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RESEARCH ARTICLE

Will elevated atmospheric CO_2 boost the growth of an invasive submerged macrophyte *Cabomba caroliniana* under the interference of phytoplankton?

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Abstract The growth of most submerged macrophytes is likely to be limited by the availability of carbon resource, and this is especially true for the obligatory carbon dioxide (CO_2) users. A mesocosm experiment was performed to investigate the physiological, photophysiological, and biochemical responses of *Cabomba caroliniana*, an invasive macrophyte specie in the Lake Taihu Basin, to elevated atmospheric CO_2 (1000 μmol mol^{-1}); we also examined the possible impacts of interferences derived from the phytoplankton proliferation and its concomitant disturbances on the growth of *C. caroliniana*. The results demonstrated that elevated atmospheric CO_2 significantly enhanced the biomass, relative growth rate, and photosynthate accumulation of *C. caroliniana*. *C. caroliniana* exposed to elevated atmospheric CO_2 exhibited a higher relative maximum electron transport rate and photosynthetic efficiency, compared to those exposed to ambient atmospheric CO_2. However, the positive effects of elevated atmospheric CO_2 on *C. caroliniana* were gradually compromised as time went by, and the down-regulations of the relative growth rate (RGR) and photosynthetic activity were coupled with phytoplankton proliferation under elevated atmospheric CO_2. This study demonstrated that the growth of *C. caroliniana* under the phytoplankton interference can be greatly affected, directly and indirectly, by the increasing atmospheric CO_2.

Keywords Elevated atmospheric carbon dioxide · Relative growth rate · Biochemical · Photophysiological · Carbon fertilization

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Introduction

The dominant drivers for the ongoing increasing atmospheric carbon dioxide (CO_2) emissions are fossil fuel combustion and land use changes (Intergovernmental Panel on Climate Change (IPCC) 2013). Due to these activities, the atmospheric CO_2 concentration has increased by more than 41% over the past 250 years and reached 399.4 μmol mol^{-1} in 2015 (Dlugokencky and Tans 2016). According to the IPCC (2013), the atmospheric CO_2 concentration is expected to range between 421 and 936 μmol mol^{-1} in the 2100 s under the representative concentration pathway (RCP) scenarios. The rising atmospheric CO_2 concentration will probably result in an elevated CO_2 partial pressure of freshwater (Hasler et al. 2016), which is often accompanied by an increased availability of dissolved carbon dioxide (DIC, which includes H_2CO_3, HCO_3^-, and CO_3^{2-}) and a reduced pH (Schippers et al. 2004; Schellnhuber et al. 2006; Deng et al. 2013). A strong adaptive capacity to environmental changes will be crucial for the growth and development of aquatic plants. A better understanding of how the invasive macrophyte *Cabomba caroliniana* (obligatory CO_2 user) will respond to the ongoing increasing atmospheric CO_2 loading induced by industrialization and urbanization is, therefore, needed.

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CO_2 is an essential substance involved in photosynthesis by primary producers and plays an important role in the growth, distribution, and development of submerged macrophytes (e.g., Bagger and Madsen 2004; Hu et al. 2011). CO_2 concentrations in most lakes are often oversaturated due to catchment inputs (Cole et al. 1994; Jansson et al. 2012; Maberly et al. 2013) or the release of internal carbon loading (Cole et al. 1994). However, DIC still fluctuates from approximately 0 to ≥ 5 mmol L^{-1} (Madsen and Sand-Jensen 1991; Maberly 1996). Due to the high diffusion resistance and the relative low amount of bioavailable carbon (Olesen and Madsen 2000), the supply of photosynthetic inorganic carbon varied notably in natural aquatic environments (Vadstrup and Madsen 1995; Jones et al. 1996; Sand-Jensen and Gordon 1984). Many submerged macrophytes have developed strategies, utilizing HCO_3^- for example (Pierini and Thomaz 2004; Dou et al. 2013), to adapt the notable variations in carbon availability. The ability to use HCO_3^- is not an indispensable life skill possessed by all the submerged plants (e.g., *C. caroliniana*) (Sand-Jensen 1983; Sand-Jensen and Gordon 1984; Maberly and Madsen 1998; Bagger and Madsen 2004; James 2011; Mendonça et al. 2013; Maberly et al. 2015; Yin et al. 2016). The plants that could use only CO_2 could suffer from more severe carbon limitation than the plants could utilize free CO_2 and HCO_3^- simultaneously (Olesen and Madsen 2000; Pedersen et al. 2013; Hussner et al. 2016). Natural habitats cannot provide sufficient carbon sources to support submerged macrophyte growth (Maberly and Spence 1983; Sand-Jensen and Gordon 1984), and elevated atmospheric CO_2 may ameliorate the impacts of CO_2 limitation on the photosynthesis and the productivity of submerged macrophytes, especially the obligate CO_2 users (Olesen and Madsen 200; Dhir 2015).

Plant responses to elevated atmospheric CO_2 often interacted with phytoplankton and other related disturbances. Increased DIC availability also favors phytoplankton proliferation, which can compromise the positive effects of CO_2 enrichment on plant growth (Xie et al. 2013; Burnell et al. 2014). This is partially regulated by phytoplankton density and biomass. Algae cells can contribute up to 13–17% of the light attenuation of the underwater climate in aquatic ecosystems (Fleming-Lehtinen and Laamanen 2012). Light regime fluctuations, benefited phytoplankton on gaining a relatively competitive advantage over submerged macrophytes, frequently led to the loss of submerged macrophytes (Sayer et al. 2010; Olsen et al. 2015; Phillips et al. 2016). Furthermore, submerged macrophytes may respond differently to varying amounts of available DIC when combined with interference from phytoplankton in the context of the ongoing climate change (Schippers et al. 2004; Bornette and Puijalon 2011; Maberly et al. 2015).

C. caroliniana typically thrives in acidic, slow-flowing water at an optimal growth temperature of 13–27 °C (Matthews et al. 2013). This species, with notable advantages in self-reproduction and habitat exploitation competition, has been defined as a persistent and competitive nuisance in parts of Europe and other areas around the world (Ding et al. 2007; Hogsden et al. 2007; Schooler et al. 2006; Wilson et al. 2007; Bickel 2012; Matthews et al. 2013). *C. caroliniana* is currently invading rivers, ponds, and lakes in eastern China and, consequently, dominates the macrophyte community in these areas (Yu et al. 2004; Jin et al. 2005; Ding et al. 2007; Hou et al. 2012; Zhang et al. 2013). Numerous studies have been carried out to investigate the distribution, environmental remediation, or ecological impacts of *C. caroliniana* (Yu et al. 2004; Jacobs and Macisaac 2009; Hou et al. 2012; Matthews et al. 2013; Bickel 2017). However, only a few studies have been conducted to estimate the potential effects of climate changes on the growth, development, and range expansion of *C. caroliniana* (Close et al. 2012; Trahan-Liptak and Carr 2016). Specifically, how *C. caroliniana* will respond to elevated atmospheric CO_2 is still largely unknown. The main objective of this study was to evaluate the combined effects of elevated atmospheric CO_2 and CO_2-induced phytoplankton proliferation on the growth of *C. caroliniana*. We hypothesized that elevated atmospheric CO_2 could substantially stimulate the growth of *C. caroliniana*, and we further expected that stimulations of elevated atmospheric CO_2 on the growth of *C. caroliniana* would be compromised by the CO_2-induced phytoplankton proliferation.

Materials and methods

Cultivation system

The experiment was performed in an improved artificial semi-closed cultivation system (Fig. 1a) containing three subsystems: a gas supply system, a gaseous transportation system, and a culture system. The gas supply system was applied to provide elevated atmospheric CO_2 produced by a CE100C-3 CO_2 enricher (Wuhan Ruihua Instrument and Equipment Co., LTD., Wuhan, China), while ambient CO_2 was supplied by an electromagnetic air pump (Sensen Group Co., Ltd. Zhejiang, China) connected to outside fresh air. The concentration of atmospheric CO_2 in the outflow of the enricher fluctuated slightly with a precision of ± 5%, and the CO_2 concentration variation could be further decreased by injecting the gas into an air bag (50 L) with an extended retention time. The gaseous transportation system transmitted the enriched CO_2 from the bag to the semi-closed culture system through an air tube with an inner diameter of 6 mm. Meanwhile, two gas flow meters (Tianchuan Co., LTD., Shanghai, China) were applied to maintain the CO_2 input at a stable rate of 1.5 L/min (Fig. 1a). A PVC pipe, with holes drilled at the interval of 30 mm, was placed 300 mm above the water surface to serve

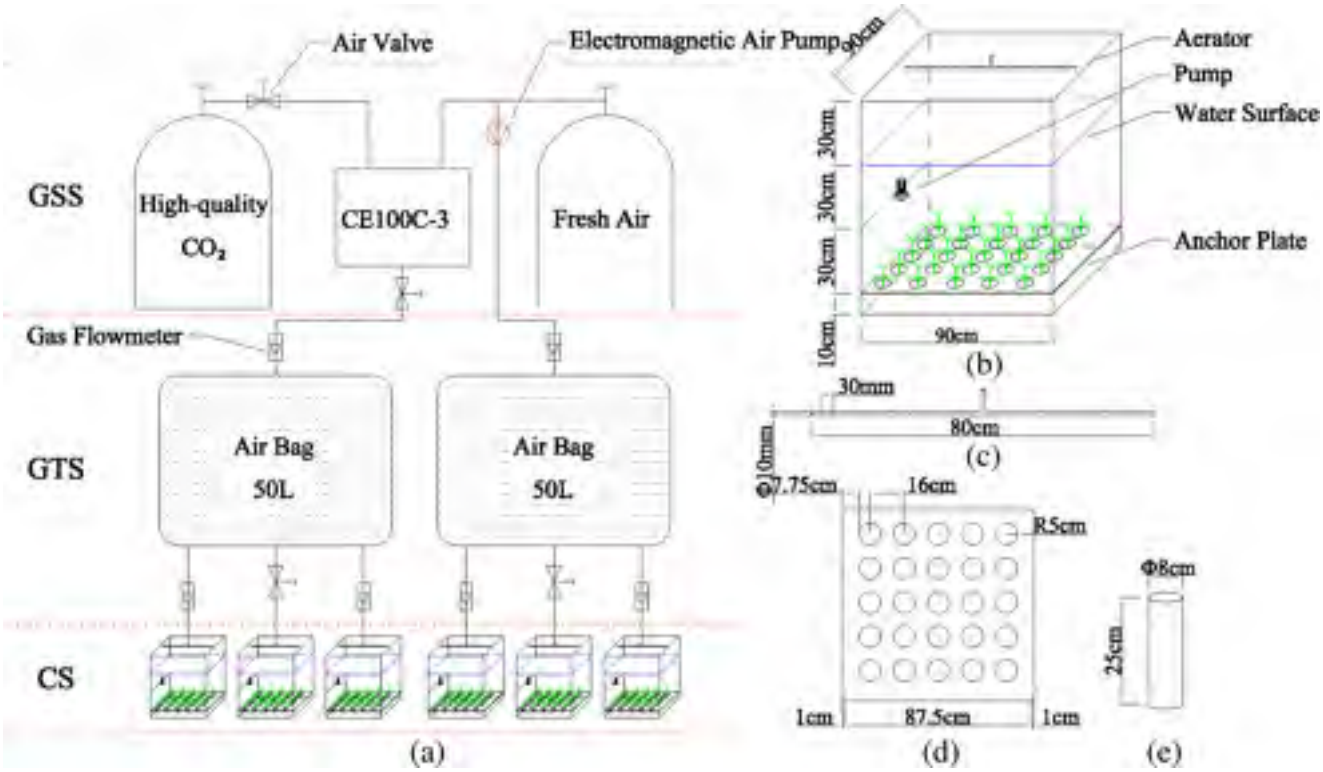

Fig. 1 Schematic diagram of the cultivation system. GSS, GTS, and CS represent gas supply system, gaseous transportation system, and culture system, respectively (**a**). **b** Semi-closed Plexiglas chamber. **c** An aerator. **d** An anchor plate. **e** Cylindrical bucket

as an aerator (Fig. 1c). Each Plexiglas chamber of the culture system (900 × 900 × 1000 mm ($L \times W \times H$)) (Fig. 1b) contained 24 cylindrical buckets (Fig. 1e) fixed to an anchor plate (Fig. 1d); a total of six chambers were built. The buckets provided relatively independent growth units for the plants and also facilitated the exchange of plant positions while concomitantly minimizing disturbances. The buckets were randomly assigned to the anchor plate and repositioned every 2 weeks to avoid position effects within a chamber. A pump, fixed to the chamber side (30 cm underwater), was used to reinforce the water column circulation and concurrently lessen the adverse effects of periphyton on the growth of *C. caroliniana*.

Plant material and the mesocosm experiment

C. caroliniana was collected from the East Lake Taihu Bay in the nearshore of Dongshan, a suburban area of Suzhou City. Since *C. caroliniana* preferred to grow in a slow-moving or stagnant water bodies (Bickel 2017), sundries and apparent epiphytes that adhered to the *C. caroliniana* leaves were removed before transplanting. Rootless apices were transferred to temporary dishes and pre-cultured with tap water for 2 days. The apices were chosen based on biomass (4.2 ± 0.79 and 4.5 ± 0.93 g for ambient and elevated atmospheric CO_2, respectively) and length (30 ± 1 cm). The culture system was directly and constantly exposed to different concentrations of atmospheric CO_2: ambient and elevated atmospheric CO_2 (1000 μmol mol^{-1}). The elevated atmospheric CO_2 concentration was chosen to simulate the projected atmospheric CO_2 concentration under the most extreme scenario RCP8.5 (representative concentration pathways, RCPs for short) with the highest greenhouse gas emissions (Moss et al. 2010; Riahi et al. 2011), according to the Fifth Assessment Report (AR5) of the IPCC (2014). The CO_2 concentration was monitored every day in each chamber, using a portable infrared CO_2 detector with a precision of less than ± 3% (Testo 535, Testo Instruments Corp., Lenzkirch, Germany; 0–10,000 μmol mol^{-1}). The mean atmospheric CO_2 concentrations were 391.2 ± 12.6 and 883.5 ± 34.5 μmol mol^{-1} for the ambient and elevated atmospheric CO_2 groups, respectively. Additionally, 1.0 mg L^{-1} N and 0.05 mg L^{-1} P were added weekly to ensure that the basic requirements for the growth of the plant and phytoplankton were met.

In order to simulate the real situations in the field, phytoplankton was not excluded from the culture systems with a mean biomass of approximately 2.00 ± 0.10 μg L^{-1} (expressed as chlorophyll *a* concentration) prior to the incubation experiment. The coexistence of macrophytes and phytoplankton is a common phenomenon in natural ecosystems. It

is reasonable and necessary to evaluate the potential effects of phytoplankton growth on the responses of *C. caroliniana* to different atmospheric CO_2 concentrations.

The experiment was conducted in a glass greenhouse at Dongshan Observation Station, which is located in East Taihu Lake (31° 2 ′ 1.08″ N, 120° 25′ 18.37″ E). The experiment lasted for 58 days from September 6 to November 3, 2015. Triplicates were run for each treatment, i.e., with a total of six chambers. Chambers 1–3 and chambers 4–6 were used for the elevated and ambient atmospheric CO_2, respectively. The rootless apices chosen were transplanted into the cylindrical buckets, and 24 buckets were randomly placed to the anchor plate of each chamber. A total of 144 plants (12 plants each chamber) were cultured.

Water quality and carbon

All the measurements including water temperature (WT), pH, turbidity, and water sample collections were performed at a depth of approximated 30 cm underwater. Observations were conducted to monitor the dynamics of water quality during the fastest growth of plant and phytoplankton on day 0 (D0), day 9 (D9), and day 14 (D14). Subsequent observations were conducted weekly for the determination of relationships between water quality and the growth of plant and phytoplankton. As Maberly (1996) investigated the amplitude of variations in pH and carbon chemistry parameters exposed to different CO_2 concentrations, hence, the carbon parameters were determined weekly to reflect the carbon dynamics under the consideration of the long growth period of *C. caroliniana*. The WT was automatically monitored every 20 min with a self-contained instrument (HOBO Pendant Temperature Loggers, Onset Corp., Bourne, MA, USA). The pH and water column turbidity were determined at 7:30 a.m. using a Hydrolab multiprobe (Hach/Hydrolab, Loveland, CO, USA), facilitating the comparisons of different treatment groups. The WT was automatically monitored every 20 min with a self-contained instrument (HOBO Pendant Temperature Loggers, Onset Corp., Bourne, MA, USA). The pH and water column turbidity were determined at 7:30 a.m. using a Hydrolab multiprobe (Hach/Hydrolab, Loveland, CO, USA), facilitating the comparisons of different treatment groups. Unfiltered water samples were digested with alkaline potassium persulfate to determine total nitrogen (TN) and total phosphorous (TP) using an ultraviolet spectrophotometer (TU-1810PC, Purkinje General Instrument Co., LTD., Beijing, China) (Jin and Tu 1990). The DIC content was determined with a TOC-L CPH carbon analyzer (Shimadzu, Japan), while carbon species were calculated from the DIC, pH, and the temperature-corrected equilibrium constants k_1 and k_2, following the methods detailed in Hu et al. (2011) and Stumm and Morgan (2013).

Plant growth

Nine out of 12 plants (three replicates in each chamber) were randomly selected to evaluate growth status on an established schedule on the 16th day (D16), 26th day (D26), 43rd day (D43), and 58th day (D58) from the initiation of the experiment. To minimize the influence of the initial status of the plant and to determine biomass accumulation over a specific period of days (Hunt 2012; Bankaji et al. 2016), the initial and time-dependent (t) biomasses (as fresh weight) were measured to calculate the relative growth rate (RGR), using Eq. (1):

$$\mathrm{RGR}\left(\mathrm{day}^{-1}\right) = \frac{\mathrm{Ln}(\mathrm{FW}_t) - \mathrm{Ln}(\mathrm{FW}_0)}{\Delta T} \quad (1)$$

where FW_0 and FW_t are the fresh weights of the *C. caroliniana* at times of $t = 0$ and t in days, respectively (e.g., Lapointe 1981; Xu et al. 2015).

Biochemical analysis

The variations in total soluble protein and total soluble sugar could be applied to trace the status of plant growth and development (Boriboonkaset et al. 2013). The apices of *C. caroliniana* were harvested for the determination of total soluble protein and total soluble sugar. Approximately 0.5 g of fresh leaves was homogenized in 5 ml of precooled normal saline, which was submersed in an ice-water mixture during the process. After centrifugation (3000 rpm for 20 min), the total soluble protein in the supernatant was determined with a spectrophotometer based on the method proposed by Bradford (1976). The total soluble sugar was extracted from 0.2 g of fresh leaves in a boiling water bath for 30 min, according to the anthrone-sulfuric acid colorimetric assay methodology (Yemm and Willis 1954). The total soluble protein and total soluble sugar contents were analyzed in triplicate for each chamber and reported as milligrams per gram fresh weight (FW).

Rapid light curves

Photosynthetic potential of *C. caroliniana* exposed to ambient and elevated atmospheric CO_2 were evaluated using rapid light curves (RLC) by a Diving-PAM (Walz, Effeltrich, Germany) during the different growth periods. The determination of RLC in *C. caroliniana* was collected between 8:00 and 11:00 a.m. This time period offers the most stable RCL results facilitating the comparisons between different treatments and growth periods, since *C. caroliniana* recovered fully from the short-term history of environmental conditions and adapted sufficiently to the instantaneous light conditions. Healthy leaves growing approximately 4 cm from the canopy were selected and then exposed to nine programmed actinic

light levels, ranging from 0 to 1344 μmol photons m^{-2} s^{-1} on D16, D26, and D43 and from 0 to 575 μmol photons m^{-2} s^{-1} on D58. The selected leaves were shaded by a purpose-built 90° acrylic clip for less than 10 s before the RLC determination, ensuing that no change has happened to the current light acclimation state in the PSII reaction centers (Ralph and Gademann 2005). Each period of actinic light exposure lasted for 10 s, and 20-s interval was set between two adjacent actinic light levels. The actinic light intensities and the corresponding relative electron transfer rate (rETRs) were fitted to generate the RLCs, according to Platt et al. (1981) and Ralph and Gademann (2005). The rETR was derived from the effective quantum yield (Y), the incident irradiance (PAR), a constant that assumes that light is harvested equally by the photosystems, and the fraction of incident light absorbed by leaf (AF), using the formula: rETR = $Y \times PAR \times 0.5 \times AF$. The photosynthetic efficiency (α) and the maximum relative electron transport rate ($rETR_{max}$) derived from the RCLs enabled us to compare the variations of the photosynthetic performance of *C. caroliniana* under different harvests. The α could be applied to estimate the light harvesting efficiency while the $rETR_{max}$ to estimate the capacity of the photosynthetic apparatus to utilize the absorbed light energy (e.g., Belshe et al. 2007; Jiang et al. 2010).

Phytoplankton growth

The phytoplankton biomass was expressed as the concentration of chlorophyll *a* (Chla) (Asaeda et al. 2004). Two hundred-milliliter water samples from each replicate were filtered through a GF/F glass fiber filter (0.7 μm, Whatman, UK) to determine the Chla concentration. The residue on the filter was extracted in 5 ml of an 80% ethanol solution for 6–8 h at room temperature in the dark and then analyzed spectrophotometrically (Sartory and Grobbelaar 1984; Chen et al. 2006). At the initial and final stages of the experiment, a few drops of buffered Lugol's solution were added to 1000 ml of each water sample to fix the phytoplankton for at least 24 h, and phytoplankton density was determined by the inverted microscope method according to Utermöhl (1958).

Statistical analyses

All data were analyzed with Microcal Origin software (version 9.2; Microcal Software Inc., Northampton, MA, USA), and *t* tests and paired *t* tests were applied to identify the effects of elevated atmospheric CO_2 on the growth of *C. caroliniana*. The normality of the data and the homogeneity of variances (e.g., RGRs and photosynthetic parameters) were tested using Shapiro-Wilk's test and Levene's tests, respectively, and the p values for the tests were 0.074 and 0.083, respectively, indicating that the data were appropriate for one-way ANOVA. One-way ANOVA were therefore applied to determine the effects of the treatments on the growth of *C. caroliniana*, and if significant differences were detected, multiple comparisons were performed using Tukey's honestly significant difference test. A general least squares linear model was applied to determine the influence of phytoplankton proliferation on the turbidity of the water column and the growth of *C. caroliniana*. A p value < 0.05 in *t* test, one-way ANOVA, and linear regression analyses were reported as statistically significant.

Results

Water quality and general CO_2 results

The results of key water quality parameters were shown in Table 1. The water temperature ranged from 17.99 to 27.89 °C, which was well within the optimal temperature range for *C. caroliniana* growth (Matthews et al. 2013). The pH responded weakly to the elevated atmospheric CO_2 with a slightly lower value of 8.96 ± 0.33, compared to ambient CO_2 (paired *t* test, $p > 0.05$). The pH increased to 9.48 ± 0.16 and 9.31 ± 0.28 in the elevated and ambient atmospheric CO_2 groups, respectively (Table 1). The atmospheric elevated atmospheric CO_2 significantly increased the water column turbidity with a higher averaged turbidity of 10.43 ± 5.60 nephelometric turbidity units (NTU), compared with the chambers exposed to ambient CO_2 (4.25 ± 1.57 NTU) (paired *t* test, $p < 0.01$). The concentrations of TN and TP in the ambient CO_2 treatment were slightly higher than those under the elevated atmospheric CO_2 (1.11- and 1.23-fold for TN and TP, respectively) (Table 1). However, the elevated atmospheric CO_2 significantly enhanced the TP removal rate (1.28-fold, $p < 0.05$) but not for TN (1.03-fold, $p > 0.05$).

The elevated atmospheric CO_2 significantly increased the water column DIC content (paired *t* test, $p < 0.01$) (Table 2) and also slightly increased the averaged H_2CO_3 content with no statistical difference (paired *t* test, $p > 0.05$), compared to the ambient CO_2 treatment. The H_2CO_3 contents fluctuated considerably over time under the elevated atmospheric CO_2 (Table 2). Therefore, we monitored the variations in H_2CO_3 as well as the growth of *C. caroliniana* and found that the enhanced *C. caroliniana* growth led to a notable decrease in H_2CO_3 during the first harvest (D16).

Growth of *C. caroliniana*

Compared with the ambient CO_2 group, the biomass of *C. caroliniana* exposed to elevated atmospheric CO_2 was significantly higher on D16 and D26 (*t* test, $p < 0.001$ for both) but significantly lower on D58 (*t* test, $p < 0.01$). No significant difference was detected on D43 between the ambient and elevated atmospheric CO_2 groups, although the biomass was

Table 1 Water quality in the experimental and control treatments during the experiment (means ± SD)

Treatment	Temperature °C	pH	Turbidity NTU	TN mg L^{-1}	TP μg L^{-1}
Ambient CO_2	24.06 ± 2.56	9.00 ± 0.46	4.25 ± 1.57	2.73 ± 1.07	40.32 ± 20.15
Elevated CO_2	24.25 ± 2.54	8.96 ± 0.33	10.43 ± 5.60	2.45 ± 1.02	32.67 ± 20.43

slightly lower under the elevated atmospheric CO_2 (Fig. 2). The RGR of *C. caroliniana* was significantly affected by the elevated atmospheric CO_2 on D16, D43, and D58 (*t* test, $p < 0.01$ for all three) but not for D26 (*t* test, $p > 0.05$) (Fig. 2). The RGR of *C. caroliniana* exposed to elevated atmospheric CO_2 fluctuated at different harvests in the following order: D16 > D43 > D26 > D58 (one-way ANOVA, $p < 0.01$), while that exposed to ambient CO_2 remained relatively stable growth (one-way ANOVA, $p > 0.05$) (Fig. 2).

Photosynthetic performance of *C. caroliniana*

The maximum relative electron transport rate ($rETR_{max}$) values observed in the elevated atmospheric CO_2 group were significantly higher than those in the ambient CO_2 group at both D16 and D26 (*t* test, $p < 0.001$ and $p < 0.01$, respectively) but were significantly lower at D43 and D58 (*t* test, $p < 0.001$ for both) (Fig. 3). Elevated atmospheric CO_2 also improved the light harvesting efficiency of *C. caroliniana*, which corresponded to a significantly higher photosynthetic efficiency (α) (*t* test, $p < 0.05$) on D16 and D26. Similar to the $rETR_{max}$, the α values observed in *C. caroliniana* were decreased at the subsequent harvests and significantly lower than those exposed to ambient CO_2 (Fig. 3).

Accumulations of total soluble sugar and total soluble protein

The total soluble sugar and total soluble protein contents exposed to different CO_2 concentrations showed continuously increases as the culture time extended (one-way ANOVA, $p < 0.001$ for both); positive effects induced by atmospheric CO_2 enrichment were still detected. Elevated atmospheric CO_2 significantly promoted the accumulations of total soluble sugar and total soluble protein in *C. caroliniana* leaves (Fig. 4). The mean foliar total soluble sugar content in the elevated atmospheric CO_2 group was 1.46 times higher than that in the ambient CO_2 group when all harvests were combined (paired *t* test, $p < 0.05$). A similar situation was observed for the mean foliar total soluble protein content, which showed a 1.54 times higher accumulation (paired *t* test, $p < 0.05$). The positive effects of elevated atmospheric CO_2 on the total soluble sugar and total soluble protein contents were recorded during each harvest time point except for the first one (Fig. 4).

Phytoplankton growth and its impacts

No significant difference was recorded between the initial phytoplankton biomass (expressed by Chla concentration) of the elevated and ambient atmospheric CO_2 groups (*t* test, $p > 0.05$). Elevated atmospheric CO_2 significantly promoted phytoplankton growth, with 2.93 times higher phytoplankton biomass under the elevated atmospheric CO_2 (*t* test, $p < 0.001$) (Fig. 5). Over the four harvests of this study, mean phytoplankton density was significantly higher in the elevated atmospheric CO_2 group (3.01×10^8 cells·L^{-1}), compared to the ambient atmospheric CO_2 group (2.12×10^7 cells·L^{-1}).

The turbidity in the water column increased significantly with the phytoplankton biomass, as indicated by a positive

Table 2 Variations in dissolved inorganic carbon (DIC) and dissolved carbon dioxide (H_2CO_3) during the experiment (means ± SD)

	Ambient CO_2		Elevated atmospheric CO_2	
	DIC (mM L^{-1})	H_2CO_3 (μM L^{-1})	DIC (mM L^{-1})	H_2CO_3 (μM L^{-1})
D0	0.78 ± 0.01	10.01 ± 1.21	0.78 ± 0.01	11.22 ± 0.15
D4	0.84 ± 0.12	8.17 ± 1.64	0.97 ± 0.12	5.70 ± 0.05
D9	1.17 ± 0.24	7.15 ± 0.97	1.25 ± 0.14	5.79 ± 0.21
D14	0.77 ± 0.21	1.84 ± 0.28	0.74 ± 0.16	1.29 ± 0.07
D24	0.86 ± 0.08	1.49 ± 0.17	0.94 ± 0.09	1.66 ± 0.24
D31	0.98 ± 0.05	1.26 ± 0.08	1.04 ± 0.2	2.09 ± 0.34
D38	1.06 ± 0.06	0.85 ± 0.12	1.10 ± 0.06	1.75 ± 0.04
D45	1.00 ± 0.19	1.91 ± 0.21	1.20 ± 0.08	2.40 ± 0.31
D52	1.12 ± 0.12	0.88 ± 0.04	1.20 ± 0.09	1.95 ± 0.04
D58	1.22 ± 0.15	0.95 ± 0.06	1.28 ± 0.41	1.52 ± 0.04

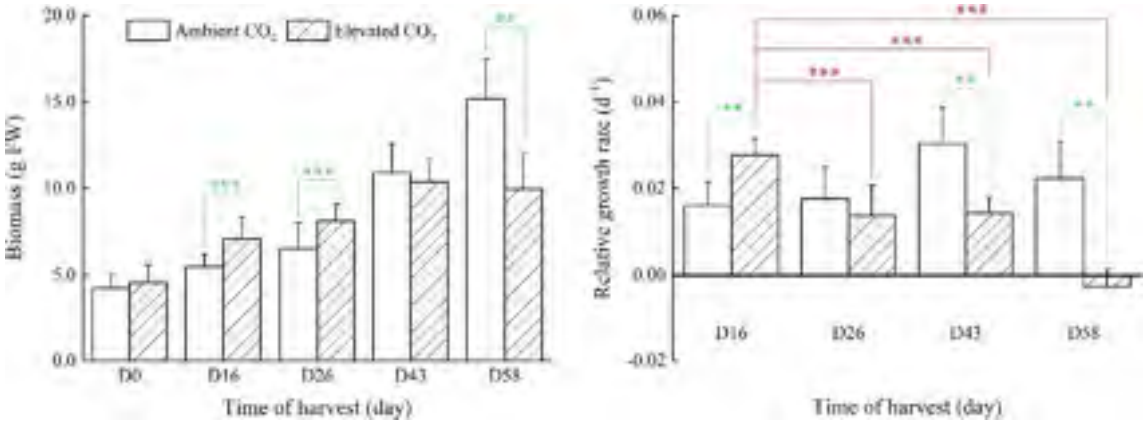

Fig. 2 Variations of biomass accumulation and RGR of *Cabomba caroliniana* exposed to different atmospheric CO_2 (means ± SD). Red refers to the results of one-way ANOVA and green for *t* test. *$0.05 \geq p > 0.01$, **$0.01 \geq p > 0.001$, and ***$p < 0.001$ for the tests of significant differences in *t* test and one-way ANOVA

linear correlation between the phytoplankton biomass and the turbidity ($R^2 = 0.93$, $p < 0.001$). However, the initial growth stage was excluded because the biomasses of both the phytoplankton and the plant were relatively low at this stage and there is no competition for resources including nutrients and light availability. The RGR of the plant was significantly decreased with phytoplankton growth for the elevated atmospheric CO_2 group but not for the ambient atmospheric CO_2 group (Fig. 6).

Discussion

Carbon chemistry

The elevated atmospheric CO_2 have failed to evoke any significant differences in both pH and H_2CO_3 but significantly increased the DIC concentration. Previous studies using various manipulation techniques estimated the potential effects of elevated CO_2 on the growth of aquatic plants and

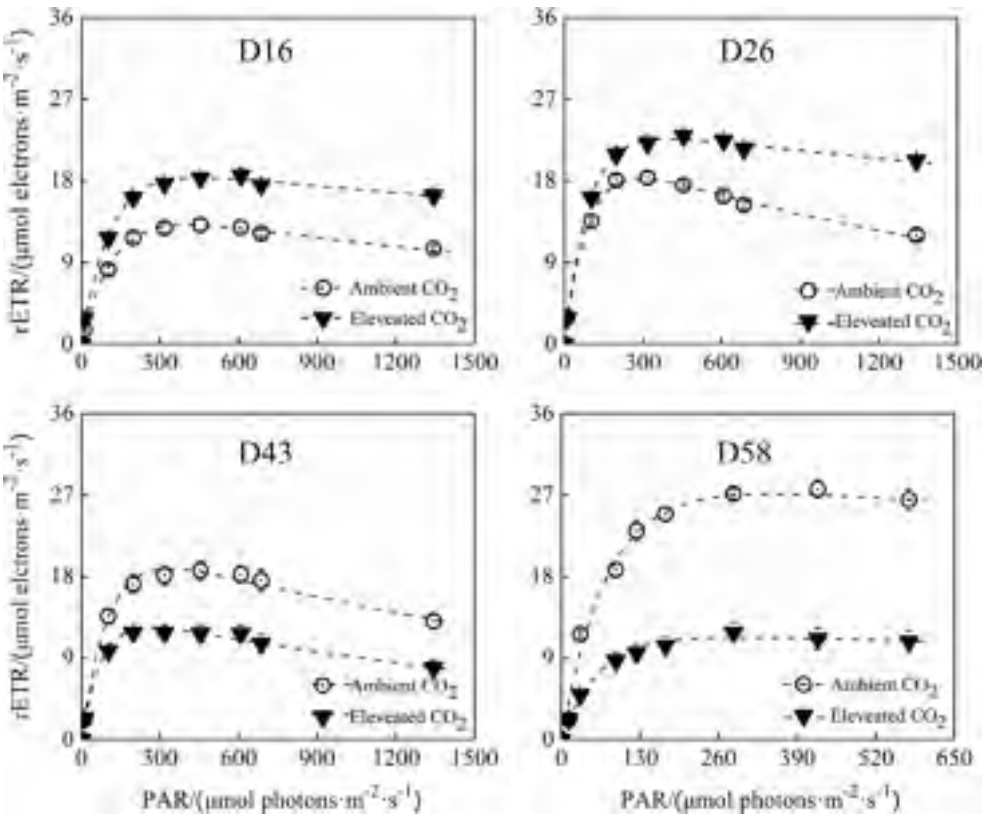

Fig. 3 Variations of rapid light curves in *Cabomba caroliniana* leaves exposed to different CO_2 concentrations. The data are shown as the means ± SE ($n = 9$)

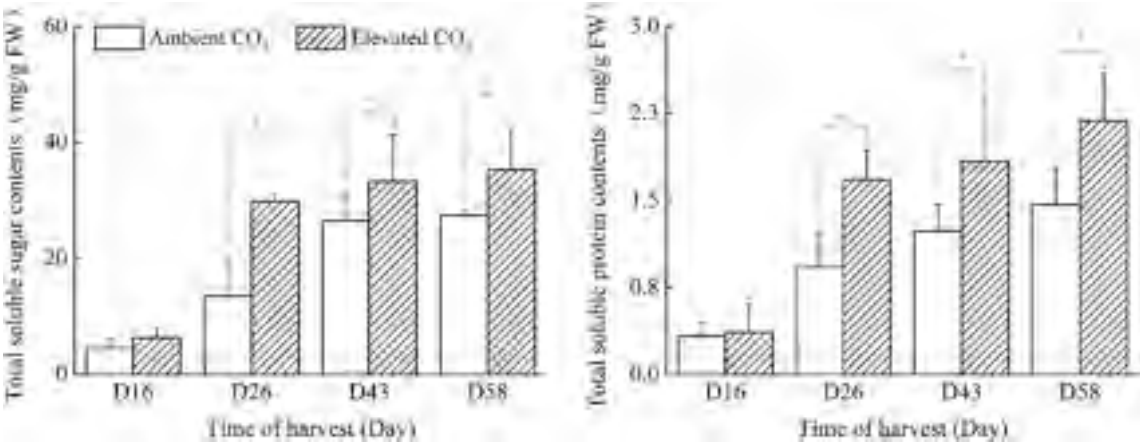

Fig. 4 Variations of the total soluble sugar and protein contents in *Cabomba caroliniana* leaves exposed to different atmospheric CO_2 concentrations during the experiment (means ± SD). Bars followed by asterisk indicate that the differences were significant between treatments ($p < 0.05$). Otherwise, the differences were not significant ($p > 0.05$)

phytoplankton and could be summarized as three pathways: direct CO_2 addition (Hein and Sand-Jensen 1997; Yan et al. 2006; Madsen et al. 1998; Deng et al. 2013), indirect carbonate addition (Riebesell et al. 1993; Burkhardt et al. 1999), and a combination of the aforementioned two (Hussner et al. 2016; Dülger et al. 2017). For example, Hussner et al. (2016) and Dülger et al. (2017) elevated the H_2CO_3 concentration in water column through a combination of carbonate and CO_2 addition. To simulate and assess the effects of predicted elevated atmospheric CO_2 on the growth of *C. caroliniana* under a condition close to natural habitats, artificial regulations on pH and initial addition of carbonate were not used in the present study. Our results of H_2CO_3 concentrations differed largely from the cited studies (Deng et al. 2013; Hussner et al. 2016; Dülger et al. 2017), and direct injection of elevated atmospheric CO_2 only caused a slightly increase of H_2CO_3 content in the water column. The growth of *C. caroliniana* and phytoplankton was closely correlated with the fluctuations of the DIC and H_2CO_3 contents in the water column. Only the H_2CO_3 content decreased notably in all the treatments during the period from D0 to D16, while *C. caroliniana* and phytoplankton experienced the most notable growth with high biomass accumulations. The DIC content, however, fluctuated slightly at the same time. This indicated that a new equilibrium with atmospheric CO_2 might establish concomitant with sufficient supplement of elevated atmospheric CO_2, which could which could replenish and elevate the DIC content in the non-closed culture system (Mook et al. 1974). The release of OH^- and the equivalent consumption of CO_2 and protons during the process of biomass accumulations could lead to the alkalinization of the water body with an increased pH (Pedersen et al. 2013). These indicated that the growth of *C. caroliniana* and phytoplankton played an important role in the fluctuations of pH and the DIC pool (Talling 1976; Morales-Williams et al. 2017).

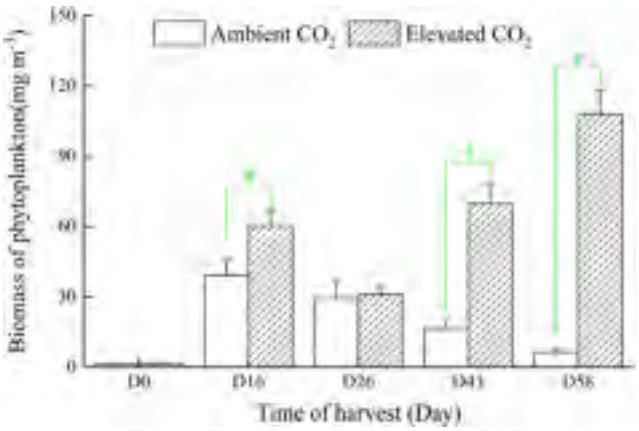

Fig. 5 Variations of the phytoplankton biomass in culture chambers exposed to different atmospheric CO_2 concentrations. The bars represent the means ± SD. Bars followed by asterisk indicate significant differences between treatments ($p < 0.05$). Otherwise, the differences were not significant ($p > 0.05$)

Growth response

Positive effects induced by the elevated atmospheric CO_2 have been observed on the growth of *C. caroliniana* (e.g., biomass; RGR). This indicated that *C. caroliniana* benefitted from the general stimulation of CO_2 enrichment on growth (Deng et al. 2013; Burnell et al. 2014; Cao and Ruan 2015; van Kempen et al. 2016). Our results agree with previous studies that CO_2 enrichment could substantially enhance the growth of aquatic plants with different enhanced ratios (ER) in the terms of biomass. This further suggests that aquatic plants suffered from different carbon limitation extents under ambient CO_2 (Yan et al. 2006; Deng et al. 2013; Cao and Ruan 2015). For *Vallisneria spinulosa*, elevated atmospheric CO_2 (1000 ± 50 μmol mol^{-1}) led to an overall increased biomass

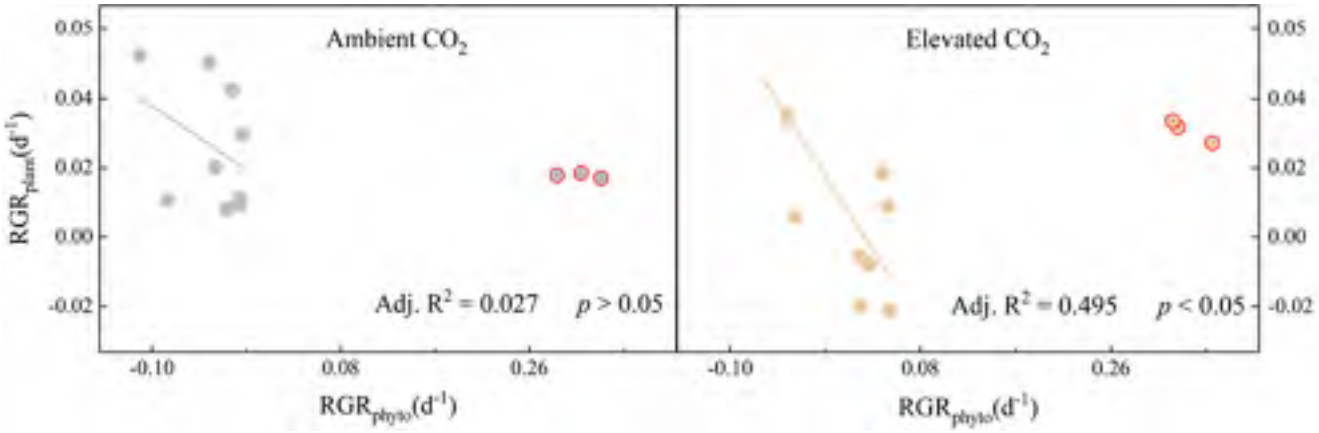

Fig. 6 The relationships of relative growth rate between *Cabomba caroliniana* and phytoplankton exposed to different atmospheric CO_2. The red circle dots are excluded from the linear fitting in both panels. RGR_{plant} represents the RGR of *Cabomba caroliniana*, and RGR_{phyto} represents the RGR of the phytoplankton

with a ER of 130% (Yan et al. 2006), while for the ER of aquatic plants reported by Wetzel and Grace (1983) is approximately 25%. The ER observed in our study is higher than general aquatic plant community but lower than *V. spinulosa*, although the phytoplankton proliferation might dramatically deplete the carbon resources. The difference reflected that *C. caroliniana* suffered from carbon limitation and showed more intense responses to CO_2 enrichment than the general aquatic plant community with the ability to utilize HCO_3^- (Wetzel and Grace 1983; Schippers et al. 2004).

As an obligatory CO_2 user, *C. caroliniana* is likely to function well in photosynthesis at high pH levels with a very low H_2CO_3 availability (Matthews et al. 2013). *C. caroliniana* have possibly developed C4-like metabolism with a reduced carbon compensation point (Saitoh et al. 1970; Salvucci and Bowes 1982; Boston et al. 1989; Matthews et al. 2013). Under a stressful environment, *C. caroliniana* could increase the activities of PEP carboxylase reinforcing one or all the following mechanisms for concentrating carbon: dark fixation of respiratory CO_2, light fixation, and refixation of photorespiratory CO_2 (Salvucci and Bowes 1982). These could reduce the respiratory CO_2 loss and increase the internal CO_2 level enhancing the growth of *C. caroliniana*, and even elevated atmospheric CO_2 only slightly increased the availability of H_2CO_3. This is also substantiated by the increased P removal rate under the elevated atmospheric CO_2 confirming the enhanced nutrient requirement for the photosynthetic and metabolic processes during the vigorous vegetative growth (Cernusak et al. 2010; Lewis et al. 2010). The increase of the RGR in *C. caroliniana* is consistent with the findings observed in *Callitriche cophocarpa*, an obligatory CO_2 user (Madsen et al. 1998; Olesen and Madsen 2000). The inducible C4-like mechanism enabled *C. caroliniana* to maintain a relatively stable photosynthetic rate even under a lowered carbon availability condition, which might be the trigger for the positive responses of *C. caroliniana* to elevated atmospheric CO_2.

Photophysiological and biochemical responses

Exposure to the elevated atmospheric CO_2 significantly improved the photosynthetic performance of *C. caroliniana*. *C. caroliniana* exposed to elevated atmospheric CO_2 showed a significantly higher relative maximum electron transport rate ($rETR_{max}$) and more efficient photosynthesis (higher α) under a limiting light condition, compared to plant exposed to ambient CO_2. This is consistent with previous studies that CO_2 enrichment enhanced the plant growth by transferring more electrons and investing more light energy for carbon fixation (Jiang et al. 2010). These might be explained by the accelerated accumulation of Rubisco and improved ribulose-bisphosphate carboxylase carboxylation efficiency in the presence of elevated atmospheric CO_2 (Jiang et al. 2010; Alexandre et al. 2012). A higher of CO_2/O_2 ratio under elevated atmospheric CO_2 could reinforce carboxylation while suppressing oxygenation (Alexandre et al. 2012), which could lead to the increased photosynthesis characterized by enhanced total soluble sugar and total soluble protein production. These results indicated that *C. caroliniana* could benefit from elevated atmospheric CO_2 loading with an enhanced photosynthesis, suggesting *C. caroliniana* suffered from a continuous carbon limitation in natural habitats.

The enhanced growth observed in *C. caroliniana* could be largely attributed to the accelerated total soluble sugar and total soluble protein accumulations. This is consistent with the biochemical responses of plants to increased CO_2 loadings (Campbell and Fourqurean 2013). The increases of total soluble sugar and total soluble protein in *C. caroliniana* leaves were not completely coupled to an equivalent response in terms of growth, while the continuous increase of total soluble protein contradicted the notion that elevated atmospheric CO_2 usually caused a reduction of total soluble protein content (Sicher and Bunce 1997; Teng et al. 2006; Zhao et al. 2011). In contrast, the observed sustainable increase in total soluble

protein might indicate a large requirement for the production of Rubisco, as Rubisco accounts for up to 50% of the foliar soluble protein (MacIntyre et al. 2000; Lin et al. 2014). The increase in carbohydrates content did not reach or surpass a critical level that would lead to the acclimation of *C. caroliniana* to elevated atmospheric CO_2. The enhanced production of carbohydrates might have an osmoprotectant effect, strengthening the tolerance of the plants to environmental stresses (Rosa et al. 2009; Jiang et al. 2010) rather than acting as a trigger down-regulating the *C. caroliniana* growth.

Recession of carbon fertilization on *C. caroliniana*

The pH, reduced H_2CO_3 availability, and phytoplankton proliferation are likely to collectively weaken *C. caroliniana* response to CO_2 enrichment. This indicated that the decreased RGR do not necessarily imply an acclimation of *C. caroliniana* to elevated atmospheric CO_2 loading. Our results verified the hypothesis that the positive responses of *C. caroliniana* are significantly compromised or negated by phytoplankton proliferation and its concomitant disturbances. The growth trend observed under the elevated atmospheric CO_2 was analogous to the results of Xie et al. (2013) and Burnell et al. (2014). They found that the positive effects of resources enrichment on the growth of opportunistic species might overwhelm the stimulatory effects on plants, limiting the response of plants to CO_2 enrichment.

The combination of elevated atmospheric CO_2 and the addition of nutrients allowed for a robust phytoplankton growth, led to carbon limitation on the growth of *C. caroliniana*. The RGR of *C. caroliniana* decrease significantly with the increase of phytoplankton RGR under the elevated atmospheric CO_2, whereas it was not significant under ambient CO_2. Since dense populations of phytoplankton might greatly deplete HCO_3^- and H_2CO_3 in the culture medium, the release of OH^- and the concomitant consumption of protons during the photosynthesis resulted in increased pH (pH > 9), as supported by Talling (1976). This further exacerbated the growth conditions for the photosynthesis of *C. caroliniana*. The decreases in $rETR_{max}$ and α in *C. caroliniana* exposed to elevated atmospheric CO_2, which substantiated the inhibition and down-regulation of *C. caroliniana* photosynthesis under heavy phytoplankton proliferation (Liang et al. 2006). These conditions suppressed the conversion of light into chemical energy and could lead to the gradual recession of carbon fertilization for *C. caroliniana*.

The growth of *C. caroliniana* was also limited by the light condition due to the phytoplankton proliferation by increasing the turbidity of the water column. Phytoplankton proliferation could substantially reduce light availability through the water column, which served as the primary reason for limiting the growth of submerged plants (Radke and Gaupisch 2005; Kosten et al. 2011; Arthaud et al. 2012; Asaeda and Rashid 2016; Li et al. 2017). High phytoplankton density and biomass notably reduced the light availability accompanied with a high turbidity (10.43 ± 5.60 NTU) under the elevated atmospheric CO_2. This high turbidity exceeded *C. caroliniana*'s optimal light range. *C. caroliniana*, characterized by high light compensation point of 55 μmol m^{-2} s^{-1} (Canfield et al. 1985) can tolerate a very limited turbidity of between 2 and 6 NTU (Radke and Gaupisch 2005; Matthews et al. 2013). Hence, the elevated atmospheric CO_2 also indirectly affected the growth of *C. caroliniana* through the lowered light availability, ascribed to the shading by the CO_2-induced phytoplankton proliferation.

Conclusions

The effects of elevated atmospheric CO_2 on *C. caroliniana* growth depend on the degree of carbon deficiency as well as the capacity of the plant to cope with the carbon-induced disturbances. Our results verified that *C. caroliniana* could benefit from the general stimulation of elevated atmospheric CO_2 on plant growth, but the positive effects were gradually compromised. The phytoplankton proliferation induced by elevated atmospheric CO_2 loading substantially depleted the carbon resources and concomitantly increased pH and increased light attenuation. Reductions in the RGR and photosynthetic capacity of *C. caroliniana* were coupled with phytoplankton proliferation. These biotic disturbances can modify the positive effects of elevated atmospheric CO_2 on plant growth and reduce the capability of *C. caroliniana* to respond to elevated atmospheric CO_2. However, the predicated increasing atmospheric CO_2 associated with the capacity to concentrate carbon may result in an enhanced growth of *C. caroliniana*. This will possibly further amplify its competitive advantages over the native species leading to a decline in the biodiversity of freshwater ecosystems.

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