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Algae mediate submerged macrophyte response to nutrient and dissolved inorganic carbon loading: A mesocosm study on different species

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HIGHLIGHTS

- Algea-mediated macrophytes responses to nutrients and DIC enrichments were studied.
- Increase nutrients resulted in algea growth and increased light attenuation.
- DIC enrichment resulted in a heavily algea state when combined with nutrients.
- The changes in algal and water chemistry are related to performances of plants.
- Species-specific performances occured when plants in high abundance of algea.

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ABSTRACT

Nutrient and dissolved inorganic carbon are two important factors that influence the development and species composition of submerged macrophyte communities in shallow lake ecosystems. Yet little is known about their potential interactive effects on the submerged macrophytes and competition outcome of macrophyte–phytoplankton. We performed a mesocosm experiment to investigate the growth and photosynthetic performance of three submerged macrophytes in relation to phytoplankton/epiphyton with nutrient and bicarbonate enrichment. During the experimental period (42 d), increase in nutrient loading in water column resulted in a substantial burst of phytoplankton and epiphyton growth and increased light attenuation. When combined with nutrient loading, bicarbonate enrichment also resulted in a heavily phytoplankton- and epiphyton-dominated state, although bicarbonate enrichment per se does not cause the phytoplankton and epiphyton growth. However, increase in nutrient loading in water column had significant negative impact on individual performances (growth and photosynthesis) of the three submerged macrophytes and bicarbonate enrichment increased the effect of eutrophication on two dissected-leaf macrophytes (*M. spicatum* and *E. nuttallii*). Furthermore, our results also suggest that species-specific photosynthetic performances occurred when submerged macrophytes in an environment with high abundance of phytoplankton/epiphyton. This study highlighted that increase in nutrient loading and bicarbonate in water column is likely to interactively impact both abiotic and biotic properties of a freshwater ecosystem. The interactions of these two factors could select macrophyte species that are able to resist the shading from phytoplankton/epiphyton, making these species more dominant in natural freshwater ecosystems.

1. Introduction

Nutrients and dissolved inorganic carbon (DIC) can influence the development and ultimately lead to the species composition changes of submerged macrophyte communities in shallow lakes (Barko et al., 1986; Lacoul and Freedman, 2006; Bornette and Puijalon, 2011). Submerged macrophytes are able to take up N and P through roots (Denny, 1972; Carignan and Kalff, 1980; Carignan, 1982); and they also can utilize nutrients from water column via shoots (Madsen and Cedergreen, 2002; Takayanagi et al., 2012). In aquatic ecosystems, eutrophication, caused by excessive anthropogenic nutrient loading (i.e., NH_4^+, NO_3^- and PO_4^{3-}), has many negative consequences, such as algal blooms, dissolved oxygen (DO) shortages and loss of biodiversity (Carpenter et al., 1998), and therefore can predominantly cause the floristic changes in the affected ecosystems (Bornette and Puijalon, 2011). In addition, although high levels of NH_4^+ may be toxic to submerged macro-

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phytes (Cao et al., 2004; Nimptsch and Pflugmacher, 2007), the toxic effect of NH_4^+ ions are not found to be a main threat to submerged macrophytes in natural eutrophic aquatic ecosystems (Li et al., 2008). In fact, previous studies implied that loss or degradation of submerged macrophytes in eutrophic aquatic ecosystems is generally the result of decreased photosynthetic rates due to reduced photosynthetically active radiation (PAR) caused by the dominance of phytoplankton/epiphyton (Phillips et al., 1978; Li et al., 2008; Bornette and Puijalon, 2011; Cao et al., 2011; Arthaud et al., 2012). These results indicate that the outcome of macrophyte–phytoplankton competition may also be determined by the performance of submerged macrophyte. However, most previous studies in aquatic habitats regarding such process only focus on single factors (e.g., nutrient availability), whereas evidence for multiple factors is scarce.

On the global scale, climate change has not only induced ascending atmospheric CO_2 but may also modify the DIC equilibrium (between free CO_2 and bicarbonate/carbonate) in aquatic ecosystems (Parry et al., 2007). In general, free CO_2 and bicarbonate are the main carbon sources for submerged macrophytes: all submerged macrophytes can use free CO_2 in water column/sediment (CO_2 users), and many macrophytes can also utilize bicarbonate (bicarbonate users) (Spence and Maberly, 1985; Sand-Jensen and Gordon, 1986; Madsen and Sand-Jensen, 1991; Jones et al., 1993). Bicarbonate users are more sensitive in photosynthetic performances than CO_2 users in response to DIC enrichment (Pagano and Titus, 2007). For example, increased bicarbonate availability enhances the rate of gross photosynthesis in submerged species such as *Egeria densa* (Planch.), *Potamogeton schweinfurthii* (Benn.) and *Potamogeton lucens* L. (Kahara and Vermaat, 2003). However, a recent study also showed that the photosynthetic rates of *Ceratophyllum demersum* L., *E. densa* and *Lagarosiphon major* Ridl. were the highest when these plants were grown under low alkaline condition (Cavalli et al., 2012). These results indicate that submerged species vary in their abilities to exploit carbon source under DIC enrichment, and the differences may potentially determine their positions in the competitive hierarchy in freshwater ecosystems (Schippers et al., 2004; Pagano and Titus, 2007; Spierenburg et al., 2009; Bornette and Puijalon, 2011).

In terrestrial habitat, plants would show their maximal response to CO_2 enrichment only in cases without environmental constraints or stresses (reviewed by Poorter and Navas, 2003). Similarly, previous studies have also demonstrated that if the sediments contained enough nutrients, an increase in DIC could result in replacement of isoetids by elodeid species (Spierenburg et al., 2009). Moreover, increased resource (e.g., nutrient loading) promotes phytoplankton/epiphyton growth (Neckles et al., 1993; Jones et al., 2002), which incurs a negative relationship with plant performance, as has been shown in both laboratories or fields (Irfanullah and Moss, 2004; Chen et al., 2007; Kosten et al., 2011; Arthaud et al., 2012). Therefore, increase in nutrient loading and DIC concentration could alter competitive interactions between periphyton and submerged macrophytes, causing the latter more prone to loss the battle (Jones et al., 2000, 2002). These results suggested that nutrient availability and DIC concentrations may interact with each other and potentially influence community structure in shallow lakes. However, less attention has been given to submerged macrophytes in response to induced phytoplankton/epiphyton abundance due to the interaction between nutrient and DIC in aquatic habitats.

Species-specific morphological and physiological traits may also modify the competition outcome of macrophyte–phytoplankton. For instance, many submerged macrophytes have thin or feather-like leaves to maximize uptake of nutrient ions and dissolved gases from water column (Sculthorpe, 1967). However, these leaves may also act as attaching sites for epiphyton to grow, which are commonly found in eutrophic condition (Carignan and Kalff, 1982; Saunkaew et al., 2011). In this study, three submerged macrophytes were used for comparisons in two nutrient and bicarbonate levels. We hypothesized that (1) increased nutrient availability would increase phytoplankton/epiphyton abundance which in turn would restrict the growth of submerge macrophytes; (2) bicarbonate enrichment might mitigate the effect of eutrophication on macrophytes (e.g., phytoplankton/epiphyton shading); and (3) species-specific photosynthetic performances would occur when plants were restricted by the abundant phytoplankton/epiphyton.

2. Methods

2.1. Experimental site and species

The study was conducted at the National Field Station of Freshwater Ecosystem of Liangzi Lake, China (30°5′–30°18′ N, 114°21′–114°39′ E). Three species (*Myriophyllum spicatum* L., *Elodea nuttallii* (Planch.) H. St. John and *Potamogeton crispus* L.) from three families (Haloragaceae, Hydrocharitaceae and Potamogetonaceae, respectively) were used in this experiment. All of these species are able to use bicarbonate from water column as an alternative carbon resource (Madsen and Maberly, 2003; Jones, 2005). In mid-March, 300 plants of the three species with fine apical shoots (100 5-cm long apical shoots per species) were collected from different sites (total nitrogen, TN: 0.22–0.56 mg g^{-1} and total phosphorus, TP: 0.04–0.08 mg g^{-1}) in Liangzi Lake. The apical shoots were rinsed and transplanted into containers filled with sand and water (20 cm depth) in a greenhouse for approximately 2 wk until the plants had developed root systems. After developed roots, 24 apical shoots of each species with similar sizes were used for the experiment (72 apical shoots in total).

2.2. Experimental design

A mesocosm study was conducted during a 42 d period, from 4th April to 15th May 2010. In 28th March 2010, 24 mesocosms were constructed (fiberglass tanks, 100 × 50 × 60 cm), each containing 300 L lake water from the Liangzi Lake. The 24 mesocosms were located outdoor and the lake water was added to them every day to compensate for evaporation and to maintain a constant water level (60 cm) during the experimental period. Mean air temperature and the PAR during the experimental period (42 d) were measured at noon everyday (temperature 28 ± 1 °C; PAR 545 ± 43 μmol photons m^{-2} s^{-1}, mean ± SD). To minimize the influence of macroinvertebrate and zooplankton, the water was filtered through a plankton net. Each fragment was planted into a plastic pot (diameter 5 cm, height 7 cm) along with 5 cm of substratum (obtained from Liangzi Lake, TN 2.45 mg g^{-1} dry weight (DW), TP 0.23 mg g^{-1} DW and 12.5% organic matter), and the three different species were put into a mesocosm.

The experimental treatments were performed using a randomized block design to minimize microsite effects. A factorial experimental design was employed that included two levels of water column nutrient treatment (ambient nutrient level and enriched nutrient level) and two levels of bicarbonate treatment (ambient bicarbonate level and enriched bicarbonate level) in six blocks, with one replicate per block. The nutrient values for the enriched nutrient treatment were consistent with a eutrophic lake (by adding N and P into the water of Liangzi Lake) which is approximately 30 km away from Liangzi Lake (Yanglan Lake, 30°00′–30°06′ N, 114°32′–115°05′ E) (TN 2.53 ± 0.17 mg L^{-1}, TP 0.13 ± 0.03 mg L^{-1}). The ambient nutrient treatment used the lake water from Liangzi Lake (TN 0.42 ± 0.07 mg L^{-1}, TP 0.06 ± 0.01 mg L^{-1} during the

experimental period). Within each nutrient treatment, two different bicarbonate levels were also included: the ambient bicarbonate concentration in Liangzi Lake (total dissolved carbon 15.74 ± 0.54 mg L^{-1}, pH value 6.53–7.64) and a carbon concentration twice the ambient level (31.00 ± 0.52 mg L^{-1}, pH value 6.09–7.64) by addition of $NaHCO_3$. To maintain constant concentrations of nutrient and bicarbonate, $NaHCO_3$, NH_4NO_3 and KH_2PO_4 were added in a 3 d interval (after the nutrient and bicarbonate concentrations were measured).

Nutrient concentrations (TN and TP) and DIC concentration of each mesocosm were measured in a 3 d interval using an IL-500 N nitrogen analyzer, an IL-500P phosphorus analyzer and an IL-500 TOC analyzer (Hach Company, Loveland, USA). The following parameters of water in each mesocosm were recorded every 7 d (at noon): chlorophyll *a* (chl *a*) concentration, subsurface light intensity (0.5 cm beneath the water surface), water light intensity, DO and pH value. All parameters (except subsurface light intensity) were measured at a depth of 20 cm. The K_d was calculated using the following model proposed by Kirk (1994): $K_d = -1/z \ \ln E(z)/E(0)$, where z is the measurement depth (0.2 m), $E(z)$ is the water light intensity at a depth of 0.2 m, $E(0)$ is the subsurface light intensity.

2.3. Chlorophyll and photosynthetic characteristics measurement

In this study, all photosynthetic characteristics (The maximal photochemical efficiency of PSII in dark, F_v/F_m; rapid light curves, RLC) were measured using a Diving-PAM fluorometer (Walz GmbH, Effeltrich, Germany). Before the final harvest, the plant apical shoots in each mesocosm were firstly removed the epiphytic algae with a soft toothbrush and then acclimated in dark for 20 min using leaf clips (Diving-LC, Walz, Effeltrich, Germany). After the dark acclimation, RLCs were measured using a pre-installed software (Wincontrol, Walz GmbH, Effeltrich, Germany) routine, where photosynthetic photon flux density of PAR (PPFD) was increased in nine steps (0–1325 μmol photons m^{-2} s^{-1}) over a 90 s period. The relative electron transport rates (rETRs, which were calculated as $rETR = \Phi_{PSII} \times PPFD$; where Φ_{PSII} is the quantum efficiency of PSII) were used to determine the following photosynthetic parameters: α (photosynthetic efficiency under non-saturating irradiances), E_k (the irradiance at the onset of saturation) and $rETR_{max}$ (the relative maximum rate of electron transfer to photosystem II under saturation irradiances), using the model proposed by Platt et al. (1980).

Following measurements of photosynthetic performance, pigments of leaves were extracted from approximately 0.02 g of leaves from the apical shoots of the plants by grinding in 80% (v/v) acetone for several minutes. Samples were then centrifuged and the supernatants analyzed for their absorption spectra (663 and 645 nm for chlorophyll *a* and *b*) using an UV–Vis spectrophotometer (UV-2800A, Unico, Shanghai, China). Chlorophyll concentration was calculated using the formula proposed by Arnon (1949).

Phytoplankton and epiphyton biomass were assayed as total extractable chl *a*. After the macrophytes were harvested, 100 mL water in each mesocosm was sampled and filtered onto a GF/F filter to separate the phytoplankton. The epiphyton was separated from 5–20 leaves (approximately 5–7 cm from apical shoot) of each species by vigorously shaking for 3 min in a 550 mL plastic bottle containing 100 mL of tap water, followed by filtering onto a GF/F filter for chl *a* analysis. Chl *a* of both phytoplankton and epiphyton were measured using the acetone method (Smoot et al., 1998). The chl *a* concentrations were calculated as μg L^{-1} and μg g^{-1} sampled DW plant leaves. All of the plant tissue samples were dried at 80 °C for 72 h and then weighed to obtain dry-weight biomass. The relative growth rate (RGR) was calculated as RGR = [ln(*Final DW*) − ln(*Initial DW*)]/d.

2.4. Statistical analyses

All of the water quality data, including the chl *a*, K_d, DO and pH values, were analyzed using repeated ANOVA, with the nutrient and bicarbonate as fixed factors. All growth and photosynthetic data were analyzed using three-way ANOVA, with the nutrient, bicarbonate and species as fixed factors. We hypothesized that enrichment of nutrient and bicarbonate in water column may cause phytoplankton/epiphyton development and negatively affect the performance of submerged macrophytes. Therefore, to quantify the performance of the macrophyte species in response to nutrient/bicarbonate treatments, we calculated the natural-log of response ratio ($lnRR_{treatment}$ = ln (treatments performances/control performances)) (Hedges et al., 1999) to test the deviation. Duncan tests were used to compare the levels for significance ($P < 0.05$). All of the experimental data were first transformed using a log(x) function, to meet homogeneity of variance or normal distribution of residuals and then analyzed. All data were analyzed using the SPSS 19.0 program (SPSS, Chicago, IL, USA).

3. Results

3.1. Impact of treatments on water quality

During the 42 d treatment, the increase of nutrient in water column (+(N,P) treatment) strongly increased the chl *a* concentration, and consequently, indirectly increased the light attenuation (K_d) and the DO concentration in the mesocosms. However, the enrichment of bicarbonate in water column (+C treatment) only had significant effect on the chl *a* concentration. The water quality characters were significantly affected when +C treatment combined with the +(N,P) treatment (+(N,P)+C treatment) (Fig. SM-1a–1c and Table SM-1 in Supplementary Material (SM)). The +(N,P) treatment significantly increased the water pH in mesocosms, whereas the +C treatment significantly decreased the water pH and significant interaction between these two factors occurred during the experimental period (Fig. SM-1d). In addition, the +(N,P) and +C treatments significantly increased the epiphyton on leaves of the most macrophytes (positive values of ln $RR_{treatment}$) and more epiphyton on leaves of *M. spicatum* were observed compared with the other two macrophytes.

3.2. Impact of treatments on targets species

Both biomass and RGR of macrophytes varied among species during the experimental period and these two traits were significantly affected by the enrichment of nutrient and bicarbonate in water column (Fig. 1a and b and Table 1). ln $RR_{treatment}$ biomass and ln $RR_{treatment}$ RGR were negative for all species in +(N,P) conditions, reflecting the strong adverse effects of +(N,P) conditions on these species; in +C and +(N,P)+C conditions, these two parameters either increased or turned positive (*P. crispus* in +C condition), indicating that the negative effects of +(N,P) condition on these macrophytes were mitigated by bicarbonate enrichment (Fig. 2a and b and Table 2).

Significant variations were also detected for biomass and RGR with different species (Table 1 and 2). In all the treatments (+(N,P),+C and +(N,P)+C), both biomass and RGR of *M. spicatum* were significantly lower than *E. nuttallii* and *P. crispus* indicating growth performances of *M. spicatum* were more affected in +(N,P) and/or +C condition than the latter two submerged macrophytes (Figs. 1a,b and 2a,b).

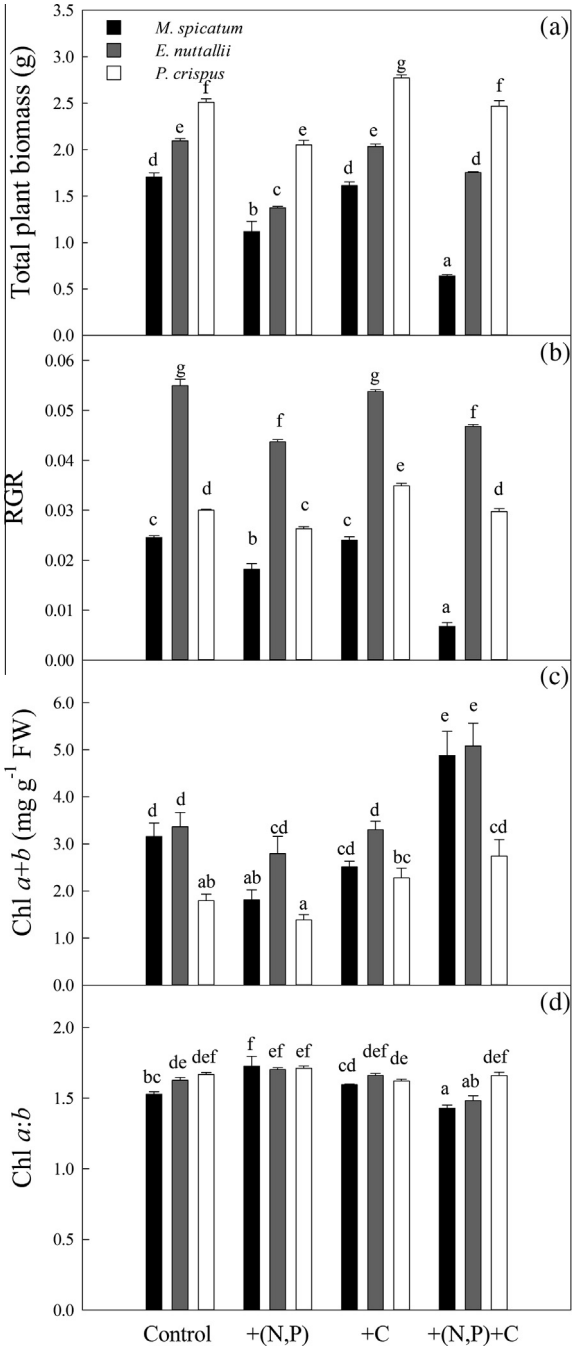

Fig. 1. Differences in plant biomass (a), relative growth rate (RGR) (b), leaf chlorophyll concentration (chl $a + b$) (c) and ratios of leaf chlorophyll a and chlorophyll b (chl a:b) (d) of the three macrophytes upon the nutrient and/or bicarbonate treatments ($n = 6$). Bars sharing the different letters indicate significant differences among the treatments (Duncan test).

Similarly, the enrichment of nutrient and bicarbonate significantly affected the chlorophyll concentration (chl $a + b$) in the three submerged macrophytes (Fig. 1c and Table 1). In the +(N,P) condition, the chl $a + b$ of the three macrophytes were negatively affected (negative ln $RR_{treatment}$ values); and the chl $a + b$ were also negatively affected in +C condition (except for *P. crispus*) (Fig. 2c and Table 2). The chl $a + b$ of three macrophytes were all positive and similar among species in +(N,P)+C condition (Fig. 2c). In addition, while the nutrient enrichment had no significant effect on the ratio between chlorophyll a and chlorophyll b (chl a:b), bicarbonate enrichment did (Fig. 1d and Table 1). Positive ln $RR_{treatment}$ chl a:b values were observed in both +(N,P) and +C conditions (except for *P. crispus*), whereas negative chl a:b values were observed in +(N,P)+C condition (Fig. 2d and Table 2). In both of *M. spicatum* and *E. nuttallii*, chl a:b values decreased significantly in +(N,P)+C condition (Fig. 2d).

Table 1
F-values for effects of nutrient (N), bicarbonate (B) on different species (S) for growth performances (biomass, RGR, Chl $a + b$ and chl a:b) and photosynthetic performances (F_v/F_m, $rETR_{max}$, α and E_k) (Three-way ANOVA).

	d.f.	Biomass	RGR	chl $a + b$	chl a:b
Growth performances					
Nutrient (N)	1, 60	422.65***	241.02***	4.82*	0.04^{ns}
Bicarbonate (B)	1, 60	7.00**	23.82***	38.34***	30.36***
Species (S)	2, 60	642.87***	704.55***	28.38***	13.79***
$N \times B$	1, 60	1.68^{ns}	39.83***	43.91***	46.18**
$N \times S$	2, 60	19.20***	76.71***	1.05^{ns}	3.13^{ns}
$B \times S$	2, 60	47.44***	68.55***	0.24^{ns}	1.61^{ns}
$N \times B \times S$	2, 60	20.31***	48.45***	5.53**	11.86*
	d.f.	F_v/F_m	$rETR_{max}$	α	E_k
Photosynthetic performances					
Nutrient (N)	1, 60	14.60***	685.83***	30.38***	37.06***
Bicarbonate (B)	1, 60	32.89***	185.68***	10.71**	8.23**
Species (S)	2, 60	21.00***	470.81***	401.92***	81.36***
$N \times B$	1, 60	17.77***	74.43***	4.41*	3.23^{ns}
$N \times S$	2, 60	12.02***	206.57***	24.21***	4.13*
$B \times S$	2, 60	0.99^{ns}	233.91***	3.32*	21.04***
N × B × S	2, 60	5.13**	123.68***	1.17^{ns}	12.22***

All data were transformed to log(x).
* $0.01 < P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
^{ns} non-significant ($P > 0.05$).

The maximal photochemical efficiency of PSII in dark (F_v/F_m) was also affected by the enrichment of nutrient and bicarbonate (Fig. 3a and Table 1). In *M. spicatum*, F_v/F_m values in all treatment conditions were lower than that of control condition (negative ln $RR_{treatment}$ values). In contrast, In *P. crispus*, the F_v/F_m values in all treatments were higher than that of the control condition (positive ln $RR_{treatment}$ values). For *E. nuttallii*, negative ln $RR_{treatment}$ F_v/F_m values were only observed in +(N,P) condition (Fig. 3b and Table 2). The RLCs of three macrophytes decreased significantly in +(N,P) and +C conditions (Fig. 4 and Table 1). Most RLC parameters ($rETR_{max}$, α and E_k) of all tested species (except for α of *P. crispus*) decreased significantly in the +(N,P) condition. In the +C condition, the RLC parameters of two macrophytes (*M. spicatum* and *E. nuttallii*) decreased. In the +(N,P) and +C combination, RLC parameters of all species decreased significantly (Fig. 3c–h, Table 1 and 2).

4. Discussion

Resource enrichment (e.g., nutrient and DIC enrichment in water column) commonly results in increased competition and loss of macrophyte species (e.g., Titus and Andorfer, 1996; Jones et al., 2002). Although the effects of nutrient or bicarbonate enrichment on aquatic macrophytes have already been recognized (see Prins and Elzenga, 1989; Petchey et al., 2004; Bornette and Puijalon, 2011 for reviews), to our best knowledge, few evidence was available regarding their interactions in aquatic ecosystems (except for Jones et al., 2002). In this study, we demonstrated that individual performances (growth and photosynthesis) of three submerged macrophytes and phytoplankton/epiphyton growth were significantly affected by enrichment of nutrient and DIC (bicarbonate). Our results supported the proposal by Arthaud et al. (2012) that "in eutrophic condition, nutrients do not limit plant growth, but phytoplankton biomass is often high and light availability low, and these affect recruitment and growth of all

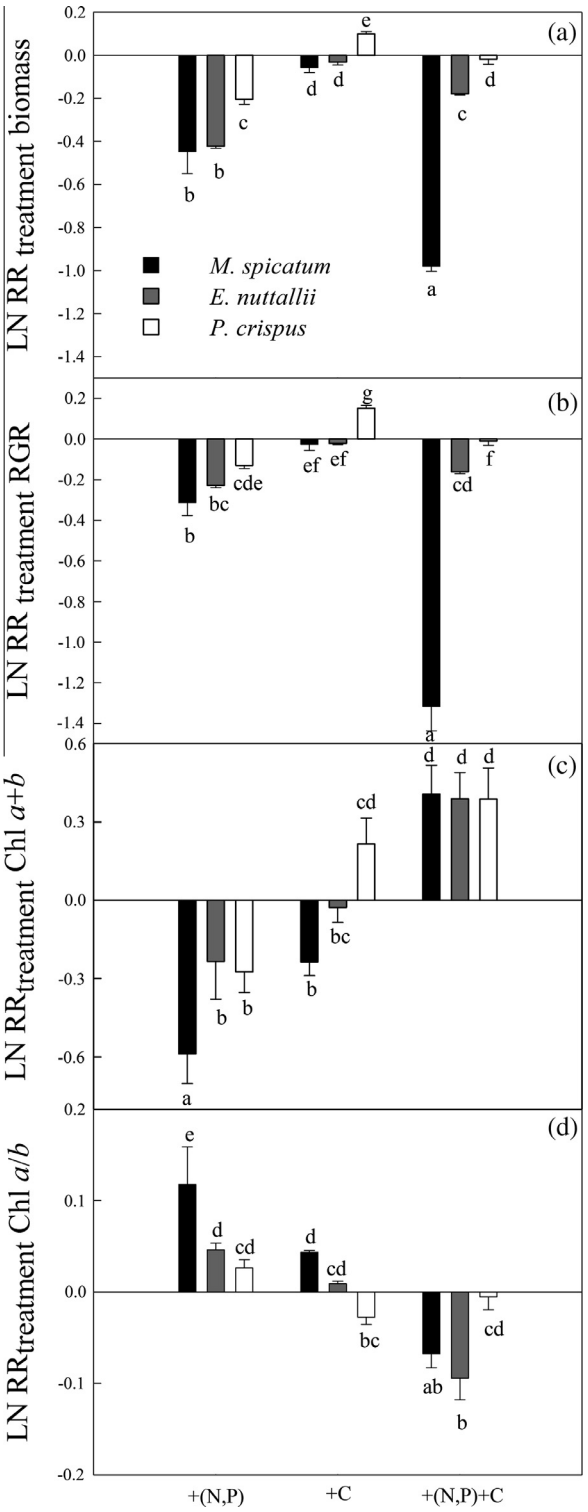

Fig. 2. Differences in the natural-log of response ratio of biomass (ln $RR_{treatment}$ biomass) (a), RGR (ln $RR_{treatment}$ RGR) (b), chl *a* + *b* (ln $RR_{treatment}$ chl *a* + *b*) (c) and chl *a*:*b* (ln $RR_{treatment}$ chl *a*:*b*) (d) of the three macrophytes upon the nutrient and/or bicarbonate treatments ($n = 6$). Bars sharing the different letters indicate significant differences among the treatments (Duncan test).

submerged aquatic vegetation" and highlighted the importance of bicarbonate concentration in water, which can influence the effect of eutrophication (e.g., low light condition induced by phytoplankton/epiphyton growth) on submerged macrophytes. Furthermore, our result that bicarbonate enrichment favored *P. crispus* compared with *M. spicatum* and *E. nuttallii* against eutrophication in a controlled experiment, indicating the species-specific traits (e.g., leaf morphology) of submerged macrophytes may change the outcome of macrophyte–phytoplankton competition in freshwater ecosystems.

Nutrient enrichment has a complex effect on the water column by altering the outcomes of biotic interactions. Nutrient enrichment in water column resulted in a substantial burst of phytoplankton growth (phytoplankton chl *a* $39 \pm 1\ \mu g\ L^{-1}$ at last harvest) and therefore increased light attenuation. Bicarbonate enrichment itself did not cause phytoplankton growth (maybe due to the nutrient deficiency, Kosten et al., 2011), but enrichment of both nutrient and bicarbonate resulted in a heavy phytoplankton state (phytoplankton chl *a* $55 \pm 2\ \mu g\ L^{-1}$ at last harvest). In both the +(N,P) and +(N,P)+C conditions, the growth performances (RGR and biomass) of all three macrophyte species were strongly affected. These results were consistent with previous studies showing that an increased nutrient loading, which subsequently increased the growth of phytoplankton/epiphyton and light attenuation (Falkowski and Raven, 2007; Le Bagousse-Pinguet et al., 2012), could inhibit the growth of submerged macrophytes (Asaeda et al., 2004; Arthaud et al., 2012). However, the depth of our mesocosm is relatively shallow (60 cm), which may not reflect the light environment in the natural water bodies. Furthermore, the influences of nutrient and bicarbonate on both DO and pH values may reflect increasing biological activity. On one hand, high phytoplankton growth in +(N,P) condition may cause an increase in oxygen production. On the other hand, high phytoplankton growth may also deplete free CO_2 concentration in water column, thereby increasing the pH values (>9 in our experiment) and consequently affecting the photosynthesis and growth of submerged macrophytes (Mvungia et al., 2012).

When light stress was induced by phytoplankton growth in +(N,P) condition, growth of submerged macrophytes in our study was severely hindered due to reduction in photosynthesis, indicating a significant negative impact of nutrient loading on these macrophytes, especially *M. spicatum*. Extended light stress can cause degradation of photosynthetic pigments and enzymes in submerged macrophytes (Brouquisse et al., 1998) and subsequently inactivate the PSII reaction center through decreased efficiency of primary quinone electrons acceptors.

Besides phytoplankton, other microorganisms such as epiphyton, developing on the leaves of macrophytes also played a crucial role at reducing photosynthesis in our experiment. Aquatic microorganisms may create a microfilm on leaves of macrophytes and cause erosion and peeling of leaf cuticular (Sand-Jensen, 1977; Howard-Williams and Davies, 1978). The abundance of epiphyton would lead to a decrease in the strength of the specific wavelength light needed by chl *a* in the macrophytes, which would force these macrophytes to compensate chl *a*, thereby increasing the chl *a*:*b* subsequently at least for a short-period (Chen et al., 2007). It is also well known that dissected-leaf species (e.g., *Myriophyllum* spp.) may suffer more from the effects of epiphyton than lanceolate-leaf species (e.g., *Potamogeton* spp.) (Hinojosa-Garro et al., 2010). Consistent to this, *M. spicatum* exhibited a greater reduction in photosynthesis and growth than *E. nuttallii* and *P. crispus* in +(N,P) condition in our experiment.

Bicarbonate usage can increase the availability of inorganic carbon, leading to positive net photosynthesis in submerged macrophytes (Maberly and Madsen, 1998; Pagano and Titus, 2007). Two macrophyte species in our study (*M. spicatum* and *E. nuttallii*) had reduced photosynthetic performances in +C condition compared with the control condition, partly consistent with previous studies that the reduction of bicarbonate uptake rates was responsible for the reduced photosynthetic rates in high bicarbonate concentration environment (Madsen et al., 1996; Cavalli et al., 2012).

Table 2
F-values for effects of treatment (*T*) and species (*S*) on natural-log of response ratios of growth performances (ln $RR_{treatment}$ biomass, ln $RR_{treatment}$ RGR, ln $RR_{treatment}$ chl *a* + *b* and ln $RR_{treatment}$ chl *a*:*b*) and photosynthetic performances (ln $RR_{treatment}$ F_v/F_m, ln $RR_{treatment}$ $rETR_{max}$, ln $RR_{treatment}$ α and ln $RR_{treatment}$ E_k) (Three-way ANOVA).

	d.f.	ln $RR_{treatment}$ biomass	ln $RR_{treatment}$ RGR	ln $RR_{treatment}$ chl *a* + *b*	ln $RR_{treatment}$ chl *a*:*b*
Growth performances					
Treatment (*T*)	2, 45	98.31***	92.76***	42.22***	32.98***
Species (*S*)	2, 45	107.15***	110.01***	4.84*	4.94*
T × *S*	4, 45	45.08***	61.60***	1.91ns	6.30***
	d.f.	ln $RR_{treatment}$ F_v/F_m	ln $RR_{treatment}$ $rETR_{max}$	ln $RR_{treatment}$ α	ln $RR_{treatment}$ E_k
Photosynthetic performances					
Treatment (*T*)	2, 45	24.94***	58.43***	22.87***	16.62***
Species (*S*)	2, 45	23.09***	98.02***	55.89***	13.15***
T × *S*	4, 45	4.19**	35.83***	14.06***	18.10***

All data were transformed to log(*x*).
* $0.01 < P < 0.05$;
** $P < 0.01$;
*** $P < 0.001$;
ns non-significant ($P > 0.05$).

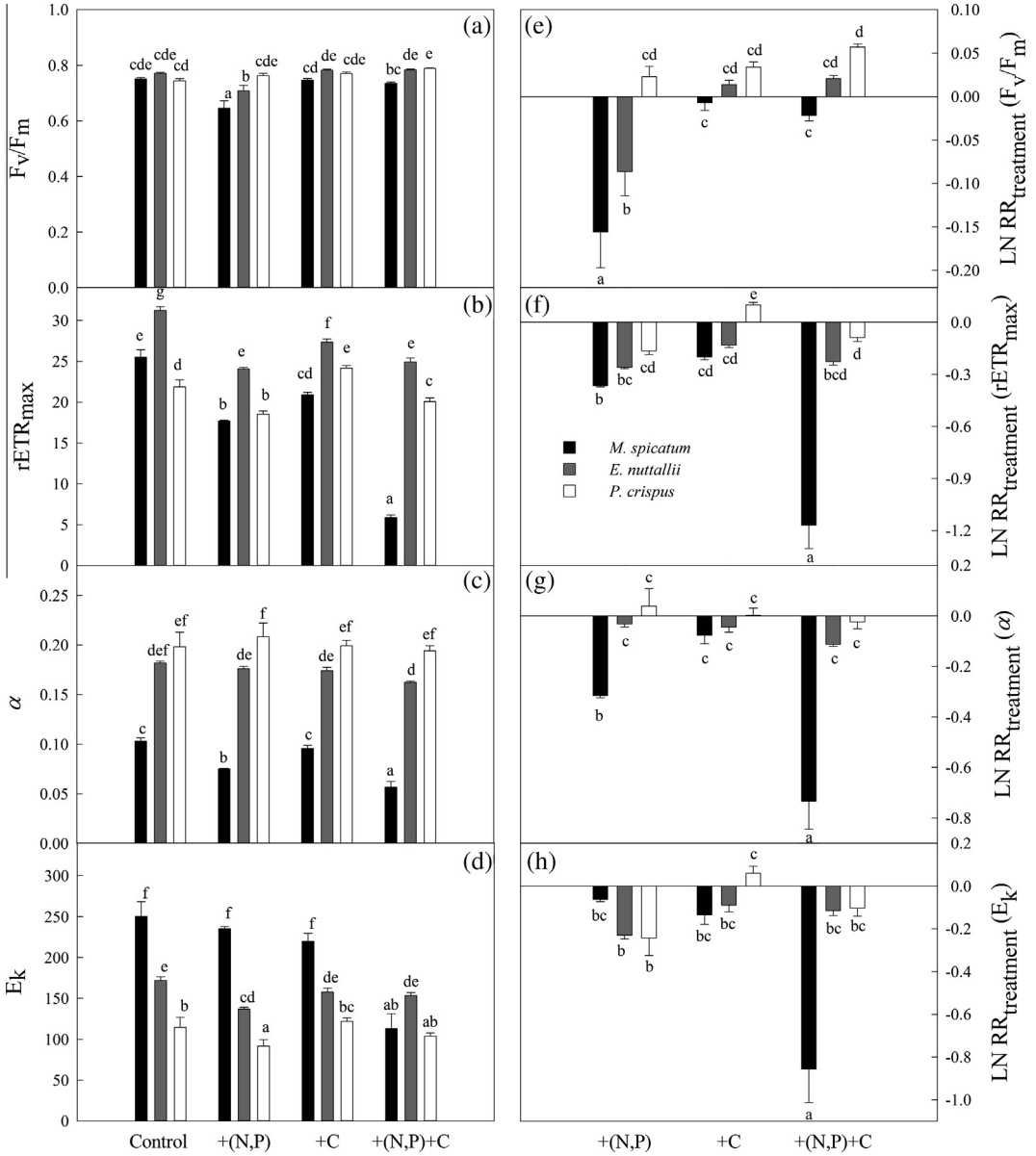

Fig. 3. Differences in photosynthetic performances: F_v/F_m (a), $rETR_{max}$ (b), α (c), E_k (d); and the natural-log of response ratio of these photosynthetic performances: ln $RR_{treatment}$ F_v/F_m (e), ln $RR_{treatment}$ $rETR_{max}$ (f), ln $RR_{treatment}$ α (g) and ln $RR_{treatment}$ E_k (h), of the three macrophytes upon the nutrient and/or bicarbonate treatments ($n = 6$). Bars sharing the different letters indicate significant differences among the treatments (Duncan test).

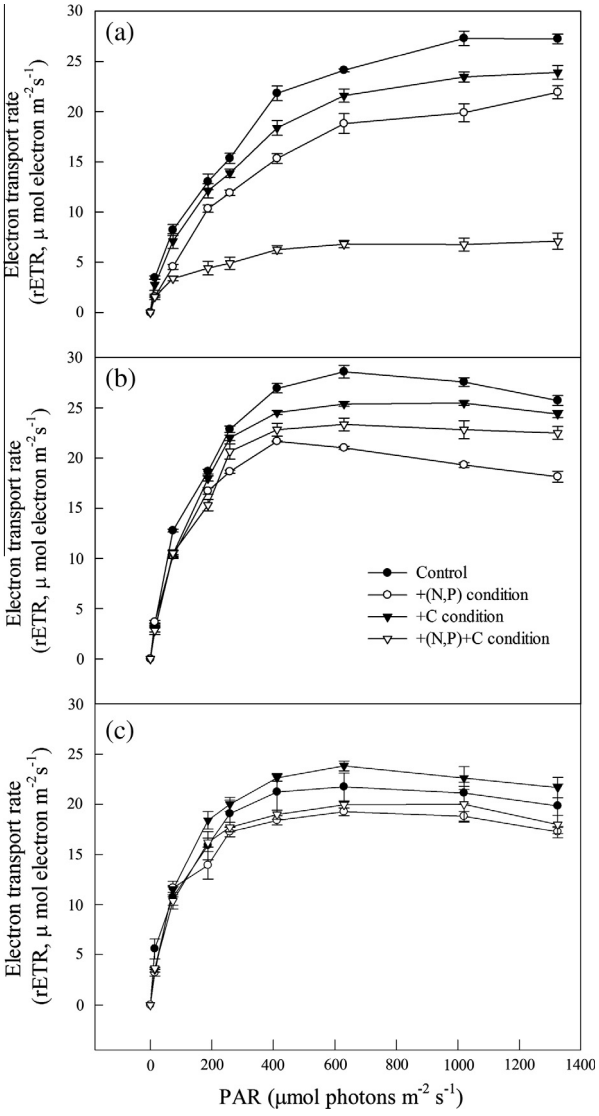

Fig. 4. Relative electron transport rate (rETR) curves for *M. spicatum* (a), *E. nuttallii* (b) and *P. crispus* (c) upon the nutrient and/or bicarbonate treatments. Each curve represents the mean of six replicate samples.

Indeed, enhanced photosynthesis in response to elevated carbon resource may diminish when other factors became limiting (e.g., the availability of nutrients or self-shading) (Kirschbaum, 2011). In the +C condition, although both phytoplankton growth and light stress were low, epiphyton grew rigorously probably due to their ability to directly absorb nutrients from the host plants (Poulíčková et al., 2008). This would accentuate the competition for inorganic carbon between the host plants (i.e., *M. spicatum* and *E. nuttallii*) and epiphyton (Jones et al., 2000, 2002).

DIC and nutrients enrichment may have the potential to affect the community structure of freshwater ecosystems (Moss, 1973; Jones et al., 2002). Our results are consistent with those in the previous studies and also indicated that in a short time period (42 d in our experiment), an increase in bicarbonate concentration would increase the negative impact of the increased nutrient loading on dissected-leaf species (i.e., *M. spicatum* and *E. nuttallii*). Under the +(N,P)+C condition, since the macrophytes were not limited by nutrient availability, an excess of nutrients may accumulate in the leaf tissues, leading to additional carbon expenditure for nutrient metabolism in macrophytes (Cao et al., 2004, 2011; Zhang et al., 2010). Conversely, additional carbon resource (i.e., bicarbonate) could be used only if nutrients were available concurrently. Because such use of bicarbonate in submerged macrophytes would incur considerable physiological cost (Jones, 2005). Similarly, DIC enrichment in water column can also shift the growth conditions of phytoplankton from carbon limitation towards nutrient limitation and change phytoplankton community structure (Moss, 1973; Verschoor et al., 2013). Therefore, the growth of phytoplankton and epiphyton were also stimulated in the +(N,P)+C condition, expressed as low light intensity, which could result the variations in growth and photosynthesis among different species. It is possible that the heavy coating of epiphyton on the leaves of *M. spicatum* and *E. nuttallii* significantly blocks light and reduces the bicarbonate uptake rates of these two macrophytes (Jones et al., 2002). In the long term (450 d), an increase in bicarbonate concentration will cause the increase in the N and P concentrations in natural water bodies due to the acceleration of organic matter decay (Smolders et al., 2006), which will increase the phytoplankton/epiphyton abundance. This trend could alter the competitive outcomes between submerged macrophytes and phytoplankton/epiphyton and eventually cause the loss of submerged macrophytes (Jones et al., 2000, 2002; Arthaud et al., 2012). However, under natural conditions, phytoplankton/epiphyton abundance is also determined by the population densities of grazing invertebrates (Jones et al., 2002). Therefore, further studies will involve resource and biotic interactions (e.g., influence of algae grazer) on both phytoplankton/epiphyton and submerged macrophyte and a long-term perspective should be considered.

5. Conclusions

In this paper, we studied the algae (phytoplankton/epiphyton) mediated growth and photosynthetic responses of three different submerged macrophytes to enriched nutrient and bicarbonate in mesocosms. Our result showed that increase in nutrient and/or bicarbonate loading in water column resulted in a substantial burst of phytoplankton and epiphyton growth and increased light attenuation, which had significant negative impact on growth and photosynthesis performances of the three submerged macrophytes. Our results also demonstrated that at least in a short period (42 d), DIC (i.e., bicarbonate) in water column may increase the negative impact of induced phytoplankton/epiphyton abundance on *M. spicatum* and *E. nuttallii* (two dissected-leaf species) under enriched nutrient condition. In addition, we found that the macrophyte *P. crispus* (a lanceolate-leaf species) outperformed the other two species in macrophyte–phytoplankton/epiphyton competition, highlighting a crucial role of leaf morphology, besides other external forces (e.g., nutrient and DIC), in the success of submerged macrophytes in freshwater ecosystems.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2013.07.008.

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