C:N:P = 106:16:1) is considered the average value of marine phytoplankton C, N, and P ratios, but several studies have found that this ratio is not suitable for freshwater systems (Elser et al., 2000; Hecky et al., 1993). Previous studies have shown that nutrient limitations of phytoplankton can be observed in pure-species continuous cultures when the nutrient ratios deviate from the Redfield ratio (Howarth, 1988). Changes in C:N:P in the water body change the elemental proportions, enzyme activities and composition of chemical substances in aquatic plants, thereby restricting their growth and affecting the composition of phytoplankton communities (Hall et al., 2005; Kilham et al., 1997). More importantly, phytoplankton taxa exhibit a wide range of capacity for absorbing inorganic C (Spijkerman et al., 2005). Some of

these are carbon-concentrating mechanisms (CCMs) with carbonic anhydrase (CA), such as in Cyanobacteria, which can overcome low CO₂ conditions (Badger and Price, 1994; Vogt et al., 2017). In contrast, Bacillariophyta and Chlorophyta favor higher CO₂ conditions with their greater C requirements (Low-Décarie et al., 2011, 2014; Shapiro, 1973).

Previous studies have focused on the standing stock (absolute concentrations) of nutrients in surface water to relate primary production to hydrophyte abundance (Kragh and Sand-Jensen, 2018). Numerous hydrological and biogeochemical processes can change the concentration of nutrient (C, N, and P) concentrations in lakes. Moreover, as noted above, the nutrient limitation paradigm has been largely based on P and/or N studies, rather than C. Nutrient limitation is related to the regular growth rates of phytoplankton populations, possible shifts in phytoplankton species composition, and the net production of the ecosystem (Howarth et al., 1988). In particular, the absolute nutrient concentrations of the water samples measured were those after consumption by hydrophytes (i.e., the result), which do not represent the original limiting factors (i.e., the reason). Moreover, the load rate or initial concentration is generally difficult to obtain for natural fresh water ecosystems. Therefore, both the absolute concentration and relative availability of C, total N, and total P (C/N, C/P and N/P) were used to better understand the co-limitation of nutrients on primary production, and biological structure/composition.

Cyanobacteria have been known for over 140 years to be harmful algae (Francis, 1978). Some of them have ability to fix nitrogen (Dokulil and Teubner, 2000; Klemer et al., 1995), concentrate CO₂ (CCM) (Huisman et al., 2018), and adjust buoyancy (Carmichael, 1994; O'Neil et al., 2012), as well as low light intensity requirements and anti-predation ability (Chislock et al., 2013; O'Neil et al., 2012). Comparatively, Chlorophyta and Bacillariophyta cause less harm to water quality (Van de Waal et al., 2011), and can be used for many purposes, such as aquaculture (Marella et al., 2020; Sergejevová and Masojídek, 2011). Eutrophication results from excessive algal growth owing to increased availability of limiting growth factors needed for photosynthesis, such as sunlight, CO₂, and nutrient fertilizers (Howarth and Marino, 2006; Low-Décarie et al., 2015; Schindler, 2006; Shi et al., 2017). Dense algal blooms are generally caused by toxigenic cyanobacteria (Catherine et al., 2013; Monchamp et al., 2018; Svrcek and Smith, 2004). Thus, we suggest that the ratio of Cyanobacterial abundance/(Chlorophyta abundance + Bacillariophyta abundance) (Cya./(Chl. + Bac.)) indicates the degree of water quality deterioration by cyanobacterial bloom in the lake (Bao et al., 2022). When the ratio increases, the lake water quality deterioration intensifies and is alleviated when the ratio decreases.

Studies on the effects of seasonal variability on the coupling of C, N, and P limitation and the response of phytoplankton community composition in karst aquatic ecosystems are scarce. Therefore, this study assessed the seasonal changes in BCP effects with CO₂ fertilization in a typical shallow karst surface water ecosystem, Caohai Lake, with a particular emphasis on the effect of C abundance. Physicochemical parameters, phytoplankton and the main submerged macrophytes samples were collected from 12 sites in the karst lake during the four seasons of 2020. Our objectives were to (1) understand the limitations of concentration and stoichiometric elemental ratios (C/N/P) on the growth of phytoplankton and submerged macrophytes in water, (2) explore the coupling mechanisms and relationships between C/N/P and the community structures of phytoplankton and submerged macrophytes, and (3) reveal the effects of BCP on the mitigation of cyanobacteria blooms and enhancement of carbon sequestration in these waters. Specifically, we sought to verify the hypothesis that carbon sink enhancement and reduction of cyanobacteria blooms by BCP with CO₂ fertilization in karst water provides new scientific support for this win-win situation and a new "paradigm" of C neutralization and cyanobacteria blooms control in aquatic ecosystems with low pCO₂ (high pH and DIC).

2. Methods

2.1. Site description

This study was conducted at 12 sites in Caohai Lake (26°49′–26°53′N, 104°12′–104°18′E), and samples were collected in January 2020, May 2020, July 2020 and October 2020, over four seasons (Fig. 1). Caohai Lake, a typical karst shallow lake with abundant submerged macrophytes, is located in the karst area of southwest China. It has a surface water area of 25 km² and an average depth of 2.4 m (Zhu et al., 2013). It experiences a typical subtropical humid monsoon climate, with a mean annual precipitation of approximately 1000 mm and a mean annual temperature of 10.6 °C (Yang et al., 2017). The lake is fed by groundwater and precipitation without visible stream channels entering it (Cao et al., 2016; Zhu et al., 2013). We investigated the lake by subdividing it into two regions: (i) heavy pollution (annual average Chl-a concentration of sample sites in 2020: 13.2 ± 7.4 µg/L); (ii) mild pollution (annual average Chl-a concentration of sample sites in 2020: 3.7 ± 2.4 µg/L). The northeastern region of the lake (C1, C2, C3, and C4) suffers from high external nutrient loading with relatively weaker development of submerged macrophyte coverage because it is adjacent to an urban area and cultivated land, although no distinct point source of pollution was noted. The remainder of the lake is closer to the forest and meadowland, with less pollution (Hu et al., 2020). *Potamogeton lucens* L., *Chara braunii* Gmel. and *Myriophyllum spicatum* L. are the dominant submerged macrophytes in the lake. The dominant phytoplankton species were Cyanobacteria, Chlorophyta, and Bacillariophyta.

2.2. Lake monitoring and sampling

One water sample was collected from the lake in each season at each of the spatially representative sampling sites, C1–C12 (Fig. 1), a total of 48 samples. Temperature (T), pH, dissolved oxygen (DO) and electrical conductivity (EC) were measured using a multi-parameter water quality analyzer (Ponsel Odeon, France) at a 0.5-m depth below the surface. The parameters of the analyzer were calibrated as follows: pH (4.01, 7.00, and 10.00), DO (0 % and 100 % air), and EC (1412 µS/cm). The measurement accuracies of the device for the T, pH, DO and EC were 0.01 °C, 0.01, 0.01 mg/L and 0.01 µS/cm, respectively. Simultaneously, transparency (Secchi depth, SD) was determined in situ using a Secchi disc (SD-30), and DIC was immediately titrated in the field using an Aquamerck alkalinity test kit (Merck, Germany) with an estimated accuracy of 0.05 mmol/L (Zeng et al., 2019). The lake water samples were passed through membrane filters (0.45 µm) into 20-mL acid-cleaned HDPE bottles and preserved with 2 % HNO₃ for cation analysis. The cation concentrations (Ca²⁺ and Si⁴⁺) of the filtered samples were determined using an inductively coupled plasma emission spectrometer (ICP-OES, USA). Total nitrogen (TN) and total phosphorus (TP) were determined on unfiltered samples preserved in 100-mL acid-cleaned glass bottles at

–4 °C; they were determined within two days using the persulfate oxidation method (Greenberg et al., 2005). Bulk water samples (1 L) were also collected at 0.5 m, immediately passed through 0.45-µm GF/C filters, and frozen until analysis. Chlorophyll *a* (Chl-*a*) was extracted using 90 % hot ethanol and measured spectrophotometrically using a method described by Sartory and Grobbelaar (1984). The CO₂ concentration was calculated using the aqueous geochemical modeling software PHREEQC with on-site records of pH, temperature, and alkalinity (Parkhurst and Appelo, 2013). For δ¹³C_{DIC} analyses, 100 mL of each filtered water sample was injected into a pre-cleaned glass bottle without any headspace and preserved with saturated HgCl₂ at 4 °C. For their measurement, they were acidified with pure phosphoric acid, and the extracted CO₂ was purified using a vacuum line and analyzed on a Finnigan MAT253 mass spectrometer (Thermo Fisher, USA). For δ¹³C_{POC} analyses, 2-L water samples were filtered through pre-combusted (450 °C, 5 h) 0.7-µm Whatman GF/F filters, and the filter was acidified to remove any inorganic carbon before preservation at –20 °C. They were then directly tested in a Flash 2000 HT device coupled with the MAT253 Plus instrument (Thermo Fisher, ±0.2 ‰, analytical error).

2.3. Analysis of phytoplankton and submerged macrophytes

Phytoplankton samples for the qualitative study were collected directly from the water using a phytoplankton net (mesh size = 0.064 mm) in an ∞ shape for at least ten minutes, and then preserved in 50 mL centrifuge tubes fixed with a formaldehyde solution (4 %). Surface water samples (1.5 L) were concentrated to 30 mL using Lugol iodine for quantitative analysis. To identify genera, all subsamples were inspected using an inverted microscope at 400× magnification (Hillebrand et al., 1999). For analysis, the genera were aggregated at the levels of Cyanobacteria, Chlorophyta, Bacillariophyta, Pyrrophyta, Englenophyta, and Chrysophyta, because these six groups made up most of the community biomass.

The submerged macrophytes were collected using a Pettersen grab bucket (port-area 0.19 m²) along one or two quadrats of 1 m² each. All the macrophytes were collected within 0.19 m², which was repeated three to six times within the transect to reduce the error caused by uneven growth, kept in the dark in a cooling box in the field, and frozen for 24 h to avoid deterioration. All samples were cleaned with ultrapure water and placed in a drying oven (60°C) until they reached constant weight (dry weight, at least 48 h). The dry weight of the plants was used as a proxy for the macrophytes biomass.

2.4. Statistical analysis

The mean and standard deviation were used to calculate the average and range of the values. Data analyses were performed using SigmaPlot 14.0, to investigate the relationships between lake nutrient chemical parameters (C, N, and P concentrations and their relative availabilities

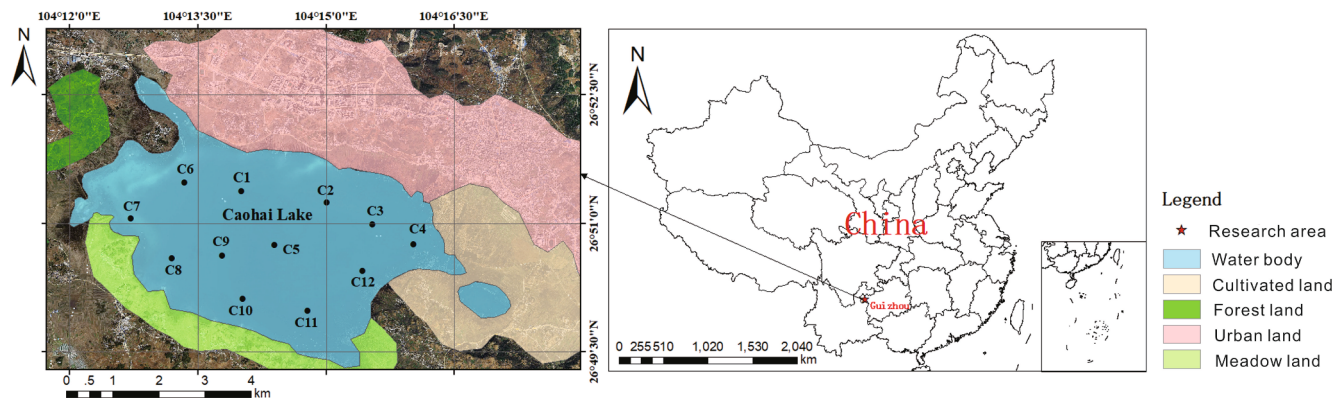


Fig. 1. Sampling site locations in Caohai Lake, southwest China.

of DIC/TN, DIC/TP, TN/TP, CO₂/TN, and CO₂/TP across reaches within each site), primary productivity of planktonic algae (Chl-a and community structure), and submerged macrophytes dry weight.

One-way analysis of variance (ANOVA) was performed to determine any significant differences among the data, using SPSS 22.0. Differences were considered statistically significant at $P < 0.05$. CANOCO 5.0 was used to conduct multivariate analysis, using redundancy (Yan et al., 2016) to determine the relationship between environment (relative availability, temperature), species (Cyanobacterial abundance, Chlorophyta abundance, Bacillariophyta abundance, and submerged macrophytes dry weight), and sample sites across the three datasets with all variables during the four seasons (Šmilauer and Lepš, 2014).

3. Results

3.1. Seasonal variations in general physicochemical parameters

Large seasonal fluctuations and differences between individual sample sites were observed in T, pH, DO, EC, SD and Ca²⁺ measurements (Fig. 2 and Table S1). In spring (20.70 ± 1.1 °C) and summer (21.95 ± 0.80 °C), the temperature was higher than in autumn (14.19 ± 0.35 °C), while the winter temperature (9.42 ± 0.45 °C) was the lowest. Compared to other three seasons, pH was significantly lower in winter ($P < 0.001$), with an average value of 8.19 ± 0.17. The DO (%) increased by approximately 12 % between spring and summer ($P = 0.09$). The EC and Ca²⁺ concentrations were higher in winter and spring than in summer and autumn ($P < 0.01$ and $P < 0.001$, respectively). Distinct seasonal differences in SD were also observed ($P < 0.01$), with the average in autumn increasing substantially to 1.39 ± 0.45 m ($P < 0.001$) compared to 0.89 ± 0.27 m in summer. The SD and DO values of sites C5-C12 (where there was more growth of submerged macrophytes) were higher than those of the other sites in most seasons. Site C4, which is close to a busy wharf, had the lowest average pH (8.02–8.30) and SD (0.41–0.64 m) and highest EC (459.50–582.30 μS/cm) and Ca²⁺ (71.19–95.82 mg/L) during all seasons.

DIC concentrations at the investigated sites varied from 8.4 to 43.2 mg/L-C (a wide range), as did the surface water pCO₂ from 15 to 1740 μatm (Fig. 3 and Table S1). Large seasonal fluctuations were observed in the DIC ($P < 0.01$). The average DIC of all sites except C4 was much higher in winter and spring (9929.0 ± 6.1 mg/L-C and 28.0 ± 6.1 mg/L-C, respectively) than in the summer (20.0 ± 8.6 mg/L-C) and autumn (19.0 ± 7.4 mg/L-C). Most sites were undersaturated with CO₂ ($n = 18$) in the summer and autumn, indicating that there was “carbon sequestration”. In contrast, the lake displayed CO₂ super-saturation ($n = 12$) in winter, i.e. it was a “carbon source”. No significant seasonal differences were observed for Chl-a, TP and TN ($P > 0.05$), but the average TP was

highest in winter (0.054 ± 0.034 mg/L), while the average TN was highest in autumn (1.56 ± 0.39 mg/L) (Fig. 3 and Table S1). The Chl-a concentration peaked from late spring to summer, but it was likely that the absolute Chl-a concentration was also high during autumn, being compensated by higher grazing pressures and larger submerged plants biomass. Sampling Site C4 had the highest Chl-a (20.58–26.29 μg/L) and TP (0.071–0.154 mg/L) during all seasons (Fig. 3 and Table S1). δ¹³C_{DIC} ranged from −7.20 to −1.28 ‰, with an average of −3.97 ‰, and δ¹³C_{POC} ranged from −33.65 to −19.88 ‰, with an average of −25.36 ‰. These variations were synchronous, with the highest in summer and lowest in winter.

3.2. Spatial variations in phytoplankton community composition and submerged macrophytes dry weight in the study lake

Phytoplankton composition and abundance are shown in Fig. 4 and Table S2. For this analysis, genera were aggregated at the Cyanobacteria, Chlorophyta, Bacillariophyta, Pyrrophyta, Euglenophyta, and Chrysophyta levels, as these six groups constituted the majority of the biomass. The community was dominated by Cyanobacteria, Chlorophyta, and Bacillariophyta; however, there were large differences between the sample sites. Phytoplankton abundance ranged from 557 to 4716 cells/mL. A seasonal peak was observed at Site C4 in summer (4716 cells/mL) and autumn (4264 cells/mL). These peaks were attributed mainly to Chlorophyta (*Coelastrum*, *Chlamydomonas*, *Scenedesmus*, and *Selenastrum bibratianum*) and Bacillariophyta (*Cyclotella*, *Melosira*, and *Synedra*). In contrast, the lowest average annual abundance was observed at Site C7 (932 ± 464 cells/mL), which had a relatively high submerged macrophytes dry weight (Figs. 4 and 5). Cyanobacteria developed best in summer, with the most common taxa being *Microcystis ichthyoblabe*, *Pseudanabaena*, *Merismopedia* sp., and *Gomphosphaeria lacustris*. Furthermore, the concentration of Chl-a was consistent with phytoplankton abundance throughout the year ($r = 0.79$, $P < 0.001$), indicating that both parameters represented the primary production of phytoplankton very well. The dry weight of the submerged macrophytes (mainly *Potamogeton lucens*, *Chara braunii*, and *Myriophyllum spicatum*) was determined by measuring the samples from the 12 sites during winter and summer. We defined winter as the dormant season and summer as the growing season (Fig. 5). The submerged macrophytes dry weight ranged from 15.05 to 53.94 g/m² in the dormant season and from 9.22 to 116.73 g/m² in the growing season ($n = 12$). The average submerged macrophytes dry weight increased by approximately 38 % from the dormant season (33.2 ± 10.9 g/m²) to the growing season (52.5 ± 30.8 g/m²). In the growing season, the variability of dry weight between the sample sites was relatively high, with the lowest average dry weight being observed at C4 (9.2 ± 2.8 g/m²)

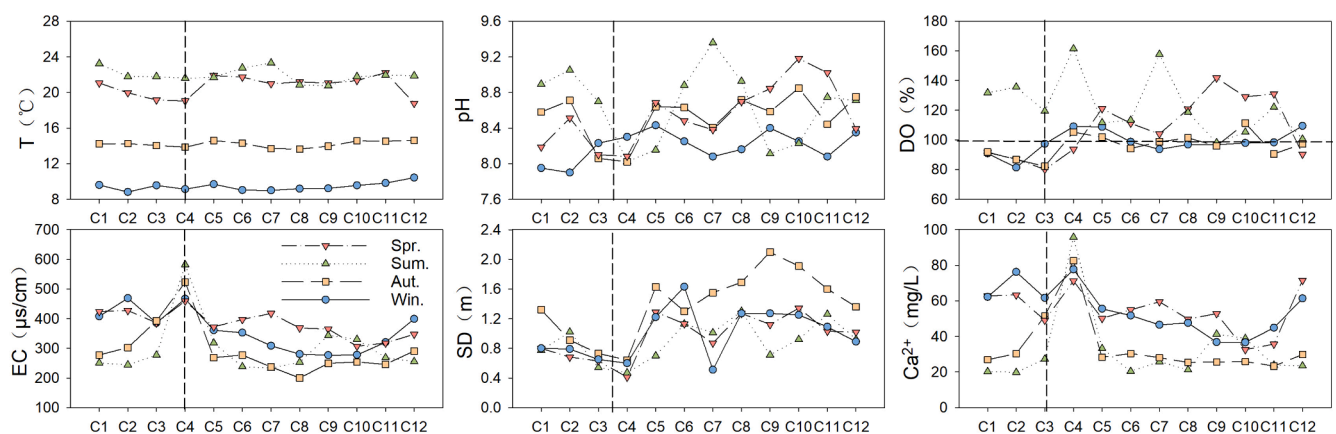


Fig. 2. Spatiotemporal variations in physicochemical parameters in Caohai Lake during 2020, the year of investigation. C1 to C12: Sampling sites; Spr.: Spring; Sum.: Summer; Aut.: Autumn; Win.: Winter. T: Temperature; DO: Dissolved oxygen; EC: Electrical conductivity; SD: Secchi Depth. The horizontal dashed line shows DO at 100%, and the vertical dashed line shows the boundary between the heavy pollution sites (C1–C4) and the mild pollution sites (C5–C12).

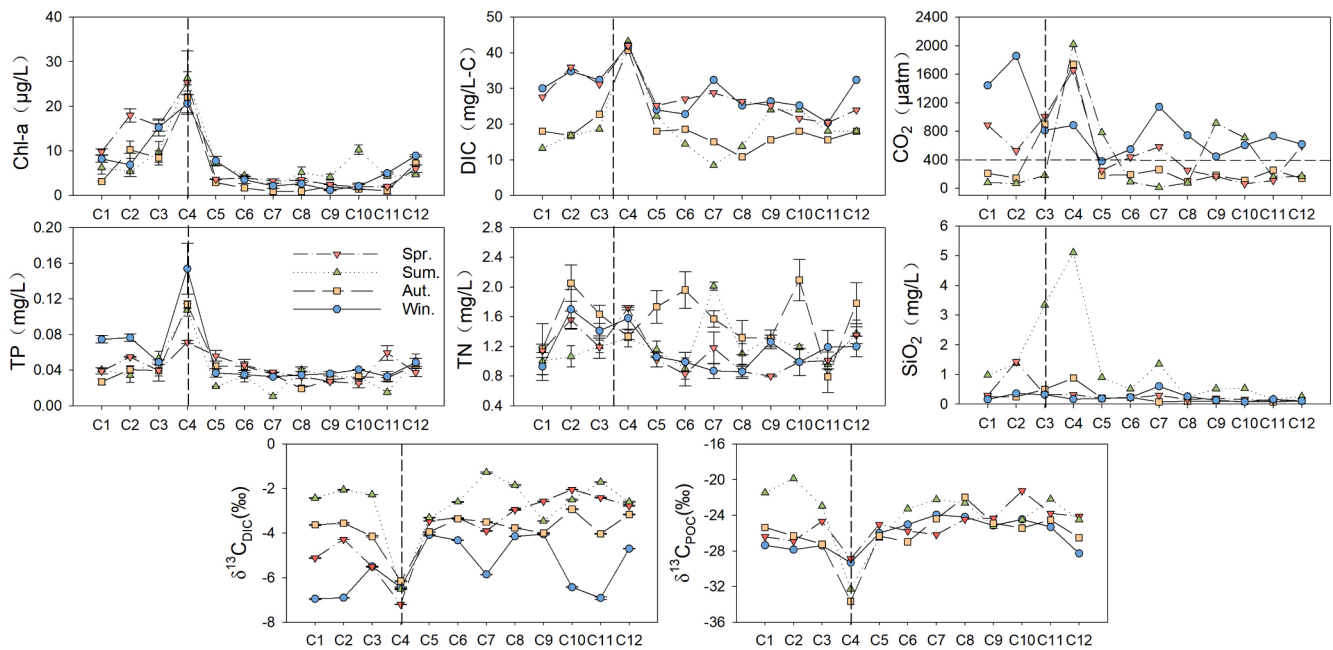


Fig. 3. Spatiotemporal variations in nutrient concentrations and C isotope measurements in Caohai Lake during 2020. C1 to C12: Sampling sites; Spr.: Spring; Sum.: Summer; Aut.: Autumn; Win.: Winter. The horizontal dashed line shows the average atmospheric CO₂ concentration, and the vertical dashed line shows the heavy pollution (C1–C4)–mild pollution (C5–C12) boundary.

and the highest at C11 ($116.7 \pm 17.0 \text{ g/m}^2$). The dry weight of submerged macrophytes were the lowest at sites C1 to C4, which were close to urban and cultivated lands (Fig. 5). At the highest external load of nutrients (Site C4), macrophyte cover was exceptionally low, with large unvegetated areas and a high Chl-a concentration.

3.3. Effects of nutrient concentrations and ratios on phytoplankton and submerged macrophytes

The relationships between Chl-a and C, N or P concentrations were examined. The results are shown in Fig. S1. There were positive correlations between Chl-a and dissolved CO₂ during all seasons, except winter (spring: $r^2 = 0.76$, $P < 0.01$; summer: $r^2 = 0.72$, $P < 0.01$; autumn: $r^2 = 0.74$, $P < 0.01$; Fig. S1b). Meanwhile, the correlations between Chl-a and TN during spring and summer were positive (Fig. S1c). The positive correlations between Chl-a and TP during the four seasons are shown in Fig. S1d. These results indicate that C, N, and P affect the primary productivity of phytoplankton in the lakes. To further investigate the co-limitation of C/N/P and the type of nutrients that play a significant role in phytoplankton primary productivity, we analyzed the correlation between Chl-a and nutrient ratio. The correlation between Chl-a and nutrients (C/N, C/P, and N/P ratios) was largely influenced by season (Fig. 6). There was a significant positive linear correlation between Chl-a concentrations and DIC-C to TN ratios during summer ($r^2 = 0.72$, $P < 0.001$) and autumn ($r^2 = 0.48$, $P < 0.01$), but a negative linear correlation during spring ($r^2 = 0.12$, $P < 0.01$; Fig. 6a). In contrast, Chl-a did not show any positive or negative correlation with the ratios of DIC-C to TP in these three seasons ($r^2 = 0.01$, $P = 0.86$; $r^2 = 0.06$, $P = 0.43$; and $r^2 = 0.19$, $P = 0.15$, respectively; Fig. 6b). Our study also revealed a significant positive linear relationship between CO₂/TN and Chl-a in the spring, summer and autumn datasets ($r^2 = 0.58$, $P < 0.01$; $r^2 = 0.71$, $P < 0.001$; and $r^2 = 0.70$, $P < 0.001$, respectively; Fig. 6d). CO₂/TP displayed the same trend, but with a low r^2 (Fig. 6e). However, CO₂/TN and CO₂/TP had little or no negative effect on Chl-a concentration in winter ($r^2 = 0.02$, $P = 0.68$; $r^2 = 0.24$, $P = 0.10$), when pCO₂ was over 400 µatm at most sites.

Our research classifies Cyanobacteria (Cya.) as harmful algae, and Chlorophyta (Chl.) and Bacillariophyta (Bac.) as harmless algae and

compared their ratios (Cya./ (Chl. + Bac.)) with the nutrient concentrations during the different seasons (Fig. S2). The results revealed a significant negative correlation between the CO₂ and Cya./ (Chl. + Bac.) ratios during summer and autumn ($r^2 = 0.55$, $P < 0.01$ and $r^2 = 0.33$, $P < 0.05$; Fig. S2a), indicating that CO₂ may influence cyanobacteria blooms during the rapid phytoplankton growth period in karst ecosystems. There was almost no correlation between TN, TP, and Cya./ (Chl. + Bac.) ratios throughout the year ($P > 0.05$; Fig. S2a and 2b). Furthermore, we analyzed the relationship between phytoplankton communities and nutrient molar ratios (Fig. 7). The increase in the CO₂/TN and CO₂/TP ratios is attributed to a decrease in the Cya./ (Chl. + Bac.) ratios, especially in summer ($r^2 = 0.57$, $P < 0.01$ and $r^2 = 0.49$, $P < 0.01$, respectively) and winter ($r^2 = 0.51$, $P < 0.01$ and $r^2 = 0.37$, $P < 0.05$, respectively). There was little or no seasonal correlation between Cya./ (Chl. + Bac.) and the TN/TP ratio, except in summer, which yielded a significant positive relationship ($r^2 = 0.54$, $P < 0.01$; Fig. 7c). The correlations between the submerged macrophytes dry weight variables and nutrient ratios are shown in Fig. 8. The DIC-C/TN ratio did not affect submerged macrophytes dry weight ($P > 0.05$, Fig. 8a). However, there was a significant positive linear relationship between the submerged macrophytes dry weight DIC-C/TP ratio in both seasons, especially during the growing season ($r^2 = 0.79$, $P < 0.001$, Fig. 8b).

4. Discussion

4.1. Limitation of elemental stoichiometric ratios (C/N/P) on phytoplankton and submerged macrophyte growth

Several studies on karst shallow water ecosystems have focused on N or P (Camacho et al., 2003; Elser et al., 2009), however, our investigation of the nutrient concentrations and stoichiometric ratios of the principal elements emphasized the role of C, reflecting the unique physicochemical characteristics of carbonate karst surface water ecosystems (high pH but low CO₂). As noted, productivity limits are determined by rate-limiting resources, such as C, or the yield-limiting resources, such as P and N (Low-Décarie et al., 2014; Riebesell et al., 1993). The pH of most of the Caohai sampling sites reached >8.0 ($n = 34$) during the growing season (Spring, Summer and Autumn),

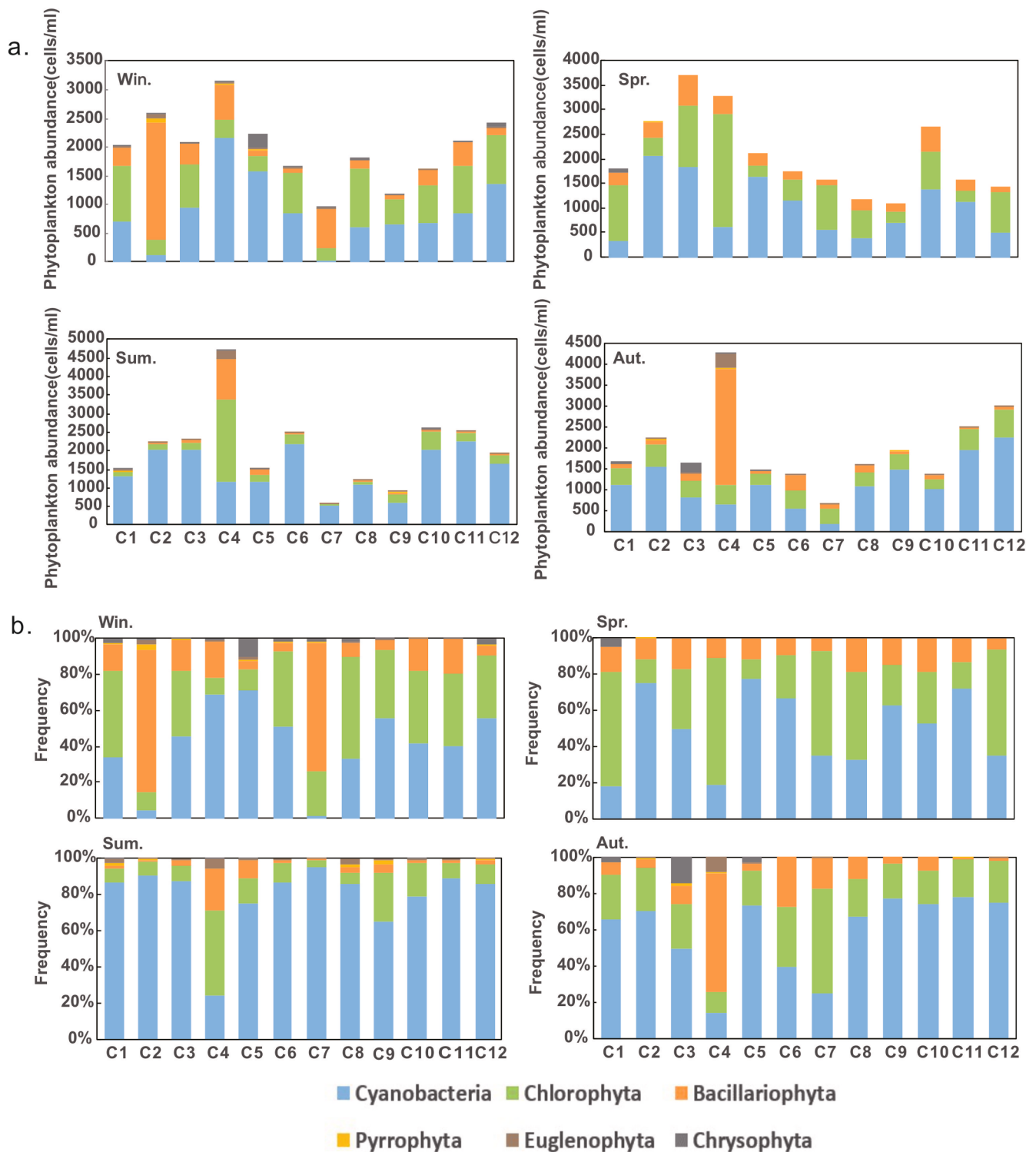


Fig. 4. Spatiotemporal variations in (a) the phytoplankton abundance and (b) the relative contributions (percentage) of each major taxonomic group of phytoplankton found in Caohai Lake during 2020. C1 to C12: Sampling sites; Spr.: Spring; Sum.: summer; Aut.: Autumn; Win.: Winter.

indicating that dissolved CO₂ was <1 % DIC (Riebesell et al., 1993; Zeng et al., 2019). This carbon limitation may occur in aquatic ecosystems when TN and TP are relatively abundant (Bogard et al., 2017; Hammer et al., 2019; Verspagen et al., 2014; Zagarese et al., 2021), at least locally or over a short timescale (e.g. lakes or reservoirs) (Schindler and Fee, 1973). Mitigating eutrophication in already eutrophic lakes by controlling N and P concentrations may not be effective in the short term (Huser et al., 2016). There are many forms of C in water bodies

(Riebesell et al., 1993) and dissolved CO₂, which is used preferentially by most plants (Bao et al., 2022; Hillebrand et al., 1999; Low-Décarie et al., 2014; Riebesell et al., 2007; Schindler, 1977; Zeng et al., 2019), is extremely scarce in karst water bodies during the growing season. Surface water CO₂/TN and CO₂/TP molar ratios ranged from 0.01 to 1.19 and 0.44 to 11.49 over the four seasons (Fig. 6d and e), far below the Redfield ratio (Redfield et al., 1963). We found a significant effect of CO₂/TN and CO₂/TP on Chl-a (Fig. 6d and e), indicating that C, N, and P

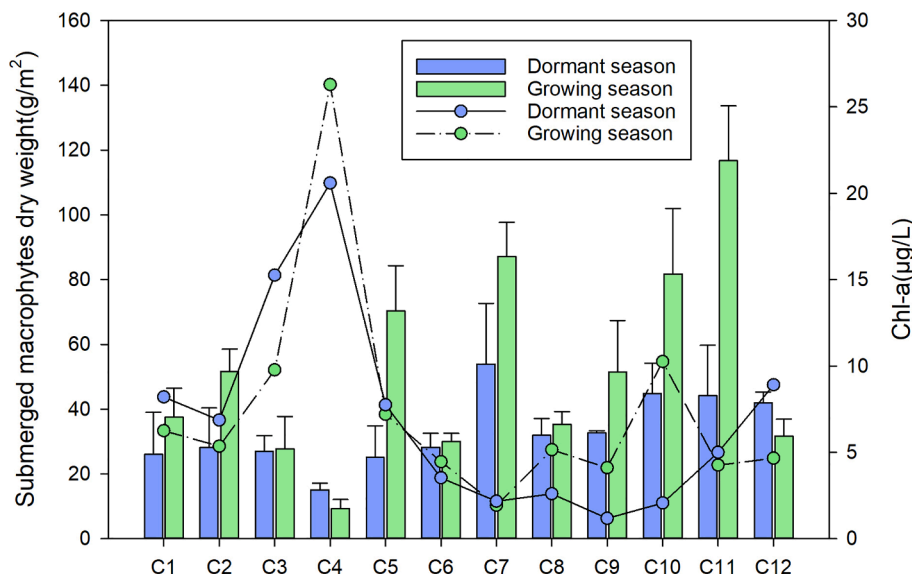


Fig. 5. Spatiotemporal variations in submerged macrophytes dry weight and Chl-a in Caohai Lake during the dormant (winter) and growing (summer) seasons. C1 to C12: Sampling sites; the bar chart shows submerged macrophytes dry weight, and the point chart shows Chl-a.

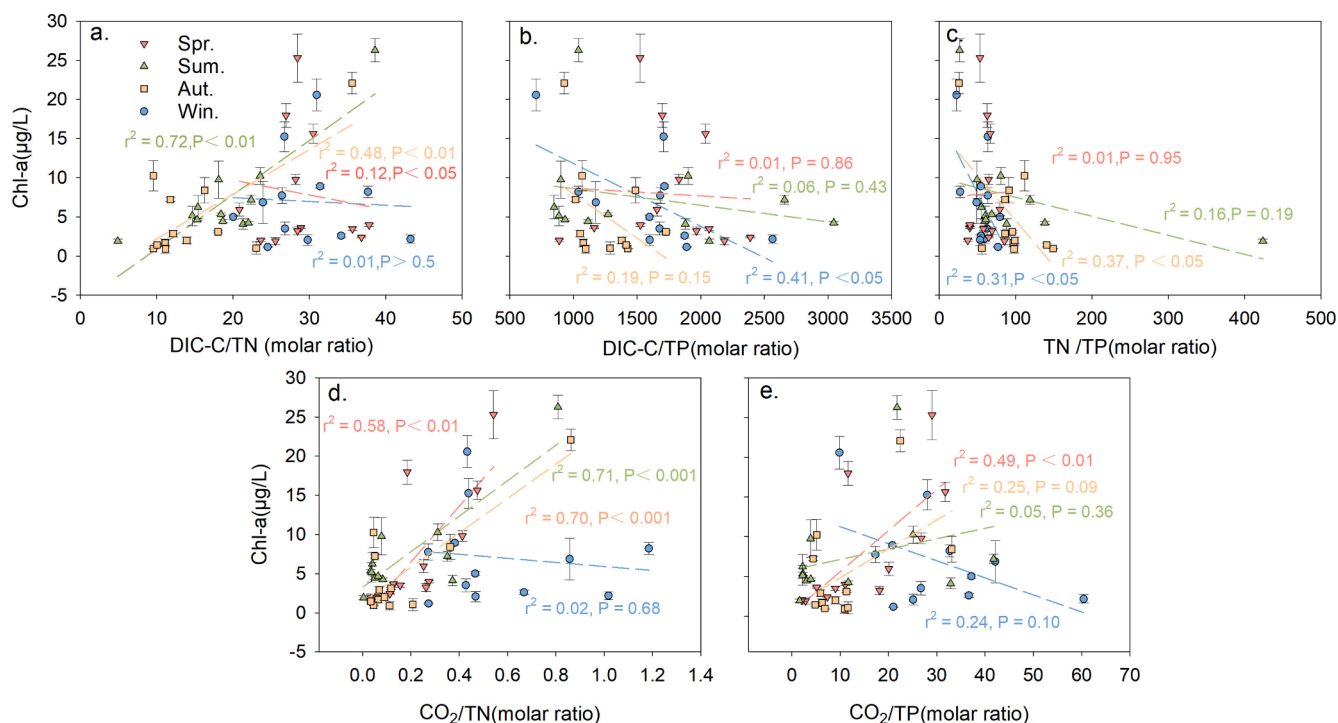


Fig. 6. Relationships between chlorophyll a (Chl-a) and the nutrient molar ratios - DIC-C/TN(a), DIC-C/TP(b), TN/TP(c), CO₂/TN(d), and CO₂/TP (e) in Caohai Lake in 2020. Spr.: Spring; Sum.: Summer; Aut.: Autumn; Win.: Winter.

co-limited primary productivity in these karst shallow surface waters. The TN to TP ratio is the most commonly used index for describing the nutrient limitations of phytoplankton (Phillips et al., 2008; Prairie et al., 1989). A decreasing trend in TN/TP with Chl-a was observed in three of the seasonal datasets (Fig. 6c), which is consistent with previous research (Dove and Chapra, 2015; Downing and McCauley, 1992; Liang et al., 2020; Yan et al., 2016). This study confirms that inorganic C promotes an increase in primary production (Chl-a) in shallow karst lakes (Andrade et al., 2021; Hamdan et al., 2018; Jansson et al., 2012; Schippers et al., 2004), indicating a higher capacity for carbon sequestration (Anderson et al., 2014). Although several studies have suggested

that temperature increases the mineralization of organic carbon (Gudasz et al., 2010), the deposition rate of organic carbon (i.e., the high deposition flux) could offset the mineralization effect caused by temperature, which has been demonstrated in numerous studies, such as those in Fuxian Lake, China (He et al., 2020) and global lakes (Cole et al., 2007; Tranvik et al., 2009). An important finding was that the nutrient limitation of phytoplankton in the karst surface water ecosystem was in the order C > P > N, except in winter. Therefore, it has been suggested that carbon should be emphasized as a rate-limiting factor (Low-Décarie et al., 2014) in mesotrophic and eutrophic freshwater (Valentyne, 1974; Zeng et al., 2019). Light limitations have been emphasized in other

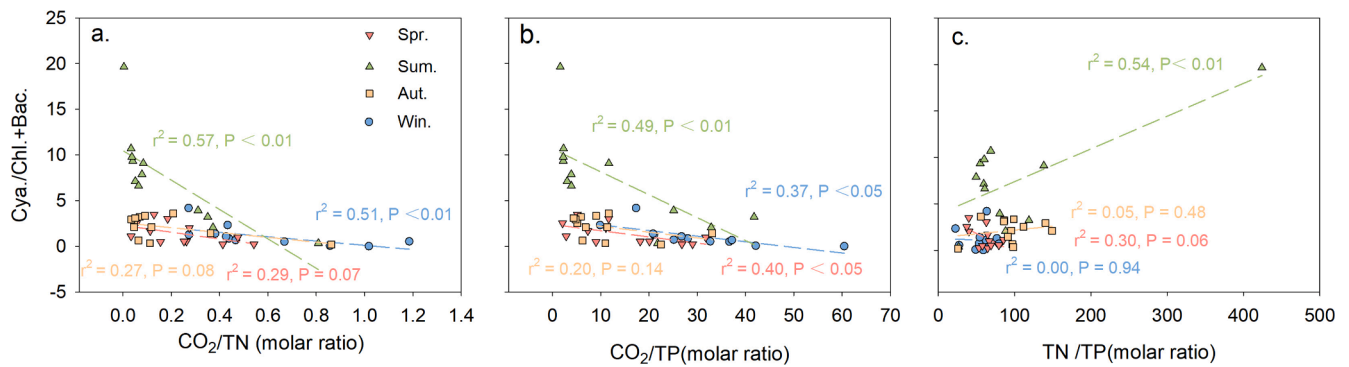


Fig. 7. Relationships between phytoplankton communities and nutrient molar ratios (CO_2/TN (a), CO_2/TP (b), and TN/TP (c)) in Caohai Lake in 2020; $\text{Cya.}/(\text{Chl.} + \text{Bac.})$: Cyanobacterial abundance/(Chlorophyta abundance + Bacillariophyta abundance). Spr.: Spring; Sum.: Summer; Aut.: Autumn; Win.: Winter.

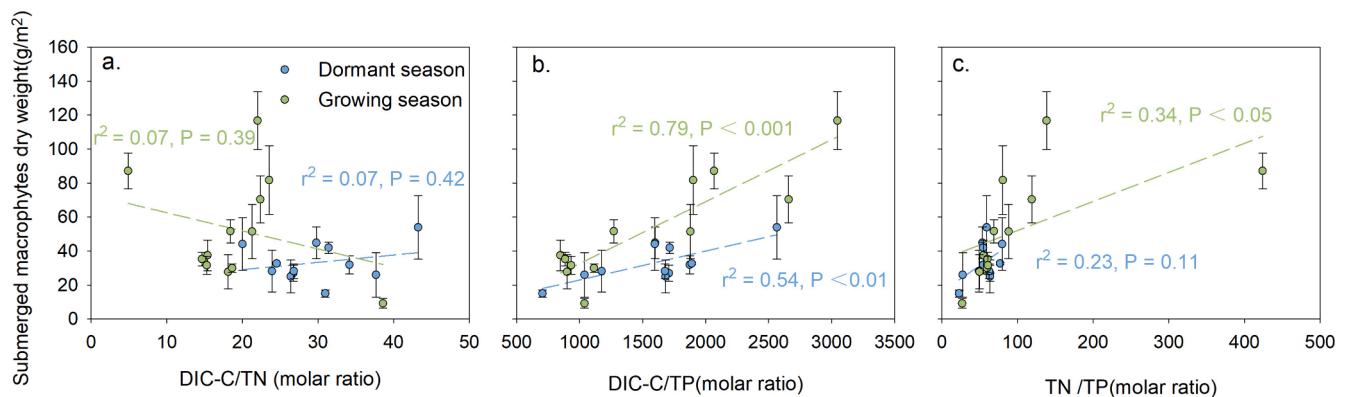


Fig. 8. Relationships between the dry weight of submerged macrophytes and the nutrient molar ratios ($\text{DIC-C}/\text{TN}$ (a), $\text{DIC-C}/\text{TP}$ (b), and TN/TP (c)) measured in Caohai Lake in 2020.

studies (Havens et al., 2003; Huisman et al., 2002; Karlsson et al., 2009; Lehman et al., 1975), but most have been in oligotrophic lakes, which are light-limited by definition (Karlsson et al., 2009). Caohai Lake is a mesotrophic-to-eutrophic water body where light limitation is not a factor. Nutrient limitation was detected within individual seasons when light penetration was similar at all sampling sites.

Both carbon dioxide (CO_2) and bicarbonate (HCO_3^-) are available for use by submerged macrophytes (Maberly and Gontero, 2017; Pedersen et al., 2013). We considered DIC to be of prime importance in macrophyte growth because of the presence of submerged macrophyte species in the study area (Pagano and Titus, 2007). The increase in submerged macrophytes dry weight by $\text{DIC-C}/\text{TP}$ was mentioned above, indicating that relatively high DIC concentrations promoted growth. Nutrient limitation was in the order of $\text{C} > \text{P}$ and $\text{N} > \text{P}$. Thus, we showed that the growth of both phytoplankton and submerged macrophytes was limited by the availability of inorganic C during the study period. Similar trends were observed at the Shawan Karst Test Site, SW China (Zeng et al. 2019; Bao et al., 2022), showing carbon limitation on aquatic photosynthesis. Thus, we suggest that C limitation in small karst water bodies, such as lakes and reservoirs, should be emphasized in further research, and the comparative availability of nutrients (C/N , C/P and N/P) and available C for aquatic plants should also be considered.

4.2. Coupling relationships and mechanisms between C/N/P and community structures of the phytoplankton and submerged macrophytes

We have previously reported that a low ratio of $\text{Cya.}/(\text{Chl.} + \text{Bac.})$ indicated that the lake cyanobacteria blooms were alleviated. Cyanobacteria, such as *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*, are the primary groups responsible for algal blooms during eutrophication (Paerl, 2008). After growth and death, some

Cyanobacteria release toxic substances into host water bodies which are detrimental to aquatic ecosystems and human health (Huisman et al., 2018; Ross et al., 2006). Thus, even if blooms occur, we hope that they are diatom or green algae blooms rather than cyanobacteria blooms. Our study revealed a significant negative relationship between $\text{Cya.}/(\text{Chl.} + \text{Bac.})$ for CO_2/TN and CO_2/TP , respectively (Fig. 7a and b). As expected, Cyanobacteria tended to grow best at low CO_2/TN and CO_2/TP ratios, whereas Chlorophyta and Bacillariophyta preferred high CO_2/TN and CO_2/TP ratios, particularly when the ratios were higher than 0.2 and 10. Cyanobacteria have been observed to bloom when the excess of N:P is low (Downing et al., 2001; Smith, 1983), which is consistent with the results in Caohai Lake. In addition, the use of HCO_3^- by different phytoplankton can result in changes in $\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{13}\text{C}_{\text{POC}}$ in the euphotic layer. Cyanobacteria have a higher capacity to utilize HCO_3^- , which allows them to thrive more than Chlorophyta and Bacillariophyta when $\delta^{13}\text{C}$ is positive (Xiao et al., 2021). There was a significant negative correlation between $\delta^{13}\text{C}_{\text{DIC}}$, $\delta^{13}\text{C}_{\text{POC}}$ and the abundance of Chlorophyta + Bacillariophyta (Fig. 9a); when $\text{CO}_2(\text{aq})$ concentrations were lower than $30 \mu\text{mol/L}$, the dominant species shifted from Chlorophyta and Bacillariophyta to Cyanobacteria (Fig. 9b). Thus, an appropriate increase in $\text{CO}_2(\text{aq})$ concentrations can significantly enhance photosynthetic carbon sequestration and reduce the proportion of cyanobacteria when controlling N and P.

Seasonal samples from all 12 sites were used in a Redundancy Analysis (RDA) of the effects of environmental factors (CO_2/TN , CO_2/TP , DIC/TN , DIC/TP , TN/TP and temperature) on phytoplankton communities (Cyanobacterial abundance, Chlorophyta abundance and Bacillariophyta abundance), Chl-a, and the submerged macrophytes dry weight. RDA confirmed that the response of Cyanobacteria to nutrients differs from that of Chlorophyta and Bacillariophyta. In the growing season, Chlorophyta and Bacillariophyta abundance increased when the

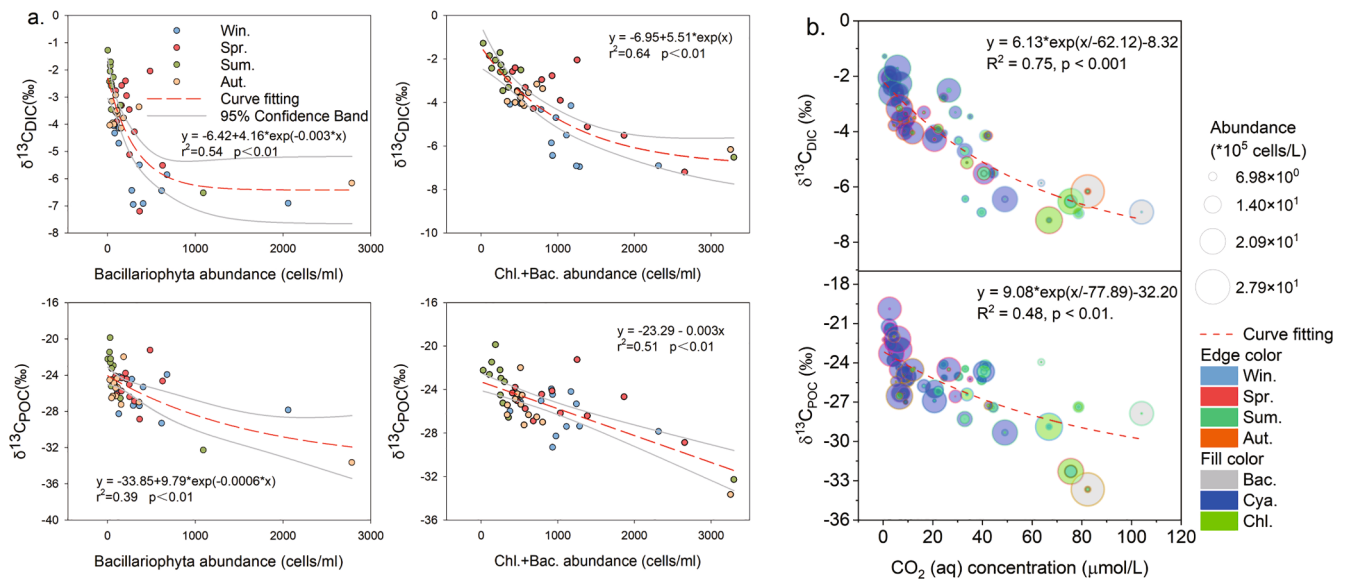


Fig. 9. (a) $\delta^{13}C_{DIC}$ and $\delta^{13}C_{POC}$ plotted against the abundances of Chlorophyta and Bacillariophyta (Chl. + Bac. abundance) in Caohai Lake in 2020. (b) $\delta^{13}C_{DIC}$ and $\delta^{13}C_{POC}$ vs CO_2 (aq) concentrations at different abundances of dominant phyla in the lake. Blue circle: Cyanobacteria; green circle: Chlorophyta; gray circle: Bacillariophyta; Circle size indicates measured abundances. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

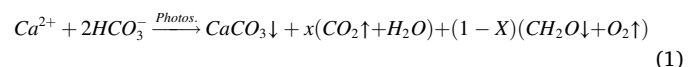
C/N and C/P ratios increased, whereas the Cyanobacteria mass decreased. In the Spring, Summer and Autumn samples, Site C4 differed from the other sampling sites. The sample taken near the wharf had the highest pollution level of all the Caohai Lake samples, as indicated by high Chl-a, C/N and C/P values. The analysis revealed that Cyanobacteria favored low CO_2 conditions, whereas Chlorophyta and Bacillariophyta favored higher CO_2 conditions.

Previous studies have indicated that P and N concentrations dominate phytoplankton growth and strongly influence community composition and structure (Guildford and Hecky, 2000; Kangur et al., 2003; Wejnerowski et al., 2018), however, the possible role of abundant C is ignored. The principal phytoplankton taxa differ in their ability to fix inorganic C and adjust their growth response to elevated CO_2 concentrations (Falkowski and Raven, 2013; Hare et al., 2007; Verschoor et al., 2013). Chlorophyta have higher C:N:P ratios (375:23:1) than Cyanobacteria (160:22:1), as shown by King (1970); that is, Chlorophyta requires more carbon than Cyanobacteria to grow (Shapiro, 1973). The strongest cyanobacteria blooms tend to occur in water bodies with low CO_2 concentrations owing to the CCMs that exploit both CO_2 and HCO_3^- for carbonic anhydrase photosynthesis on cell surfaces (Aizawa and Miyachi, 1986; Burnap et al., 2015; Price et al., 1998; Reinfelder, 2011; Shapiro, 1990; Van Dam et al., 2018). Phytoplankton CCM is species-specific (Raven et al., 2020). Chlorophyta and Bacillariophyta favor higher CO_2 concentrations, with low CCMs and ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO) specificity (Low-Décarie et al., 2011, 2015; Tortell et al., 2002). It has been demonstrated that Cyanobacteria has a higher RubisCO maximum carboxylation rate than Chlorophyta and Bacillariophyta (Savir et al., 2010; Young et al., 2016). Consequently, the limited availability of CO_2 may lead phytoplankton to shift from being dominated by Chlorophyta and Bacillariophyta, which indicate relatively good water quality, to being dominated by Cyanobacteria, which are associated with bad water quality, thus forcing phytoplankton to use HCO_3^- as an inorganic C source (Riebesell, 2004; Shi et al., 2017). This finding may explain the increase in Chlorophyta and Bacillariophyta with increasing CO_2 concentrations (Reinfelder, 2011; Low-Décarie et al., 2015), and the competitive advantages of low concentrations of CO_2 for the growth of Cyanobacteria. In summary, Cyanobacteria were more competitive than the other two phyla under low CO_2 conditions.

In addition, changes in the concentrations of inorganic C and nutrients in the water significantly affect the growth of submerged plants (Dülger et al., 2017). The relationship between DIC/TP, TN/TP (Fig. 8), and submerged macrophytes dry weight in Caohai Lake revealed that more C or N promoted macrophytes growth. This finding is consistent with that of O'Hare et al. (2010), who found that macrophyte biomass increased with an increase in the amount of carbon present as HCO_3^- in 14 rivers across the UK, improving the water quality and promoting the carbon sink. More P inhibited the growth of submerged macrophytes, which was not conducive to good water quality. Thus, C/N/P ratios play a significant role in regulating phytoplankton and the biological structure/composition of the submerged macrophyte biomass. Finally, the negative correlation between submerged macrophytes dry weight and Chl-a observed in Figs. 5 and 10 demonstrates that submerged macrophytes can inhibit phytoplankton growth, which can occur directly via allelopathy or indirectly via competition for nutrients (Dahlgren and Kautsky, 2004; Körner and Nicklisch, 2002; Vanderstukken et al., 2011). This phenomenon offers another way to mitigate cyanobacteria blooms in freshwater environments.

4.3. Possibilities and implications of mitigating cyanobacteria blooms and enhancing carbon sequestration by the BCP with CO_2 fertilization

The below processes reflect the nature of photosynthesis in the aquatic ecosystem and the BCP concept (Liu et al., 2010, 2018). This emphasizes the role of C fertilization in photosynthesis and as a mechanism for the sequestration of atmospheric CO_2 .



The BCP effect refers to photosynthesis caused by DIC concentration (DIC fertilization effect), which increases the aquatic biomass and enhances its carbon sequestration (Liu et al., 2010, 2018). The presence of inorganic C promotes an increase in primary production (Chl-a) and submerged macrophyte biomass in shallow karst lakes (Brothers et al., 2013; Hamdan et al., 2018; Jansson et al., 2012; Schippers et al., 2004; Vogt et al., 2017), as verified in this study.

Our analysis of the elemental ratios (CO_2 /TN, CO_2 /TP, and TN/TP) and phytoplankton relationships revealed that the composition of

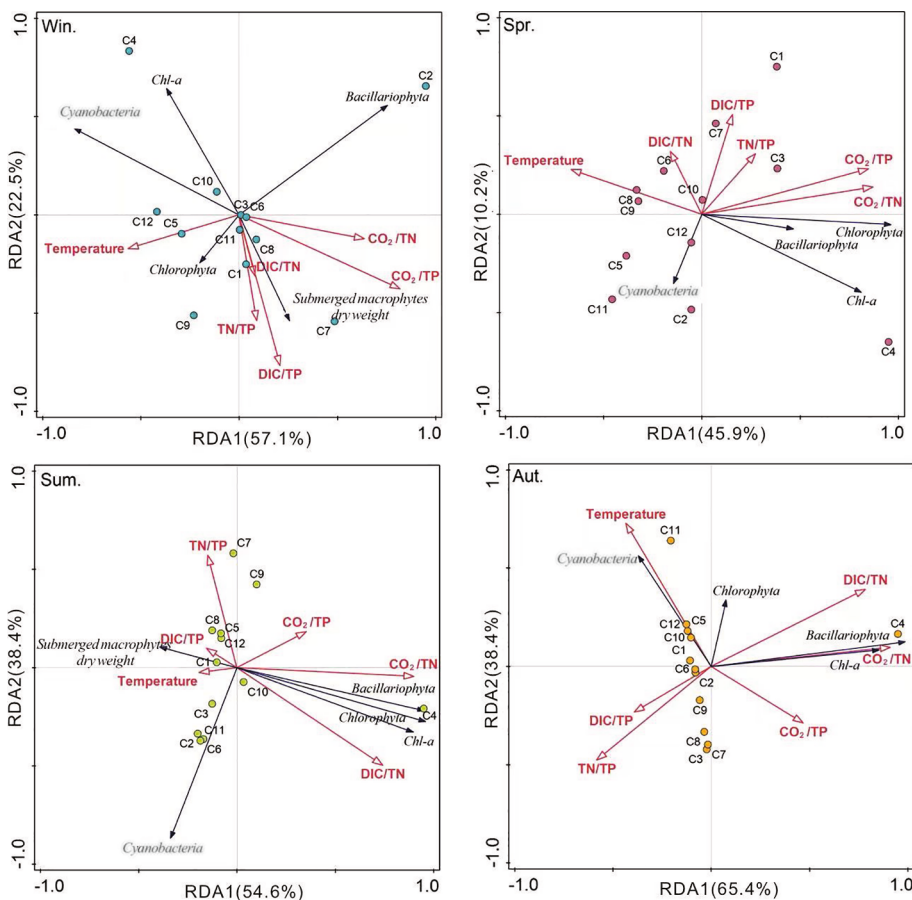


Fig. 10. Redundancy analysis to show the influence of environmental factors on phytoplankton communities (Chl-a, Cyanobacteria, Chlorophyta and Bacillariophyta) and submerged macrophytes dry weight in Caohai Lake during each sampling season in the lake. The symbols indicate different sampling sites in the lake. Environmental factors (CO₂/TN, CO₂/TP, DIC/TN, DIC/TP, TN/TP, and Temperature) are indicated by red arrows. Species (phytoplankton communities, Chl-a, and submerged macrophytes dry weight) are indicated with blue arrows. Spr.: Spring; Sum.: summer; Aut.: Autumn; Win.: Winter. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

phytoplankton communities varied as a function of CO₂ concentrations and ratios, which is consistent with our hypothesis and the predictions of previous theoretical and experimental studies (Low-Décarie et al., 2011, 2014, 2015; Shi et al., 2016; Wolfe and Siver, 2013). Efforts to decrease N and/or P inputs into aquatic ecosystems to control eutrophication often result in reduced agricultural yields globally (Hossain and Singh, 2000; Lu and Tian, 2017). Our observations suggest that cyanobacteria blooms can be mitigated by regulating phytoplankton composition by injecting CO₂ into water (Bao et al., 2022), which has no impact on agricultural productivity. Our findings in Caohai Lake show that C, N and P co-limit primary production and regulate the biological structure/composition of phytoplankton and submerged macrophytes by the BCP effect, which has significant implications for cyanobacteria bloom control and carbon neutralization—a win-win situation.

5. Conclusions

In this study, we investigated seasonal variations in the absolute and relative availability of nutrient concentrations (C/N, C/P, and N/P), physicochemical parameters, submerged macrophyte biomass, and phytoplankton composition in Caohai Lake, a typical karst lake in southwest China. Our results indicate that dissolved inorganic carbon promotes the primary production of phytoplankton (Chl-a) and macrophyte biomass; therefore, primary production may be limited not only by P and/or N but also by C, as a consequence of the distinct water chemistry characteristics of carbonate karst regions. Furthermore, submerged macrophytes that inhibited phytoplankton growth were identified in this lake. The increase in C:N and C:P ratios led to a reduction in the proportion of Cyanobacteria over Chlorophyta and Bacillariophyta (Cya./Chl. + Bac.) throughout the year, particularly when the CO₂/TN and CO₂/TP ratios were higher than 0.2 and 10, respectively. Based on

isotopic evidence, the abundance of Cyanobacteria was reduced by CO₂ fertilization, especially when the CO₂ exceeded 30 μmol/L.

Our study revealed that BCP on CO₂ fertilization in karst surface water ecosystems have synergistic effects on biological structure/composition and carbon sequestration, potentially a win-win situation. In contrast to the traditional approach of reducing N and/or P, we suggest that this is a possible step towards alleviating cyanobacteria blooms by adding emitted industrial CO₂ to water bodies after they are thoroughly treated from a variety of pollutants with a simple, low-cost, and passive option in low pCO₂ aquatic ecosystems. However, further controlled experiments are needed to examine the effects of artificial addition of CO₂ on biological composition and carbon sequestration in freshwater ecosystems.

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.

Data availability

The authors do not have permission to share data.

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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.2023.129111>.

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