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Physiological changes of submerged macrophytes in response to a floating filamentous green algae bloom in clear-water conditions*

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Abstract The aim of this study was to evaluate the physiological responses of a submerged macrophyte to a floating filamentous green algal bloom in clear-water conditions. *Elodea nuttallii* was grown with floating *Cladophora* sp. at four different levels (0, control; 140, 280, 560 g FW/m^2) in an outdoor experimental system, and its photosynthetic and antioxidant systems were evaluated. The presence of floating *Cladophora* sp. significantly changed the water environment by decreasing light intensity and increasing dissolved oxygen and the pH value. The photosynthetic parameters of *E. nuttallii* (e.g. $\Delta F/F_m'$, F_v/F_m, total chlorophyll) were higher in the presence of floating *Cladophora* sp. than in the control at the beginning of experiment. Because of the increasing dissolved oxygen concentration and pH value, the values of these indicators decreased (except for photosynthetic pigments) during the experiment. Compared with *E. nuttallii* in the control, *E. nuttallii* growing in the presence of floating *Cladophora* sp. showed higher malondialdehyde content, catalase activity, and peroxidase activity. The biomass of *E. nuttallii* was decreased by about 30% in the presence of high biomasses of floating *Cladophora* sp. (280 and 560 g FW/m^2). These results suggest that floating *Cladophora* had complex effects on the biomass of *E. nuttallii* and that changes in water quality resulting from floating *Cladophora* sp. may be more important than its direct shading effect.

Keyword: floating *Cladophora* sp.; *Elodea nuttallii*; growth; physiology

1 INTRODUCTION

Mass filamentous green algae are frequently found in the littoral zones of lakes, ponds, and wetlands (Auer et al., 1982; Irfanullah and Moss, 2005a; Gallego et al., 2014). When filamentous green algae become dominant, they directly or indirectly influence the growth of submerged macrophytes. This can lead to a decrease in, or the disappearance of, the submerged macrophytes (Irfanullah and Moss, 2005a). Some studies have suggested that nutrient-rich shallow lakes may be dominated by a particular group, such as epiphytic or filamentous algae or free-floating macrophytes (Morris et al., 2003; Scheffer et al., 2003; Scheffer and van Nes, 2007). Filamentous green algae may be dominant in a clear water state representing an alternative state in high-nutrient shallow temperate lakes (Trochine et al., 2011). But, the overgrowth of filamentous green algae is not conducive to maintain the clear-water state in which submerged macrophytes are the primary producers.

Submerged macrophytes enhance water quality and biodiversity by competing for resources and producing allelochemicals that suppress phytoplankton blooms in shallow lakes. Consequently, the recovery of submerged macrophytes is an important goal for the

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restoration of eutrophic lakes (Pot and ter Heerdt, 2014; Vanderstukken et al., 2014; Velthuis et al., 2017). Filamentous green algae can affect the growth of submerged macrophytes by shading, allelopathy, mechanical destruction, changing water conditions, and competing for nutrients (Phillips et al., 1978; Simpson and Eaton, 1986; Irfanullah and Moss, 2004). Previous studies have reported that filamentous algae (mainly *Cladophora glomerata*) did not affect *Elodea canadensis* when the ratio of *E. canadensis* biomass to filamentous algae biomass was 1:1, but larger biomasses of decomposing algae limited *E. canadensis* growth (Tarmanowska, 1995; Pieczyńska and Tarmanowska, 1996). Other studies showed that overgrowth of filamentous green algae resulted in light competition with submerged macrophytes, and strongly inhibited the recovery of *Elodea nuttallii* (Irfanullah and Moss, 2004, 2005a). In another study, filamentous green algae (mainly *Spirogyra* sp.) did not influence the number of shoot divisions, shoot length, or growth rate of *E. nuttallii* by allelopathy under nutrient-rich conditions (Irfanullah and Moss, 2005b). Filamentous green algae such as *Cladophora* can dislodge from substrates and form a floating mat (Stevenson and Stoermer, 1982; Ólafsson et al., 2013), which provides an anaerobic environment for the growth of bacteria. The growth of *Clostridium botulinum* in a floating mat was shown to negatively affect water quality (Byappanahalli and Whitman, 2009)

Several studies have focused on the effects of living or decomposing filamentous green algae on submerged macrophytes, but few have investigated the effects of living floating algae mats on submerged macrophytes. To study the potential effects of floating algae on a submerged macrophyte, we selected *Cladophora* as the floating alga and *Elodea* as the macrophyte in this experiment. Our objectives were as follows: (1) to determine the effects of different amounts of floating *Cladophora* on the photosynthetic and antioxidant systems of *Elodea*; and (2) to determine the amount of floating *Cladophora* that negatively affects *Elodea*.

2 MATERIAL AND METHOD

2.1 Growth of test species under field conditions

Restoration of submerged macrophytes in Caohai Lake, a eutrophic lake (102.642°E, 24.982°N; Kunming, China) is one of the main goals of the Major Science and Technology Program for Water Pollution Control and Treatment. Therefore, we constructed different-sized enclosures in the lake to restore submerged macrophytes. The submerged macrophytes (mainly *E. nuttallii*) initially grew well. However, when the environmental conditions were appropriate, such as water temperature and transparency, *Cladophora* sp. settled on the *E. nuttallii* plants and grew rapidly, about a week later, covering the entire enclosure or accumulating in the corner and floating on the surface of the water. After a few days, the submerged macrophytes showed a drastic decrease in growth rate or disappeared.

2.2 Collection of alga and submerged macrophyte

Cladophora sp. and *E. nuttallii* were collected from Caohai Lake and cultured outdoors in clean tap water (total nitrogen=1.957±0.053 mg/L, total phosphorus=0.065±0.008 mg/L) for 4 days under natural conditions before the experiment.

2.3 Experimental design

The experiment was performed from 20th August to 7th September 2014 at the field station near Caohai Lake. Twelve clear acid-washed plastic tanks (length 45 cm×width 32 cm×height 25 cm; volume ca. 36 L each) were used to establish four treatments in triplicate: control, low, middle, high, containing floating *Cladophora* sp. at 0, 20 g (140 g fresh weight (FW)/m^2), 40 g (280 g FW/m^2), and 80 g (560 g FW/m^2), respectively. The tanks were arranged side by side to ensure that all groups were subjected to the same external conditions. Healthy apical shoots of *E. nuttallii* (~10 cm length, fully expanded leaves, and without lateral shoots; three per bundle) were weighed and planted in individual plastic pots (12 cm diameter, 8 cm high) containing 7 cm sediment from Caohai Lake (Fig.1). To ensure that the submerged macrophyte reached a certain biomass and was rooted in the sediment, *E. nuttallii* plants were cultured for 4 days before adding floating *Cladophora* sp. into the system. The floating *Cladophora* sp. were weighed weekly to determine fresh biomass and adjusted to the initial value to maintain the biomass ratio in each treatment. At the end of the experiment, the FW of *E. nuttallii* in each tank was measured.

2.4 Data collection

2.4.1 Environmental and physical parameters

During the experiment, environmental variables including water temperature, salinity, conductivity,

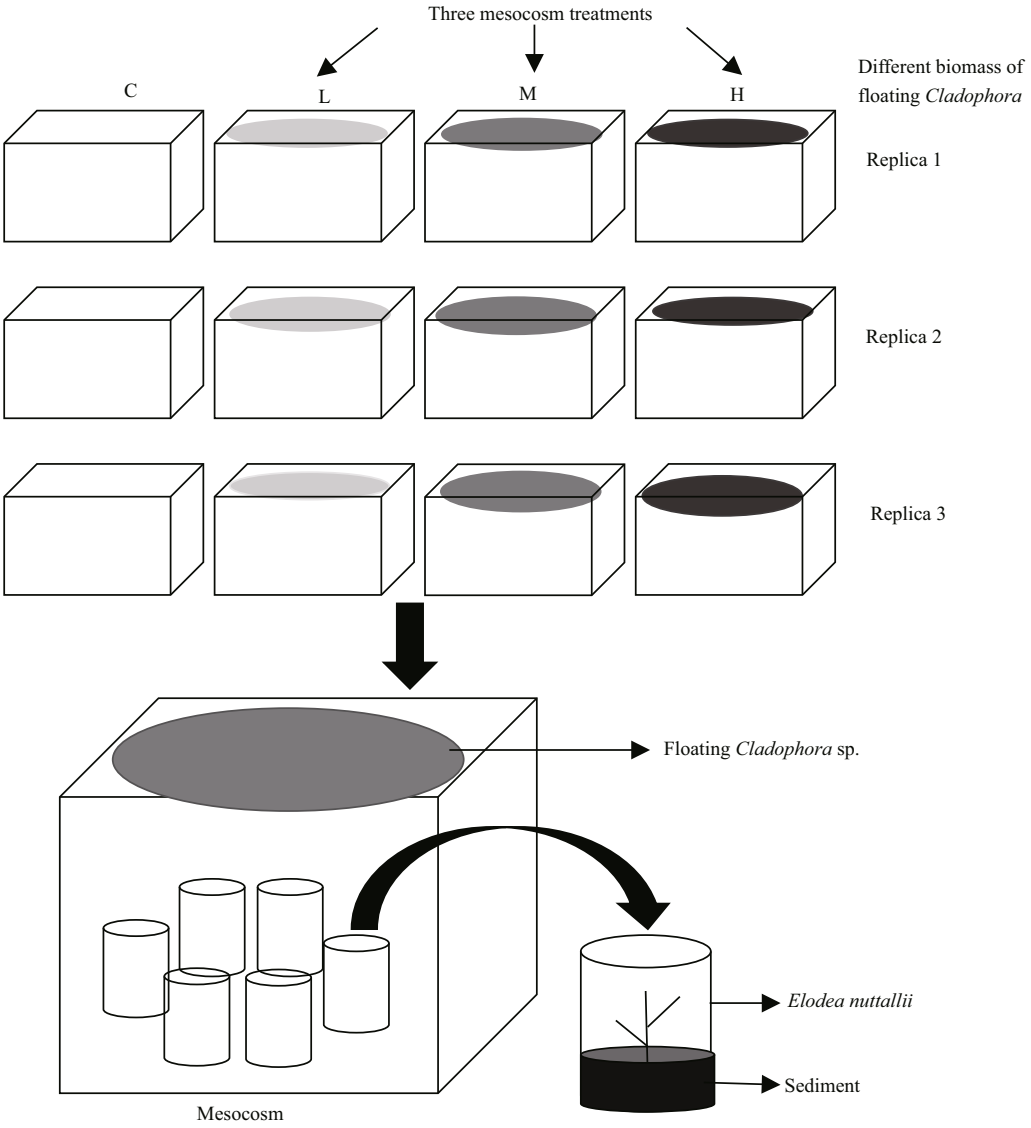

Fig.1 Schematic diagram of experimental design

C: control, no floating *Cladophora* sp.; L, M, H: 20, 40, and 80 g (fresh weight) floating *Cladophora* sp., respectively.

dissolved oxygen, and pH were measured by a multi-parameter water quality analyzer (YSI Inc., Yellow Springs, OH, USA) at 12.00 PM every 6 days. The underwater light intensity was measured at a depth of 5 cm using a LI-250 light meter (LI-COR, Lincoln, NE, USA) at 15:00 to 16:00 every 6 days. The percentage of shading was calculated using Eq.1:

$$\text{shading}\ (\%)=((LC_t-LN_t)/LC_t)\times 100, \quad (1)$$

where LC_t (μmol/(m^2·s)) and LN_t (μmol/(m^2·s)) are the underwater light intensities in the control and the treatment at time t, respectively.

2.4.2 Determination of lipid peroxidation and peroxidase and catalase activities

Weighed plant samples (0.025±0.002 g) were ground in liquid nitrogen and extracted in 5 mL 5% (v/v) trichloroacetic acid. The mixture was centrifuged for 15 min at 13 000×g at 4°C. Then, 2 mL 0.67% thiobarbituric acid was added to 2 mL supernatant, and the mixture was boiled in a 100°C water bath for 30 min. The mixture was cooled to room temperature, centrifuged for 10 min at 13 000×g, and then the absorbance of the supernatant was measured at 450, 532, and 600 nm. The absorbance values were used to calculate the malonaldehyde (MDA) concentration (Heath and Packer, 1968).

To determine catalase (CAT) and peroxidase (POD) activities, weighed plant samples (0.025± 0.002 g) were ground on ice using a tissue homogenizer and extracted in 5 mL 50 mmol/L potassium phosphate buffer (pH 7.8) containing 10% polyvinyl pyrrolidine (PVP). The extract was centrifuged for 15 min at

13 000×*g* at 4°C. The supernatant was used to determine CAT (Góth, 1991) and POD (Maehly, 1955) activities, as well as total soluble protein (TSP) content (Bradford, 1976). Activity was expressed as units of CAT/POD activity per milligram TSP. One unit (U) of CAT (POD) activity was defined as an absorbance change of 0.01 units per minute at 240 nm (470 nm) under these conditions.

2.4.3 Extraction and quantification of photosynthetic pigments

Chlorophylls and carotenoids were extracted from leaves (0.025±0.002 g fresh weight) in 25 mL 95% (v/v) ethanol in darkness for 48 h at 4°C. The absorbance of the mixture was determined at 470, 649, and 665 nm and these values were used to calculate the concentrations of chlorophyll *a*, *b*, and carotenoids (Lichtenthaler and Buschmann, 2001).

2.4.4 Chlorophyll fluorescence

The chlorophyll fluorescence parameters of the fourth leaf from the apex were measured using a WATER-PAM (Walz, Effeltrich, Germany) equipped with a Water-EDF fiber optic unit. The efficiency of PSII photochemistry ($\Delta F/F_m'$) was calculated as follows: $(F_m'-F_t)/F_m'$. After the samples were dark-adapted for 10 min, the maximum quantum efficiency of photosystem II (F_v/F_m) was measured and calculated as follows $(F_m-F_0)/F_m$. F_m' and F_m are the maximal fluorescence from a light-adapted leaf and a dark-adapted leaf, respectively; F_t is the instantaneous steady state fluorescence (sometimes called F_s); and F_0 is the minimal fluorescence from a dark-adapted leaf (Maxwell and Johnson, 2000; Baker, 2008).

Rapid light-response curves (RLC) were constructed by applying light every 10 s at nine increasing light intensity levels (30, 64, 96, 143, 219, 324, 495, 738, and 1 048 μmol/(m^2·s)). The relative electron transport rate (rETR) was calculated as the product of effective quantum yield and light intensity. Relative ETR was plotted against light intensity and fitted with a function from which the light-response curve parameters light utilization efficiency (α) and maximum rETR ($rETR_{max}$) were determined. The light saturation parameter (E_k) was calculated as follows: $rETR_{max}/\alpha$ (Jassby and Platt, 1976). The α, $rETR_{max}$, and E_k determined from the RLC reflect the ability of plants to use light efficiently, the maximum photosynthetic rate, and the ability to tolerate high light, respectively (Ralph and Gademann, 2005).

2.5 Data analysis

The MDA content, enzyme activity, photosynthetic pigments, F_v/F_m, and $\Delta F/F_m'$ were measured at indicated times during the experiment, and treatment (df=3) and time (df=3) effects were determined using repeated-measures analyses of variance (RM-ANOVA) with the treatment (control, low, middle, high) as the fixed factor and time as the repeated factor. The sphericity assumption was tested using Mauchly's test, and the degrees of freedom were Greenhouse-Geisser adjusted when the data did not meet the assumption of sphericity. One-way analyses of variance (ANOVA) with Tukey's multiple comparisons were used to detect significant differences in each variable among treatments and time points separately. If heterogeneity of variance was detected, Dunnett's T3 multiple comparisons were used to test the differences. Pearson's correlation analysis was used to test correlations between environmental variables. Paired *t*-tests were used to test the significance of differences in RLC parameters (α, $rETR_{max}$, E_k) between day 6 and day 18. The data were Log_e-transformed when necessary. All statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Significance was accepted at $P<0.05$. Data are presented as mean±standard deviation.

3 RESULT

3.1 Environmental physicochemical parameters

During the 18-day experiment, the biomass of floating *Cladophora* sp. decreased in the low treatment, slightly increased in the high treatment and was almost unchanged in middle treatment. Compared with the water in the control, the water in the treatments with *Cladophora* sp. showed higher pH values (Fig.2a) and dissolved oxygen concentrations (Fig.2b). The pH increased during the experiment. On day 18, the average pH of water in the middle and high treatments was 0.80 and 0.89 higher than that in the control, respectively ($P<0.001$). The dissolved oxygen concentration tended to fluctuate during the experiment and peaked on day 6 at 16.44±0.48 and 17.11±1.10 mg/L in the middle and high treatments, respectively ($F=25.35$, $P<0.001$). The underwater light intensity decreased substantially as the biomass of floating *Cladophora* sp. increased. The final percentages of shading in the four groups (Fig.2c) were as follows: control, 0%; low, 34%±1%; middle,

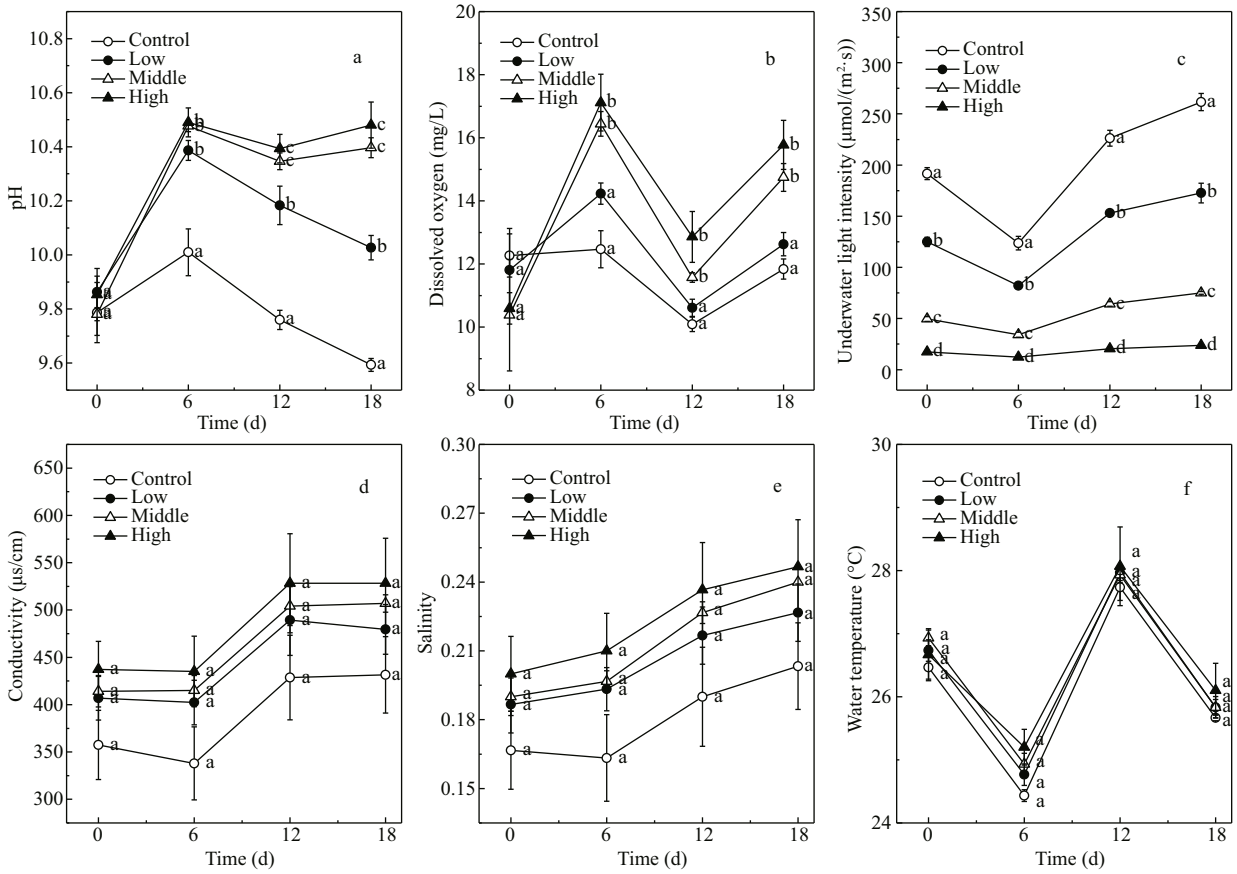

Fig.2 Changes in pH (a), dissolved oxygen (b), underwater light intensity (c), conductivity (d), salinity (e), and water temperature (f) in control (no floating *Cladophora* sp.) and low, middle, and high (20 g, 40 g, and 80 g fresh weight floating *Cladophora* sp., respectively) treatment groups

Error bars represent standard deviation (n=3). Different letters indicate significant difference (P<0.05).

Table 1 Pearson's correlations between physicochemical parameters

Parameter	pH	Dissolved oxygen	Underwater light intensity	Conductivity	Salinity	Water temperature
pH	1	0.685**	-0.439**	0.458**	0.519**	-0.106
Dissolved oxygen	0.685**	1	-0.058	0.112	0.276	-0.570**
Underwater light intensity	-0.439**	-0.058	1	-0.639**	-0.493**	-0.441**
Conductivity	0.458**	0.112	-0.639**	1	0.866**	0.464**
Salinity	0.519**	0.276	-0.493**	0.866**	1	0.147
Water temperature	-0.106	-0.570**	-0.441**	0.464**	0.147	1

* correlation significant at 0.05 level (2-tailed); ** correlation significant at 0.01 level (2-tailed).

72%±2%; and high, 90%±1% (P<0.05).

There were no significant differences in conductivity (Fig.2d), salinity (Fig.2e), and water temperature (Fig.2f) among the four treatment groups (P>0.05). Correlation analyses revealed a negative correlation between dissolved oxygen concentration and water temperature (R=-0.57, P<0.001), and positive correlations between pH and dissolved oxygen concentration (R=0.69, P<0.001) and between pH and salinity (R=0.52, P<0.001) (Table 1).

3.2 Lipid peroxidation and antioxidant enzymes

From day 6 onwards, the MDA content of *E. nuttallii* differed significantly among the four treatments (F=2.75, P=0.023). At the end of the experiment, the average MDA content in the middle and high treatments was 60.4% and 49.1% higher, respectively, than that in the control (P<0.05). There was no significant difference in the MDA content of *E. nuttallii* between the low treatment and the control

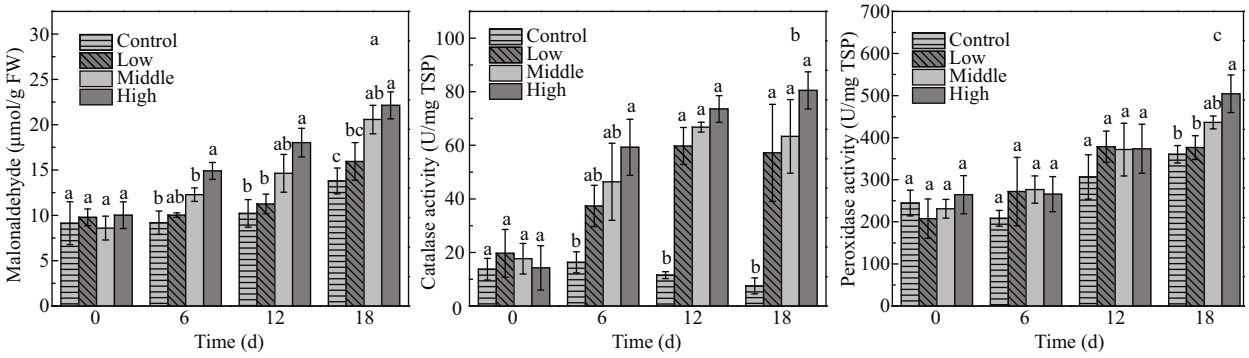

Fig.3 Changes in malonaldehyde concentration (μmol/g FW) (a), catalase activity (U/mg TSP) (b), and peroxidase activity (c) (U/mg TSP) in *Elodea nuttallii*

Control: no floating *Cladophora* sp.; low, middle, and high: 20 g, 40 g, and 80 g (fresh weight) floating *Cladophora* sp., respectively. Error bars represent standard deviation (n=3). Different letters indicate significant difference (P<0.05).

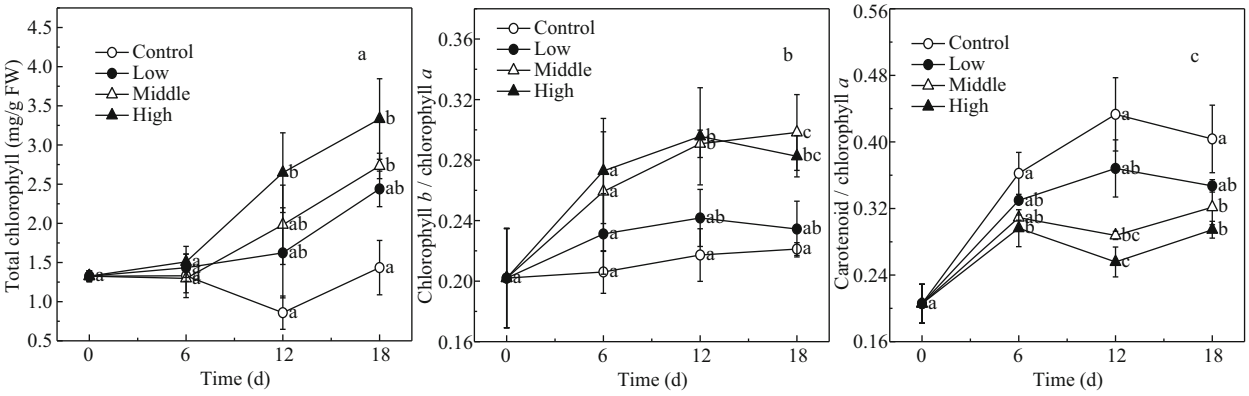

Fig.4 Changes in total chlorophyll (mg/g FW) (a), chlorophyll *b*/chlorophyll *a* (b) and carotenoids/chlorophyll *a* (c) in *Elodea nuttallii* during experiment

Control: no floating *Cladophora* sp.; low, medium, and high: 20 g, 40 g, and 80 g (fresh weight) floating *Cladophora* sp., respectively. Error bars represent standard deviation (n=3). Different letters indicate significant difference (P<0.05).

(P>0.05) (Fig.3a), suggesting that greater biomass of floating *Cladophora* sp. resulted in greater oxidative stress in *E. nuttallii*.

The presence of floating *Cladophora* sp. stimulated CAT activity in *E. nuttallii*, and CAT activity increased over time (F=4.82, P=0.001). The average CAT activity of *E. nuttallii* in the high treatment was 3.63 times that in the control on day 6 (P=0.011). On day 18, the CAT activity of *E. nuttallii* in the low, middle, and high treatments was 6.62, 7.44, and 9.73 times that in the control, respectively (P=0.014, P=0.007, P<0.001, respectively) (Fig.3b).

The POD activity of *E. nuttallii* increased over time (F=27.93, P<0.001), and differed significantly between the high treatment and the other treatments by day 18 (F=9.84, P=0.05). The POD activity in the high treatment was 39.9% higher than that in the control, but did not differ significantly among the control, low, and middle treatments (P>0.05) (Fig.3c).

3.3 Photosynthetic pigments

In the presence of floating *Cladophora* sp., the total chlorophyll content (total chl) of *E. nuttallii* increased over time (F=27.82, P<0.001) and was proportional to the amount of *Cladophora* sp. (F=18.73, P=0.001) (Fig.4a).

The response of chlorophyll *b*/chlorophyll *a* (chl *b*/chl *a*) in *E. nuttallii* to floating *Cladophora* sp. was similar to that of total chl. The chl *b*/chl *a* increased with increasing biomass of floating *Cladophora* sp. (F=8.10, P=0.008; Fig.3b). The *Cladophora* sp. had a strong shading effect, and so *E. nuttallii* was in a shade-adapted state in the middle and high treatments (Fig.4b).

The trend in carotenoids/chlorophyll *a* (car/chl *a*) was almost opposite to that of total chl and chl *b*/chl *a*. The car/chl *a* decreased with increasing biomass of floating *Cladophora* sp. (F=21.87, P<0.001). Between

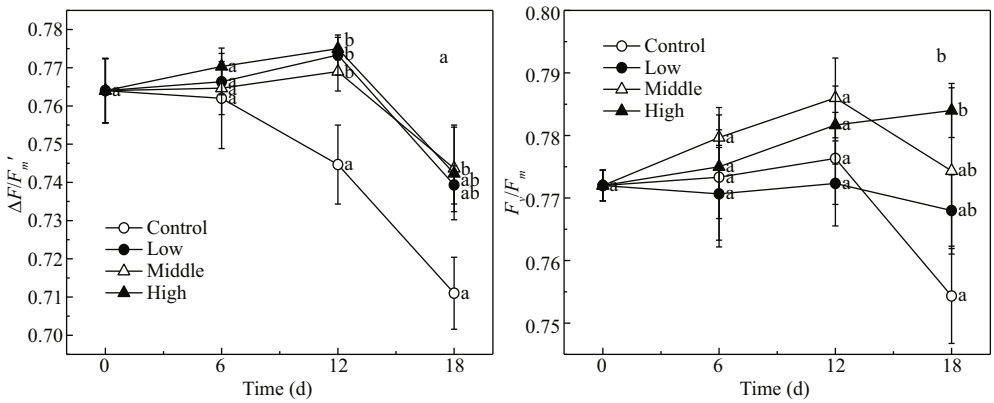

Fig.5 Changes in $\Delta F/F_m'$ (a) and F_v/F_m (b) in *Elodea nuttallii* during experiment

Control: no floating *Cladophora* sp.; low, medium, high: 20 g, 40 g, and 80 g (fresh weight) floating *Cladophora* sp., respectively. Error bars represent standard deviation (n=3). Different letters indicate significant difference (P<0.05).

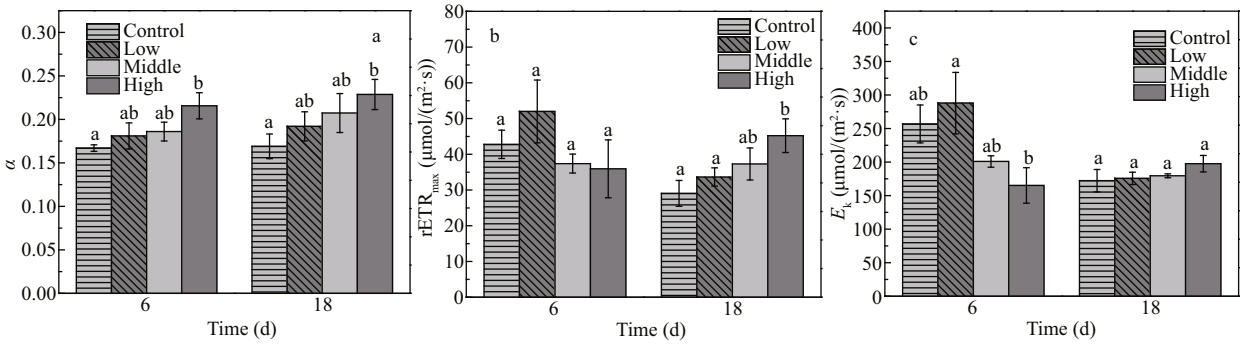

Fig.6 Changes in α (a), $rETR_{max}$ (μmol/(m²·s)) (b) and E_k (μmol/(m²·s)) (c) in *Elodea nuttallii* during experiment

α: maximum light utilization efficiency; $rETR_{max}$ (μmol electrons/(m²·s)): maximum relative electron transport rate; E_k (μmol electrons/(m²·s)): light saturation parameter. Control: no floating *Cladophora* sp.; low, middle, high: 20 g, 40 g, and 80 g (fresh weight) floating *Cladophora* sp., respectively. Different letters indicate significant difference (P<0.05).

days 6 and 18, the car/chl *a* in the control (0.40±0.050) was higher than those in the middle and high treatments (0.31±0.020 and 0.28±0.027, respectively), suggesting that *E. nuttallii* in the control was in a light-protective state (Fig.4c).

3.4 Chlorophyll fluorescence parameters

The $\Delta F/F_m'$ values of *E. nuttallii* were higher in the treatments with *Cladophora* sp. than in the control (F=9.60, P=0.005). The $\Delta F/F_m'$ first fluctuated over a certain range and then decreased during the experiment (F=31.21, P<0.001) (Fig.5a). However, the F_v/F_m value of *E. nuttallii* was not affected by treatments or time (F=0.96, P=0.466). On day 18, the F_v/F_m value of *E. nuttallii* was 0.754±0.008 in the control group (Fig.5b). As indicated by the $\Delta F/F_m'$ and F_v/F_m values, *E. nuttallii* in the four treatment groups maintained photosynthesis during the experiment.

The photosynthetic parameters (α, E_k and $rETR_{max}$) of *E. nuttallii* were influenced by the increasing biomass of floating *Cladophora* sp. to different extents. The α values of *E. nuttallii* were higher in the treatments with floating *Cladophora* sp. than in the control (F=12.855, P=0.002), indicating that *E. nuttallii* improved its light utilization to adapt to low light intensity (Fig.6a). The $rETR_{max}$ and E_k values of *E. nuttallii* were affected by the amount of *Cladophora* sp. and time (F=5.84, P=0.021; F=9.68, P=0.005, respectively). The $rETR_{max}$ value of *E. nuttallii* was 29.1±4.44 μmol/(m²·s) in the control, lower than that in the high treatment (45.2±5.77 μmol/(m²·s)) on day 18 (P=0.015). (Fig.6b). The E_k value of *E. nuttallii* was higher on day 6 when the biomass of floating *Cladophora* was lower (F=6.62, P=0.015), but there were no differences in E_k among the four treatment groups on day 18 (F=1.96, P=0.20) (Fig.6c). In the control, the values of $rETR_{max}$ and E_k for *E. nuttallii* were lower on day 18 than on day 6 (t=4.91, P=0.039; t=9.74, P=0.010, respectively).

3.5 Growth of submerged macrophyte

Floating *Cladophora* sp. negatively affected the growth of *E. nuttallii* (F=27.39, P<0.001). In the middle and high *Cladophora* sp. treatments, the

biomass of *E. nuttallii* was decreased by 30.5% and 38.3%, respectively, compared with that in the control (P<0.001). However, the low *Cladophora* sp. treatment did not affect the biomass of *E. nuttallii* (P=0.083) (Fig.7).

4 DISCUSSION

The effects of floating *Cladophora* sp. on the submerged macrophyte *E. nuttallii* were rather complex, and included positive and negative effects. The percentage of shading, dissolved oxygen, and pH value increased with increasing biomass of *Cladophora* sp., while dissolved oxygen and pH values fluctuated in a small range in the control. During the day, the floating *Cladophora* sp. caused the dissolved oxygen concentration increasing and carbon dioxide concentration decreasing in the water column through photosynthesis. Decreased carbon dioxide concentration in turn raised the pH value in the water. Conversely, the floating *Cladophora* sp. were only respiring at night, decreasing dissolved oxygen concentration in the water column and producing carbon dioxide that lowered pH level. The dissolved oxygen concentration decreased with increasing water temperature, which implied that high water temperature negatively affected the photosynthesis of both *E. nuttallii* and *Cladophora* sp. Netten et al. (2010) found that the free-floating macrophyte *Salvinia natans* showed increased growth with an increase in water temperature, and it out-competed *E. nuttallii* to take advantage of the environmental change (Netten et al., 2010). However, the water temperature was not the main factor limiting the growth of *E. nuttallii* in our experiment. Several studies have shown that *E. nuttallii* has strong phenotypic plasticity to adapt to changing conditions (Ozimek et al., 1993; Baldy et al., 2015). Velthuis et al. (2017) reported that the aboveground biomass of *E. nuttallii* showed no difference between 10°C and 25°C, but the C:N ratio was lower at 25°C (Velthuis et al., 2017). The conductivity and salinity of each group increased over time, but did not differ significantly among the four groups. Aquatic plants can produce secondary metabolites such as organic acids and terpenoids (Irfanullah and Moss, 2005b; Gao et al., 2014; Wang et al., 2014), which might explain this phenomenon.

Larger biomasses of *Cladophora* sp. at the water surface resulted in increased shading, and the total chlorophyll content and chl *b*/chl *a* of *E. nuttallii* were positively correlated with the degree of shading.

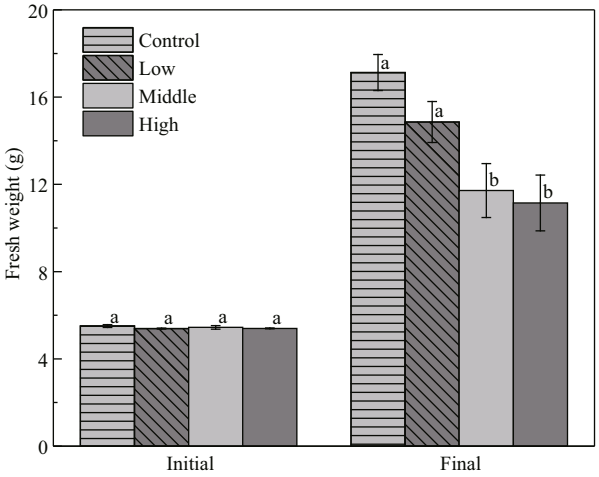

Fig.7 Fresh weight (g) of *Elodea nuttallii* at the end of the experiment

Control: no floating *Cladophora* sp.; low, middle, high: 20 g, 40 g, and 80 g (fresh weight) floating *Cladophora* sp., respectively. Different letters indicate significant difference (P<0.05).

These results suggest that the decrease in light intensity led to greater chlorophyll accumulation to produce sufficient energy for plant growth. An increase in the relative chlorophyll b content in leaves has been shown to be beneficial for absorbing shorter wavelengths (Lichtenthaler et al., 1981). The high total chlorophyll content of *E. nuttallii* increased the values of several photosynthetic parameters (including $\Delta F/F_m'$ and the RLC parameters). Some studies have shown that the total chlorophyll content affects the efficiency of photosystem II at the genetic level (Vijayalakshmi et al., 2010; Czyczyło-Mysza et al., 2013). Under shaded conditions, the *E. nuttallii* leaf cells showed an accelerated electron transfer rate and had a higher proportion of open reaction centers to increase their photosynthetic activity. These are strategies that allow plants to tolerate short-term low light intensity (Ralph and Gademann, 2005).

In a previous study, the chlorophyll fluorescence of a submerged macrophyte remained high under prolonged shading (30 d) (Lu et al., 2012). However, we observed a slight decrease in the $\Delta F/F_m'$ and F_v/F_m values of *E. nuttallii* from day 12 to day 18. These decreases were related to the high pH values and dissolved oxygen concentration. Simpson et al. (1980), Simpson and Eaton (1986) found that high O_2, high pH, and low CO_2 conditions led to major decreases in the photosynthetic activity of *E. canadensis* (Simpson et al., 1980; Simpson and Eaton, 1986). In the control, the chlorophyll fluorescence parameters ($\Delta F/F_m'$, F_v/F_m, $rETR_{max}$, and E_k) of *E. nuttallii* decreased and the car/chl *a* increased

as a result of the high light intensity. Hussner et al. (2010) found that the effective quantum yield of photosystem II in *E. nuttallii* decreased when photosynthetically active radiation was higher than 100 μmol/(m^2·s) (Hussner et al., 2010). In addition, the large amount of UV radiation decreased the photosynthetic efficiency of *E. nuttallii* at a field station located at 1 950 m above sea level (Chen et al., 2012). Since the photosynthetic activity of *E. nuttallii* was higher in the presence *Cladophora* sp. than in its absence, we suggest that the presence of floating *Cladophora* sp. had a stimulating effect on the photosynthetic system of *E. nuttallii*.

The impact of floating macrophytes appears to be more complex than just simple physical shading (Lu et al., 2012). Drastic changes in the external environment in the presence of floating *Cladophora* sp. caused an increase in free radicals, increased MDA content, and increased antioxidant enzyme activities in *E. nuttallii* leaves. In the present experiment, POD activity increased significantly by day 18, whereas CAT activity increased throughout the experiment, suggesting that it was more sensitive than POD activity to the ROS induced by the presence of *Cladophora* sp. Butow et al. (1994) found that CAT activity remained unchanged within the range of pH 6 to pH 10 in in vitro and in vivo conditions, and that high pO_2/pCO_2 increased CAT activity (Butow et al., 1994). Zhang et al. (2010) and Song et al. (2017) found that low light intensity resulted in increased POD and SOD activities in plants (Zhang et al., 2010; Song et al., 2017). We did not observe large changes in POD activity in our experiment, indicating that shading was not the reason for the increased enzymes activities. Instead, the high dissolved oxygen concentration and pH values were the main contributors to oxidative stress.

Elodea nuttallii can adapt to low light conditions, but very low light is not conducive to the growth of *E. nuttallii* over a long period (Li et al., 2015; Zefferman, 2015). The light intensity and UV radiation were high at the experimental site; therefore, shading protected/promoted photosynthetic activity during this 18-day experiment. Although photosynthesis is one of the main factors affecting plant growth, there was no direct correlation between the growth rate and photosynthetic rate. The growth rate of *E. nuttallii* was likely influenced by other factors, for example, water level fluctuations, life-cycle, and competitive ability (Korner, 1991; Dollerup et al., 2013; Grudnik et al., 2014; Wang et al., 2016). In the present study, the increasing concentration of dissolved oxygen and pH value were the main factors affecting the biomass of *E. nuttalllii* via their negative effects on oxidative stress and enzyme activities. The increased antioxidant enzyme activities consumed energy, which meant that less energy was available for biomass accumulation and reproduction of *E. nuttallii*. Thus, *E. nuttallii* had a low biomass in the high *Cladophora* sp. treatments (280 and 560 g FW/m^2). In addition, the high pH decreased the amount of dissolved inorganic carbon (Choo et al., 2004; Falkowski and Raven, 2013), negatively affecting the growth of *E. nuttallii*.

5 CONCLUSION

Floating *Cladophora* sp. changed the water environment by shading and increasing the dissolved oxygen concentration and pH value. Shading promoted the photosynthetic activity of *E. nuttallii*, while the increases in pH value and dissolved oxygen concentration reduced photosynthetic activity and increased the antioxidant enzyme activity of *E. nuttallii*. These changes resulted in decreased biomass of *E. nuttallii* in the presence of floating *Cladophora* sp. The results indicated that the indirect effects of floating *Cladophora* sp. on water quality may be more important than the direct effects of shading. Consequently, in the ecological restoration of submerged macrophytes, managers should pay more attention to changes in water quality than to shading by floating *Cladophora* sp.

6 DATA AVAILABILITY STATEMENT

The datasets obtained in this study are available from the corresponding author on request.

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