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Effect of submerged plant coverage on phytoplankton community dynamics and photosynthetic activity *in situ*

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ABSTRACT

Restoration of submerged plants in eutrophic lakes can reduce nutrients and phytoplankton biomass in the water body. However, the effect of submerged plants on phytoplankton communities and their photosynthetic activity *in situ* are still poorly understood. Here, we studied the response of phytoplankton community structure and fluorescence parameters to different submerged plants coverage, the relationship of phytoplankton community and fluorescence parameters with submerged plants coverage and water physicochemical parameters were analysed in sampling area of Hangzhou West Lakes. The results showed that the coverage and biomass of submerged plants were negatively correlated with nitrogen and phosphorus contents in water body but positively correlated with total phenol content. The ratio of nitrogen to phosphorus in the study site changed greatly (32.25–124.54). In spring and summer, *Oscillatoria* and *Leptolyngbya* (Cyanophyta) were the dominant species, while in autumn and winter, the dominant species were *Cyclotella* (Chlorophyta), and *Melosira* and *Cymbella* (Bacillariophyta). Compared with Chlorophyta and Bacillariophyta, fluorescence parameters of Cyanophyta were more sensitive to total phosphorus, N:P ratio, total phenols, pH, and electric conductivity. Fluorescence parameters of Chlorophyta and Bacillariophyta were only affected by underwater light. Total phosphorus (TP) and N:P had a negative effect on the maximum photochemical electron yield of Cyanophyta. Furthermore, Cyanophyta was inhibited by total phenols from submerged plants. When phytoplankton were under stress, photochemical electron yield decreased significantly, whereas non-photochemical quenching increased. The structural equation model showed that the coverage of submerged plants might indirectly affect the fluorescence parameters of Cyanophyta by affecting nitrogen, phosphorus, and total phenol contents in the water body. These findings contribute to the understanding of the mechanisms underlying the impact of submerged plant restoration on phytoplankton community dynamics in subtropical eutrophic shallow lakes and provide a theoretical basis for the management of lakes.

1. Introduction

During the past decades, eutrophication of water bodies has become a major problem for many aquatic ecosystems around the world due to human activities (Zeng et al., 2017). Submerged macrophytes, as important primary producers of lake ecosystems, play a significant role in stabilising clear-water conditions by certain mechanisms, such as reducing the nutrient load in a lake, inhibiting endogenous release of nitrogen and phosphorus, or by the allelopathic inhibition of algae (Wang et al., 2019; Wu et al., 2019). The restoration of submerged macrophytes has been used as an important ecological engineering measure to control and reduce eutrophication in shallow lakes due to degradation of submerged plants (Gao et al., 2017; Ke et al., 2019).

Submerged plants of certain coverage can improve water quality and impact the biomass and community structure of phytoplankton in lake and wetland systems (Ferreira et al., 2018). There are different theories regarding the impact of submerged plant coverage on phytoplankton. One such theory proposes that submerged plants compete with

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phytoplankton for resources such as nutrients and light, which can impact the growth of the two competitors because they are in the same niche (Xu et al., 2019a). Thus, for example, when the N:P ratio decreased from 93.6 to 28.0 in the water body of the submerged plant recovery area, the biomass of cyanobacteria was significantly reduced (Chen et al., 2009). However, some studies have found that phytoplankton community changes and biomass reduction still occur under conditions of nutrient saturation in eutrophic lakes where submerged plants have been recovered (Vanderstukken et al., 2011; Amorim and Moura, 2020). This is presumably due to the release of allelochemicals by submerged plants to suppress phytoplankton biomass (Pakdel et al., 2013; Kaminski et al., 2015). Indeed, field observations and laboratory experiments have confirmed that submerged plants such as *Vallisneria natans*, *Ceratophyllum demersum*, and *Myriophyllum spicatum* all have allelopathic algae-inhibiting effects (Chen et al., 2012; He et al., 2016). Among the allelochemicals of plant origin that have been reported to date, polyphenols are an important category. Phenolic acid allelochemicals can be detected in many submerged plants and their surrounding waters, whereby the growth of algae is reportedly inhibited (Nakai et al., 2000). Three types of polyphenols from the extract of *M. spicatum* (pyrogallic acid, gallic acid, and ellagic acid) can inhibit the growth of cyanobacteria (Zhu et al., 2010). In addition, phytoplankton show different degrees of resistance to allelochemicals from submerged plants (Hilt, 2006; Hilt and Gross, 2008), and previous studies have shown that the allelopathic influence of submerged plants on phytoplankton may be species-specific (Vanderstukken et al., 2011). Thus, for instance, in co-culture, allelochemicals of submerged plants had a stronger inhibitory effect on cyanobacteria (*Microcystis aeruginosa*) than on green algae (*Desmodesmus armatus*) (Chang et al., 2012). Similarly, when Švanys et al. (2014) studied the influence of *M. spicatum* on the natural phytoplankton community, they found that the inhibitory effect on cyanobacteria was stronger; this indicates that the community composition of phytoplankton is related to the level of resistance of submerged plants to different types of microalgae.

Photosynthesis is the basis for phytoplankton survival and plays an ecological role. Chlorophyll molecules in phytoplankton absorb light energy, part of which is funnelled into chemical energy through photosynthesis, while the rest is dissipated as heat as well as chlorophyll fluorescence emission. These three processes compete with each other (Hanelt, 2018). Fluorescence parameters can be used as indicators to reflect the impact of environmental stress on photosynthetic efficiency. Thus, maximum photochemical quantum yield (Fv/Fm) of algal cells usually decreases under environmental stress (Higo et al., 2017), while non-photochemical quenching (NPQ) reflects the ability of plants to dissipate excess light energy into heat, as a photoprotective mechanism (Xu et al., 2019b). For some species of phytoplankton, when nutrients such as nitrogen and phosphorus decrease, Fv/Fm shows a downward trend (Beardall et al., 2001). Consistently, chlorophyll *a* content and PSII activity of phytoplankton such as *Thalassiosira pseudonana* and *Coscinodiscus* sp. were significantly reduced under nitrogen and phosphorus deficiency (Geider et al., 2010; Loebl et al., 2010). Further, the photosystem of phytoplankton may be an important target for allelochemicals such as polyphenols (Bussotti et al., 2020; Zhu et al., 2021), which significantly inhibited PSII activity of *M. aeruginosa* (Huang et al., 2020). However, these studies have focused on a single species of algae (He et al., 2016). The effect of the restoration of submerged plants on *in situ* photosynthesis of phytoplankton is still poorly understood. In addition, *in situ* studies on the mechanism (allelopathy or nutrient competition) responsible for the impact of submerged plant restoration on phytoplankton biomass and community composition are scarce.

In this study, we chose the West Lake in Hangzhou, China, a typical subtropical shallow lake, as the research site to study the dynamics of phytoplankton and photosynthetic activity in different submerged plant-coverage areas. The relationship between phytoplankton community, photosynthetic activity and physicochemical parameters, and phenolic acid released from submerged plants were explored. The main objectives of our study were to investigate: (1) dynamics of phytoplankton communities and biomass, (2) *in situ* fluorescence response of phytoplankton, (3) change in submerged plant biomass and total phenol content in the water body, and (4) relationship between phytoplankton dynamics, changes in its fluorescence levels, and environmental factors. We hypothesised that the results will objectively reflect the response of phytoplankton to submerged plants and will be helpful for understanding the mechanism underlying the effect of submerged plants on phytoplankton under different environmental conditions. The study findings might serve as a guide for the recovery of submerged plants in subtropical, eutrophic shallow lakes.

2. Materials and methods

2.1. Study design and sample collection

The Hangzhou West Lake, a typical urban-landscape lake in eastern China and a UNESCO world heritage site, was chosen as the research location. The lake consists of seven sub-lakes that are connected by bridges and boat passes. These sub-lakes include Maojiabu Lake, Wuguitan Lake, Xiaonan Lake, Yuhuwang Lake, Yuehu Lake, Xilihu Lake, and Beilihu Lake. The West Lake in Hangzhou was eutrophicated and only few submerged plants were found in the lake before 2009. Therefore, to improve the function of the water ecosystem and construct a stable ecosystem in the Hangzhou West Lake, restoration project of submerged macrophytes was undertaken during 2009–2016 in the sub-lakes, including Maojiabu Lake, Wuguitan Lake, and Xiaonan Lake. The pioneer species of submerged plants such as *V. natans* and *C. demersum* were planted in areas with a water depth of 1.5 m along the shores of three sub-lake mentioned above and the different coverage submerged macrophyte standshad formed by growth and propagation in different sites every sub-lake over several years.

Maojiabu Lake, Wuguitan Lake, and Xiaonan Lake of West Lake were chosen as the study areas. The submerged plants in the three sub-lakes were mainly *V. natans*, *Hydrilla verticillata*, and *C. demersum*. According to the extent of coverage by the submerged plants, the study sites were divided into high-coverage (HC), medium-coverage (MC), and low-coverage (LC) areas. HC, MC, and LC were defined as submerged plant coverage greater than 50%, between 20% and 50%, and less than 20%, respectively (Fig. 1). Submerged plant coverage was monitored using the MX Echosounder System (BioSonics, United States). The echo detection of the coverage of submerged plants in the three sub-lakes was performed by means of cross-section navigation. Echo detection recorded data were processed by the submerged plant analysis software Visual Habitat of BioSonics to obtain the percentage coverage of submerged plants.

The sampling was performed in December 2017 (winter), April 2018 (spring), July 2018 (summer), and October 2018 (autumn). At each sampling time point, sampling began from 8:00 to 11:00 in the morning to consider the effect of illumination on phytoplankton photosynthesis, and the sampling order was Maojiabu Lake, Wuguitan Lake, and Xiaonan Lake.

Submerged plants were collected three times at each sampling point using a submerged plant harvesting clip with an area of 30 × 47 cm. All submerged plants were cleaned and absorbed on the surface water, and then weighed to determine the average biomass fresh-weight per unit area (g/m^2 FW).

Water samples were collected at 0.5 m below water surface at each sampling point and stored in 1 L brown glass bottles. Samples were immediately transported to the laboratory and stored at 4 °C. All sample analyses were completed within 48 h.

2.2. Analysis of water quality parameters

Total nitrogen (TN, mg/L) was analysed using the alkaline potassium persulfate-digestion, UV spectrophotometric method. Total phosphorus

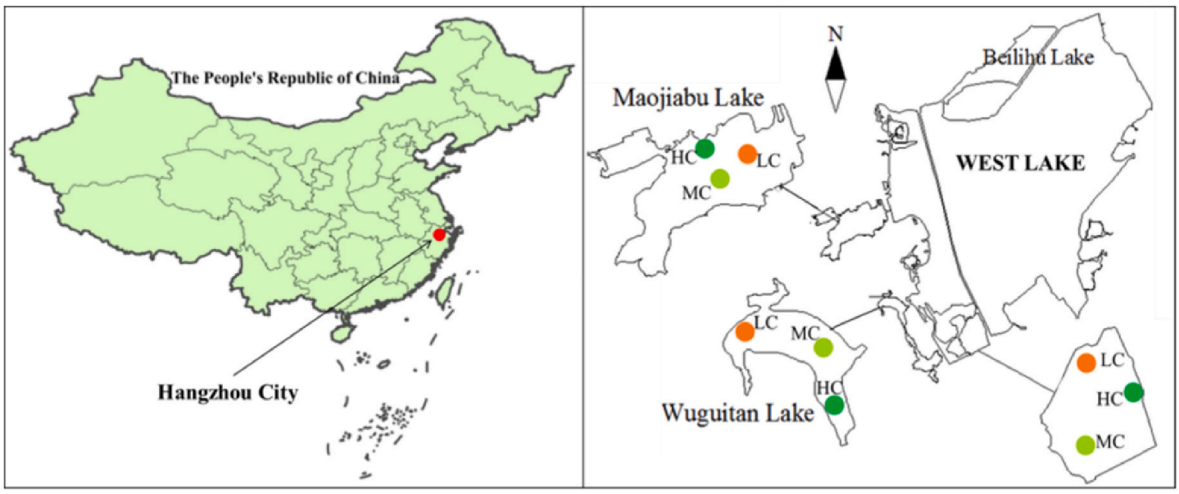

Fig. 1. The experiment sampling sites in Maojiabu Lake, Wuguitan Lake and Xiaonan Lake.

(TP, mg/L) was determined using the potassium persulfate-digestion, ammonium molybdate spectrophotometric method (Yu et al., 2017). Dissolved oxygen (DO, mg/L), electric conductivity (EC, μs/cm), pH, and water temperature (WT, °C) were all measured *in situ* using a YSI-Pro-Plus multi-parameter water quality metre (YSI ProPlus, USA). An underwater illuminance meter (ZDS-10W-2D, China) was used to measure the illuminance on the water surface (OWI, lx) and the level of illuminance at 0.5 m below water surface (UWI, lx).

2.3. Total phenol content

Total phenol content was determined using the Folin-Ciocalteau method (Ali et al., 2014). Each water sample (10 mL) was mixed with 2 mL Folin-Ciocalteau reagent. After 3 min, 2 mL of sodium carbonate solution (7.5%) was added to the mixture. Absorbance was measured at 760 nm after incubation in the dark for 90 min. A standard curve was prepared using a solution of gallic acid in methanol. Using the equation obtained from the standard gallic acid scatter diagram, total phenol concentration (μg/L) was determined as micrograms of gallic acid equivalent in the water sample.

2.4. Chlorophyll fluorescence monitoring and phytoplankton identification

Phytoplankton chlorophyll fluorescence was measured *in situ* using a FastOcean APD fluorometer (Chelsea Technologies Group Ltd, UK). A peristaltic pump capable of extracting phytoplankton samples from different layers in the water column was used to pump water samples to the sample chamber for measurement. Each sampling area was monitored three times, each time for approximately 3 min, for a total of 90 data points. Real-time fluorescence parameters, including Fv/Fm, NPQ, and Chl were obtained, and the average values were used as proxy of each fluorescence parameter. Based on the different absorption wavelengths (Chlorophyta and Bacillariophyta: 450 nm; Cyanophyta: 624 nm), the fluorescence values of different types of phytoplankton were obtained. Water samples collected at each sampling point were filtered through a 0.22 μm polycarbonate filter to obtain "baseline" fluorescence, which was used to correct subsequent F0 and Fm measurements.

Phytoplankton samples (1 L) were collected 0.5 m below the water surface at every site and fixed *in situ* with 10 mL Lugol's iodine solution (1.5% v/v). Phytoplankton samples were precipitated for 48 h and concentrated to 50 mL with a siphon. Algae cell counts were calculated under a microscope (Olympus BX51), using a 0.1 mL counting box (Palmer counting cell). Phytoplankton identification and counting were performed according to previously described methods (Hu and Wei, 2006). Phytoplankton were identified at the genus level. We determined the dominant genera of phytoplankton according to the degree of dominance, which was calculated using the following formula (Huo et al., 2019):

$$Y = (n_i / N) \times f_i$$

where N is the total abundance of phytoplankton, n_i is the total abundance of the i-th genera in the sample, *and* f_i is the frequency of occurrence of this species in all samples. If Y is greater than 0.02, it is considered to be the dominant genus of phytoplankton.

2.5. Data analysis

One-way analysis of variance (ANOVA) and Spearman correlation analysis were performed using the SPSS 20 software. GraphPad Prism 8 software was used to create the graphs.

Canonical correspondence analysis (CCA) was used to determine the influence of environmental variables on the dominant phytoplankton genus. Prior to the analysis, we used $\log_{10}(x + 1)$ to convert species abundance data and environmental variables data (except for pH values) and performed a collinearity analysis on environmental variables to eliminate variables with inflation coefficients >20. Non-metric multidimensional scaling (NMDS) was used to analyse the differences in phytoplankton dominant genera in different seasons and at different sites; CCA and NMDS were performed using the Canoco 5 software.

The main assumption of the structural equation model (SEM) was that submerged plants altered TP, TN, and total phenol content of the water body, which in turn affected the fluorescence parameters of cyanobacteria. Environmental variables for the SEM were selected based on the results of the Spearman correlation analysis. Model fit was evaluated using the χ^2 test ($P > 0.05$), goodness of fit (GIF) index (>0.9), and approximate root mean square error (RMSEA) index (<0.08) (Liu et al., 2017). SEM was conducted using the Amos 24 software.

3. Results

3.1. Physicochemical characteristics at different submerged plant-coverage sites

Physicochemical characteristics of different submerged plant-coverage areas in the four sampling seasons showed that TN and TP contents in HC areas were significantly lower than those in LC areas, except in winter ($P < 0.05$) (Table 1). TN and TP were higher in spring and winter than in summer and autumn. The N:P ratio ranged from 32.35 to 124.54 during the four seasons, with significant seasonal

Table 1
Seasonal mean (±standard deviation) of physicochemical variables at all sampling sites according to coverage types during the study period.

Season	Area	WT (°C)	TN (mg/L)	TP (mg/L)	N: P	pH	EC (μs/cm)	DO (mg/L)	OWI (lx)	UWI (lx)
Spring	HC	19.5 ± 0.3 (a)	2.05 ± 0.16 (a)	0.050 ± 0.03 (a)	50.11 ± 20.24 (a)	7.83 ± 0.13 (a)	209.03 ± 10.88 (c)	8.30 ± 0.50 (a)	51067 ± 1020 (b)	32340 ± 2150 (b)
	MC	19.7 ± 0.2 (a)	2.10 ± 0.25 (a)	0.057 ± 0.03 (a)	43.15 ± 23.25 (a)	7.85 ± 0.20 (a)	198.67 ± 13.05 (b)	8.73 ± 1.62 (b)	45033 ± 2300 (a)	23270 ± 2300 (a)
	LC	19.5 ± 0.4 (a)	2.36 ± 0.16 (b)	0.053 ± 0.02 (a)	46.89 ± 11.73 (a)	7.90 ± 0.09 (a)	191.27 ± 13.27 (a)	10.07 ± 1.51 (c)	58100 ± 1900 (c)	34800 ± 1793 (c)
Summer	HC	30.4 ± 0.2 (a)	1.17 ± 0.11 (b)	0.014 ± 0.00 (a)	89.76 ± 48.59 (c)	7.86 ± 0.39 (a)	244.83 ± 12.47 (b)	5.17 ± 1.04 (a)	48667 ± 2100 (a)	23667 ± 2190 (b)
	MC	30.3 ± 0.3 (a)	0.78 ± 0.18 (a)	0.015 ± 0.01 (a)	62.91 ± 34.99 (b)	7.83 ± 0.46 (a)	265.73 ± 14.78 (c)	6.15 ± 0.61 (c)	66100 ± 2890 (b)	30500 ± 2240 (c)
	LC	30.2 ± 0.1 (a)	1.13 ± 0.24 (b)	0.023 ± 0.00 (b)	49.62 ± 9.25(a)	7.85 ± 0.51 (a)	230.83 ± 9.28 (a)	5.33 ± 0.91 (b)	47600 ± 2200 (a)	21400 ± 1870 (a)
Autumn	HC	20.4 ± 0.7 (a)	1.23 ± 0.03 (a)	0.014 ± 0.00 (a)	92.18 ± 30.37 (a)	7.81 ± 0.20 (a)	219.37 ± 4.76 (a)	7.01 ± 0.58 (b)	51967 ± 2150 (a)	18367 ± 1800 (a)
	MC	20.2 ± 0.2 (a)	1.19 ± 0.19 (a)	0.012 ± 0.00 (a)	109.09 ± 38.49 (b)	7.73 ± 0.18 (a)	221.23 ± 3.51 (b)	6.89 ± 0.71 (a)	58000 ± 3200 (b)	29867 ± 2140 (b)
	LC	20.3 ± 0.3 (a)	1.34 ± 0.23 (b)	0.012 ± 0.00 (a)	124.54 ± 42.23 (c)	7.84 ± 0.22 (a)	221.33 ± 6.98 (b)	7.33 ± 0.30 (c)	63200 ± 2500 (c)	34333 ± 2210 (c)
Winter	HC	9.7 ± 0.2 (a)	2.43 ± 0.20 (c)	0.038 ± 0.00 (a)	63.99 ± 3.13(b)	8.70 ± 0.37 (a)	337.20 ± 10.65 (b)	11.80 ± 1.15 (a)	7940 ± 1500 (a)	4293 ± 720(a)
	MC	9.6 ± 0.3 (a)	2.10 ± 0.25 (b)	0.063 ± 0.01 (b)	33.68 ± 1.74(a)	8.77 ± 0.15 (a)	339.17 ± 14.38 (b)	11.77 ± 1.50 (a)	8227 ± 2100 (b)	5510 ± 580(b)
	LC	9.9 ± 0.5 (a)	2.06 ± 0.39 (a)	0.072 ± 0.03 (c)	32.35 ± 1.85(a)	8.73 ± 0.16 (a)	331.93 ± 14.49 (a)	11.87 ± 0.92 (a)	15163 ± 2673 (c)	8207 ± 490(c)
P values among seasons		**	**	**	**	**	**	**	**	**

differences ($P < 0.05$). Among the different sites, pH differences were not significant ($P > 0.05$); however, pH was significantly higher in winter than in any other season ($P < 0.05$). EC ranged from 191.27 to 339.17 μs/cm, which was higher in winter than in any other season. DO was significantly higher in LC than in HC areas ($P < 0.05$); furthermore, it was higher in winter than in any other season. There were significant differences in WT among seasons ($P < 0.05$), but WT was not significantly different among sampling sites. The highest value for UWI was found in spring (30137 ± 6073 lx), followed by autumn (27522 ± 8237 lx) and summer (25189 ± 4737 lx). The changing trend of OWI was consistent with that of UWI values.

3.2. Changes in submerged plant biomass and total phenol content

The highest biomass of submerged plants in HC areas (994.20 ± 306.05 g/m^2 FW) was recorded in summer, which was more than three-fold higher than that recorded in spring (Fig. 2a). In autumn, the highest biomass was found in MC (764.57 ± 273.13 g/m^2 FW) and the lowest in LC (397.47 ± 271.68 g/m^2 FW). Finally, in winter, the biomass of submerged plants in HC, MC and LC decreased to 684.33 ± 298.42, 390.00 ± 235.86, and 131.00 ± 33.96 g/m^2 FW, respectively.

Total phenol content was lowest in winter (29.99 ± 12.55 μg/L), while the highest (104.87 ± 15.86 μg/L) was found in autumn (Fig. 2b). The highest total phenol content (82.13 ± 23.86 μg/L) in spring occurred in HC areas, while the lowest value (54.51 ± 13.83 μg/L) occurred in MC areas. Meanwhile, in summer, total phenol content varied significantly among areas ($P < 0.05$). The highest value for total phenol content (107.86 ± 33.07 μg/L) was recorded in HC areas, followed by MC areas (77.49 ± 30.86 μg/L). In autumn, total phenol content in HC areas (114.83 ± 18.11 μg/L) was significantly higher than that in MC (105.87 ± 18.11 μg/L) or LC areas (93.92 ± 4.48 μg/L). In winter, the highest value for total phenol content (43.68 ± 23.84 μg/L) was found in HC areas, followed by MC areas (27.25 ± 13.36 μg/L).

3.3. Variations in phytoplankton biomass and community

In spring, the phytoplankton cell density in HC areas (24.84 × 10^5 cells/L) was significantly lower than that (42.86 × 10^5 cells/L) in LC areas (Fig. 3a); furthermore, species of Cyanophyta were dominant, followed by those of Bacillariophyta. Dominant genera in HC areas included *Leptolyngbya*, *Oscillatoria*, *Ulothrix*, and *Synedra*; *Pseudanabaena*, *Leptolyngbya*, *Chlorella*, *Melosira*, and *Synedra* were dominant in MC and *Leptolyngbya*, *Oscillatoria*, *Ulothrix*, *Chlorella*, *Fragaria*, *Cyclotella*, *and Navicula,* were dominant in LC areas (Fig. 4).

Phytoplankton cell density ranged from 1.35 × 10^5 cells/L to 8.56 × 10^5 cells/L (Fig. 3b) in summer. Cyanophyta dominated in HC, MC, and LC areas, accounting for 81.8%, 79.1%, and 97.5% of total cell density, respectively. *Pseudanabaena*, *Leptolyngbya*, and *Oscillatoria* had an absolute advantage at different areas (Fig. 4). The dominant genera in HC areas were *Glenodinium* (3256.00 cells/L) and *Gymnodinium* (3738.67

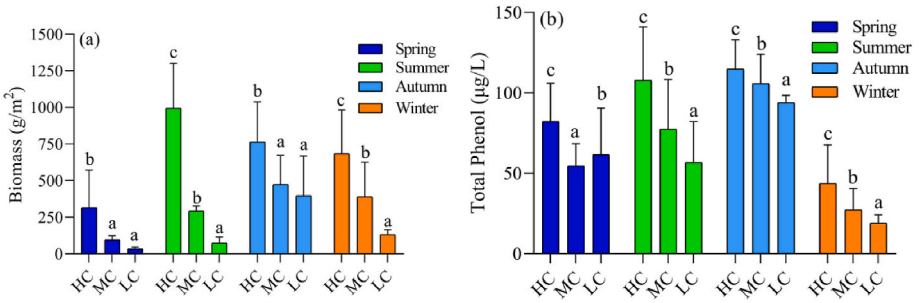

Fig. 2. The biomass and total phenol content of submerged plants in different seasons and sites (Mean ± S. D, N = 3).

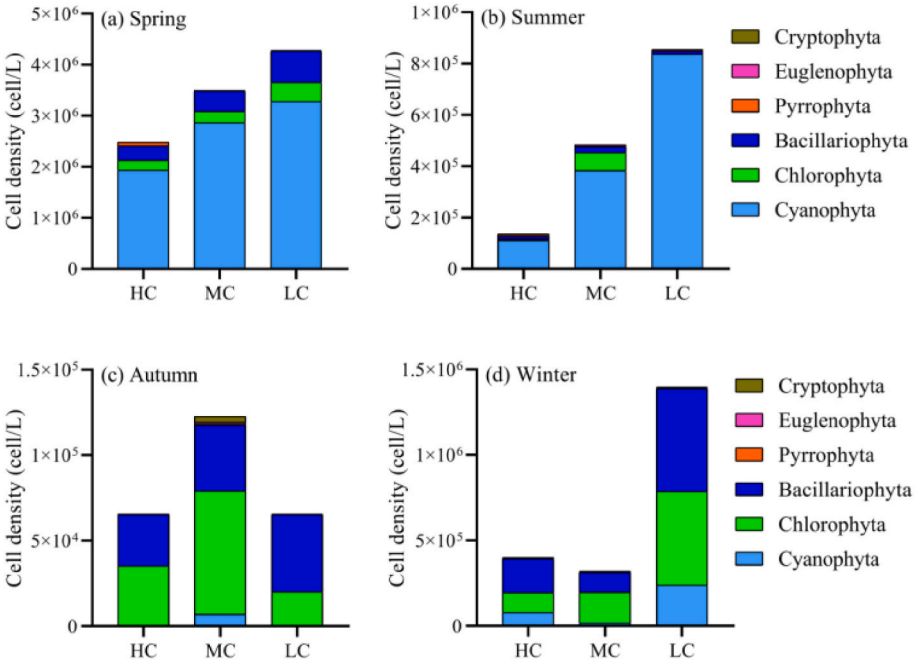

Fig. 3. Phytoplankton cell density in different sites and seasons.

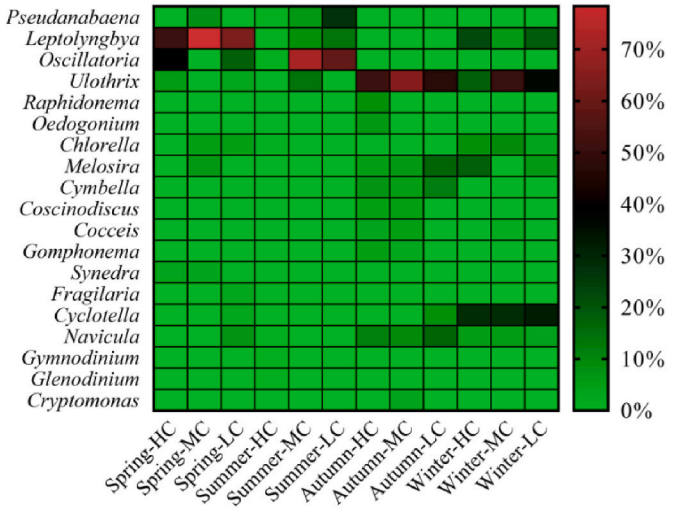

Fig. 4. Heatmap of dominant genera level of the phytoplankton community in different sites among various seasons. (The color intensity in each box represents the relative percentage of a genus in each sample.). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cells/L).

Chlorophyta and Bacillariophyta were dominant in autumn, and phytoplankton cell density was highest (1.23×10^5 cells/L) in MC areas (Fig. 3c). Dominant phytoplankton genera in HC and MC areas included *Ulothrix*, *Raphidonema*, *Oedogonium*, *Melosira*, *Cymbella*, *Coscinodiscus*, *Cocceis*, *Gomphonema*, and *Navicula*. There were relatively few dominant genera in LC areas, mainly *Ulothrix*, *Melosira*, *Cymbella*, *Cyclotella*, and *Navicula* (Fig. 4).

Finally, in winter, Chlorophyta (5.47×10^5 cells/L) and Bacillariophyta (6.04×10^5 cells/L) were dominant at the different sampling areas. The highest phytoplankton cell density (13.98×10^5 cells/L) was observed in LC areas (Fig. 3d). Dominant genera included *Leptolyngbya*, *Ulothrix*, *Chlorella*, *Melosira*, *Cyclotella*, and *Navicula* in HC, MC, and LC areas (Fig. 4).

The Shannon Wiener diversity index values were higher in HC than in LC areas (Fig. S1a, $P < 0.05$). The highest value for diversity index (1.99 ± 0.34) was recorded in HC areas in autumn and the lowest (1.01 ± 0.39) in summer, in LC areas. Meanwhile, the highest value for the Shannon Wiener diversity index (1.57 ± 0.45) was recorded in autumn and the lowest in summer (1.09 ± 0.28).

Highest Chl content occurred in autumn (18.58 ± 9.16 μg/L), followed by winter and spring at 15.73 ± 9.16 and 14.02 ± 3.25 μg/L, respectively, and the lowest value (7.59 ± 2.38 μg/L) was recorded in summer (Fig. S1b). The seasonal variation trend of Chl content was consistent with the Shannon Wiener diversity index. Chl content was significantly higher in LC areas than in HC or MC areas (Fig. S1b, $P < 0.05$).

3.4. Characteristics of chlorophyll fluorescence in phytoplankton

The range of Fv/Fm at 450 nm in the four seasons was 0.30–0.50 in different sites (Fig. 5a). Fv/Fm values in LC areas were significantly higher than those in HC or MC areas in different seasons ($P < 0.05$). Fv/Fm values were not significantly different between MC and HC areas in spring, summer, or winter. The changes in Fv/Fm in the three cover areas were marked in autumn, and the values in LC areas were 1.64 times higher than those in HC areas. The change trend of NPQ in the different seasons was opposite to the change trend of Fv/Fm at 450 nm. NPQ values in HC areas were higher than those in MC or LC areas (Fig. 5b).

The Fv/Fm of Cyanophyta (624 nm) was highest in winter (mean = 0.40) and lowest in autumn (mean = 0.28) (Fig. 5c), indicating that, although cyanobacteria had low biomass in winter, they displayed high-rate photosynthetic activity. NPQ was highest in autumn (mean = 2.88) and lowest in winter (mean = 1.82) (Fig. 5c).

3.5. Influence of environmental factors on phytoplankton community parameters and dominant genera

Spearman correlations between community parameters of phytoplankton and environmental factors showed that the total cell density of phytoplankton was significantly and positively correlated with TN, TP, pH, and DO, and was significantly and negatively correlated with

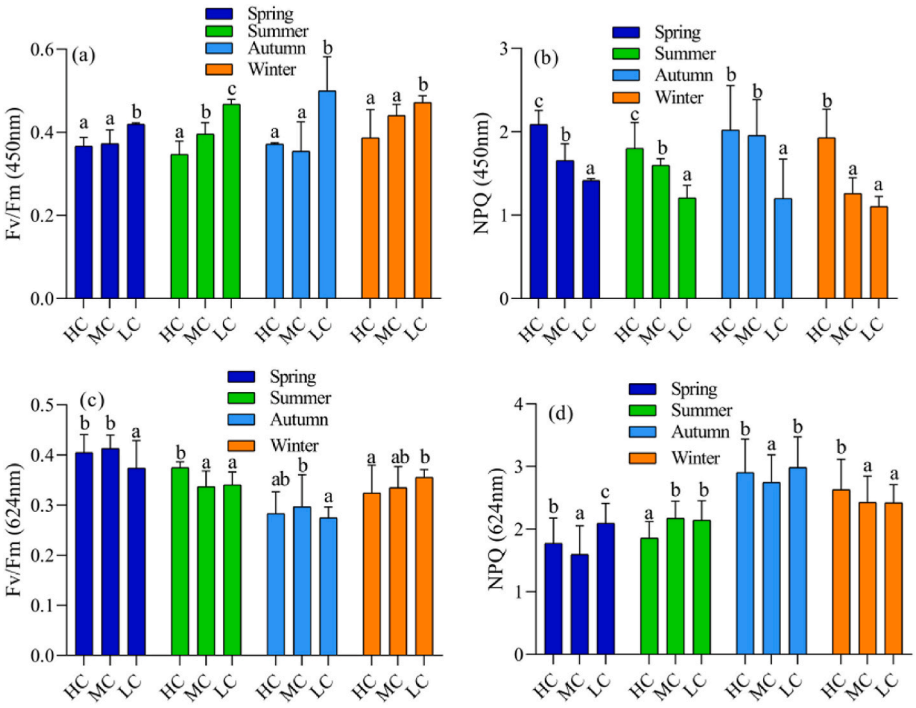

Fig. 5. Fluorescence parameters of phytoplankton in different seasons and sites (Mean ± S. D, N = 3).

biomass of submerged plants, N:P ratio, and WT (Table 2, $P < 0.05$). The Shannon Wiener diversity index was significantly and negatively correlated with TP and significantly and positively correlated with N:P ratio, total phenols, and coverage area. Further, there was a significantly negative relationship between Chl and coverage area of submerged plants ($P < 0.1$). Cell density of Cyanophyta was negatively related to the biomass of submerged plants, N:P ratio, and EC. Cell density of Chlorophyta and Bacillariophyta was significantly and positively correlated with TN, TP, pH, and DO ($P < 0.05$) and was significantly and negatively correlated with biomass of submerged plants, N:P ratio, and WT.

From the NMDS plot (Fig. 6a), it was observed that the cell densities of the dominant genera were similar in spring and summer, and that dominant genera in autumn and winter were similar. According to the results of CCA, the first axis explained 32.69% of the dominant genera variation, and the second axis explained 17.93%. *Pseudanabaena*, *Oscillatoria*, and *Leptolyngbya* were positively correlated with WT, and negatively correlated with N:P ratio, biomass of submerged plants, and total phenol content (Fig. 6b). *Oedogonium*, *Gomphonema*, *Coscinodiscus*, and *Cymbella* were positively correlated with biomass, coverage, and N: P ratio, while negatively correlated with WT and TN (Fig. 6b).

3.6. Influence factors on fluorescence parameters

The results of Spearman correlation analysis of fluorescence at 450 nm and environmental factors are shown in Table 3. Fluorescence parameters, namely, Fv/Fm and NPQ of Chlorophyta and Bacillariophyta were only affected by UWI. Fv/Fm was negatively correlated with UWI ($r = -0.536$, $P < 0.01$). NPQ only had a significant correlation with UWI ($r = 0.517$, $P < 0.001$).

Correlation analysis between fluorescence parameters at 624 nm and environmental factors showed that Fv/Fm was more affected by environmental factors than at 450 nm. The Fv/Fm of Cyanophyta was significantly and positively correlated with TP content ($r = 0.385$, $P < 0.05$), pH ($r = 0.439$, $P < 0.01$), and EC ($r = 0.409$, $P < 0.05$). Fv/Fm was significantly and negatively correlated with N:P ratio ($r = -0.390$, $P < 0.05$), total phenol content ($r = -0.593$, $P < 0.01$), and UWI ($r = -0.466$, $P < 0.01$). NPQ had a significant positive correlation with total phenol and UWI but a negative correlation with pH and EC (Table 3).

Based on the results of correlation analysis, SEM was used to explore the direct and indirect effects of environmental factors on fluorescence parameters of Cyanophyta. The model equation obtained was $\chi^2 = 22.417$, df = 11, $P = 0.021$, GFI = 0.869, indicating that the model fitted the data well. The coverage of submerged plants had a positive effect on N:P ratio, with a path coefficient of 0.32. Coverage of submerged plants had a positive effect on total phenol, with a path coefficient of 0.47.

Table 2
Spearman correlations between community parameters of phytoplankton and environmental factors.

	TN	TP	N: P	Total phenol	pH	EC	DO	WT	Biomass	Coverage	UWI	OWI
Total cell density	**0.478****	**0.656****	**−0.560****	−0.306	**0.416***	−0.150	**0.565****	**−0.444****	**−0.464****	−0.314	−0.25	−0.14
Shannon Index	0.093	**−0.344***	**0.516****	**0.350***	0.101	−0.043	0.039	−0.063	**0.376***	**0.395***	−0.011	−0.086
Chl	0.237	0.043	0.074	−0.098	−0.047	−0.126	0.235	−0.269	−0.092	**−0.359***	0.075	0.136
Cyanophyta	0.063	0.304	**−0.383***	−0.056	−0.050	**−0.422***	0.024	0.059	**−0.545****	−0.201	−0.297	0.133
Chlorophyta	**0.658****	**0.598****	**−0.361***	−0.263	**0.391***	−0.043	**0.702****	**−0.621****	−0.159	−0.141	0.090	−0.073
Bacillariophyta	**0.581****	**0.658****	**−0.434****	−0.329	**0.498****	−0.083	**0.754****	**−0.669****	**−0.383***	−0.296	−0.043	−0.202
Pyrrophyta	0.017	0.063	−0.085	0.036	−0.164	−0.169	−0.094	0.091	−0.164	0.094	−0.144	0.175
Euglenophyta	0.109	0.176	−0.257	−0.124	0.298	**0.355***	0.288	−0.235	0.118	0.16	−0.015	−0.215
Cryptophyta	−0.122	0.056	−0.122	−0.066	0.244	0.161	0.169	−0.138	−0.119	−0.067	−0.265	**−0.332***

Note: *: $P < 0.05$; **: $P < 0.01$.

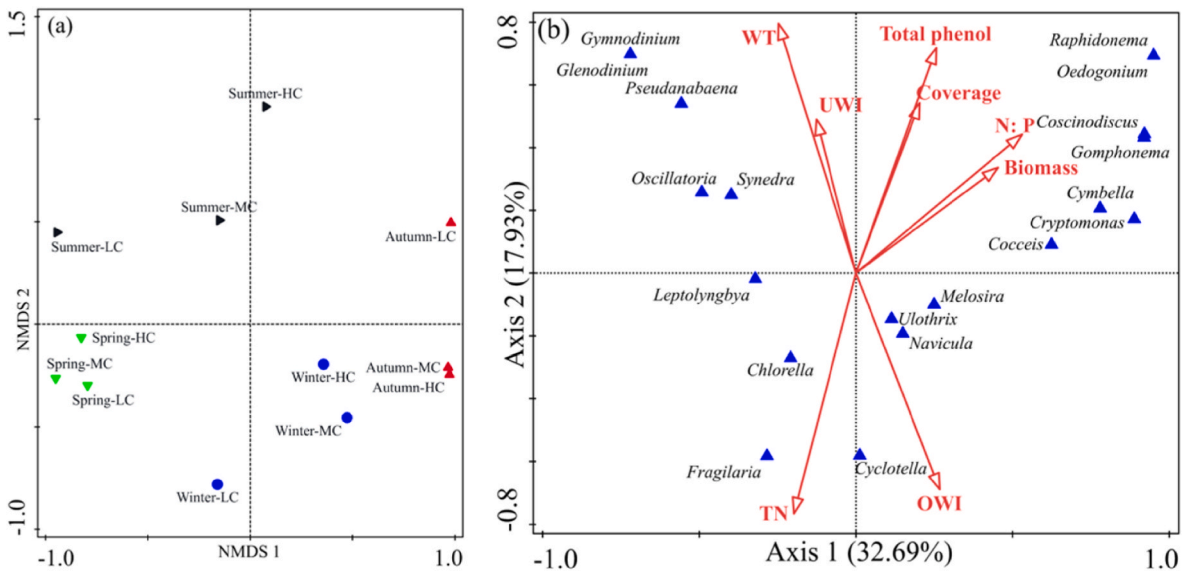

Fig. 6. NMDS and RDA ordination plot of phytoplankton dominant genera and their relation to environment parameters. (a: NMDS ordination plot, b: RDA ordination plot).

Table 3
Spearman correlations between fluorescence parameters at 450 nm and 624 nm of phytoplankton and environmental factors.

	450 nm (Chlorophyta and Bacillariophyta)		624 nm (Cyanophyta)	
	Fv/Fm	NPQ	Fv/Fm	NPQ
TN	0.064	−0.08	0.275	−0.142
TP	0.043	−0.043	**0.385***	−0.238
N: P	−0.119	0.101	**−0.390***	0.290
Total phenol	−0.231	0.229	**−0.593****	**0.519****
pH	0.327	−0.308	**0.439****	**−0.370***
EC	0.193	−0.183	**0.409***	**−0.424****
DO	0.116	−0.094	0.327	−0.184
WT	−0.082	0.089	−0.289	0.133
Biomass	−0.057	0.072	−0.134	0.106
Coverage	−0.084	0.102	−0.126	0.072
OWI	−0.323	0.322	0.029	−0.085
UWI	**−0.536****	**0.517****	**−0.466****	**0.372***
Total cell density	0.103	−0.151	0.219	−0.064
Chl	0.031	−0.014	−0.105	0.140

Note: *: $P < 0.05$; **: $P < 0.01$.

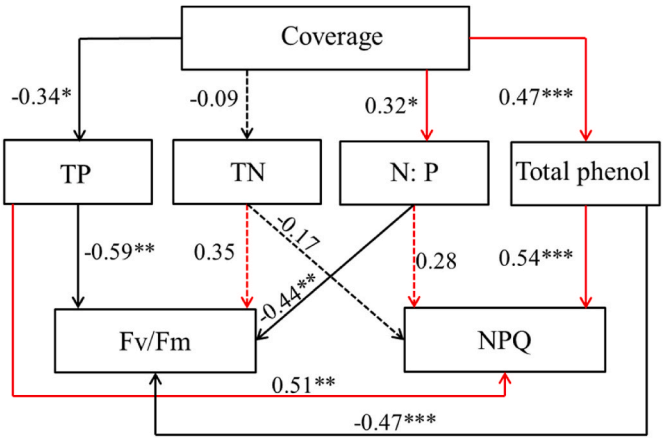

Fig. 7. Structural equation modeling (SEM) showing the direct or indirect effects of environment factors on the fluorescence parameters of Cyanophyta. (The red arrows represents the significant positive effects, and black arrows represents negative effects.**$P < 0.05$, ***$P < 0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Conversely, TN had no significant effect on Fv/Fm or NPQ. TP and total phenol had significant negative effects on Fv/Fm, and the effect coefficients were −0.59 ($P < 0.01$) and −0.47 ($P < 0.001$), respectively. TP and total phenols had significant positive effects on NPQ, and the effect coefficients were 0.51 ($P < 0.01$) and 0.54 ($P < 0.001$), respectively (Fig. 7).

4. Discussion

4.1. Influence of submerged plant coverage on total phenol and nutrient contents

The biomass of submerged plants peaked in autumn and was lowest in spring, presumably owing to the influence of temperature on the growth of submerged plants. In winter, the WT decreases, resulting in poor growth of submerged plants (Zeng et al., 2017). Total phenol content was consistent with the biomass of submerged plants. Previous studies have shown that as the density of submerged plants increases, the phenolic acid content released by submerged plants into the water gradually increase (Gao et al., 2014). Total phenol content was lowest in winter, likely due to the biomass and physiological status of submerged plants in winter. Fabrowska et al. (2018) found that the phenolic compound content in natural lakes decreased with ageing of *Cladophora glomerata*. Phenolic compounds are secondary metabolites of submerged plants under abiotic stress. When nutrients are limited, submerged plants secrete more phenolics to improve their competitive advantage (Bauer et al., 2009). This might be one of the reasons why total phenol content was higher in summer and autumn than in the other seasons.

Submerged plants can intercept and absorb soluble nutrients present in the water through the stems and leaves during their growing season (Xu et al., 2019a). Our research *in situ* found that the TN and TP contents in the HC and MC areas were significantly lower than those in the LC areas, but there was no significant difference between the HC and MC areas. These results showed that when the restoration coverage of submerged plants in shallow lakes reached a certain level, further submerged plant coverage had little effect on improving water quality, which was consistent with previous research results (Chen et al., 2009; Dai et al., 2012). In winter, which is the season of decay for some

submerged plants, the decomposition of submerged plant debris in HC areas may increase the nitrogen and phosphorus contents in the water body, thus resulting in an increase in phytoplankton biomass (Zhang et al., 2018, 2021). Combining the economic factors involved in restoring submerged vegetation and navigation convenience, submerged plants with MC (20%–50%) may be more conducive to the optimal coverage of submerged vegetation in shallow lakes.

4.2. Impact of submerged plant coverage on phytoplankton community parameters

The influence of submerged plants on phytoplankton community has been emphasised in many previous studies (Jeppesen et al., 1997). Here, we found that the density of phytoplankton decreased as the coverage of submerged plants increased. This may be partially explained by allelopathic inhibition of phytoplankton by submerged plants or by nutritional competition between phytoplankton and submerged plants. The negative correlation between submerged plant coverage and TN and TP indicates that submerged plants may indirectly reduce nutrient availability for phytoplankton. In addition, the presence of submerged plants increased the diversity of phytoplankton. In areas with high coverage of submerged plants, the biomass of cyanobacteria was reduced. Instead, dominant phyla were Bacillariophyta and Pyrrophyta including the following genera: *Melosira*, *Cocceis*, *Navicula*, *Gymnodinium*, and *Glenodinium*. Combined with the analysis of the results of our SEM, this may be due to the total phenols secreted by submerged plants, which can inhibit the growth of cyanobacteria biomass, reduce their competitive advantage, and benefit the growth of other species (Chen et al., 2012).

The diversity and dominant species of phytoplankton are important indicators for evaluating ecosystems. A larger number of dominant species and a higher biodiversity indicate that phytoplankton are more evenly distributed in the aquatic ecosystem, which thereby becomes more stable. The biodiversity and dominant species in the HC sites were significantly higher than that in the LC sites, likely because submerged plants inhibit the growth of cyanobacteria, thus reducing the competitive advantage of cyanobacteria over other algae, and, thereby increasing phytoplankton diversity (Wang et al., 2017).

4.3. Effect of submerged plant coverage on the fluorescence parameters of phytoplankton

The most used and most representative indices of photosynthetic activity in the study of photosynthetic characteristics of phytoplankton are Fv/Fm and NPQ, which can reflect the influence of various external environmental factors on the photosynthetic activity of algae (Dewez et al., 2008; Miller et al., 2017). Our research found that the photosynthetic system of Cyanophyta was more affected than those of Chlorophyta and Bacillariophyta by TP, N:P ratio, total phenol, pH, and EC, presumably because of the differences between the photosynthetic systems of Cyanophyta and Chlorophyta (Hu and Wei, 2006). Cyanophyta cells do not have chloroplasts, and their photosynthesis mainly depends on phycobiliprotein in the light-harvesting antenna system (Tooley et al., 2001). Studies have shown that the release of allelochemicals from submerged plants can reduce phycobiliprotein content of cyanobacteria, thereby affecting its photosynthesis activity (Wu et al., 2008). In addition, the decomposition of submerged plants releases organic acids and phenols, which might affect the biomass and photosynthesis of phytoplankton (Gross et al., 1996; Zhang et al., 2018). Our results showed a negative correlation between total phenols of submerged plants and Fv/Fm and NPQ of cyanobacteria, whereas Fv/Fm and NPQ of green algae and diatoms were weakly correlated with total phenol content. When coexisting with other algae, submerged plant allelochemicals inhibit cyanobacteria photosynthesis more strongly (Zhu et al., 2021), presumably because photosynthetic electron transfer in cyanobacteria is blocked, and the photochemical efficiency is reduced (Zhu et al., 2010). This might explain why the biomass of cyanobacteria decreased sharply during autumn, and might be related to the higher total phenol content in the water bodies in this season (Fig. 2b; Fig. 3c). It is reasonable to expect that total phenols have a high inhibitory effect on the photosynthesis of cyanobacteria in natural aquatic ecosystems, whereby, the reconstruction of submerged plants can effectively inhibit photosynthesis and growth of cyanobacteria.

Nitrogen and phosphorus are generally considered restrictive nutrients for primary production. A N:P ratio <10 may be nitrogen-limiting, whereas if >20 it is usually phosphorus-limiting (Maberly et al., 2020). In our study, the N:P ratio was in the range of 32.35–124.54, which is relatively high, implying that the phytoplankton was in a phosphorus-limited state. A phosphorus limitation reduces ATP synthesis in phytoplankton cells, which leads to a decrease in Fv/Fm (Lippemeier et al., 2001). Our correlation analysis and SEM results showed that the N:P ratio was negatively correlated with Fv/Fm and positively correlated with NPQ of Cyanophyta. This was likely due to the low phosphorus content, which reduced the FV/Fm of cyanobacteria and activated the NPQ mechanism to consume the excess energy absorbed by the algal cells (Lippemeier et al., 2001). To prevent damage to the photosynthetic system, especially to the electron transport chain, NPQ plays an important role in protecting the normal progress of photosynthesis when algal cells are under environmental stress (Chorus and Spijkerman, 2020). Harke et al. (2017) found that the photosynthetic efficiency of *M. aeruginosa* was significantly reduced in a phosphorus-free culture medium, and many important genes encoding photosynthesis-related enzymes were differentially expressed, indicating that phosphorus regulates photosynthesis in cyanobacteria. In addition, total phenols released by submerged plants can form chemical complexes with many enzymes, especially alkaline phosphatase produced by phytoplankton and bacteria, which causes algal cells to be limited by phosphorus due to the inactivation of phosphatase (Wetzel, 1992).

5. Conclusions

We conducted *in situ* experiments to determine the effects of different levels of submerged plant coverage on water quality parameters, phytoplankton community composition, and fluorescence parameters. The results showed that nitrogen and phosphorus contents in submerged plant HC areas were significantly lower than those in LC sites. Further, total phenol content of submerged plants in HC areas was significantly higher than that in MC or LC areas. In spring and summer, *Pseudanabaena*, *Leptolyngbya*, and *Oscillatoria* of Cyanophyta were dominant, and were positively correlated with temperature and negatively correlated with N:P ratio, coverage of submerged plants, total phenols, and biomass. By contrast, *Ulothrix*, *Cyclotella*, *Melosira*, and *Cymbella* were dominant in autumn and winter, and were negatively correlated with temperature and UWI. Total phenol content of submerged plants had a lower effect on the Fv/Fm and NPQ of Bacillariophyta and Chlorophyta, and a greater impact on Cyanophyta. TP, N:P ratio, and total phenols had a negative effect on Fv/Fm and a positive effect on NPQ of Cyanophyta. Coverage of submerged plants indirectly affected fluorescence parameters of Cyanophyta by affecting nitrogen, phosphorus, and total phenol content.

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.jenvman.2021.113822.

Credit author statement

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