



Responses of the submerged macrophyte *Vallisneria natans* to elevated CO₂ and temperature

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ABSTRACT: Inorganic carbon and temperature are 2 important factors that regulate the growth of submerged macrophytes. However, experimental evidence regarding the eco-physiological changes that occur in submerged macrophytes in response to elevated CO₂ and temperature is still limited. To investigate how the submerged macrophyte *Vallisneria natans* (Hydrocharitaceae), a common species in the waters of the middle and lower reaches of the Yangtze River, responds to these factors, we conducted a mesocosm experiment using simulated CO₂ elevation (by bubbling CO₂ into experimental water) and ambient temperature warming systems. During the 60 d experiment, CO₂ elevation significantly increased the inorganic carbon concentration in the water column. The warming systems elevated average water temperature by approximately 3°C. The elevation of CO₂ levels significantly enhanced the photosynthetic performance, growth and clonal propagation of *V. natans*. When combined with an increase in CO₂, elevated temperatures also promoted photosynthesis and growth. The individual ramet biomass of *V. natans* decreased with increasing temperature, but only significantly under ambient CO₂ levels. CO₂ elevation increased both stolon elongation and bud number. At elevated CO₂ concentration, more biomass was allocated to the stolons, roots and buds, while less biomass was allocated to the leaves. These results indicate that the eco-physiological responses of *V. natans* should increase its stress tolerance in aquatic plant communities under future spatial and temporal variation in CO₂ levels, however, further research is required.

KEY WORDS: CO₂ elevation · Biomass allocation · Photosynthetic performance · Submerged macrophyte · Warming

INTRODUCTION

Since the first records in 1958, the concentration of CO₂ in the atmosphere has increased at an annual rate of 0.45%, and the present atmospheric CO₂ concentration is >360 ppm (Parry et al. 2007). If no emission reduction measures are taken, the atmospheric CO₂ concentration is estimated to reach 450 ppm by 2040 and 700 ppm by 2100 (Houghton et al. 1996, 2001). According to the IPCC (2001, 2007), the greenhouse effect caused by such high CO₂ concentrations will increase the global average temperature by 1.4 to 5.8°C. Sustained increases in inorganic carbon lev-

els and temperature impact the photosynthesis and carbon balance of plants, thereby influencing their growth, development and functioning (Ahuja et al. 2010, Wang et al. 2012). However, most previous studies have focused on the impacts of elevated atmospheric CO₂ and ambient temperature in terrestrial habitats, while relatively few studies have considered such processes in aquatic habitats (Olesen & Madsen 2000). Although the effects of each factor alone on submerged macrophytes have been studied extensively (i.e. Rooney & Kalff 2000, Madsen & Maberly 2003, Lacoul & Freedman 2006, Pagano & Titus 2007), studies on the influence of the interaction

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between these 2 factors on submerged macrophytes are rare.

Submerged macrophytes are important to aquatic ecosystems and play an important role in regulating the structure and function of these systems (Cook 1990, Bornette & Puijalon 2011). Macrophytes are one of the primary producers in aquatic ecosystems and are an important part of the aquatic food chain (van Donk et al. 1990). Furthermore, the complex habitats created by submerged macrophytes offer food resources and shelter to organisms in and around the water body (Sheldon 1987). The effect of temperature on submerged macrophytes is well known (Madsen & Brix 1997, Rooney & Kalff 2000). Most macrophytes require a temperature higher than 10°C to maintain growth (Lacoul & Freedman 2006). Barko & Smart (1981) studied 3 common submerged macrophytes and demonstrated that they increased production of aboveground biomass as temperature increased, thus increasing their total biomass. *Potamogeton crispus* begins turion production at temperatures above 20°C (Rogers & Breen 1980). You et al. (2013b) found that as temperatures increase, the invasive ability (i.e. production of large stem bases) of *Eichhornia crassipes* increases, with its distribution spreading from southern China into central and northern China.

All submerged macrophytes can directly use the CO₂ dissolved in the water column/sediment pore-water, and some can also use bicarbonate as an alternative carbon source (Spence & Maberly 1985, Sand-Jensen & Gordon 1986, Madsen & Sand-Jensen 1991, Yan et al. 2006, Xie et al. 2013). Submerged macrophytes whose carbon source is restricted to free CO₂ in the water column have a higher affinity for CO₂ than bicarbonate users (Maberly & Madsen 1998, Pagano & Titus 2007). Additionally, increased CO₂ availability enhances the growth of submerged species such as *Vallisneria americana*, *V. spiralis* and *Myriophyllum verticillatum* (Titus & Andorfer 1996, Madsen & Maberly 2003, Nishihara & Ackerman 2007). However, other studies have also shown that utilization of bicarbonate increases when plants are grown under conditions of low CO₂ availability (Sand-Jensen & Gordon 1986, Jones 2005). These results indicate that the inorganic carbon content of water is an important resource for submerged macrophytes, and that macrophytes vary in their ability to exploit carbon sources under inorganic carbon enrichment (Lacoul & Freedman 2006, Bornette & Puijalon 2011, Wang et al. 2012).

The increase of inorganic carbon content in water caused by CO₂ elevation can significantly increase

the photosynthetic rate of submerged macrophytes (Madsen et al. 1996, Zimmerman et al. 1997, Maberly & Madsen 1998, Pagano & Titus 2007). Similarly, temperature can also directly influence the metabolism of submerged macrophytes. For example, elevated temperatures increase photorespiration because of the reduced solubility of CO₂ compared with O₂, and the reduced specificity of rubisco for CO₂ at higher temperatures (Sage & Monson 1999, Wang et al. 2012). However, in contrast to terrestrial plants, the responses of submerged macrophytes to increases in CO₂ and temperature are poorly understood (Wang et al. 2012).

Vallisneria natans (Lour.) Hara (Hydrocharitaceae) is a perennial submerged macrophyte that is widely distributed in the waters of the middle and lower Yangtze River basin in China (Xie et al. 2005, Ke & Li 2006, Cao et al. 2011). *V. natans* can produce stolons, spread horizontally above the sediment surface, and form ramets at the nodes. Each ramet has a basal rosette of leaves which may extend to the surface but does not form a canopy. It is widely used in ecological restoration because of its extensive adaptability, high reproductive capacity and high adsorption capacity for pollutants and nutrients (Ke & Li 2006, Cao et al. 2011). In addition to CO₂, *V. natans* can also use bicarbonate as a carbon resource (Dou et al. 2013). In this study, *V. natans* was used to examine the eco-physiological responses of submerged macrophytes to CO₂ and ambient temperature elevation. Two hypotheses were tested: (1) *V. natans* can adapt to elevated CO₂ levels and temperature via alteration of photosynthetic performance and growth plasticity, and (2) elevated CO₂ levels and temperature promote plant growth and vegetative production.

MATERIALS AND METHODS

Experimental design

In March 2013, similarly sized buds of *Vallisneria natans* were collected from Gonghu Bay (31° 31' N, 120° 16' E) in Taihu Lake, the third largest freshwater lake in China. Upon collection, the buds were placed in aluminum pans filled with sand substratum for sprouting in a laboratory at Nanjing Forestry University in Jurong City, Jiangsu Province, China. After sprouting, 24 plantlets of similar sizes (length: 4.8 ± 0.24 cm; fresh weight: 1.0 ± 0.031 g) were selected as experimental material. To ensure the full development of the creeping stems, one individual plantlet was placed into each mesocosm block (L × W × H =

50 × 30 × 30 cm) containing 8 cm of substratum (sand and clay mixture); the mesocosms were then left *in situ* to acclimate for 1 wk before the experiment began.

The study was conducted at an experimental site of Nanjing Forestry University. The experiment lasted for 60 d, from 21 May until 20 July 2013, which was consistent with the growing season of *V. natans* in eastern China. A 2-way factorial experimental design was employed which included 2 levels of CO₂ (ambient and elevated CO₂) and 2 levels of temperature (ambient and elevated temperature) in 6 blocks, with 1 replicate per block. Elevated CO₂ concentration (1000 ppm) was obtained by bubbling ambient air (CO₂ concentration: 370 ppm) mixed with CO₂ stored in gas cylinders (99.99% purity) (Yan et al. 2006) into a semi-enclosed system; the control treatment was obtained by bubbling ambient air into the system (see Fig. 1 for detailed experimental setup). In both treatments, the air velocity was 4 l min⁻¹, and the gases (both ambient air and air mixed with CO₂) were pumped into the water system. The elevated CO₂ levels significantly increased the free CO₂ and bicarbonate concentrations in the water column, and reduced the pH and CO₃²⁻ concentrations ($p < 0.001$, Table 1). The dissolved inorganic carbon concentrations (CO₂, HCO₃⁻ and CO₃²⁻) in our experiment were consistent with previous *in situ* studies in many lakes in China (590 to 2000 μmol l⁻¹) (Xiao et al. 2007b, Zhai et al. 2007, Zhang et al. 2009). The ambient temperature elevation was simulated using far infrared quartz heating tubes (15 cm from the mesocosm, 2000 W, 12 h d⁻¹). The water temperature was increased by approximately 3°C compared with the control treatment (*in situ* water temperature) during the experimental period. The temperature elevation did not have a significant effect on water pH ($p > 0.05$, Table 1). The treatments were replicated 6 times, for a total of 24 mesocosms. The water depth in each mesocosm was 20 cm (Fig. 1; to minimize the influence of phytoplankton, the water was filtered using a plankton net) (Cao et al. 2012). The physical and chemical characteristics of the water during the

experimental period are given in Table 1. Water quality characteristics were analyzed using Hach® portable water quality meters. The soluble inorganic carbon level was analyzed using a carbon dioxide-selective electrode (Orion™, 9502BNWP, Thermo Scientific). All water quality characteristics (i.e. temperature, pH, dissolved oxygen, turbidity, CO₂, HCO₃⁻ and CO₃²⁻ concentrations) were recorded at noon every third day.

Plant harvest

One week before harvest, the photosynthetic activity of *V. natans* under different treatments was measured using a Diving-PAM fluorometer (Walz), and the data were collected using WinControl software (Walz). Healthy leaves on the second-youngest of the ramets in each daughter plant were chosen and dark-acclimated for 20 min using leaf clips (Diving-LC, Walz) (You et al. 2013a). After the acclimation period, the rapid light curves (RLCs) were measured. The photosynthetic photon flux density (PPFD) was increased in 9 steps (0, 11, 30, 56, 96, 147, 275, 419 and 715 μmol photons m⁻² s⁻¹) over a 90 s period. The relative electron transport rates (rETR_s, calculated as rETR = ΦPSII × PPFD, where ΦPSII is the quantum efficiency of PSII) were used to determine the following photosynthetic parameters: α (photosynthetic efficiency under nonsaturating irradiance), E_k (irradiance at the onset of saturation) and rETR_{max} (maximum rETR to E_k). These parameters were estimated using the following model proposed by Platt et al.

(1980): $P = P_m \left[1 - e^{-\left(\frac{\alpha \text{PAR}}{P_m}\right)} \right] e^{-\left(\frac{\beta \text{PAR}}{P_m}\right)}$, where P_m is the maximum photosynthetic rate, α is the initial slope of rETR–PAR response curve, and β is the slope of the rETR–PAR response curve after saturation. E_k was calculated by dividing P_m (maximum electron rate) by α (Mvungi et al. 2012).

The following characteristics were measured at the time of harvest: ramet number, creeping stem length

Table 1. Water quality characteristics and inorganic carbon concentrations (means ± SE) in the water column during the experimental period

Treatment	Water temperature (°C)	pH	Dissolved oxygen (mg l ⁻¹)	Turbidity (NTU)	[CO ₂] (μmol l ⁻¹)	[HCO ₃ ⁻] (μmol l ⁻¹)	[CO ₃ ²⁻] (μmol l ⁻¹)
Ambient temperature and CO ₂	17.8 ± 0.22	7.92 ± 0.12	5.66 ± 0.15	1.02 ± 0.06	13.4 ± 2.8	420.6 ± 25.7	7.30 ± 0.42
Elevated CO ₂	17.7 ± 0.22	7.73 ± 0.09	5.95 ± 0.22	1.07 ± 0.06	35.1 ± 4.5	801.5 ± 59.9	5.14 ± 0.55
Elevated temperature	20.6 ± 0.31	8.01 ± 0.08	5.94 ± 0.10	1.20 ± 0.09	13.6 ± 4.3	430.4 ± 37.1	7.07 ± 0.5
Elevated temperature and CO ₂	20.8 ± 0.36	7.76 ± 0.16	5.69 ± 0.26	1.09 ± 0.10	35.8 ± 5.2	814.9 ± 54.8	5.05 ± 0.32

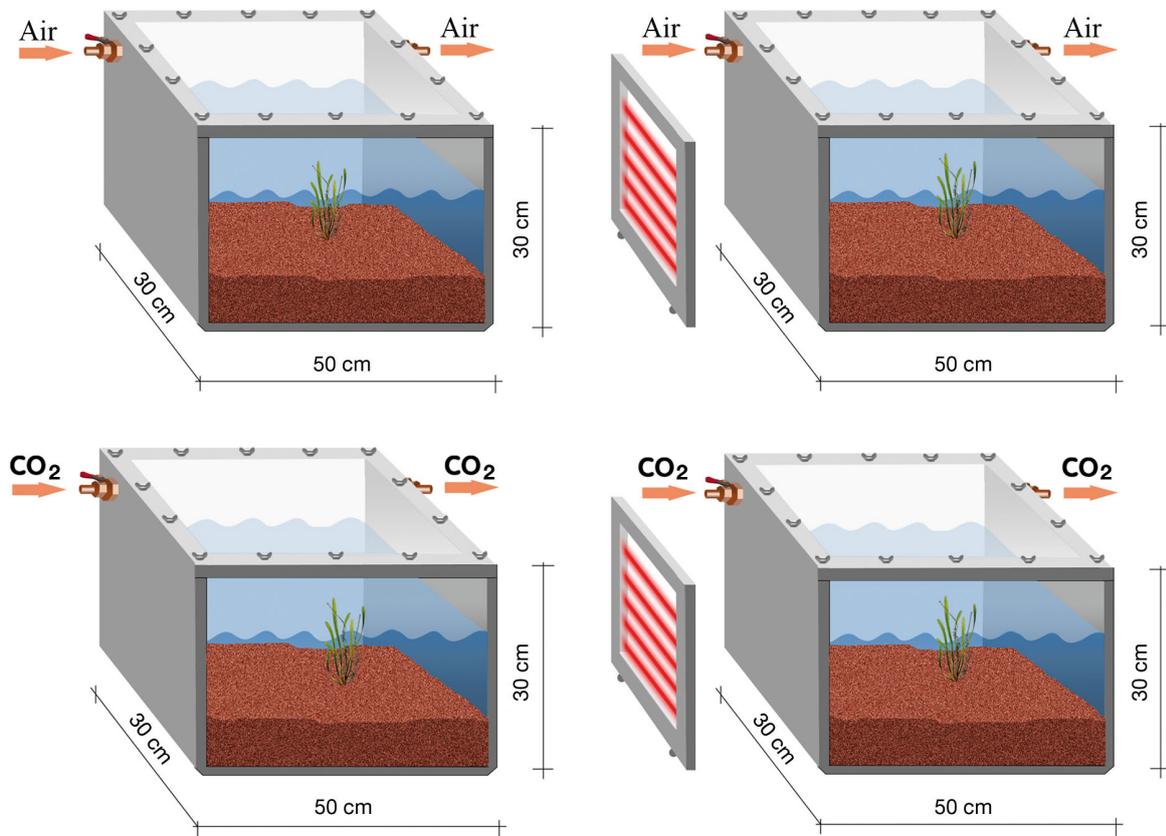


Fig. 1. Experimental design showing one of the experimental blocks. *Vallisneria natans* plantlets were placed in mesocosms that were subjected to different treatments. Two CO₂ levels (ambient and elevated) and 2 temperature levels (ambient and elevated; elevation achieved with far infrared quartz heating tubes) were employed (n = 6)

and bud number per plant. The plants were divided into roots, leaves, creeping stems and buds, and then dried at 70°C for 72 h and weighed to calculate the total plant biomass (per mesocosm) and individual ramet biomass.

Statistical analysis

Before analysis, all data were log-transformed to meet the conditions of homogeneity of variance and normal distribution of residuals. No significant differences were detected among blocks ($p > 0.05$), therefore the effects of CO₂ and elevated temperature on photosynthesis, growth and reproduction of *V. natans* (e.g. total plant biomass, ramet number, individual ramet biomass, creeping stem length and bud number) were analyzed by 2-way ANOVA. A 2-way multivariate ANOVA (MANOVA) was employed to investigate the effects of CO₂ and elevated temperature on the biomass allocation parameters. When a significant treatment effect was detected, post hoc

pairwise comparisons of means were conducted to examine the differences between treatments using a studentized Tukey's HSD test for multiple comparisons at the 0.05 significance level. All data were analyzed using SPSS v.18.0.

RESULTS

During the experimental period, the values of all photosynthetic parameters significantly increased with elevated CO₂ levels. The RLCs of the plants under elevated CO₂ were significantly higher than those of the plants in ambient air (Fig. 2). However, the effects of elevated temperature were only significant when interacting with the effects of elevated CO₂ (Fig. 2). The equation fitting revealed that elevated CO₂ levels significantly increased P_m , α and E_k of the RLCs (Table 2). When interacting with the elevated CO₂ levels, temperature elevation also increased the photosynthetic parameters (α and E_k , but not P_m) (Table 2).

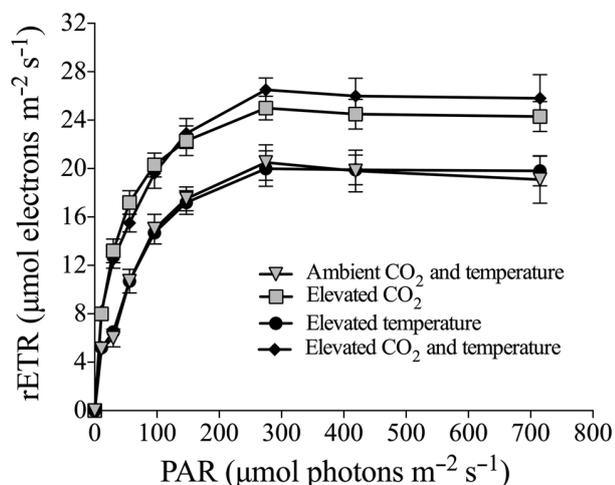


Fig. 2. Relative electron transport rate (rETR) curves for *Vallisneria natans* under ambient and elevated CO₂ and temperature. Each curve represents the mean of 6 replicate samples

Elevation of CO₂ increased total biomass and ramet number. Elevated temperature increased total biomass accumulation and ramet number but reduced the individual ramet biomass (only significant at ambient CO₂) (Fig. 3a–c, Table 3). Creeping stem length and bud number were significantly higher under elevated CO₂, but these traits were not affected by temperature or by the interaction between temperature and elevated CO₂ (Fig. 3d,e, Table 3).

Generally, the biomass allocation pattern of *Vallisneria natans* was only affected by elevated CO₂ (Table 4a). Under increased levels of CO₂, leaf allocation was significantly reduced (Fig. 4a, Table 4b), and more biomass was allocated to the production of creeping stems, roots and buds (Fig. 4b–d, Table 4b). However, neither elevated temperature nor its interaction with elevated CO₂ had a significant effect on biomass allocation (Fig. 4, Table 4).

Table 2. Effects of elevated CO₂ and elevated temperature on light response curve parameters in *Vallisneria natans*. Significant differences ($p < 0.05$) among treatments are indicated by different letters; P_m = maximum photosynthetic rate, α = initial slope of rETR–PAR response curve, E_k was calculated by dividing P_m by α ; R^2 is regression coefficient of rETR–PAR response curves

Treatment	P_m ($\mu\text{mol electrons m}^{-2} \text{s}^{-1}$)	α	E_k ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	R^2
Ambient temp. and CO ₂	20.1 ± 1.5 ^a	0.28 ± 0.02 ^a	72.2 ± 4.1 ^a	0.991
Elevated CO ₂	24.3 ± 2.3 ^b	0.32 ± 0.03 ^b	87.0 ± 2.5 ^b	0.985
Elevated temperature	20.6 ± 1.6 ^a	0.28 ± 0.04 ^a	74.6 ± 3.2 ^a	0.971
Elevated temp. and CO ₂	25.9 ± 1.7 ^b	0.33 ± 0.02 ^c	93.3 ± 5.1 ^c	0.989

DISCUSSION

Compared with terrestrial plants, submerged macrophytes can use a wider range of carbon sources. Many submerged macrophytes can use both free CO₂ and bicarbonate in the water column as carbon sources for photosynthesis (Madsen et al. 1996, Maberly & Madsen 1998, Jones 2005, Cavalli et al. 2012). However, submerged macrophytes always prefer CO₂ due to the additional metabolic cost of bicarbonate utilization (Jones 2005). The use of bicarbonate is believed to be an active process that requires a protein in the plasmalemma membrane to ‘pump’ in bicarbonate ions (Prins & Elzenga 1989). In the present study, the elevation of ambient CO₂ significantly increased the total biomass and clonal growth (ramet number) of *Vallisneria natans*, which is consistent with previous studies suggesting that CO₂ enrichment stimulated *Vallisneria* species (bicarbonate user) (Titus et al. 1990, Titus & Andorfer 1996). Moreover, changes in aquatic pH values can also alter the inorganic carbon balance, indirectly affecting the growth and reproduction of submerged macrophytes (Schippers et al. 2004, Baatrup-Pedersen et al. 2013).

For submerged macrophytes that also use bicarbonate, internal (or recycled) CO₂ concentration is the dominant component that causes a reduction of CO₂ loss, thus maintaining high CO₂ concentration (Madsen & Maberly 2003). Therefore, the ability to use bicarbonate may increase the internal concentration of CO₂, and hence suppress or reduce the oxygenation reactions of rubisco, which may promote the photosynthetic activity of bicarbonate users such as *V. natans* (Maberly & Madsen 1998, Cavalli et al. 2012, Xie et al. 2013). In addition, abiotic and biotic factors (e.g. water velocity and shoot morphology) also affect CO₂ diffusion through a boundary layer around the leaves of submerged macrophytes (Nielsen et al. 2006). Although several studies have suggested that prolonged exposure to high CO₂ concentrations can reduce photosynthesis to basal or even lower levels (Madsen et al. 1996), the present experimental period was relatively short (60 d), and all plants were still in the vigorous growth period. If the duration of the present experiment were extended, photosynthetic activity may change over time under high CO₂ levels, but this hypothesis requires further evaluation.

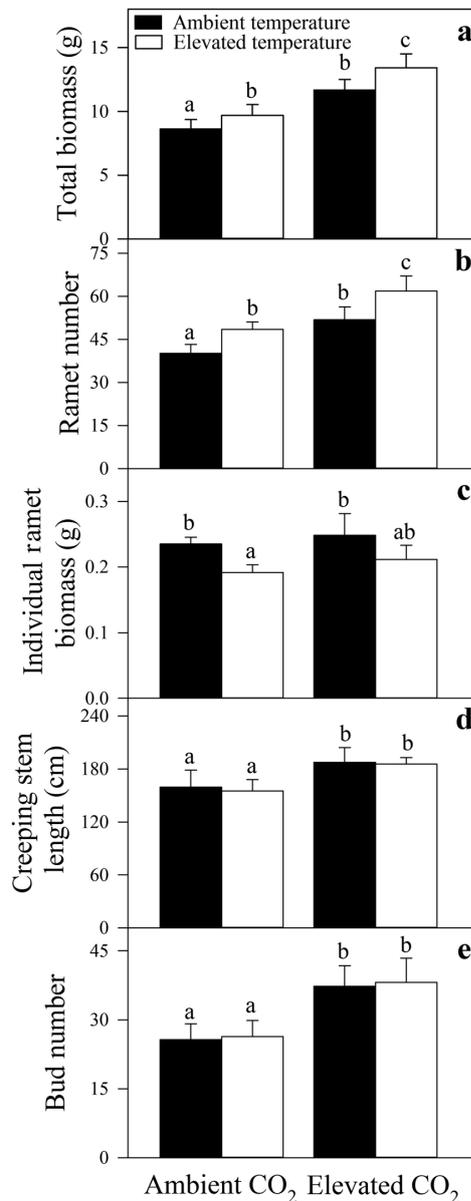


Fig. 3. Differences in (a) total plant biomass (per mesocosm), (b) ramet number, (c) individual ramet biomass, (d) creeping stem length and (e) bud number of *Vallisneria natans* under different CO₂ and temperature conditions (n = 6). Bars with different letters indicate significant differences among the treatments (studentized Tukey's HSD)

Additionally, the moderate increase in water temperature in combination with elevated CO₂ may also have increased the activity of photosynthetic enzymes and carbonic anhydrase (Olesen & Madsen 2000). However, the maximum temperature dependence of growth has been observed only at saturating inorganic carbon availability (Olesen & Madsen 2000); as the temperature increases, the proportion of carbon lost via photorespiration increases, and the

Table 3. *F*-values of a 2-way ANOVA for the effects of elevated CO₂ and elevated temperature on growth (total plant biomass, ramet number, individual ramet biomass, total stem length and bud number) in *Vallisneria natans*. Significance levels: ns *p* > 0.05; **p* < 0.05; ****p* < 0.001; df = 1, 20

Dependent variable	Elevated CO ₂ (C)	Elevated temperature (T)	C × T
Total plant biomass	123.56***	5.11*	0.46 ^{ns}
Ramet number	59.34***	31.91***	0.26 ^{ns}
Individual ramet biomass	5.66*	21.33***	0.32 ^{ns}
Stem length	28.84***	0.29 ^{ns}	0.11 ^{ns}
Bud number	46.12***	0.27 ^{ns}	0.09 ^{ns}

positive effect of temperature on enzymatic processes and inorganic carbon diffusion is counterbalanced or even reversed (Long 1991). Furthermore, dark respiration rates also increase with temperature; to maintain growth, photosynthesis should increase in response to warmer temperatures at elevated CO₂ conditions (Morison & Lawlor 1999).

Growth and clonal reproduction of *V. natans* were significantly promoted under elevated CO₂ conditions, as indicated by the higher biomass, higher ramet production and longer creeping stems. These effects likely resulted from the increased supply of inorganic carbon in the water, which likely increased the available photosynthetic substrate and thus enhanced plant biomass accumulation (Maberly & Madsen 1998). Our results agree with those of many previous studies (e.g. Maberly & Madsen 1998, Nielsen et al. 2006, Pagano & Titus 2007). Titus & Andorfer (1996) found that the aboveground biomass of submerged macrophytes increased by 5.5 to 7 times when the CO₂ level was elevated in an acidic aquatic environment (pH 5). Indeed, a CO₂ increase of 2 to 10 times the ambient level was previously shown to promote biomass accumulation of submerged macrophytes in multiple previous studies at various pH levels (Titus 1992, Olesen & Madsen 2000, Yan et al. 2006, Malheiro et al. 2013). Clonal reproduction is the major reproduction and dispersal method of *Vallisneria* species (Xiao et al. 2006, 2007a, 2011). In the present study, longer creeping stems and a greater ramet number were observed under elevated CO₂ conditions, which is consistent with previous studies; this clonal growth enables *Vallisneria* species to occupy more habitats (Yan et al. 2006).

An increase in temperature also increased growth rate and ramet number in *V. natans*. Temperature elevation can increase the activity of photosynthetic

Table 4. MANOVA summary for the effects of elevated CO₂, elevated temperature and their interaction on the percentage of biomass allocation in *Vallisneria natans*. Significance level: ns $p > 0.05$; *** $p < 0.001$

(a) Multivariate test statistics and exact <i>F</i> -statistic				
	Wilk's lambda	<i>F</i>	df	<i>p</i>
Elevated CO ₂ (C)	0.12	32.15	4,17	<0.001
Elevated temperature (T)	0.83	0.89	4,17	0.50
C × T	0.93	1.10	4,17	0.63
(b) Univariate test statistics (<i>F</i> -values, df = 1, 20)				
Trait	Elevated CO ₂ (C)	Elevated temperature (T)	C × T	
Leaf biomass allocation	17.23***	0.59 ^{ns}	1.01 ^{ns}	
Creeping stem biomass allocation	22.04***	1.21 ^{ns}	0.94 ^{ns}	
Root biomass allocation	21.18***	2.41 ^{ns}	0.10 ^{ns}	
Bud biomass allocation	58.00***	2.63 ^{ns}	1.05 ^{ns}	

enzymes and carbonic anhydrase, which likely caused the increases of biomass and ramet number in our experiment. These results are in agreement with a previous study in which higher temperatures increased growth under higher inorganic carbon availability (Olesen & Madsen 2000). Additional inorganic carbon resources can be used only if other resources (e.g. water soluble nutrients) are concurrently available (Xie et al. 2013). In addition, the temperature increase in the present experiment was relatively minor (approximately 3°C), which may have been insufficient to affect the composition and balance of inorganic carbon in the water column and thus may have further influenced the utilization of inorganic carbon in *V. natans*. A recent study produced similar results, indicating that soil warming had little effect on the gas exchange of plants at the alpine treeline (Streit et al. 2014).

The individual ramet biomass of *V. natans* decreased with elevated temperature, but only significantly at ambient CO₂. CO₂ elevation may promote the assimilation process, as reflected by the observation of increased ramet sizes, which reflects a positive response to an increase in inorganic carbon levels in the water (Titus & Andorfer 1996, Yan et al. 2006, Xie et al. 2013). However, temperature ele-

vation reduced the individual ramet size of *V. natans*. Many studies have evaluated the relationship between temperature and individual size, employing theoretical frameworks such as Bergmann's rule, James' rule and the temperature-size dependent rule. Daufresne et al. (2009) studied ectothermic aquatic organisms (bacteria, phyto- and zooplankton and fish) from the individual scale to the community scale and found that global warming increased the proportion of small and young individuals, thereby decreasing the size-age relationship. Individual miniaturization may therefore be a third general rule for the response of aquatic ecosystems to global warming (Daufresne et al. 2009).

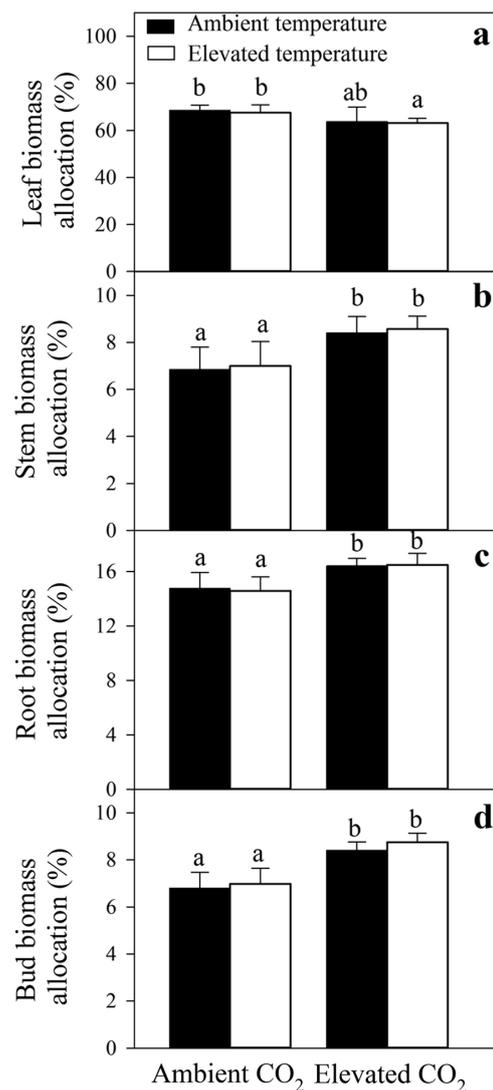


Fig. 4. Differences in (a) leaf biomass allocation, (b) stem biomass allocation, (c) root biomass allocation and (d) bud biomass allocation of *Vallisneria natans* under different CO₂ and temperature conditions (n = 6). Bars with different letters indicate significant differences among the treatments (studentized Tukey's HSD)

Biomass allocation in *V. natans* was significantly affected by CO₂ concentration, which demonstrated the plant's plasticity to some extent. Under the elevated CO₂ condition, more biomass was allocated to the roots, ramets and buds, while less biomass was allocated to the leaves. When inorganic carbon is no longer a limiting factor, nutrient intake will likely be more important than photosynthesis as a result of carbohydrate production, and roots may therefore be emphasized over leaves (Titus & Andorfer 1996, Geng et al. 2004). Additionally, greater resource allocation to creeping stems may allow *V. natans* to capture more resources, and reduce competition for soil nutrients with neighboring plants (Xiao et al. 2006, 2007a, Yan et al. 2006), and more buds will likely lead to higher productivity in waters with high CO₂ concentrations.

Different macrophytes have different underwater carbon utilization abilities (Madsen et al. 1993, Xie et al. 2013), which influence the distribution and structure of aquatic plants (Lacoul & Freedman 2006, Bornette & Puijalon 2011). However, studies on the influence of interactions between CO₂ and temperature on submerged macrophyte communities remain limited, and further research is needed.

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LITERATURE CITED

- Ahuja I, de Vos RCH, Bones AM, Hall RD (2010) Plant molecular stress responses face climate change. *Trends Plant Sci* 15:664–674
- Baatrup-Pedersen A, Madsen TV, Riis T, Cavalli G (2013) Photosynthetic performance of submerged macrophytes from lowland stream and lake habitats with contrasting CO₂ availability. *New Phytol* 198:1135–1142
- Barko JW, Smart RM (1981) Comparative influences of light and temperature on the growth and metabolism of selected submersed freshwater macrophytes. *Ecol Monogr* 51:219–236
- Bornette G, Puijalon S (2011) Response of aquatic plants to abiotic factors. *Rev Aquat Sci* 73:1–14
- Cao T, Ni L, Xie P, Xu J, Zhang M (2011) Effects of moderate ammonium enrichment on three submersed macrophytes under contrasting light availability. *Freshw Biol* 56:1620–1629
- Cao J, Wang Y, Zhu Z (2012) Growth response of the submerged macrophyte *Myriophyllum spicatum* to sediment nutrient levels and water-level fluctuations. *Aquat Biol* 17:295–303
- Cavalli G, Riis T, Baatrup-Pedersen A (2012) Bicarbonate use in three aquatic plants. *Aquat Bot* 98:57–60
- Cook CDK (1990) *Aquatic plant book*. SPB Academic Publishing, Amsterdam
- Daufresne M, Lengfellner K, Sommer U (2009) Global warming benefits the small in aquatic ecosystems. *Proc Natl Acad Sci USA* 106:12788–12793
- Dou Y, Wang B, Chen L, Yin D (2013) Alleviating versus stimulating effects of bicarbonate on the growth of *Vallisneria natans* under ammonia stress. *Environ Sci Pollut Res* 20:5281–5288
- Geng X, Yu D, Huang Y, Xie Y, Yang Y (2004) Growth and physio-biochemistry responses of *Vallisneria spiralis* L. to CO₂ enrichment. *Acta Hydrobiol Sin* 28:304–309
- Houghton JT, FilhoMeria LG, Callander BA, Harris N, Kattenberg A, Maskell K (1996) *Climate change: the science of climate change*. Cambridge University Press, Cambridge
- Houghton JT, Ding Y, Griggs D (2001) *Climate change of 2001: the scientific basis*. Cambridge University Press, Cambridge
- IPCC (2001) *Climate change 2001: the scientific basis. Contribution of Working Group I to the 3rd assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge
- IPCC (2007) *Climate change 2007: the physical science basis. Contribution of Working Group I to the 4th assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge
- Jones JI (2005) The metabolic cost of bicarbonate use in the submerged plant *Elodea nuttallii*. *Aquat Bot* 83:71–81
- Ke X, Li W (2006) Germination requirement of *Vallisneria natans* seeds: implications for restoration in Chinese lakes. *Hydrobiologia* 559:357–362
- Lacoul P, Freedman B (2006) Environmental influences on aquatic plants in freshwater ecosystems. *Environ Rev* 14: 89–136
- Long SP (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO₂ concentrations: Has its importance been underestimated? *Plant Cell Environ* 14:729–739
- Maberly SC, Madsen TV (1998) Affinity for CO₂ in relation to the ability of freshwater macrophytes to use HCO₃⁻. *Funct Ecol* 12:99–106
- Madsen TV, Brix H (1997) Growth, photosynthesis and acclimation by two submerged macrophytes in relation to temperature. *Oecologia* 110:320–327
- Madsen TV, Maberly SC (2003) High internal resistance to CO₂ uptake by submerged macrophytes that use HCO₃⁻: measurements in air, nitrogen and helium. *Photosynth Res* 77:183–190
- Madsen TV, Sand-Jensen K (1991) Photosynthetic carbon assimilation in aquatic macrophytes. *Aquat Bot* 41:5–40
- Madsen TV, Sand-Jensen K, Beer S (1993) Comparison of photosynthetic performance and carboxylation capacity in a range of aquatic macrophytes of different growth forms. *Aquat Bot* 44:373–384
- Madsen TV, Maberly SC, Bowes G (1996) Photosynthetic acclimation of submersed angiosperms to CO₂ and HCO₃⁻. *Aquat Bot* 53:15–30
- Malheiro ACE, Jahns P, Hussner A (2013) CO₂ availability rather than light and temperature determines growth and phenotypical responses in submerged *Myriophyllum aquaticum*. *Aquat Bot* 110:31–37
- Morison JIL, Lawlor DW (1999) Interactions between increasing CO₂ concentration and temperature on plant growth. *Plant Cell Environ* 22:659–682

- Mvungi EF, Lyimo TJ, Björk M (2012) When *Zostera marina* is intermixed with *Ulva*, its photosynthesis is reduced by increased pH and lower light, but not by changes in light quality. *Aquat Bot* 102:44–49
- Nielsen HD, Nielsen SL, Madsen TV (2006) CO₂ uptake patterns depend on water current velocity and shoot morphology in submerged stream macrophytes. *Freshw Biol* 51:1331–1340
- Nishihara GN, Ackerman JD (2007) The interaction of CO₂ concentration and spatial location on O₂ flux and mass transport in the freshwater macrophytes *Vallisneria spiralis* and *V. americana*. *J Exp Biol* 210:522–532
- Olesen B, Madsen TV (2000) Growth and physiological acclimation to temperature and inorganic carbon availability by two submerged aquatic macrophytes species, *Callitriche cophocarpa* and *Elodea canadensis*. *Funct Ecol* 14:252–260
- Pagano AM, Titus JE (2007) Submersed macrophyte growth at low pH: carbon source influences response to dissolved inorganic carbon enrichment. *Freshw Biol* 52:2412–2420
- Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (2007) Climate change 2007: impacts, adaptation and vulnerability. Contribution of Working Group II to the 4th assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge
- Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J Mar Res* 38:687–701
- Prins HBA, Elzenga JTM (1989) Bicarbonate utilization: function and mechanism. *Aquat Bot* 34:59–83
- Rogers KH, Breen CM (1980) Growth and reproduction of *Potamogeton crispus* in a South African lake. *J Ecol* 68:561–571
- Rooney N, Kalff J (2000) Inter-annual variation in submerged macrophyte community biomass and distribution: the influence of temperature and lake morphology. *Aquat Bot* 68:321–335
- Sage RF, Monson RK (1999) C4 plant biology. Academic Press, San Diego, CA
- Sand-Jensen K, Gordon DM (1986) Variable HCO₃⁻ affinity of *Elodea canadensis* Michaux in response to different HCO₃⁻ and CO₂ concentrations during growth. *Oecologia* 70:426–432
- Schippers P, Vermaat JE, de Klein J, Mooij WM (2004) The effect of atmospheric carbon dioxide elevation on plant growth in freshwater ecosystems. *Ecosystems* 7:63–74
- Sheldon SP (1987) The effects of herbivorous snails on submerged macrophyte communities in Minnesota lakes. *Ecology* 68:1920–1931
- Spence DHN, Maberly SC (1985) Occurrence and ecological importance of HCO₃⁻ use among aquatic higher plants. In: Lucas WJ, Berry J (eds) Inorganic carbon uptake by aquatic photosynthetic organisms. American Society of Plant Physiologists, Rockville, MD, p 125–143
- Streit K, Siegwolf RTW, Hagedorn F, Schaub M, Buchmann N (2014) Lack of photosynthetic or stomatal regulation after 9 years of elevated [CO₂] and 4 years of soil warming in two conifer species at the alpine treeline. *Plant Cell Environ* 37:315–326
- Titus JE (1992) Submerged macrophyte growth at low pH. II. CO₂ × sediment interactions. *Oecologia* 92:391–398
- Titus JE, Andorfer JH (1996) Effects of CO₂ enrichment on mineral accumulation and nitrogen relations in a submerged macrophyte. *Freshw Biol* 36:661–671
- Titus JE, Feldman RS, Grisé D (1990) Submersed macrophyte growth at low pH. *Oecologia* 84:307–313
- van Donk E, Grimm MP, Gulati RD, Klein Breteler JPG (1990) Whole-lake food-web manipulation as a means to study community interactions in a small ecosystem. *Hydrobiologia* 200–201:275–289
- Wang D, Heckathorn S, Wang X, Philpott S (2012) A meta-analysis of plant physiological and growth responses to temperature and elevated CO₂. *Oecologia* 169:1–13
- Xiao K, Yu D, Wang J (2006) Habitat selection in spatially heterogeneous environments: a test of foraging behaviour in the clonal submerged macrophyte *Vallisneria spiralis*. *Freshw Biol* 51:1552–1559
- Xiao K, Yu D, Xu X, Xiong W (2007a) Benefits of clonal integration between interconnected ramets of *Vallisneria spiralis* in heterogeneous light environments. *Aquat Bot* 86:76–82
- Xiao Y, Chen K, Dai X, Xu X (2007b) Dissolved inorganic carbon uptake in two submerged macrophytes from Taihu Lake, China. *J Plant Ecol* 31:490–496
- Xiao K, Yu D, Wang L, Han Y (2011) Physiological integration helps a clonal macrophyte spread into competitive environments and coexist with other species. *Aquat Bot* 95:249–253
- Xie Y, An S, Yao X, Xiao K, Zhang C (2005) Short-time response in root morphology of *Vallisneria natans* to sediment type and water-column nutrient. *Aquat Bot* 81:85–96
- Xie D, Yu D, You W, Wang L (2013) Algae mediate submerged macrophyte response to nutrient and dissolved inorganic carbon loading: a mesocosm study on different species. *Chemosphere* 93:1301–1308
- Yan X, Yu D, Li YK (2006) The effects of elevated CO₂ on clonal growth and nutrient content of submerge plant *Vallisneria spirulosa*. *Chemosphere* 62:595–601
- You W, Yu D, Liu C, Xie D, Xiong W (2013a) Clonal integration facilitates invasiveness of the alien aquatic plant *Myriophyllum aquaticum* L. under heterogeneous water availability. *Hydrobiologia* 718:27–39
- You W, Yu D, Xie D, Yu L (2013b) Overwintering survival and regrowth of the invasive plant *Eichhornia crassipes* are enhanced by experimental warming in winter. *Aquat Bot* 19:45–53
- Zhai W, Dai X, Guo X (2007) Carbonate system and CO₂ degassing fluxes in the inner estuary of Changjiang (Yangtze) River, China. *Mar Chem* 107:342–356
- Zhang Y, An Y, Zhu C, Yang S (2009) Physiological effects of dissolved inorganic carbon on common submerged macrophytes. *Acta Hydrobiol Sin* 33:1020–1030
- Zimmerman RC, Kohrs DG, Steller DL, Alberte RS (1997) Impacts of CO₂ enrichment on production and light requirements of eelgrass. *Plant Physiol* 115:599–607