

ORIGINAL ARTICLE

Submerged macrophytes can counterbalance the negative effects of rising temperature and eutrophication by inhibiting the photosynthetic activity of cyanobacteria and adjusting their morphology and physiology

Yu Zhao^{1,2,3}  | Rong Wang^{1,2,3}  | Erik Jeppesen^{4,5,6,7,8} | Enlou Zhang^{1,2,3,9}

¹State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, China

²University of Chinese Academy of Sciences, Beijing, China

³College of Nanjing, University of Chinese Academy of Sciences, Nanjing, China

⁴Sino-Danish College, University of Chinese Academy of Sciences, Beijing, China

⁵Department of Ecoscience, Aarhus University, Aarhus, Denmark

⁶Limnology Laboratory, Department of Biological Sciences and Centre for Ecosystem Research and Implementation, Middle East Technical University, Ankara, Turkey

⁷Institute of Marine Sciences, Middle East Technical University, Mersin, Turkey

⁸Institute for Ecological Research and Pollution Control of Plateau Lakes, School of Ecology and Environmental Sciences, Yunnan University, Kunming, China

⁹Poyang Lake Wetland Research Station, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Jiujiang, China

Correspondence

Rong Wang, State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, 73 East Beijing Road, Nanjing 210008, China.
Email: rwang@niglas.ac.cn

Funding information

National Natural Science Foundation of China; Youth Innovation Promotion Association of the Chinese Academy of Sciences; TÜBITAK program BIDE2232; Double Thousand Plan of Jiangxi Province

Abstract

1. Climate-driven changes in temperature and high nutrient inputs from anthropogenic activities significantly impact the interactions between submerged macrophytes and phytoplankton, potentially causing regime shifts in shallow freshwater lakes. Cyanobacteria, in particular, are predicted to become dominant among the phytoplankton under climate change, contributing to the collapse of submerged macrophytes. This study aimed to explore how submerged macrophytes and phytoplankton respond to rising temperatures and nutrient addition, how the presence of submerged macrophytes influences the photosynthetic activity of cyanobacteria, and how submerged macrophytes adapt their morphology and physiology to cope with excess phytoplankton.
2. We conducted a fully factorial experiment consisting of two temperature conditions (low and high temperature), two nutrient conditions (with and without nutrient addition) and two plant conditions (plant presence and absence). We analysed changes in phytoplankton biomass and diversity (taxonomic and functional) in response to rising temperatures and nutrient addition. The maximum photochemical efficiency of the photosystem II (F_v/F_m) and the maximum relative electron transport rates (rETR_{max}) of cyanobacteria were determined. We measured the response of macrophytes to environmental changes by measuring antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), as well as morphological indicators like plant height, weight, and the relative growth rate.
3. In the absence of submerged macrophytes, rising temperatures led to higher total phytoplankton biomass and cyanobacteria dominance alongside reduced taxonomic and functional diversity. Conversely, when submerged macrophytes were present and no nutrients were added, rising temperatures did not lead to increased phytoplankton biomass.
4. Macrophytes can suppress undesirable cyanobacterial proliferation by competing for nutrients and inhibiting F_v/F_m . Furthermore, under conditions of environmental stress, such as high chlorophyll *a* concentrations due to nutrient inputs, submerged macrophytes adapted by increasing height and the activity of antioxidant enzymes, such as SOD and CAT.

5. Within the context of climate warming and increased nutrient inputs, phytoplankton growth, especially of cyanobacteria, may be favoured. However, our study demonstrated the critical role of submerged macrophytes in inhibiting phytoplankton, especially when nutrients were controlled. By using these physiological indicators to assess the life activity of organisms, our research provided new insights into macrophyte-phytoplankton relationships during regime shifts in aquatic ecosystems.

KEYWORDS

climate change, photosynthesis, physiological indicators, phytoplankton, regime shift

1 | INTRODUCTION

Freshwater ecosystems around the world are nowadays strongly affected by global changes such as rising temperatures and increasing nutrient inputs (Jeppesen et al., 2014; Matzinger et al., 2007). In many lakes, this causes a shift from a clear-water state dominated by submerged macrophytes to a turbid state dominated by phytoplankton (Polst et al., 2022; Scheffer & Carpenter, 2003; Scheffer & Jeppesen, 2007), including also many shallow lakes in the middle and lower reaches of the Yangtze River in China (Xu et al., 2019). The shift affects the services and functions of the lakes by diminishing the recreational and conservation value (swimming, fishing, etc.), causing loss of habitat generated by macrophytes, and enhancing the nutrient cycling (Meerhoff & Gonzalez-Sagrario, 2022). Submerged macrophytes can act as a nutrient source, compete with phytoplankton, and provide shelter for phytoplankton-grazing zooplankton (Lauridsen et al., 1996; Vanderstukken et al., 2011; Wang et al., 2023). Phytoplankton is an important food source and can release oxygen through photosynthesis (Krause-Jensen & Sand-Jensen, 1998; Taipale et al., 2019); however, excess phytoplankton can trigger algal blooms, reduced transparency, loss of macrophytes, and even production of toxic substances (Amorim & Moura, 2021; Carmichael, 2001). The interactions between submerged macrophytes and phytoplankton with changing nutrient regimes depend, though, on the lake temperature (Kosten et al., 2011, 2012).

Temperature determines many physiological properties of organisms and can regulate primary productivity (Staeher & Sand-Jensen, 2006). An increase in temperature within a specific range can enhance enzyme activity and promote nutrient cycling, which in turn enhances photosynthetic rates, respiration, and cell growth (Raven & Geider, 1988). Some studies have shown that macrophytes benefit from the rising temperature through growth stimulation and seed germination (Ersoy et al., 2020; Rooney & Kalff, 2000). Phytoplankton and its diversity are also sensitive to temperature changes. Some experiments have shown negative effects of climate warming on phytoplankton diversity (Urrutia-Cordero et al., 2017), which may be due to competitive exclusion of some species (Burgmer & Hillebrand, 2011). However, the relationship between temperature

and phytoplankton diversity is controversial and depends on many aspects (specific temperature and environmental background conditions; Pálffy & Smeti, 2024). In addition to the taxonomic diversity, functional/trait diversity is increasingly used in phytoplankton community ecology, and the key functional traits scale with cell size/volume (Borics et al., 2021; Litchman et al., 2007). Research has shown that the reduced size of aquatic organisms is one of the ecological responses to global warming (Daufresne et al., 2009), and warming favours dominance of small and fast-growing phytoplankton (Rasconi et al., 2015). Lake stratification, exacerbated by climate change, may further favour the dominance of buoyant cyanobacteria species, which can float to the surface and consequently shade other phytoplankton taxa (Huisman et al., 2004; Johnk et al., 2008; Paerl & Huisman, 2009). As predicted by Moss et al. (2003), warming will result in a shift towards cyanobacteria dominance (Burgmer & Hillebrand, 2011), as their optimal growth rates are found at higher temperatures—usually above 25°C—compared to most other eukaryotic phytoplankton species (Paerl, 2014; Paerl & Huisman, 2009), thereby increasing the risk of submerged macrophyte collapse in shallow lakes. However, the size of the temperature effect also depends on the nutrient conditions (Gonzalez-Olalla et al., 2022; Rhee & Gotham, 1981; Staeher & Sand-Jensen, 2006).

Nutrients affect the biomass of submerged macrophytes and phytoplankton by providing essential elements and energy sources for photosynthesis, respiration, and enzyme catalysis (Elser et al., 2000). Phosphorus and nitrogen are key limiting nutrients in aquatic ecosystems and may play crucial roles in determining the growth and biomass of phytoplankton (O'Donnell et al., 2017). Phytoplankton has a higher metabolism rate and nutrient requirement than submerged macrophytes (Sand-Jensen & Borum, 1991), and this may contribute to the shift from a macrophyte- to a phytoplankton-dominated state in shallow lakes suffering from a strong nutrient input (Hargeby et al., 2007; Scheffer & Jeppesen, 2007; Smith & Schindler, 2009). The nutrient inputs, which are likely to become higher due to climate change (e.g. increased rainfall intensity and temperature-mediated phosphorus release from the sediment) and increased human activities, may, therefore, have a severe impact on lake ecosystems (Jeppesen et al., 2009; Smith & Schindler, 2009).

Temperature and nutrients often work synergistically and have profound effects on aquatic ecosystems (Gonzalez-Olalla et al., 2022; Lürling et al., 2018; Staehr & Sand-Jensen, 2006). Rising temperatures increase the nutrient uptake and nutrient-use efficiency by organisms (Cross et al., 2015; Lewington-Pearce et al., 2019) and the risk of nutrient release from sediments (Jeppesen et al., 2009). Nutrient availability can also influence the thermal response of phytoplankton (Thomas et al., 2017). This synergy may make the interactions between submerged macrophytes and phytoplankton more complex. Given the present global changes, there is a growing need to explore the mechanisms of submerged macrophytes and phytoplankton in response to the synergistic effects of temperature and nutrients. In an experiment, Maredová et al. (2021) found that extracts from submerged macrophytes had an inhibitory effect on cyanobacteria. However, the inhibiting effect of plant extracts may not solely be attributed to allelopathically active compounds; other factors like nutrient competition might also contribute (Scheffer et al., 1993; Van Donk & Van de Bund, 2002). Gross et al. (1996) proposed that plant-secreted polyphenols may play a role in allelopathic interactions, but the compounds in their study were only extracted from certain species and had species-specific allelopathic effects on phytoplankton, making them difficult to detect, especially in situ (Gross, 2003; Gross et al., 2007; Hilt & Gross, 2008). The mechanisms by which submerged macrophytes inhibit phytoplankton need further investigation.

The inhibitory effects of submerged macrophytes on phytoplankton can be characterized by the activities of photosystem II (PSII) in phytoplankton (Gross, 2003; Leu et al., 2002). The maximum photochemical efficiency of photosystem II (F_v/F_m) serves as a robust indicator of photosynthetic efficiency across various studies, including field surveys and experiments (Bussotti et al., 2020; Lürling et al., 2018). Besides, maximum relative electron transport rates ($rETR_{max}$; capacity of photosynthesis) increase as temperature increases within the optimal temperature range (Salleh & McMinn, 2011). The effect of phytoplankton on submerged macrophytes is reflected by morphological (biomass, plant length, etc.) and physiological indicators. For example, it has been shown that hypoxic stress caused by algal blooms increases the activity of antioxidant enzymes of plants, such as superoxide dismutase (SOD) and catalase to cope with the stress (CAT; Yuan et al., 2019; Zhang et al., 2011). In addition to antioxidant enzymes, malondialdehyde (MDA) is a byproduct resulting from lipid peroxidation, which can be considered as an indicator of damage to plant cells (Song et al., 2015). Incorporating physiological and morphological indicators into the study of submerged macrophyte-phytoplankton interactions may help to better understand the mechanisms involved.

Within the context of global change, characterized by climate warming and nutrient enrichment, it is necessary to identify the response mechanisms of phytoplankton (especially cyanobacteria that prefer high temperatures and nutrient enrichment) and submerged macrophytes under different combinations of environmental gradients through suitable and reliable indicators. We conducted a fully factored manipulation experiment with two temperature conditions,

two nutrient conditions, and two plant conditions. We hypothesized that in the absence of macrophytes (1) rising temperature would boost the photosynthetic activity and dominance of cyanobacteria; (2) rising temperature would reduce the taxonomic diversity and functional diversity of phytoplankton communities, but that (3) submerged macrophytes would resist adverse effects under warming and nutrient enrichment conditions by inhibiting the photosynthetic activity of cyanobacteria and nutrient competition, and (4) submerged macrophytes would adapt to excess phytoplankton by making physiological adjustments, such as increasing enzyme activity, and morphological changes, such as altering their height and weight.

2 | METHODS

2.1 | Study design

We used 24 experimental cylindrical aquaria (11 cm diameter, 65 cm height) with a sediment layer of 6.50 cm randomly positioned in two illumination incubators. The water level was maintained at 50 cm (from sediment surface to water surface: 5.23 L). The sediment and water used for the experiments were from Lake Xuanwu (32°44.71'N, 118°47'55.72'E), which is a small urban shallow eutrophicated lake with a surface area of 3.68 km² and an average water depth of 1.1 m. The total phosphorus (TP) concentrations in the water column of Lake Xuanwu ranged from 0.04 to 0.19 mg L⁻¹ (mean value 0.10 mg L⁻¹) and the total nitrogen (TN) concentrations from 0.72 to 3.55 mg L⁻¹ (mean value 1.85 mg L⁻¹) in a bi-monthly survey conducted at four sampling sites between 2021 and 2022 (Qu & Zhou, 2024). The water was filtered through a 64 μm mesh to remove macro-organisms and the sediment through a 1 mm aperture sieve to remove coarse material. The phosphorus and nitrogen contents in the sediment on day 0 were 1.44 ± 0.01 mg g⁻¹ dry weight and 2.25 ± 0.04 mg g⁻¹ dry weight, respectively. The initial water TP and TN concentrations on day 0 were 0.043 ± 0.001 mg L⁻¹ and 1.47 ± 0.03 mg L⁻¹, respectively. The evaporation losses were compensated by distilled water once a week. The experimental factors included temperature (low temperature: LT; high temperature: HT), plants (with plant: WP; no plant: NP) and nutrients (with nutrient addition: WN; no nutrient addition: NN). A full factorial design with eight treatments (LT-NP-NN, LT-NP-WN, LT-WP-NN, LT-WP-WN, HT-NP-NN, HT-NP-WN, HT-WP-NN, and HT-WP-WN) was used, with three replications of each treatment, yielding a total of 24 experimental units.

The setup of temperature and illumination is shown in Figure 1. The low temperature (25°C) was chosen based on the actual water temperature of Lake Xuanwu at the time of sampling. The high temperature (30°C, Figure S1) was based on the field survey in 2022 when China experienced a summer heatwave, especially in the middle reaches of the Yangtze River (Hua et al., 2023). In the high temperature setup, 30°C was recorded during four of the 12 h (day-time). During the night, the temperature was set to 22°C. A total of two incubators were used, corresponding to different temperature

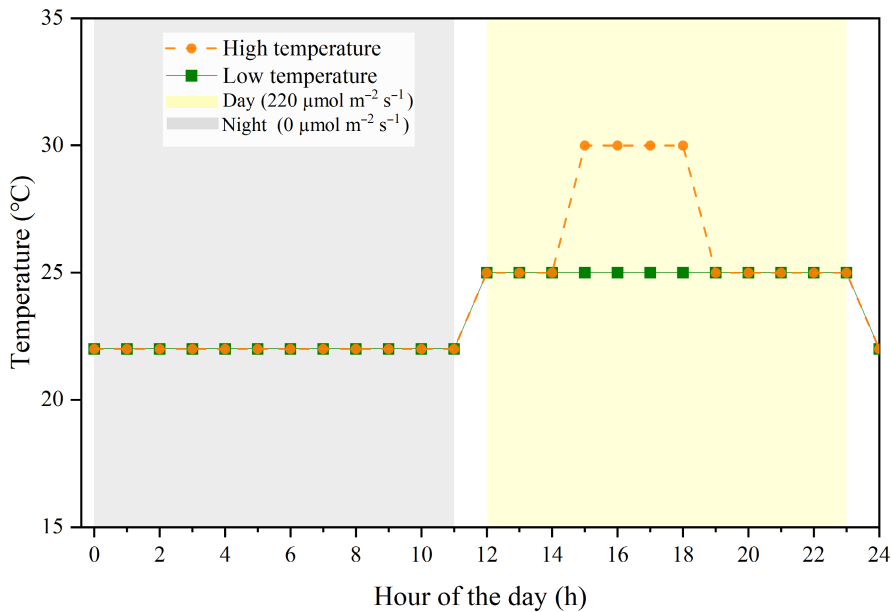


FIGURE 1 The temperature and light regime of the experiment.

settings. In both incubators, fluorescent tubes provided light from above at $220 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ photon flux density in 12:12h light: dark cycles (Szabo et al., 2022).

Phosphorus ($0.07 \text{ mg P}^{-1} \text{ L}^{-1}$ as KH_2PO_4) was added to the treatments involving nutrient addition every 5 days (days 0, 5, 10, 15). The volume of actual water column is 5.23 L; for simplicity of calculation, a volume of 5 L was selected to calculate the required phosphorus load. The nutrient was added as a 7 mL solution from KH_2PO_4 stock ($50 \text{ mg P}^{-1} \text{ L}^{-1}$) using an injector. Nitrogen was not added as a nutrient source in our experiment but the TN concentration by the end of the experiment was $0.79 \pm 0.01 \text{ mg L}^{-1}$ (Figure 2), which is close to the 0.8 mg L^{-1} threshold for nitrogen limitation in phytoplankton growth described by Xu et al. (2010).

Vallisneria natans, a typical and widely distributed submerged macrophyte in freshwater lakes of the middle and lower Yangtze River, was used in the plant treatments. The artificially planted *V. natans* in Lake Xuanwu may have been treated with cycocel, which promotes dwarf growth (Elateeq et al., 2022), before being transplanted into the lake. In order to avoid any influence of this on our measurements, the plants used for the experiments were collected in eutrophic Lake Taihu (Dong et al., 2014). Submerged macrophytes with uniform morphology were carefully chosen and gently washed before being measured and prepared for planting. The submerged macrophytes were transplanted into aquaria and acclimatized for 7 days. The initial total fresh weight of *V. natans* was $1.60 \pm 0.03 \text{ g}$ ($168.4 \pm 3.2 \text{ g m}^{-2}$). The experiment lasted for a total of 20 days, after which the height of submerged macrophytes had reached the surface of the water column.

2.2 | Sampling and processing

A total of 20 mL water was sampled to determine TP and TN concentrations. A total of 150 mL water was filtered through GF/C filters

(Whatman, Maidstone, UK), and chlorophyll α (Chl α) was extracted from the filters using 96% ethanol (Arvola, 1981). The filtered water was used to determine dissolved total phosphorus (DTP). The TP, DTP, and TN concentrations were determined using peroxodisulfate oxidation after digestion (Ebina et al., 1983). The difference between TP and DTP is particulate phosphorus (PP). The above sampling is carried out every 5 days. Water samples (50 mL) were collected from each treatment and subsequently fixed with Lugol's solution (1% v/v). After sedimentation for 48 h, the samples were concentrated to a final volume of 10 mL. Then, 0.1 mL portions of the samples were taken and placed in a 0.1 mL counting chamber at 400 \times magnification using an Olympus BX-53 microscope (Olympus Corporation, Tokyo, Japan). The phytoplankton biomass was calculated from cell size and abundance by assuming a specific gravity of 1 g cm^{-3} (Hillebrand et al., 1999). Phytoplankton was identified and counted at the genus level following the method of Hu (2006). Samples for phytoplankton identification were taken and analysed at the beginning and the end of the experiment.

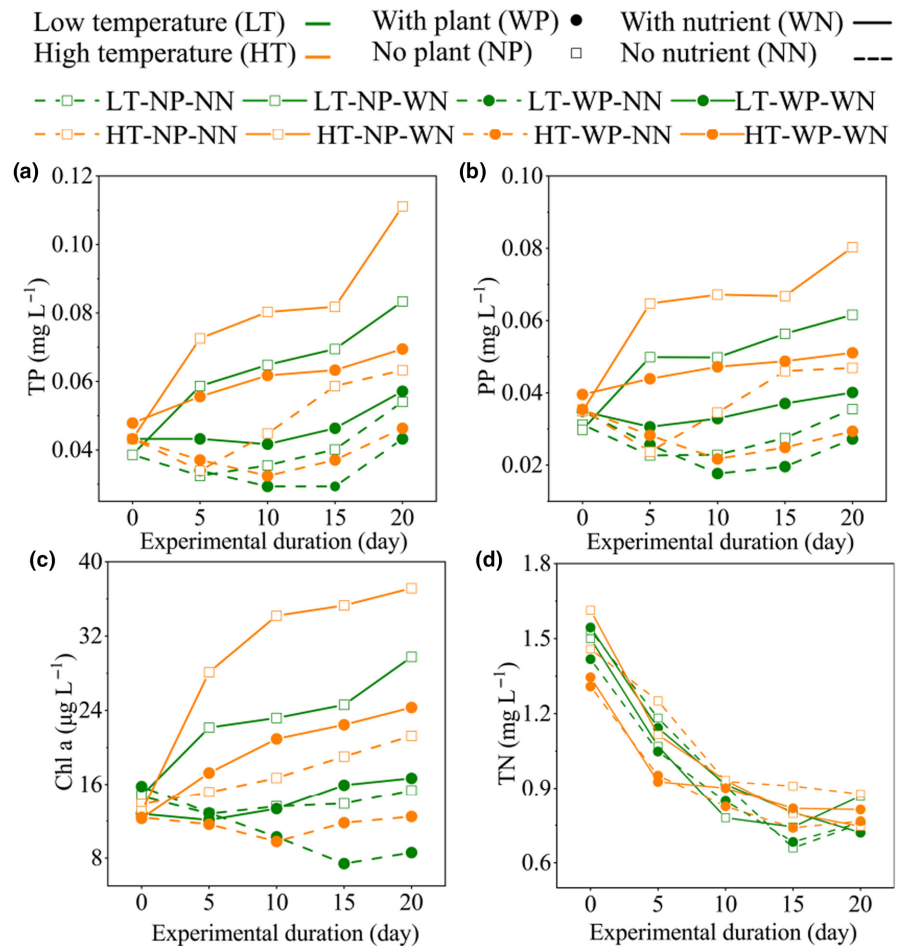
After 20 days, intact plants were collected and washed for laboratory determination of indicators. The height and weight of the individual *V. natans* were measured. The total fresh weight of *V. natans* equals the sum of the individual fresh weights. The relative growth rate (RGR) of submerged macrophytes was calculated following Miyashita and Tateno (2014):

$$\text{RGR} (\text{g g}^{-1} \text{ d}^{-1}) = \frac{\ln B_2 - \ln B_1}{t_2 - t_1} \quad (1)$$

where B_1 is the fresh weight at the initial time t_1 , and B_2 is the fresh weight after 20 days.

Superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were extracted from fresh *V. natans* tissues. About 0.5 g of fresh leaves mixed with phosphate-buffered saline solution (50 mM, pH 7.8) were ground into the homogenate using mortars and pestles. Then, the homogenates were centrifuged at 10,000 \times g

FIGURE 2 Variation of total phosphorus (a), particulate phosphorus (b), chlorophyll α (c) and total nitrogen (d) concentrations in the different treatments. Only mean values are shown. The standard errors can be found in Table S4.



at 4°C for 10 min, and the supernatants were used for subsequent analysis. The SOD activity was measured based on the fact that SOD has an inhibitory effect on superoxide anion radicals (Beauchamp & Fridovich, 1971), and one unit of SOD activity is defined as the amount required to achieve a 50% inhibition rate per milligram of protein in a 1 mL solution. The CAT activity was measured following the method of Aebi (1984), and one unit of CAT activity is defined as the amount of enzyme decomposing 1 μmol H₂O₂ per min per milligram of protein. The test for MDA content is based on the fact that MDA can react with thiobarbituric acid to form products with a maximum absorption peak at 532 nm (Heath & Packer, 1968). Protein contents were determined according to the method of Bradford (1976). The enzyme activities of SOD and CAT, as well as MDA contents, were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

As we were interested in the response of cyanobacteria, which may have a competitive advantage over other eukaryotic algae in our experimental scenario (nutrient enrichment and temperature increase; Paerl & Paul, 2012; Paerl, 2014), we measured the photosynthetic characteristics of cyanobacteria. A total of 3 mL water was sampled from each treatment at the end of the experiment. The minimum fluorescence (F_0) value and maximum fluorescence (F_m) value of cyanobacteria were measured by a Phyto-PAM phytoplankton analyser (Heinz Walz GmbH, Effeltrich, Germany),

which can distinguish between cyanobacteria and other eukaryotic algae through differences in wavelength absorption (440, 480, 540, 590, and 625 nm; Beecraft et al., 2017). Next, the maximum photochemical efficiency of the photosystem II (F_v/F_m) was calculated (Equation [2]),

$$F_v/F_m = \frac{F_m - F_0}{F_m} \quad (2)$$

A decrease in F_v/F_m is often associated with environmental stresses that result in suppression of maximum phytoplankton photochemical efficiency (Maxwell & Johnson, 2000). The maximum relative electron transport rates ($rETR_{max}$) of cyanobacteria were measured at 10 light intensities (2–855 μmol photons m⁻² s⁻¹). The calculation of $rETR_{max}$ was based on the model proposed by Ralph and Gademann (2005) and can be obtained in PhytoWin software. The high $rETR_{max}$ values indicate that phytoplankton possesses a high photosynthetic capacity (Ensminger et al., 2001).

2.3 | Data analysis

The data used for analysis were derived at the end of the experiment unless otherwise stated. Taxonomic diversity and the functional diversity of phytoplankton were calculated. Taxonomic diversity was

represented as a Shannon Wiener index. Functional diversity was represented as a community-weighted mean by using the trait of cell size (CWM.size; Equation (3); Díaz et al., 2007; Violle et al., 2007). Taxonomic and functional diversity were calculated using the R packages *Picante* (Kembel et al., 2010) and *FD* (Laliberté & Legendre, 2010),

$$\text{CWM.size} = \sum_{i=1}^s (p_i \times t_i) \quad (3)$$

where s is the number of species, p_i is the relative abundance of species i , and t_i is the trait value of species i . In our study, trait is the size (μm^3) of phytoplankton. Larger CWM values (μm^3) indicate the average larger cell size of the species in the community.

Three-way ANOVA was carried out to determine the effects of temperature, plants, and nutrients on water quality variables and phytoplankton. Two-way ANOVA was carried out to determine the effects of temperature and nutrients on submerged macrophytes. If there was an interaction between factors, independent samples t -tests were performed to analyse differences between treatments. Differences in water quality variables between the beginning (day 0) and end (day 20) were analysed using independent samples t -tests. Homoscedasticity (Levene test) and normality (Shapiro-Wilk) tests were performed before the ANOVA and t -test analysis, and data that did not satisfy the tests were log-transformed. Pearson's correlation analysis was used to analyse the relationship between Chl α concentrations and plant morphology (height, weight and RGR) and physiology (SOD, CAT and MDA). Normality was satisfied before the correlation analysis. The direct/indirect interactions between submerged macrophytes and phytoplankton and with environmental variables were examined using structural equation model (SEM) and elucidated by standardized regression coefficients (Grace et al., 2012). In order to simplify the model and improve the model fit, non-significant paths were removed from the model. The choice of model fit indices is flexible, and it is usually recommended to use at least two (Fan et al., 2016). Chi-squared/df (<2), p (χ^2 test; >0.05), and comparative fit index (CFI; >0.90) were used to evaluate the global fitting quality (Guine et al., 2016). Both phytoplankton and photosynthesis capacity refer to cyanobacteria as we only analysed changes in the photosynthesis of cyanobacteria. We used total phosphorus (TP) to represent nutrients and quantified both submerged macrophytes and cyanobacteria as biomass. The SEM analysis was performed using Amos 24.0 (IBM Corporation, Armonk, NY, USA). All data processing and statistical analyses were carried out using R version 4.3.1 (R Core Team, 2023) and Origin software (OriginLab Corporation, Northampton, MA, USA).

3 | RESULTS

3.1 | Water quality

Independent samples t -tests were used to assess whether there were differences in water quality on day 20 (last day) compared to the initial measurements (Table S1). The results showed that at high

temperatures, TP and Chl α concentrations increased significantly (Independent samples t -tests, $p < 0.05$ for all comparisons) in all treatments on day 20 compared to the initial day except for the treatment with plants and no nutrient addition (HT-WP-NN; Independent samples t -tests, $p > 0.05$ for all comparisons). At low temperatures, TP and Chl α concentrations increased significantly in the treatment with nutrient addition and no plant (LT-NP-WN; Independent samples t -tests, $p < 0.05$ for all comparisons). Among all treatments, only the LT-WP-NN treatment showed a significant decrease in Chl α concentrations (Independent samples t -test, $t_4 = 5.948$, $p = 0.004$; Figure 2).

Three-way ANOVA showed that temperature, plant, nutrient and plant-nutrient interactions significantly affected TP, PP and Chl α concentrations on day 20 (Table S2). We explored the effect of one environmental factor on water quality when fixing the levels of the remaining two factors through independent samples t -tests (Table S3). For temperature effects, the increased temperature significantly increased Chl α concentrations (Independent samples t -tests, $p < 0.05$ for all comparisons). Rising temperatures significantly increased the TP and PP concentrations when no plants were present and nutrients were added (Independent samples t -tests, $p < 0.05$ for all comparisons). For plant effects, plants significantly reduced the Chl α concentrations (Independent samples t -tests, $p < 0.05$ for all comparisons). The results showed that plants did not significantly affect TP and PP concentrations in the treatments without nutrient addition (Independent samples t -tests, $p > 0.05$ for all comparisons). However, when nutrient was added, plants significantly reduced TP and PP concentrations (Independent samples t -tests, $p < 0.05$ for all comparisons). Nutrient addition resulted in a significant increase in TP, PP and Chl α concentrations (Independent samples t -tests, $p < 0.05$ for all comparisons) except when plants were present and no nutrient was added (LT-WP-NN vs. LT-WP-WN; t -test, $t_4 = -2.714$, $p = 0.053$; Figure 2).

3.2 | Phytoplankton biomass and proportion

Six phytoplankton phyla were identified: Cryptophyta, Dinophyta, Phaeophyta, Bacillariophyta, Cyanophyta and Chlorophyta. Temperature, plant, nutrient and interactions significantly affected the total phytoplankton and Cyanophyta biomass as revealed by three-way ANOVA (Figure 3a; Table S5). Bacillariophyta, Cyanophyta and Chlorophyta formed the vast majority of the community biomass (Figure 3b). Rising temperatures did not result in significant changes in total phytoplankton biomass when plants were present and nutrients were not added (LT-WP-NN vs. HT-WP-NN; t -test, $t_4 = -0.656$, $p = 0.548$; Figure 3a). Rising temperatures significantly increased the biomass and proportion of Cyanophyta (Independent samples t -tests, $p < 0.05$ for all comparisons; Table S6), causing a significant decrease in the biomass proportions of Bacillariophyta and Chlorophyta (Independent samples t -tests, $p < 0.05$ for all comparisons; Figure 3 and Table S6). In general, Cyanophyta (biomass and proportion)

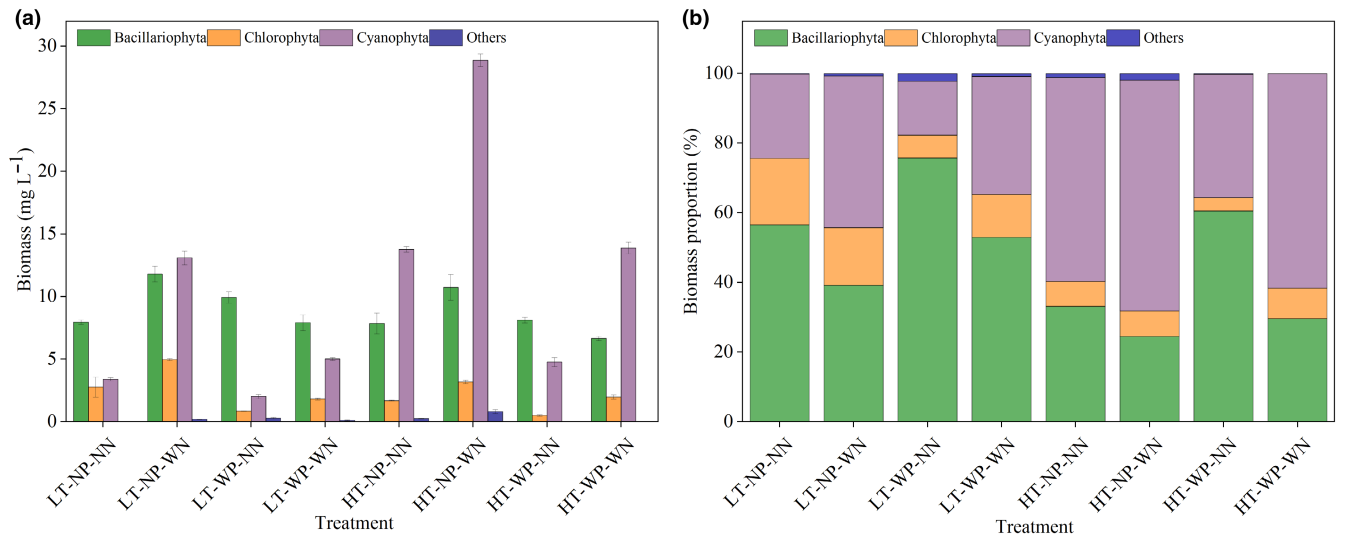


FIGURE 3 The (a) biomass and (b) proportion of Bacillariophyta, Cyanophyta and Chlorophyta under different environmental conditions at the end of the experiment. Others refer to the total biomass of Cryptophyta, Dinophyta and Phaeophyta. Error bars indicate 1 standard error. For an explanation of x axis letters—see [Figure 2](#).

dominated in the higher temperature treatments except for the HT-WP-NN treatment with plant presence and no nutrient addition ([Figure 3](#)). Plants significantly reduced the biomass and proportion of Cyanophyta (Independent samples *t*-tests, $p < 0.05$ for all comparisons). Nutrients significantly increased the biomass and proportion of Cyanophyta (Independent samples *t*-tests, $p < 0.05$ for all comparisons) and decreased the biomass proportion of Bacillariophyta (Independent samples *t*-tests, $p < 0.05$ for all comparisons).

3.3 | Phytoplankton diversity

The three-way ANOVA showed that temperature had significant effects on the Shannon Wiener index ($F_{1,16} = 229.6$, $p < 0.001$) and functional diversity ($F_{1,16} = 142.39$, $p < 0.001$). Increased temperatures resulted in a significantly lower Shannon Wiener index and functional diversity (Independent samples *t*-tests; $p < 0.05$ for all comparisons; [Table S7](#)). The presence of plants significantly reduced the Shannon Wiener index (Independent samples *t*-tests; $p < 0.05$ for all comparisons), except in the treatment with low temperature and no nutrient addition (*t*-test; $t_4 = 0.996$, $p = 0.376$). At low temperatures, plants significantly increased the functional diversity of phytoplankton (Independent samples *t*-tests; $p < 0.05$ for all comparisons). At high temperatures, plants did not significantly affect functional diversity when nutrient was added (*t*-test, $t_4 = -1.876$, $p = 0.134$).

3.4 | Macrophyte morphology

The effect of temperature on plant height was not significant ([Figure 4](#); two-way ANOVA, $F_{1,20} = 0.070$, $p = 0.794$). However,

the higher temperature significantly reduced the weight (two-way ANOVA, $F_{1,8} = 9.793$, $p = 0.014$) and RGR (two-way ANOVA, $F_{1,8} = 7.579$, $p = 0.025$) of the plants. Plant height, weight, and RGR responded differently to nutrient addition ([Figure 4](#)). Nutrient addition significantly increased the height of the plants (two-way ANOVA, $F_{1,20} = 20.476$, $p < 0.001$) but had the opposite effect on weight (two-way ANOVA, $F_{1,8} = 17.393$, $p = 0.003$) and RGR (two-way ANOVA, $F_{1,8} = 9.641$, $p = 0.015$). The results demonstrated the significant negative correlations between Chl α concentration and both weight (Pearson's correlation, $r_{12} = -0.778$, $p = 0.003$) and RGR (Pearson's correlation, $r_{12} = -0.683$, $p = 0.014$) but a positive correlation between Chl α concentration and height (Pearson's correlation, $r_{12} = 0.591$, $p = 0.043$).

3.5 | Phytoplankton and macrophyte physiology

We analysed photosynthetic activity of cyanobacteria in response to environmental changes ([Figure 5](#)). Only temperature had a significant positive impact on $rETR_{max}$ (three-way ANOVA, $F_{1,16} = 29.469$, $p < 0.001$). Conversely, plant (three-way ANOVA, $F_{1,16} = 0.476$, $p = 0.500$) and nutrient (three-way ANOVA, $F_{1,16} = 0.827$, $p = 0.377$) did not exhibit significant effects on $rETR_{max}$. The F_v/F_m was found to be most responsive to plant conditions (three-way ANOVA, $F_{1,16} = 24.637$, $p < 0.001$). Further analysis revealed that the presence of plants led to a significant reduction in F_v/F_m (Independent samples *t*-tests, $p < 0.05$ for all comparisons), except for the treatment with low temperature and no nutrient addition (LT-NP-NN vs. LT-WP-NN; *t*-test, $t_4 = 1.564$, $p = 0.193$).

The two-way ANOVA showed that the temperature had a significant effect on SOD (two-way ANOVA, $F_{1,32} = 74.24$, $p < 0.001$) and CAT (two-way ANOVA, $F_{1,32} = 45.73$, $p < 0.001$) activities

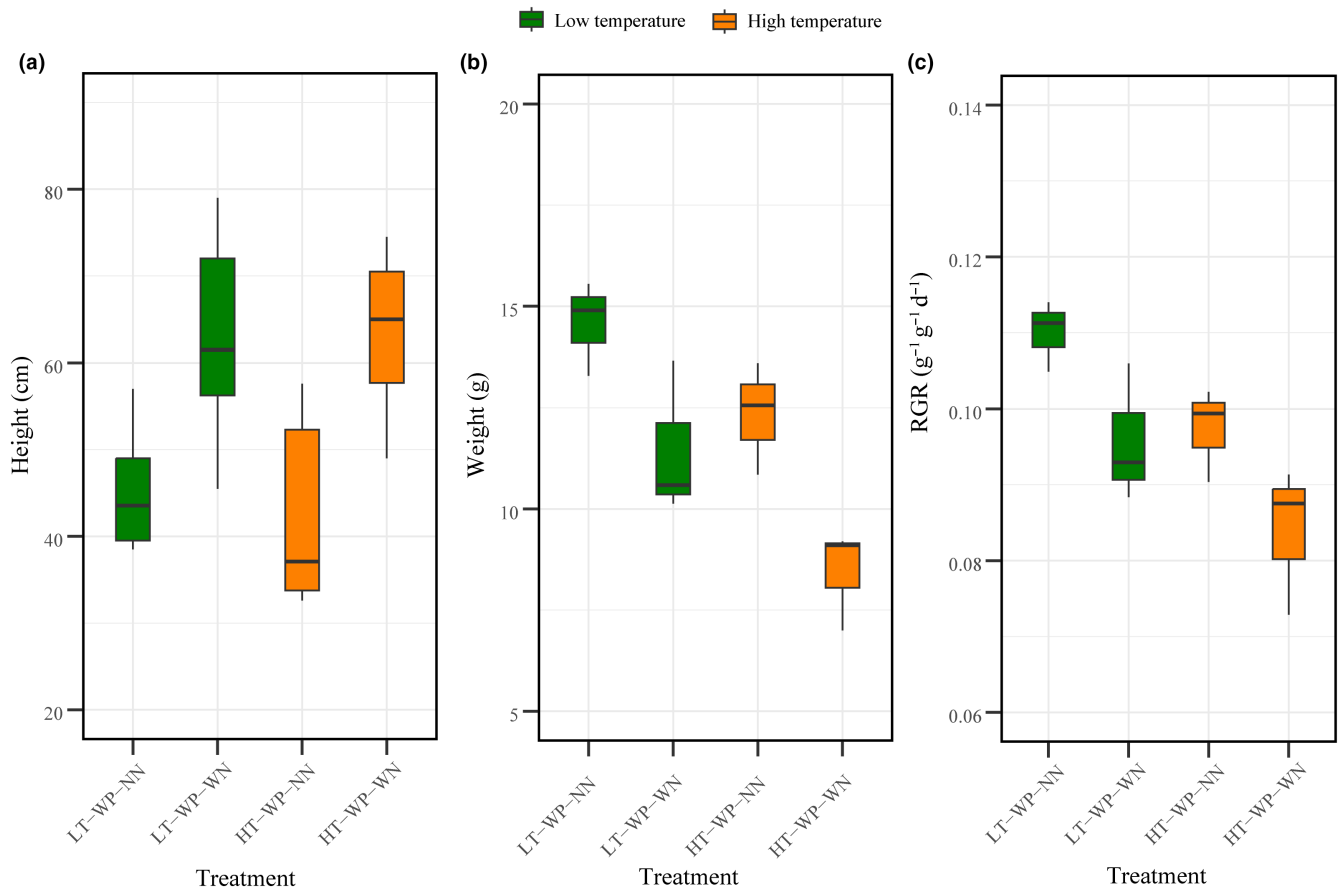


FIGURE 4 Height, weight and relative growth rate (RGR) of *Vallisneria natans* at the end of the experiment. The boxes indicate the 25th–75th percentiles, and the line in the box represents the median. For an explanation of x-axis letters—see Figure 2.

(Figure 6). Rising temperatures were associated with increased levels of SOD and CAT, as confirmed by *t*-tests (Independent samples *t*-tests, $p < 0.05$ for all comparisons). Furthermore, nutrient addition had a significant impact on SOD (two-way ANOVA, $F_{1,32} = 201.36$, $p < 0.001$), CAT (two-way ANOVA, $F_{1,32} = 129.91$, $p < 0.001$), and MDA (two-way ANOVA, $F_{1,32} = 42.77$, $p < 0.001$). The activities of SOD, CAT, and MDA were notably higher in the treatments with than without nutrient addition (Independent samples *t*-tests, $p < 0.05$ for all comparisons). The results revealed significant positive correlations between Chl α concentration and SOD (Pearson's correlation, $r_{12} = 0.871$, $p < 0.001$), CAT (Pearson's correlation, $r_{12} = 0.948$, $p < 0.001$) and MDA (Pearson's correlation, $r_{12} = 0.909$, $p < 0.001$) contents.

In the SEM analysis, the χ^2/Df and CFI values indicated that the selected model had a good fit. The SEM indicated that the rising temperatures and nutrient caused an increase in cyanobacterial biomass regardless of the submerged macrophyte condition (Figure 7). A higher photosynthetic capacity of cyanobacteria corresponded to a higher cyanobacteria biomass. The results also showed that the submerged macrophytes could indirectly inhibit cyanobacteria biomass by suppressing the photosynthetic activity and competing for nutrients (Figure 7b).

4 | DISCUSSION

Strong interactions between submerged macrophytes and phytoplankton, affecting the state of lake ecosystems (Jeppesen et al., 1997; Scheffer et al., 1993), have been observed in numerous field surveys (Phillips et al., 2016). Within the context of a changing climate, the cumulative effects of warming and eutrophication have increased the dominance of cyanobacteria (Elliott, 2012; Paerl, 2014), which may lead to collapse of submerged macrophytes and the turbid state dominated by phytoplankton (Moss et al., 2003). We experimentally investigated how the interactions between phytoplankton and submerged macrophytes changed with nutrient input in a climate warming perspective, with focus on changes in the photosynthetic activity of phytoplankton (cyanobacteria) and the physiological characteristics of submerged macrophytes.

We observed significant changes in ecosystem state in response to temperature and nutrient conditions. In the absence of plants, rising temperatures led to a marked increase in Chl α concentrations whether or not nutrients were added. This may, in part, reflect a temperature-induced stimulation of phytoplankton growth as seen in other studies (e.g. Fanesi et al., 2016). High temperatures

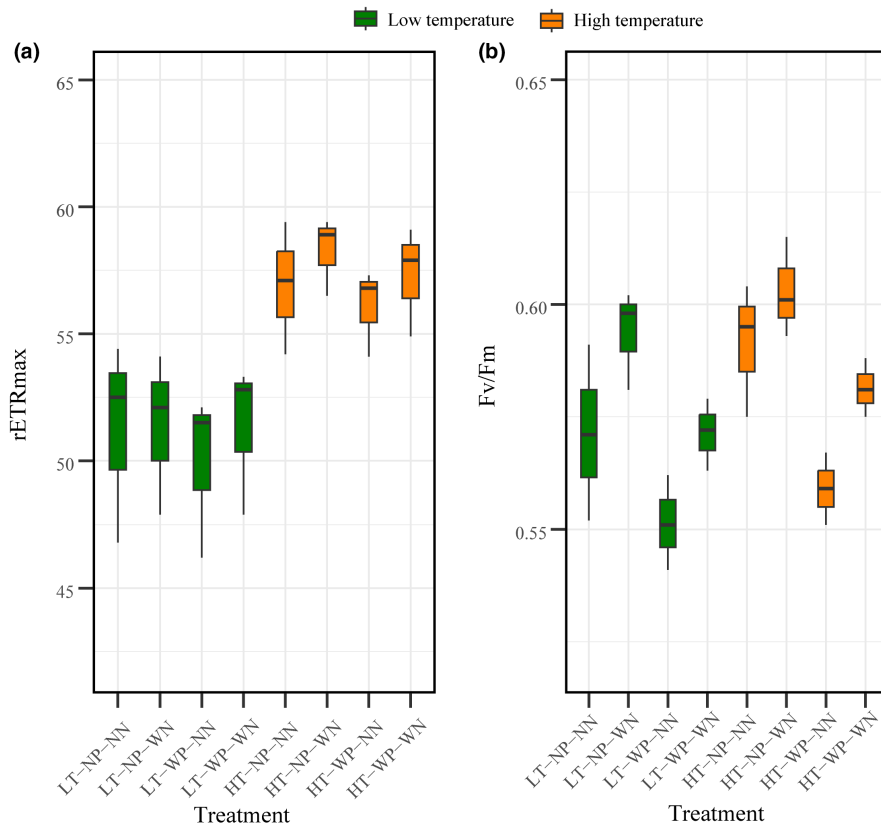


FIGURE 5 The maximum relative electron transport rates (rETR_{max}) and the maximum photochemical efficiency of the photosystem II (F_v/F_m) of cyanobacteria at the end of the experiment. The boxes indicate the 25th–75th percentiles, and the line in the box represents the median. For an explanation of x axis letters—see [Figure 2](#).

may promote cyanobacteria, augmenting their growth efficiency compared to most other phytoplankton taxa (Elliott, 2012; Paerl & Huisman, 2009). In correspondence with this, we found higher biomass and rETR_{max} of cyanobacteria under high temperature conditions in the absence of plants, leading to their dominance of the phytoplankton community. Other studies have also shown that at higher temperatures, cyanobacteria tend to dominate the phytoplankton community (Jeppesen, Audet, et al., 2021; Paerl & Huisman, 2008). Regarding phytoplankton diversity, both taxonomic and functional diversities were found to be lower at higher temperatures, as shown in [Figure 8](#). This reduction may be attributed to a shift towards a smaller, cyanobacteria-dominated phytoplankton community at higher temperatures, this being consistent with findings in an experiment by Rasconi et al. (2015). Furthermore, the decreased functional diversity could result in reduced stability of the phytoplankton community (Zhang et al., 2022). Our results suggested that, in the absence of plants, eutrophication caused by increased nutrient input may be reinforced by warming, which has also been revealed in other studies based on experiments and monitoring data (Jeppesen et al., 2020; Jeppesen, Johansson, et al., 2021).

When plants were present, rising temperatures also resulted in higher Chl α concentrations regardless of nutrient conditions, while warming did not result in significant increases in TP concentrations and total phytoplankton biomass when there was no nutrient addition, indicating that water bodies with plants and low nutrient levels

may be more resistant to the adverse effects of rising temperature. In terms of phytoplankton dynamics, rising temperatures coupled with nutrient addition significantly increased the total biomass of phytoplankton and specifically of cyanobacteria, known for their adaptability to warmer and nutrient-enriched conditions (Paerl & Huisman, 2008). However, the presence of macrophytes inhibited cyanobacteria at high temperatures without nutrient addition, supporting the role in preventing cyanobacteria dominance in a warming environment. The observed reductions in plant weight and RGR at rising temperatures and nutrient addition could be attributed to the high Chl α concentrations. Excessive phytoplankton can degrade the light environment by reducing transparency and thus shading the submerged macrophytes (Bakker et al., 2013). To compensate for this, submerged macrophytes may obtain light for growth by increasing their height (Goldsborough & Kemp, 1988). This adaptation aligns with our observations of a significant positive correlation between Chl α concentrations and plant height. Concurrently, under nutrient addition (with higher Chl α concentrations), the increased activities of SOD and CAT, as well as the elevated MDA content, also pointed to macrophyte adaptation. These adjustments may enhance the macrophytes' resilience to the combined stresses of rising temperature and nutrient enrichment within the context of climate change.

We saw clear differences when comparing the results from the plant presence and the plant absence treatments; the Chl α

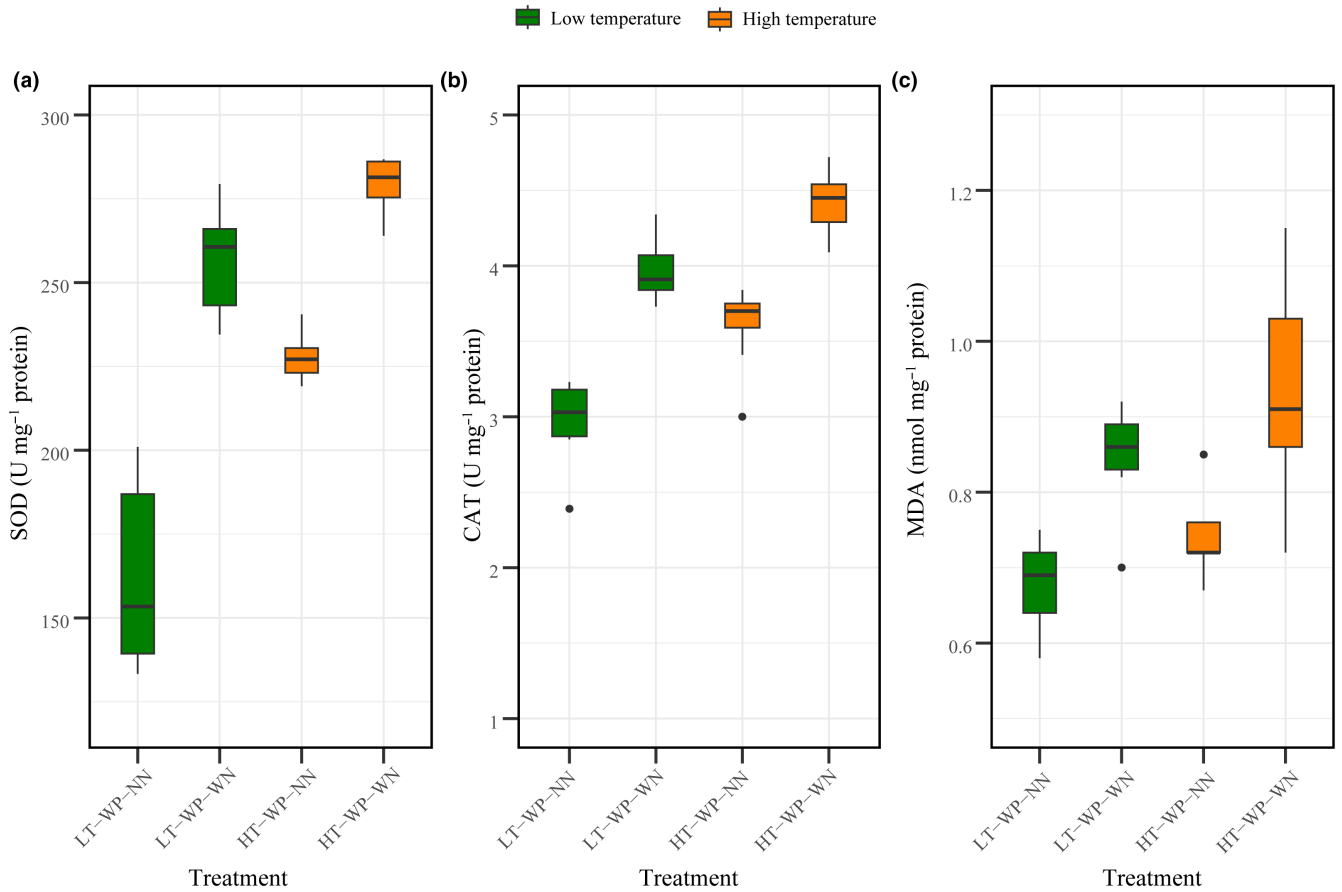


FIGURE 6 Enzyme activities of superoxide dismutase (SOD) and catalase (CAT) along with malondialdehyde (MDA) contents in *Vallisneria natans* at the end of experiment. The boxes indicate the 25th–75th percentiles, the line in the box the median, and the dots outliers. For an explanation of x-axis letters—see [Figure 2](#).

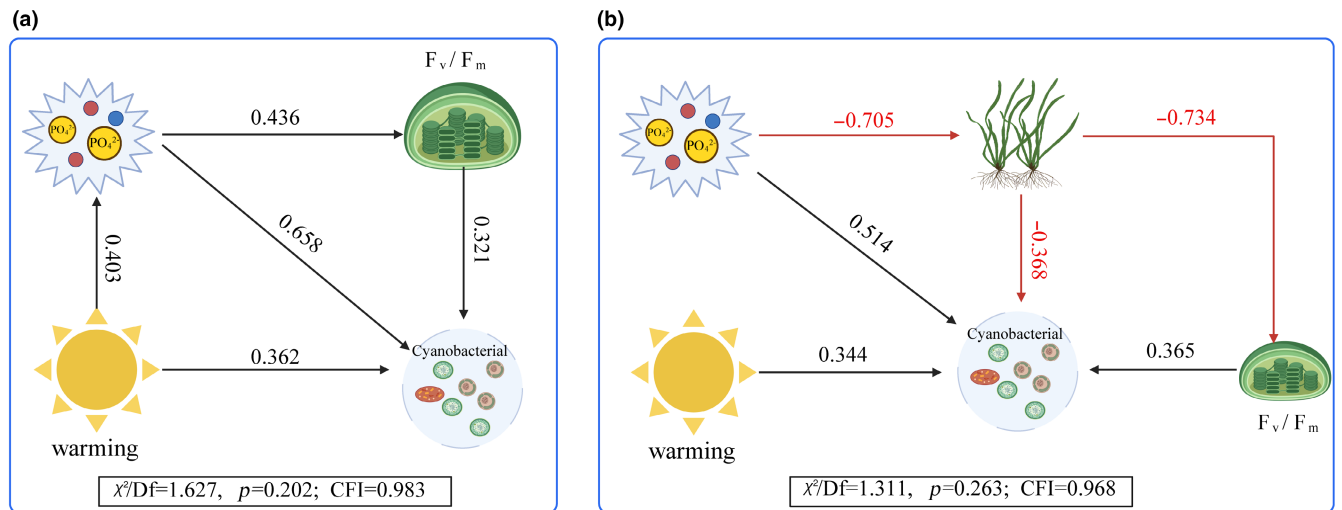


FIGURE 7 The results of structural equation models showed interactions between submerged macrophytes and phytoplankton and with environmental variables in the absence (a) and presence (b) of submerged macrophytes. The photosynthetic capacity of phytoplankton is represented by the maximum photochemical efficiency of the photosystem II (F_v/F_m). Phytoplankton, in this case, is cyanobacteria as only the F_v/F_m of cyanobacteria was measured. F_v/F_m was chosen based on the results of a three-way ANOVA (plants had a significant effect on F_v/F_m (three-way ANOVA, $F_{1,16}=24.637$, $p<0.001$) but not on the maximum relative electron transport rates ($rETR_{max}$; two-way ANOVA, $F_{1,16}=0.476$, $p=0.5$). Only statistically significant paths were retained. The standardized regression coefficients are marked next to the lines. Black lines show positive correlations and red lines negative correlations.

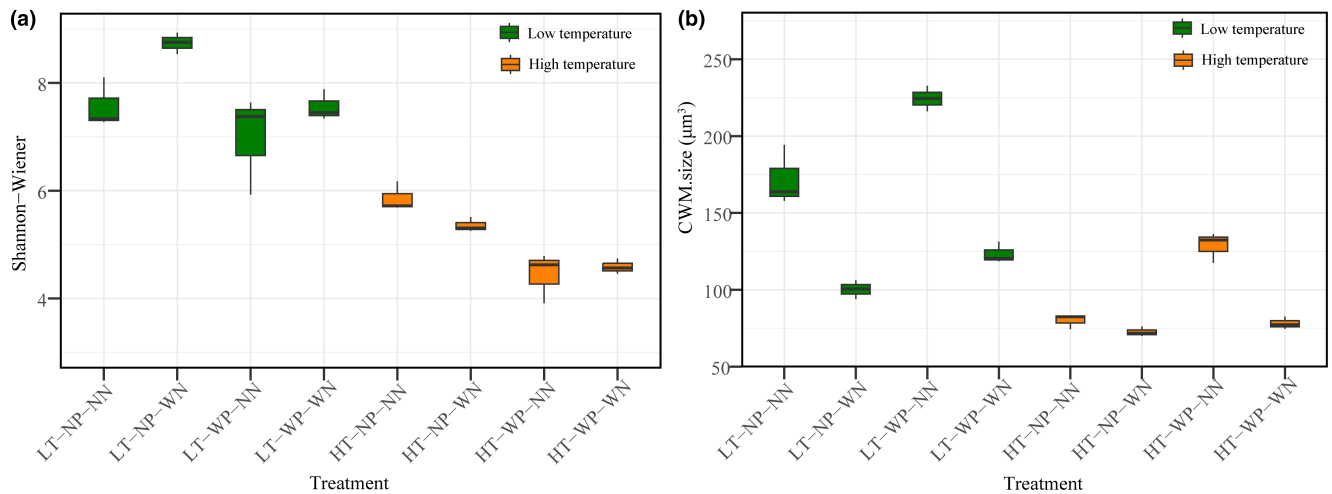


FIGURE 8 Taxonomic diversity (a) and functional diversity (b) of the phytoplankton communities at the end of the experiment under different conditions. Functional diversity is represented as community-weighted means using the cell size trait (CWM.size). The boxes indicate the 25th–75th percentiles, and the line in the box represents the median. For an explanation of x-axis letters—see Figure 2.

concentrations were lower in all temperature and nutrient scenarios in the plant-present treatment than in the plant-absent treatment. Thus, the presence of plants mitigated the impact of warming on eutrophication regardless of the nutrient conditions. And the presence of submerged macrophytes significantly reduced the biomass of cyanobacteria regardless of temperature and nutrient conditions. It is well established that submerged macrophytes indirectly or directly can suppress phytoplankton through several feedback mechanisms (Jeppesen et al., 1998; Moss, 1990; Scheffer et al., 1993). Accordingly, we found that the submerged macrophytes significantly inhibited the photosynthetic activity (F_v/F_m) of cyanobacteria. The presence of macrophytes also had different effects on the functional diversity of phytoplankton. At lower temperatures, the functional diversity of phytoplankton was higher in the presence of macrophytes without resulting in an increase in their biomass (Figure 8), suggesting a shift towards a more diverse but less dense phytoplankton community. This finding corresponds with observations by Barrow et al. (2019), that ponds with high densities of macrophytes exhibited higher functional diversity of phytoplankton. Similar effects of plants on functional diversity did not occur at high temperatures (Figure 8b), potentially reflecting that the functional diversity of phytoplankton was already significantly reduced at high temperatures.

In conclusion, higher temperature and nutrient addition increased phytoplankton biomass by increasing the photosynthetic activity and nutrient availability, respectively, as indicated by the SEM model. However, our results showed that submerged macrophytes may play a regulatory role by inhibiting phytoplankton (cyanobacteria) photosynthesis and competing for nutrients, thereby mitigating the adverse effects of eutrophication and rising temperatures. Additionally, our findings suggested that the adaptability of macrophytes, responding to environmental stress by physiological adaptations such as enzyme activity and morphological adjustments, could facilitate their survival under adverse

conditions. By using these physiological indicators to assess the life activity of organisms, our research provided new insights into macrophyte-phytoplankton relationships during regime shifts in aquatic ecosystems.

AUTHOR CONTRIBUTIONS

Conceptualization: Y.Z., R.W. and E.Z. Developing methods: Y.Z. and R.W. Conducting the research: Y.Z., E.Z. Data analysis, data interpretation, preparation of figures and tables: Y.Z. and E.Z. Writing: Y.Z., R.W., E.Z. and E.Z.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (42025707 and 42171163). R.W. acknowledges the financial support of the Youth Innovation Promotion Association of the Chinese Academy of Sciences (award Y2021086). E.Z. was supported by the TÜBITAK program BIDEB2232 (project 118C250). E.Z. acknowledges the financial support of the Double Thousand Plan of Jiangxi Province (jxsq2023101035). We thank Anne Mette Poulsen for English editions. We also thank Kexin Zhu, Hengshuai Qiu and Jianan Zheng for their help with the experiment. Finally, the authors thank the editors and two anonymous reviewers for their constructive comments.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Yu Zhao  <https://orcid.org/0000-0001-5443-9324>

Rong Wang  <https://orcid.org/0000-0003-1070-1460>

REFERENCES

- Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105, 121–126. [https://doi.org/10.1016/s0076-6879\(84\)05016-3](https://doi.org/10.1016/s0076-6879(84)05016-3)
- Amorim, C. A., & Moura, A. (2021). Ecological impacts of freshwater algal blooms on water quality, plankton biodiversity, structure, and ecosystem functioning. *Science of the Total Environment*, 758(143), 605. <https://doi.org/10.1016/j.scitotenv.2020.143605>
- Arvola, L. (1981). Spectrophotometric determination of chlorophyll *a* and phaeopigments in ethanol extractions. *Annales Botanici Fennici*, 18(3), 221–227. Retrieved from <http://www.jstor.org/stable/23725236>
- Bakker, E. S., Sarneel, J. M., Gulati, R. D., Liu, Z. W., & van Donk, E. (2013). Restoring macrophyte diversity in shallow temperate lakes: Biotic versus abiotic constraints. *Hydrobiologia*, 710(1), 23–37. <https://doi.org/10.1007/s10750-012-1142-9>
- Barrow, J. L., Beisner, B. E., Giles, R., Giani, A., Domaizon, I., & Gregory-Eaves, I. (2019). Macrophytes moderate the taxonomic and functional composition of phytoplankton assemblages during a nutrient loading experiment. *Freshwater Biology*, 64(8), 1369–1381. <https://doi.org/10.1111/fwb.13311>
- Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase—Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44(1), 276–287. [https://doi.org/10.1016/0003-2697\(71\)90370-8](https://doi.org/10.1016/0003-2697(71)90370-8)
- Beecraft, L., Watson, S. B., & Smith, R. E. H. (2017). Multi-wavelength Pulse Amplitude Modulated fluorometry (Phyto-PAM) reveals differential effects of ultraviolet radiation on the photosynthetic physiology of phytoplankton pigment groups. *Freshwater Biology*, 62(1), 72–86. <https://doi.org/10.1111/fwb.12850>
- Borics, G., Abonyi, A., Salmaso, N., & Ptasnik, R. (2021). Freshwater phytoplankton diversity: Models, drivers and implications for ecosystem properties. *Hydrobiologia*, 848(1), 53–75. <https://doi.org/10.1007/s10750-020-04332-9>
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Burgmer, T., & Hillebrand, H. (2011). Temperature mean and variance alter phytoplankton biomass and biodiversity in a long-term microcosm experiment. *Oikos*, 120(6), 922–933. <https://doi.org/10.1111/j.1600-0706.2010.19301.x>
- Bussotti, F., Gerosa, G., Digrado, A., & Pollastrini, M. (2020). Selection of chlorophyll fluorescence parameters as indicators of photosynthetic efficiency in large scale plant ecological studies. *Ecological Indicators*, 108(105), 686. <https://doi.org/10.1016/j.ecolind.2019.105686>
- Carmichael, W. W. (2001). Health effects of toxin-producing cyanobacteria: “The CyanoHABs”. *Human and Ecological Risk Assessment*, 7(5), 1393–1407. <https://doi.org/10.1080/20018091095087>
- Cross, W. F., Hood, J. M., Benstead, J. P., Huryn, A. D., & Nelson, D. (2015). Interactions between temperature and nutrients across levels of ecological organization. *Global Change Biology*, 21(3), 1025–1040. <https://doi.org/10.1111/gcb.12809>
- Daufresne, M., Lengfellner, K., & Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, 106(31), 12788–12793. <https://doi.org/10.1073/pnas.0902080106>
- Díaz, S., Lavorel, S., de Bello, F., Quetier, F., Grigulis, K., & Robson, M. (2007). Incorporating plant functional diversity effects in ecosystem service assessments. *Proceedings of the National Academy of Sciences of the United States of America*, 104(52), 20684–20689. <https://doi.org/10.1073/pnas.0704716104>
- Dong, B., Qin, B., Gao, G., & Cai, X. (2014). Submerged macrophyte communities and the controlling factors in large, shallow Lake Taihu (China): Sediment distribution and water depth. *Journal of Great Lakes Research*, 40(3), 646–655. <https://doi.org/10.1016/j.jglr.2014.04.007>
- Ebina, J., Tsutsui, T., & Shirai, T. (1983). Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulfate oxidation. *Water Research*, 17(12), 1721–1726. [https://doi.org/10.1016/0043-1354\(83\)90192-6](https://doi.org/10.1016/0043-1354(83)90192-6)
- Elateeq, A., Abou Elhassan, M., Bosila, H., Hamza, M., & Abdel-Gawad, A. (2022). Effect of cycocel and paclobutrazol on the dwarfing characteristics of *Chrysanthemum indicum* L. *Al-Azhar Journal of Agricultural Research*, 46(2), 41–50. <https://doi.org/10.21608/ajar.2022.254848>
- Elliott, J. A. (2012). Is the future blue-green? A review of the current model predictions of how climate change could affect pelagic freshwater cyanobacteria. *Water Research*, 46(5), 1364–1371. <https://doi.org/10.1016/j.watres.2011.12.018>
- Elser, J. J., Sterner, R. W., Gorokhova, E., Fagan, W. F., Markow, T. A., Cotner, J. B., & Harrison, J. F. (2000). *Ecological stoichiometry: The biology of elements from molecules to the biosphere*. Princeton University Press.
- Ensminger, I., Xyländer, M., Hagen, C., & Braune, W. (2001). Strategies providing success in a variable habitat: III. Dynamic control of photosynthesis in *Cladophora glomerata*. *Plant, Cell & Environment*, 24(8), 769–779. <https://doi.org/10.1046/j.1365-3040.2001.00725.x>
- Ersoy, Z., Scharfenberger, U., Baho, D. L., Bucak, T., Feldmann, T., Hejzlar, J., Levi, E. E., Mahdy, A., Nöges, T., Papastergiadou, E., Stefanidis, K., Šorf, M., Søndergaard, M., Trigal, C., Jeppesen, E., & Beklioglu, M. (2020). Impact of nutrients and water level changes on submerged macrophytes along a temperature gradient: A pan-European mesocosm experiment. *Global Change Biology*, 26(12), 6831–6851. <https://doi.org/10.1111/gcb.15338>
- Fan, Y., Chen, J., Shirkey, G., John, R., Wu, S. R., Park, H., & Shao, C. (2016). Applications of structural equation modeling (SEM) in ecological studies: An updated review. *Ecological Processes*, 5(1), 19. <https://doi.org/10.1186/s13717-016-0063-3>
- Fanesi, A., Wagner, H., Becker, A., & Wilhelm, C. (2016). Temperature affects the partitioning of absorbed light energy in freshwater phytoplankton. *Freshwater Biology*, 61(9), 1365–1378. <https://doi.org/10.1111/fwb.12777>
- Goldsborough, W. J., & Kemp, W. M. (1988). Light responses of a submerged macrophyte—implications for survival in turbid tidal waters. *Ecology*, 69(6), 1775–1786. <https://doi.org/10.2307/1941156>
- Gonzalez-Olalla, J. M., Medina-Sanchez, J. M., & Carrillo, P. (2022). Fluctuation at high temperature combined with nutrients alters the thermal dependence of phytoplankton. *Microbial Ecology*, 83(3), 555–567. <https://doi.org/10.1007/s00248-021-01787-8>
- Grace, J. B., Schoolmaster, D. R., Jr., Guntenspergen, G. R., Little, A. M., Mitchell, B. R., Miller, K. M., & Schweiger, E. W. (2012). Guidelines for a graph-theoretic implementation of structural equation modeling. *Ecosphere*, 3(8), 73. <https://doi.org/10.1890/es12-00048.1>
- Gross, E. M. (2003). Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences*, 22(3–4), 313–339. <https://doi.org/10.1080/713610859>
- Gross, E. M., Hilt, S., Lombardo, P., & Mulderij, G. (2007). Searching for allelopathic effects of submerged macrophytes on phytoplankton—State of the art and open questions. *Hydrobiologia*, 584, 77–88. <https://doi.org/10.1007/s10750-007-0591-z>
- Gross, E. M., Meyer, H., & Schilling, G. (1996). Release and ecological impact of algicidal hydrolysable polyphenols in *Myriophyllum spicatum*. *Phytochemistry*, 41(1), 133–138. [https://doi.org/10.1016/0031-9422\(95\)00598-6](https://doi.org/10.1016/0031-9422(95)00598-6)
- Guine, R. P. F., Duarte, J., Ferreira, M., Correia, P., Leal, M., Rumbak, I., Barić, I. C., Komes, D., Satalić, Z., Sarić, M. M., Tarcea, M., Fazakas, Z., Jovanoska, D., Vanevski, D., Vittadini, E., Pellegrini, N., Szűcs, V., Harangozó, J., El-Kenawy, A., ... Straumite, E. (2016). Knowledge about dietary fibres (KADF): Development and validation of an evaluation instrument through structural equation modelling

- (SEM). *Public Health*, 138, 108–118. <https://doi.org/10.1016/j.puhe.2016.03.031>
- Hargeby, A., Blindow, I., & Andersson, G. (2007). Long-term patterns of shifts between clear and turbid states in Lake Krankesjön and Lake Takern. *Ecosystems*, 10(1), 29–36. <https://doi.org/10.1007/s10021-006-9008-5>
- Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125(1), 189–198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1)
- Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollinger, U., & Zohary, T. (1999). Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, 35(2), 403–424. <https://doi.org/10.1046/j.1529-8817.1999.3520403.x>
- Hilt, S., & Gross, E. M. (2008). Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic and Applied Ecology*, 9(4), 422–432. <https://doi.org/10.1016/j.baae.2007.04.003>
- Hu, H. (2006). *The freshwater algae of China: Systematics, taxonomy and ecology*. Science Press.
- Hua, W., Dai, A., Qin, M., Hu, Y., & Cui, Y. (2023). How unexpected was the 2022 summertime heat extremes in the middle reaches of the Yangtze River? *Geophysical Research Letters*, 50(16), e2023GL104269. <https://doi.org/10.1029/2023gl104269>
- Huisman, J., Sharples, J., Stroom, J. M., Visser, P. M., Kardinaal, W. E. A., Verspagen, J. M. H., & Sommeijer, B. (2004). Changes in turbulent mixing shift competition for light between phytoplankton species. *Ecology*, 85(11), 2960–2970. <https://doi.org/10.1890/03-0763>
- Jeppesen, E., Audet, J., Davidson, T. A., Neif, É., Cao, Y., Filiz, N., Lauridsen, T. L., Larsen, S. E., Beklioglu, M., Tserenpil, S., & Sondergaard, M. (2021). Nutrient loading, temperature and heat wave effects on nutrients, oxygen and metabolism in shallow lake mesocosms pre-adapted for 11 years. *Watermark*, 13(2), 127. <https://doi.org/10.3390/w13020127>
- Jeppesen, E., Canfield, D. E., Bachmann, R. W., Søndergaard, M., Havens, K. E., Johansson, L. S., Lauridsen, T. L., Sh, T., Rutter, R. P., Warren, G., Ji, G., & Hoyer, M. V. (2020). Toward predicting climate change effects on lakes: A comparison of 1656 shallow lakes from Florida and Denmark reveals substantial differences in nutrient dynamics, metabolism, trophic structure, and top-down control. *Inland Waters*, 10(2), 197–211. <https://doi.org/10.1080/20442041.2020.1711681>
- Jeppesen, E., Jensen, J. P., Søndergaard, M., Lauridsen, T., Pedersen, L. J., & Jensen, L. (1997). Top-down control in freshwater lakes: The role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia*, 342, 151–164. <https://doi.org/10.1023/a:1017046130329>
- Jeppesen, E., Johansson, L. S., Tserenpil, S., Søndergaard, M., Lauridsen, T. L., & Andersen, P. (2021). Do cross-latitude and local studies give similar predictions of phytoplankton responses to warming? An analysis of monitoring data from 504 Danish lakes. *Sustainability*, 13(24), 14049. <https://doi.org/10.3390/su132414049>
- Jeppesen, E., Kronvang, B., Meerhoff, M., Søndergaard, M., Lauridsen, T. L., Liboriussen, L., Beklioglu, M., Ozen, A., & Olesen, J. E. (2009). Climate change effects on runoff, catchment phosphorus loading and lake ecological state, and potential adaptations. *Journal of Environmental Quality*, 38(5), 1930–1941. <https://doi.org/10.2134/jeq2008.0113>
- Jeppesen, E., Meerhoff, M., Davidson, T. A., Trolle, D., Søndergaard, M., Lauridsen, T. L., Beklioglu, M., Balmaña, S. B., Volta, P., González-Bergonzoni, I., & Nielsen, A. (2014). Climate change impacts on lakes: An integrated ecological perspective based on a multifaceted approach, with special focus on shallow lakes. *Journal of Limnology*, 73, 88–111. <https://doi.org/10.4081/jlimnol.2014.844>
- Jeppesen, E., Søndergaard, M., Jensen, J. P., Kanstrup, E., & Petersen, B. (1998). Macrophytes and Turbidity in Brackish Lakes with Special Emphasis on the Role of Top-Down Control. In E. Jeppesen, M. Søndergaard, M. Søndergaard, & K. Christoffersen (Eds.), *The structuring role of submerged macrophytes in lakes*. Springer New York.
- Johnk, K. D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P. M., & Stroom, J. M. (2008). Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology*, 14(3), 495–512. <https://doi.org/10.1111/j.1365-2486.2007.01510.x>
- Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, 26(11), 1463–1464. <https://doi.org/10.1093/bioinformatics/btq166>
- Kosten, S., Huszar, V. L. M., Becares, E., Costa, L. S., van Donk, E., Hansson, L.-A., Jeppesen, E., & Scheffer, M. (2012). Warmer climates boost cyanobacterial dominance in shallow lakes. *Global Change Biology*, 18(1), 118–126. <https://doi.org/10.1111/j.1365-2486.2011.02488.x>
- Kosten, S., Jeppesen, E., Huszar, V. L. M., Mazzeo, N., van Nes, E. H., Peeters, E., & Scheffer, M. (2011). Ambiguous climate impacts on competition between submerged macrophytes and phytoplankton in shallow lakes. *Freshwater Biology*, 56(8), 1540–1553. <https://doi.org/10.1111/j.1365-2427.2011.02593.x>
- Krause-Jensen, D., & Sand-Jensen, K. (1998). Light attenuation and photosynthesis of aquatic plant communities. *Limnology and Oceanography*, 43(3), 396–407. <https://doi.org/10.4319/lo.1998.43.3.0396>
- Labilberté, E., & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, 91(1), 299–305. <https://doi.org/10.1890/08-2244.1>
- Lauridsen, T. L., Pedersen, L. J., Jeppesen, E., & Søndergaard, M. (1996). The importance of macrophyte bed size for cladoceran composition and horizontal migration in a shallow lake. *Journal of Plankton Research*, 18(12), 2283–2294. <https://doi.org/10.1093/plankt/18.12.2283>
- Leu, E., Krieger-Liszka, A., Goussias, C., & Gross, E. M. (2002). Polyphenolic allelochemicals from the aquatic angiosperm *Myriophyllum spicatum* inhibit photosystem II. *Plant Physiology*, 130(4), 2011–2018. <https://doi.org/10.1104/pp.011593>
- Lewington-Pearce, L., Narwani, A., Thomas, M. K., Kremer, C. T., Vogler, H., & Kratina, P. (2019). Temperature-dependence of minimum resource requirements alters competitive hierarchies in phytoplankton. *Oikos*, 128(8), 1194–1205. <https://doi.org/10.1111/oik.06060>
- Litchman, E., Klausmeier, C. A., Schofield, O. M., & Falkowski, P. G. (2007). The role of functional traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to ecosystem level. *Ecology Letters*, 10(12), 1170–1181. <https://doi.org/10.1111/j.1461-0248.2007.01117.x>
- Lüring, M., Mello, M. M. E., van Oosterhout, F., de Senerpont Domis, L., & Marinho, M. M. (2018). Response of natural cyanobacteria and algae assemblages to a nutrient pulse and elevated temperature. *Frontiers in Microbiology*, 9, 1851. <https://doi.org/10.3389/fmicb.2018.01851>
- Maređová, N., Altman, J., & Kastovsky, J. (2021). The effects of macrophytes on the growth of bloom-forming cyanobacteria: Systematic review and experiment. *Science of the Total Environment*, 792(148), 413. <https://doi.org/10.1016/j.scitotenv.2021.148413>
- Matzinger, A., Schmid, M., Veljanoska-Sarafiloska, E., Patceva, S., Guseska, D., Wagner, B., Müller, B., Sturm, M., & Wuest, A. (2007). Eutrophication of ancient Lake Ohrid: Global warming amplifies detrimental effects of increased nutrient inputs. *Limnology and Oceanography*, 52(1), 338–353. <https://doi.org/10.4319/lo.2007.52.1.0338>
- Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—A practical guide. *Journal of Experimental Botany*, 51(345), 659–668. <https://doi.org/10.1093/jexbot/51.345.659>
- Meerhoff, M., & Gonzalez-Sagrario, M. D. (2022). Habitat complexity in shallow lakes and ponds: Importance, threats, and potential for

- restoration. *Hydrobiologia*, 849(17–18), 3737–3760. <https://doi.org/10.1007/s10750-021-04771-y>
- Miyashita, A., & Tateno, M. (2014). A novel index of leaf RGR predicts tree shade tolerance. *Functional Ecology*, 28(6), 1321–1329. <https://doi.org/10.1111/1365-2435.12290>
- Moss, B. (1990). Engineering and biological approaches to the restoration from eutrophication of shallow lakes in which aquatic plant communities are important components. *Hydrobiologia*, 200, 367–377. <https://doi.org/10.1007/bf02530354>
- Moss, B., McKee, D., Atkinson, D., Collings, S. E., Eaton, J. W., Gill, A. B., Harvey, I., Hatton, K., Heyes, T., & Wilson, D. (2003). How important is climate? Effects of warming, nutrient addition and fish on phytoplankton in shallow lake microcosms. *Journal of Applied Ecology*, 40(5), 782–792. <https://doi.org/10.1046/j.1365-2664.2003.00839.x>
- O'Donnell, D. R., Wilburn, P., Silow, E. A., Yampolsky, L. Y., & Litchman, E. (2017). Nitrogen and phosphorus colimitation of phytoplankton in Lake Baikal: Insights from a spatial survey and nutrient enrichment experiments. *Limnology and Oceanography*, 62(4), 1383–1392. <https://doi.org/10.1002/lno.10505>
- Paerl, H. W. (2014). Mitigating harmful cyanobacterial blooms in a human- and climatically-impacted world. *Life-Basel*, 4(4), 988–1012. <https://doi.org/10.3390/life4040988>
- Paerl, H. W., & Huisman, J. (2008). Climate—Blooms like it hot. *Science*, 320(5872), 57–58. <https://doi.org/10.1126/science.1155398>
- Paerl, H. W., & Huisman, J. (2009). Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports*, 1(1), 27–37. <https://doi.org/10.1111/j.1758-2229.2008.00004.x>
- Paerl, H. W., & Paul, V. J. (2012). Climate change: Links to global expansion of harmful cyanobacteria. *Water Research*, 46(5), 1349–1363. <https://doi.org/10.1016/j.watres.2011.08.002>
- Pálffy, K., & Smeti, E. (2024). Combined effect of warming, nutrients, and species pool size on the seasonal variability of phytoplankton composition: A modeling perspective. *Limnology and Oceanography*, 69, 1056–1069. <https://doi.org/10.1002/lno.12548>
- Phillips, G., Willby, N., & Moss, B. (2016). Submerged macrophyte decline in shallow lakes: What have we learnt in the last forty years? *Aquatic Botany*, 135, 37–45. <https://doi.org/10.1016/j.aquabot.2016.04.004>
- Polst, B. H., Hilt, S., Stibor, H., Holker, F., Allen, J., Vijayaraj, V., Kipferler, N., Leflaive, J., Gross, E. M., & Schmitt-Jansen, M. (2022). Warming lowers critical thresholds for multiple stressor-induced shifts between aquatic primary producers. *Science of the Total Environment*, 838(4), 156511. <https://doi.org/10.1016/j.scitotenv.2022.156511>
- Qu, S., & Zhou, J. (2024). Phytoplankton community structure and water quality assessment in Xuanwu Lake, China. *Frontiers in Environmental Science*, 11, 1303851. <https://doi.org/10.3389/fenvs.2023.1303851>
- R Core Team. (2023). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Available at: <http://www.R-project.org/>
- Ralph, P. J., & Gademann, R. (2005). Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquatic Botany*, 82(3), 222–237. <https://doi.org/10.1016/j.aquabot.2005.02.006>
- Rasconi, S., Gall, A., Winter, K., & Kainz, M. J. (2015). Increasing water temperature triggers dominance of small freshwater plankton. *PLoS One*, 10(10), e0140449. <https://doi.org/10.1371/journal.pone.0140449>
- Raven, J. A., & Geider, R. J. (1988). Temperature and algal growth. *New Phytologist*, 110(4), 441–461. <https://doi.org/10.1111/j.1469-8137.1988.tb00282.x>
- Rhee, G. Y., & Gotham, I. J. (1981). The effect of environmental-factors on phytoplankton growth—Temperature and the interactions of temperature with nutrient limitation. *Limnology and Oceanography*, 26(4), 635–648. <https://doi.org/10.4319/lo.1981.26.4.0635>
- Rooney, N., & Kalf, J. (2000). Inter-annual variation in submerged macrophyte community biomass and distribution: The influence of temperature and lake morphology. *Aquatic Botany*, 68(4), 321–335. [https://doi.org/10.1016/s0304-3770\(00\)00126-1](https://doi.org/10.1016/s0304-3770(00)00126-1)
- Salleh, S., & McMinn, A. (2011). The effects of temperature on the photosynthetic parameters and recovery of two temperate benthic microalgae, *amphora* cf. *Coffeaeiformis* and *Cocconeis* cf. *Sublittoralis* (Bacillariophyceae). *Journal of Phycology*, 47(6), 1413–1424. <https://doi.org/10.1111/j.1529-8817.2011.01079.x>
- Sand-Jensen, K., & Borum, J. (1991). Interactions among phytoplankton, periphyton, and macrophytes in temperate fresh-waters and estuaries. *Aquatic Botany*, 41(1–3), 137–175. [https://doi.org/10.1016/0304-3770\(91\)90042-4](https://doi.org/10.1016/0304-3770(91)90042-4)
- Scheffer, M., & Carpenter, S. R. (2003). Catastrophic regime shifts in ecosystems: Linking theory to observation. *Trends in Ecology & Evolution*, 18(12), 648–656. <https://doi.org/10.1016/j.tree.2003.09.002>
- Scheffer, M., Hosper, S. H., Meijer, M. L., Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution*, 8(8), 275–279. [https://doi.org/10.1016/0169-5347\(93\)90254-m](https://doi.org/10.1016/0169-5347(93)90254-m)
- Scheffer, M., & Jeppesen, E. (2007). Regime shifts in shallow lakes. *Ecosystems*, 10(1), 1–3. <https://doi.org/10.1007/s10021-006-9002-y>
- Smith, V. H., & Schindler, D. W. (2009). Eutrophication science: Where do we go from here? *Trends in Ecology & Evolution*, 24(4), 201–207. <https://doi.org/10.1016/j.tree.2008.11.009>
- Song, Y., Kong, F., Xue, Y., & Qin, B. (2015). Responses of chlorophyll and MDA of *Vallisneria natans* to nitrogen and phosphorus availability and epiphytic algae. *Journal of Freshwater Ecology*, 30(1), 85–97. <https://doi.org/10.1080/02705060.2014.989554>
- Staeher, P. A., & Sand-Jensen, K. (2006). Seasonal changes in temperature and nutrient control of photosynthesis, respiration and growth of natural phytoplankton communities. *Freshwater Biology*, 51(2), 249–262. <https://doi.org/10.1111/j.1365-2427.2005.01490.x>
- Szabo, S., Koleszar, G., Braun, M., Nagy, Z., Vicei, T. T., & Peeters, E. T. H. M. (2022). Submerged rootless macrophytes sustain a stable state against free-floating plants. *Ecosystems*, 25(1), 17–29. <https://doi.org/10.1007/s10021-021-00637-5>
- Taipale, S. J., Vuorio, K., Aalto, S. L., Peltomaa, E., & Tiirola, M. (2019). Eutrophication reduces the nutritional value of phytoplankton in boreal lakes. *Environmental Research*, 179(B), 108836. <https://doi.org/10.1016/j.envres.2019.108836>
- Thomas, M. K., Aranguren-Gassis, M., Kremer, C. T., Gould, M. R., Anderson, K., Klausmeier, C. A., & Litchman, E. (2017). Temperature-nutrient interactions exacerbate sensitivity to warming in phytoplankton. *Global Change Biology*, 23(8), 3269–3280. <https://doi.org/10.1111/gcb.13641>
- Urrutia-Cordero, P., Ekvall, M. K., Ratcovich, J., Soares, M., Wilken, S., Zhang, H., & Hansson, L. A. (2017). Phytoplankton diversity loss along a gradient of future warming and brownification in freshwater mesocosms. *Freshwater Biology*, 62(11), 1869–1878. <https://doi.org/10.1111/fwb.13027>
- Van Donk, E., & Van de Bund, W. J. (2002). Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: Allelopathy versus other mechanisms. *Aquatic Botany*, 72(3–4), 261–274. [https://doi.org/10.1016/s0304-3770\(01\)00205-4](https://doi.org/10.1016/s0304-3770(01)00205-4)
- Vanderstucken, M., Mazzeo, N., Van Colen, W., Declerck, S. A. J., & Muylaert, K. (2011). Biological control of phytoplankton by the subtropical submerged macrophytes *Egeria densa* and *Potamogeton illinoensis*: A mesocosm study. *Freshwater Biology*, 56(9), 1837–1849. <https://doi.org/10.1111/j.1365-2427.2011.02624.x>
- Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, 116(5), 882–892. <https://doi.org/10.1111/j.2007.0030-1299.15559.x>
- Wang, D. S., Gan, X. Y., Wang, Z. Q., Jiang, S. F., Zheng, X. Y., Zhao, M., Zhang, Y., Fan, C., Wu, S., & Du, L. (2023). Research status on

- remediation of eutrophic water by submerged macrophytes: A review. *Process Safety and Environmental Protection*, 169, 671–684. <https://doi.org/10.1016/j.psep.2022.11.063>
- Xu, H., Paerl, H. W., Qin, B., Zhu, G., & Gao, G. (2010). Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. *Limnology and Oceanography*, 55(1), 420–432. <https://doi.org/10.4319/lo.2010.55.1.0420>
- Xu, M., Wang, R., Dong, X. H., & Yang, X. D. (2019). A palaeolimnological perspective to understand regime-shift dynamics in two Yangtze-basin lakes. *Biology Letters*, 15(11), 20190447. <https://doi.org/10.1098/rsbl.2019.0447>
- Yuan, C., Zhu, T., Cao, T., Xi, Y., Zhang, X., & Ni, L. (2019). Antioxidant systems of aquatic macrophytes in three life forms: A case study in Lake Erhai, China. *Journal of Oceanology and Limnology*, 37(2), 665–674. <https://doi.org/10.1007/s00343-019-8025-0>
- Zhang, M., Wang, Z., Xu, J., Liu, Y., Ni, L., Cao, T., & Xie, P. (2011). Ammonium, microcystins, and hypoxia of blooms in eutrophic water cause oxidative stress and C-N imbalance in submersed and floating-leaved aquatic plants in Lake Taihu, China. *Chemosphere*, 82(3), 329–339. <https://doi.org/10.1016/j.chemosphere.2010.10.038>
- Zhang, X., Tan, L., Cai, Q., & Ye, L. (2022). Environmental factors indirectly reduce phytoplankton community stability via functional diversity. *Frontiers in Ecology and Evolution*, 10(990), 835. <https://doi.org/10.3389/fevo.2022.990835>

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How to cite this article: Zhao, Y., Wang, R., Jeppesen, E., & Zhang, E. (2024). Submerged macrophytes can counterbalance the negative effects of rising temperature and eutrophication by inhibiting the photosynthetic activity of cyanobacteria and adjusting their morphology and physiology. *Freshwater Biology*, 00, 1–15. <https://doi.org/10.1111/fwb.14346>