DOI: 10.1007/s11099-014-0055-4 PHOTOSYNTHETICA 52 (4): 484-492, 2014

Effects of salinity on temperature-dependent photosynthetic parameters of a native C_3 and a non-native C_4 marsh grass in the Yangtze Estuary, China

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Abstract

The invasion of *Spartina alterniflora* along the coasts of China has allowed this C_4 grass to outcompete often much of the native, salt marsh vegetation, such as *Phragmites australis* (C_3 grass), in the Yangtze Estuary. In this study, native grass, *P. australis*, and non-native grass, *S. alterniflora*, were grown in fresh and saline water (moderate salinity of 15‰ and high salinity of 30‰) to compare the effects of salinity on photosynthetic and biochemical parameters in combination with measurement temperatures. The C_4 grass, *S. alterniflora*, showed a greater CO_2 assimilation rate than *P. australis*, across the tested temperatures. The net photosynthetic rate declined significantly with increasing salinity as a result of inhibited stomatal conductance together with a greater decrease in the maximum rate of electron transport (J_{max}). In *P. australis*, salt treatments shifted the optimum temperatures for the maximum rate of carboxylation by Rubisco (V_{cmax}) and J_{max} to lower temperatures. *S. alterniflora* showed a greater salt tolerance to moderate stress than that of the native grass, with lower sensitivity of V_{cmax}, J_{max}, and the maximum rate of phospho*enol*pyruvate carboxylation. Both moderate and high stress decreased significantly stomatal conductance of *S. alterniflora*; high salinity reduced significantly photosynthetic efficiency and J_{max}. Our findings indicated that the combination of stomatal conductance, enzyme activity, and electron transport affected the photosynthetic performance of the plants in response to salt treatments. The success of *S. alterniflora* could be probably attributed to its C_4 photosynthetic pathway and the tolerance to moderate salinity. In this study, a modified parameterization of the photosynthetic model was suggested to support a more reasonable simulation of photosynthesis under salt stress.

Additional key words: carboxylation efficiency; coastal wetlands; gas exchange; invasive species.

Introduction

Of the various environmental stresses impacting coastal wetlands, salinity is a major constraint on the metabolism and growth of marsh vegetation (Chambers *et al*. 1998, Maricle *et al*. 2007, Yu *et al*. 2012). Furthermore, in the future, coastal wetlands may be affected by flooding and salinity because a rise in sea level is expected in various

Received 28 August 2013, *accepted* 31 January 2014.
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Abbreviations: C_a – ambient CO_2 concentration; C_c – chloroplast CO_2 concentration; C_i – intercellular CO_2 concentration; C_m – CO_2 concentration in the mesophyll cell; C_s – CO_2 concentration at the carboxylation site of Rubisco in the bundle-sheath; g_{bs} – bundle sheath cell conductance; g_m – mesophyll conductance; g_s – light-saturated stomatal conductance; H_a – enthalpy of activation; H_d – enthalpy of deactivation; HS – high salinity; J, J_t – rate of electron transport for C_3 and C_4 plants; J_{max} – maximum rate of electron transport; K_c, K_o – Rubisco Michaelis constants for CO_2 and O_2; K_p – Michaelis–Menten constants for PEP carboxylation; MS – moderate salinity; O – O_2 concentration; PEP – phospho*enol*pyruvate; PEPC – phospho*enol*pyruvate carboxylase; P_N – net photosynthetic rate; P_{Nsat} – light-saturated net photosynthetic rate; R – molar gas constant; R_D – dark respiration; RH – relative humidity; R_m – mitochondrial respiration in the mesophyll; RuBP – ribulose-1,5-bisphosphate; ΔS – entropy of the desaturation equilibrium; $S_{c/o}$ – reciprocal of Rubisco specificity; T_L – the leaf temperature; V_c – rate of Rubisco carboxylation; V_{cmax} – maximum rate of carboxylation by Rubisco; V_p – rate of PEP carboxylation; V_{pmax} – maximum rate of PEP carboxylation; α – quantum efficiency; α_1 – photosynthetically active irradiance absorbed by PSII; γ^* – half of $S_{c/o}$; θ – curvature of the light response curve; Γ^* – CO_2 compensation point (absence of dark respiration); χ – partitioning factor of electron transport.

Acknowledgments: This work was funded through the Natural Science Foundation of China (41201091), the Shanghai Pujiang Program (13PJ1402200) and the International Project (12230707500) of Shanghai Science & Technology Committee, the Global Change Scientific Research Program of China (2010CB951204), and the Project sponsored by SRF for ROCS, SEM. We thank the experts of Edanz Editing (Edanz Group Ltd.) for revising the language of this paper.

global climate change scenarios (Saha *et al*. 2011). To protect coastal dykes from tidal erosion and to promote sediment build-up for polder formation, *Spartina alterniflora* (C_4 grass) was introduced from North America to the Yangtze Estuary in 1979; it has spread rapidly along the entire Chinese coast (Ge *et al*. 2014). As a result, this non-native plant has strongly outcompeted much of the native, salt marsh vegetation including *Phragmites australis* (C_3 grass) in the Yangtze Estuary (Li *et al*. 2006, Huang and Zhang 2007).

The effects of salinity stress on leaf characteristics and gas exchange are well studied in C_3 (Sudhir and Murthy 2004, Naz *et al*. 2010, Li *et al*. 2013, Wu *et al*. 2013) and C_4 plants (Maricle *et al*. 2007, Hichem *et al*. 2009, Wang *et al*. 2013); various physiological variables have been analyzed including photosynthetic rate, stomatal conductance, transpiration rate, and water-use efficiency, as well as chlorophyll fluorescence parameters. However, less is known about modifications of biochemical parameters in relation to activities of photosynthetic enzymes under salt stress. These activities could be crucial in explaining the physiological and ecological mechanisms of *S. alterniflora* invasion with respect to its growth rate, productivity, and strong competitive ability in the Yangtze Estuary.

In C_3 photosynthesis, various factors, such as the amount of activated enzyme Rubisco, the rate of regeneration of ribulose-1,5-bisphosphate (RuBP), and the relative partial pressures of CO_2 and O_2 on the site of CO_2 fixation, control the rate of photosynthesis. Correspondingly, the biochemical parameters of the V_{cmax} and the J_{max} are believed to indicate two distinct steady states of photosynthesis (Farquhar *et al*. 1980, 2001; Sharkey *et al*. 2007, Ge *et al*. 2012). The first step in the C_4-photosynthetic pathway is the conversion of pyruvate to phospho*enol*pyruvate (PEP) by phospho*enol*pyruvate carboxylase (PEPC) in mesophyll cells. The intermediate products are then delivered to bundle-sheath cells for decarboxylation and the released CO_2 is assimilated by Rubisco in the C_3 pathway of the Benson-Calvin cycle (von Caemmerer and Furbank 1999, Massad *et al*. 2007, Yin *et al*. 2011). Therefore, the maximum rate of phospho*enol*pyruvate carboxylation (V_{pmax}) is a specific, C_4 photosynthetic parameter. Furthermore, mechanistic photosynthetic models have been developed for C_3 and C_4 plants (Farquhar *et al*. 1980, 2001; von Caemmerer and Furbank 1999, Massad *et al*. 2007, Sharkey *et al*. 2007, Yin *et al*. 2011). They consider main biochemical processes occurring under various environmental conditions. The most difficult challenge is to identify the responses of key photosynthetic parameters in terms of the biochemical mechanisms in response to environmental stress.

In this paper, the responses of photosynthetic parameters of *P. australis* and *S. alterniflora* grown in fresh water and two salinity levels (15‰ and 30‰) were measured and analyzed. The measured response curves of the net photosynthetic rate (P_N) *vs*. intercellular CO_2 concentration (C_i) and PPDF at different temperatures were used to describe the variation of the photosynthetic parameters under a salinity gradient.

Material and methods

Plant material and growth conditions: In early summer, intact blocks consisting of *P. australis* and *S. alterniflora* seedlings in soil monoliths (0.5 m × 0.5 m × 0.5 m) were obtained from the Chongming Dongtan Wetland (31°25'–31°38'N, 121°50'–122°05'E) located on the eastern fringe of Chongming Island in the Yangtze Estuary (Ge *et al*. 2010). The seedlings of vegetation with similar size were collected at the same tidal line.

The blocks were then cultivated in high-density polyethylene containers and were watered to saturation once a week with Hoagland nutrient solution. Before experimental treatments were initiated, the plants were irrigated with fresh water for 30 d to recover from being disturbed. Containers (24 in total, two species × three salt treatments × four repetitions) with *P. australis* and *S. alterniflora* were placed in a greenhouse chamber. Before the experiment, the growth characteristics of the plants in the containers were relatively homogenous. The densities of the plants were 156 ± 25 and 178 ± 26 individuals m^{-2} for *P. australis* and *S. alterniflora*, respectively. The plant height was 183 ± 16 and 159 ± 17 cm for *P. australis* and *S. alterniflora*, respectively.

Selected containers with single grass species were treated with one of three salinity levels, ranging from 0‰ (fresh water), to 15‰ (moderate salinity, MS), and 30‰ (high salinity, HS). Growth chamber conditions consisted of a 12 h (07:00–19:00 h) photoperiod with day/night temperature of 26/18°C. The humidity was controlled at 60±5% in the chamber, and the photon flux density was approximately 500 μmol m^{-2} s^{-1} near the tops of leaves. During the pre-treatment period, the salinity level (NaCl) was elevated by 5‰ and 10‰ per week until it reached the demanded salinity levels (*i.e.*, MS or HS). The water with Hoagland nutrient solution was replaced weekly.

Determination of photosynthetic parameters: During the study period (June–August), gas-exchange measurements were carried out with a 2 × 3 cm standard leaf chamber in a portable steady-state photosynthesis system (*Li-Cor 6400*, *Li-Cor Inc*., Lincoln, USA). The measurements were performed on the first fully expanded leaves below the flag leaf, and the instrument was calibrated before each set of measurements. The containers with plants were moved into an air-conditioned laboratory one hour before the start of the measurements and temperatures for measurements were set to six different temperatures ranging from 15 to 35°C with intervals of 5°C. Relative humidity (RH) was maintained at 60% in the laboratory

with a humidifier for ambient temperature above 30°C.

To calculate the photosynthetic parameters for the C_3 and C_4 grasses, two kinds of curves were assayed including P_N *vs*. C_i, and P_N *vs*. PPFD. Measurements were restricted to the hours from 08:00 to 11:00 a.m. The air flow in the leaf chamber was set at 400 mL min^{-1}, the vapor pressure deficit was kept at 1.0 ± 0.1 kPa, and RH of the air in the leaf chamber was set above 60%. The CO_2 source for the measurements was a computer-controlled CO_2 mixing system supplied with the *Li-Cor 6400*. Meanwhile, the light-saturated net photosynthetic rate (P_{Nsat}) and stomatal conductance (g_s) were recorded at 5°C intervals from 15 to 35°C, under PPFD of 1,800 μmol(photon) m^{-2} s^{-1}, and CO_2 concentration of 370 μmol mol^{-1}.

The P_N-C_i curves were carried out under saturating light intensity [1,800 μmol(photon) m^{-2} s^{-1}]. The ambient CO_2 concentration (C_a) in the leaf chamber was lowered in a stepwise manner from 400 to 20 μmol mol^{-1} (400, 240, 180, 120, 80, 40, and 20 μmol mol^{-1}) and then returned to 400 μmol mol^{-1} to re-establish the initial steady state value of photosynthesis. Gas-exchange measurements were determined as soon as the inlet air CO_2 concentration was stable (Long *et al*. 2004). Three measurements of P_N were made 15 min after reaching the desired CO_2 concentrations at 30 s intervals. The P_N-PPFD curves were carried out under 1,400 μmol mol^{-1} CO_2 concentration by decreasing PPFD in a stepwise manner from 1,800 to 20 μmol m^{-2} s^{-1} (1,800; 1,500; 1,000; 700, 500, 350, 250, 150, 100, 50, 20 μmol m^{-2} s^{-1}). Sufficient time was allowed for photosynthesis to stabilize to the new PPFD before logging the measurements. Leaves were allowed to equilibrate for 10–20 min before logging the data in the curves. Fig. 1 illustrates the examples of experimental points measured at 25°C together with the fitted polynomial for the P_N-C_i and P_N-PPFD curves.

For *P. australis* (C_3 grass), using the biochemical model of Farquhar *et al*. (1980) and Farquhar and von Caemmerer (1982), the Rubisco-limited photosynthesis is given by Eq. 1:

$$P_N = V_{cmax}\left[\frac{C_c - \Gamma^*}{C_c + K_c\,(1+\frac{O}{K_o})}\right] - R_D \qquad (1)$$

where C_c is the chloroplast CO_2 concentration, Γ* is the CO_2 compensation point in the absence of dark respiration, K_c and K_o are the Michaelis constants for CO_2 and O_2, respectively, and O is the oxygen concentration. Γ* is a function of the CO_2/O_2 specificity (K_oV_c/K_cV_o), and O, and V_o is taken as $0.21V_c$.

C_c can be calculated from the CO_2 concentrations in C_i (Eq. 2) because the reduction of C_c from C_i depends on mesophyll conductance (g_m), using the curve-fitting calculator developed by Sharkey *et al*. (2007).

$$C_c = C_i - \frac{P_N}{g_m} \qquad (2)$$

The RuBP-limited photosynthetic rate is calculated by the rate of electron transport J using Eqs. 3 and 4:

$$P_N = J\,(\frac{C_c - \Gamma^*}{4C_c + 8\Gamma^*}) - R_D \qquad (3)$$

$$J = \frac{\alpha\,\mathrm{PPFD} + J_{max} - \sqrt{(\alpha\,\mathrm{PPFD} + J_{max})^2 - 4\theta\,\alpha\,\mathrm{PPFD}\,J_{max}}}{2\theta} \qquad (4)$$

where θ is the curvature of the PPFD response curve of J and α is the quantum efficiency.

For *S. alterniflora* (C_4 grass), the von Caemmerer and Furbank (1999) photosynthesis model identified that photosynthesis can either be enzyme limited or electron transport limited. In each process, the rate of PEP (V_p) and Rubisco (V_c) carboxylation are described as a function of V_{cmax} and V_{pmax} for the former and of J_{max} for the latter (*see* Eqs. 5, 6, 7, and 8):

$$P_N = V_p - L - R_m \qquad (5)$$

$$L = g_{bs}\,(C_s - C_m) \qquad (6)$$

$$P_N = V_c\,(1 - \frac{\gamma^* O}{C_s}) - R_D \qquad (7)$$

$$V_c = \frac{C_s V_{cmax}}{C_s + K_c\,(1+\frac{O}{K_o})} \qquad (8)$$

where L is the rate of CO_2 leakage from the bundle-sheath to the mesophyll, R_m is the mitochondrial respiration occurring in the mesophyll, which for practical purposes can be set as 0.5 R_D, g_{bs} is the bundle sheath cell conductance to CO_2, C_m is the CO_2 concentration in the mesophyll cells, C_s is the CO_2 concentration at the carboxylation site of Rubisco in the bundle-sheath, and γ* is the half the reciprocal of Rubisco specificity ($S_{c/o}$).

C_m can be calculated from the values of CO_2 concentrations in C_i because the drawdown of C_m from C_i depends on g_m (Eq. 9).

$$C_m = C_i - \frac{P_N}{g_m} \qquad (9)$$

Considering that the measurements were made in conditions, where photosynthesis is enzyme limited, we can use the equations given as follows and use Eq. 10 to calculate V_p:

$$V_p = \frac{C_m V_{pmax}}{C_m + K_p} \qquad (10)$$

where K_p is the Michaelis–Menten constant for PEP carboxylation.

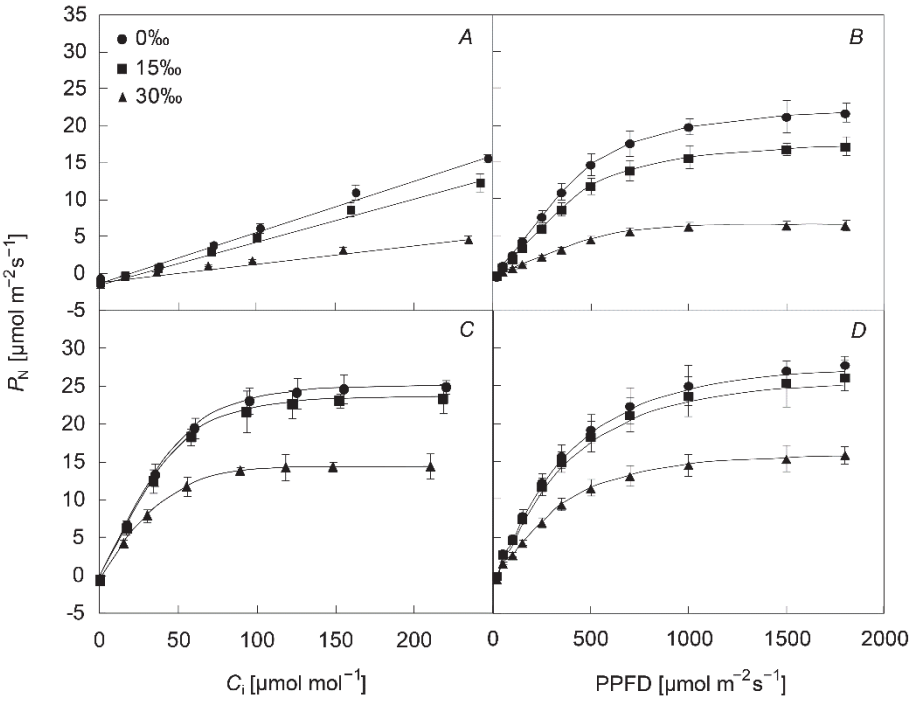

Fig. 1. Net photosynthetic rates (P_N) as a function of intercellular CO_2 concentration (C_i) and PPDF gradient at 25°C for *P. australis* (*A*,*B*) and *S. alterniflora* (*C*,*D*), together with the fitted polynomial for the P_N-C_i and P_N-PPFD curves under different salinity treatments (0‰, 15‰, and 30‰). Each point represents a measured mean (± SE), based on four replicates in each treatment.

For the calculation of J_{max}, we used the electron transport-limited expressions of V_p and the empirical, nonrectangular hyperbolic function by Farquhar and Wong (1984) using Eqs. 11 and 12:

$$V_p = \frac{\chi J_t}{2} \tag{11}$$

$$J_t = \frac{\alpha_1 \,\mathrm{PPFD} + J_{max} - \sqrt{(\alpha_1 \,\mathrm{PPFD} + J_{max})^2 - 4\theta\,\alpha_1 \,\mathrm{PPFD}\, J_{max}}}{2\theta} \tag{12}$$

where χ is a partitioning factor of electron transport, J_t is the electron transport rate, and α_1 is the photosynthetically active irradiance absorbed by PSII.

Table 1 shows the values of the parameters used in the C_3 and C_4 photosynthesis model. The temperature dependence of V_{cmax}, V_{pmax}, and J_{max} can be described using a modified Arrhenius equation in relation to an optimum temperature, based on Farquhar *et al*. (2001) and Leuning (2002) in Eq. 13:

$$\mathrm{Parameter} = \mathrm{Parameter}_{25} e^{\frac{Ha(TL-298)}{298RTL}} \frac{1+e\frac{298\Delta S - H_d}{298R}}{1+e\frac{T_L\Delta S - H_d}{298RT_L}} \tag{13}$$

where $\mathrm{Parameter}_{25}$ is the value of V_{cmax}, J_{max} or V_{pmax} at 25°C, T_L is the leaf temperature, H_a is the enthalpy of activation reflecting the rate of exponential increase of the function below the optimum; H_d is the enthalpy of deactivation describing the rate of decrease of the function above the optimum and ΔS is the entropy of the desaturation equilibrium.

Statistical analysis: Statistical analyses were performed using the analysis of variance (*ANOVA*) and the *Tukey*'s HSD test using the *SPSS v. 16.0* software package (*SPSS Inc*., Chicago, USA). For each salinity treatment, pair-wise comparisons were made with all measurement temperatures. Differences in photosynthetic parameters between treatments were assessed to be statistically significant at $p<0.05$.

Results

Gas-exchange response to salinity: Regardless of salinity treatments and measurement temperatures, P_{Nsat} and g_s of *P. australis* were lower than that of *S. alterniflora* (Fig. 2). With increasing temperatures, P_{Nsat} and g_s displayed a curvilinear response. For *P. australis*, P_{Nsat} and g_s increased with temperature starting from 15°C and peaking at 25°C; thereafter they declined at 30–35°C. For *S. alterniflora*, P_{Nsat} and g_s peaked at 30°C with a subsequent decrease.

P_{Nsat} of *P. australis* was on average 22.1 and 69.8% lower after the MS and HS treatments, respectively, over the range of temperatures used (Fig. 2) compared with that of without salt. The limitation of P_{Nsat} in *S. alterniflora* was only marginal (5.4%) under MS, but the P_{Nsat} was significantly lower by 44.8% under HS over the range of temperatures used. The inhibition of g_s by salinity was more significant relative to P_{Nsat} in both grasses (Fig. 2),

Table 1. Values for constants used in the calculations on photosynthetic parameters of C_3 and C_4 grasses.

Constant	Unit	C_3 Value	Resource	C_4 Value	Resource
K_c	μbar	270	Bernacchi *et al*. 2001,	650	
K_o	μbar	165,000	2002	450,000	
K_p	μbar	-	-	80	
θ	-	0.7	Farquhar *et al*. 2001	0.7	von Caemmerer and
a	mol mol^{-1}	0.5		-	Furbank 1999,
x	-	-	-	0.4	Cousins *et al*. 2010
a_1	mol mol^{-1}	-	-	0.361	
γ^*	-	-	-	0.000193	
$S_{c/o}$	bar bar^{-1}	-	-	2,862	

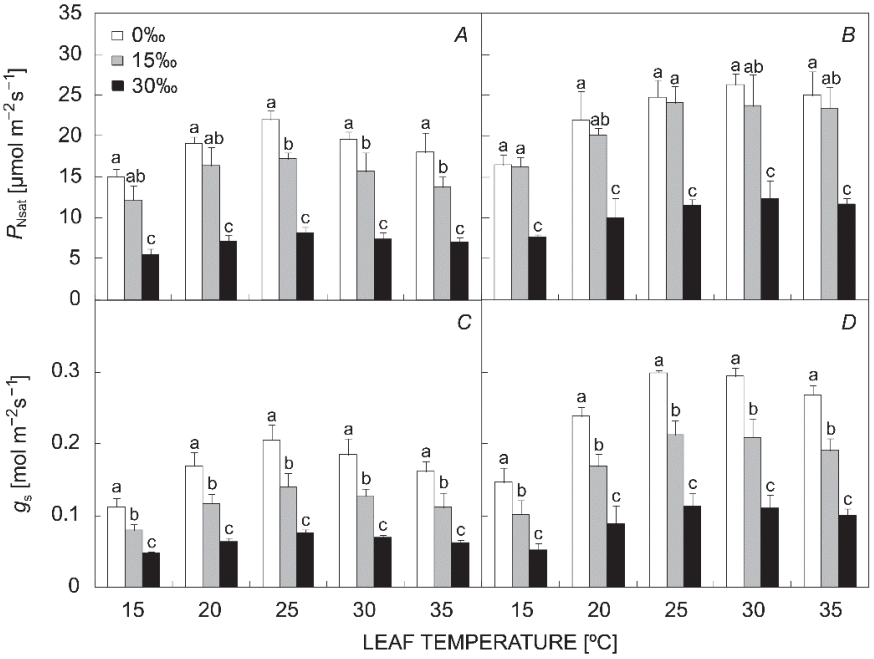

Fig. 2. Means (± SE) of the light-saturated net photosynthetic rates (P_{Nsat}) and the light-saturated stomatal conductance (g_s) in *P. australis* (*A*,*C*) and *S. alterniflora* (*B*,*D*) under different salinity treatments (0‰, 15‰, and 30‰). The measurements were conducted at six temperatures ranging from 15 to 35°C, based on four replicates in each treatment.

i.e., g_s of *P. australis* decreased on average 35.7 and 71.4%; it was 26.9 and 60.2% lower in *S. alterniflora* under MS and HS treatments, respectively, over the range of temperatures, compared with that without salt.

Response of photosynthetic parameters to salinity: Regardless of salinity and temperatures, V_{cmax} and J_{max} peaked at 25°C in *P. australis* and 30°C in *S. alterniflora*, and then declined with an increase in temperature (Figs. 3, 4). In the C_4 grass, *S. alterniflora*, V_{pmax} continued to increase with increasing temperature.

Under both salinity treatments, V_{cmax} of *P. australis* declined, by an average of 9.5% at MS and 18.2% at HS compared with the treatment without salt over the range of measured temperatures (Fig. 3). In *P. australis*, the MS treatment reduced J_{max}, on average, by 16.5%; it decreased significantly by 30.4% at HS over the range of measured responses of V_{cmax} and J_{max} in *P. australis* and *S. alterniflora*. For *P. australis*, salinity treatments shifted the optimum temperature of the photosynthetic parameters to lower temperatures, 23.5 and 22.4°C for V_{cmax} and J_{max}, temperatures. In *S. alterniflora*, the MS treatment had no effect on V_{cmax}, V_{pmax}, and J_{max} (Fig. 4). When the measurements were done after HS treatment, V_{cmax}, V_{pmax}, and J_{max} were lowered in *S. alterniflora*, on average, by 9.3, 8.5, and 19.8%, respectively, over the range of measured temperatures, compared with that without salt. Under HS, J_{max} was much lower at high temperatures, *i.e.*, 25–35°C in *P. australis*, and 30–35°C in *S. alterniflora*.

As for the relationship between J_{max} and V_{cmax}, HS resulted in lower values of the J_{max} to V_{cmax} ratio in both C_3 and C_4 grasses (Fig. 5). H_a, H_d, and ΔS were calculated as functions with inputs of the temperature dependent photosynthetic parameters, showing no difference under salt treatments regarding *S. alterniflora* (Table 2). However, H_a of J_{cmax} for *P. australis* increased significantly under HS (Table 2), compared with that without salt.

Fig. 6 shows the normalized (to 1 at 25°C) temperature respectively, under HS. The optimum temperature of photosynthetic parameters of *S. alterniflora* was not changed by the salinity treatments.

Discussion

In this study, the net photosynthetic rates of *S. alterniflora* along the C_a and PPFD gradients were higher than that of *P. australis*, reflecting the distinct properties of C_4 and C_3 pathways in relation to CO_2 fixation. The measurements confirmed that the non-native *S. alterniflora* performed photosynthesis at lower temperatures more productively than the native grass. The maximal rate of photosynthesis showed strong temperature dependence under saturated light conditions with an optimum at around 25°C in *P. australis* and 30°C in *S. alterniflora*. According to Farquhar *et al*. (2001) and Sharkey *et al*. (2007), the optimum net assimilation rate for C_3 plants should be around 25°C. Crafts-Brandner and Salvucci (2002) and Kubien *et al*. (2003) suggested a broad temperature optimum between 28°C and 37.5°C for C_4 plants.

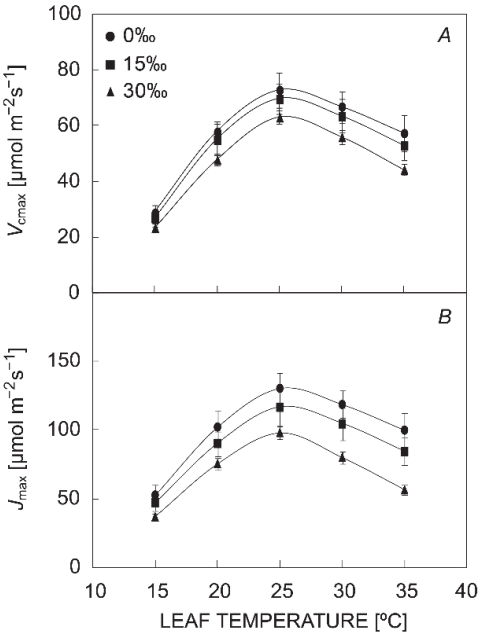

Fig. 3. Temperature responses of the maximum rate of Rubisco activity (V_{cmax}) (*A*) and the potential rate of electron transport (J_{max}) (*B*) in *P. australis* under different salinity treatments (0‰, 15‰, and 30‰).

The inhibition of P_{Nsat} in *P. australis* indicated that photosynthesis was significantly limited at MS and HS, confirming salinity as a well-known stressor of this species. As observed by Burdick *et al*. (2001), salinity stress on a salt marsh slowed down the expansion of *P. australis*, which preferred to access fresh water. The restrained gas exchange was attributed to stomatal limitation, which is a common response of leaf g_s to salinity (Farquhar *et al*. 1980, Farquhar and von Caemmerer 1982). Salt stress results in an alteration in water status and local synthesis of abscisic acid in stomatal guard cells (Munns and Tester 2008).

As measured in this study, P_{Nsat} in *S. alterniflora* was

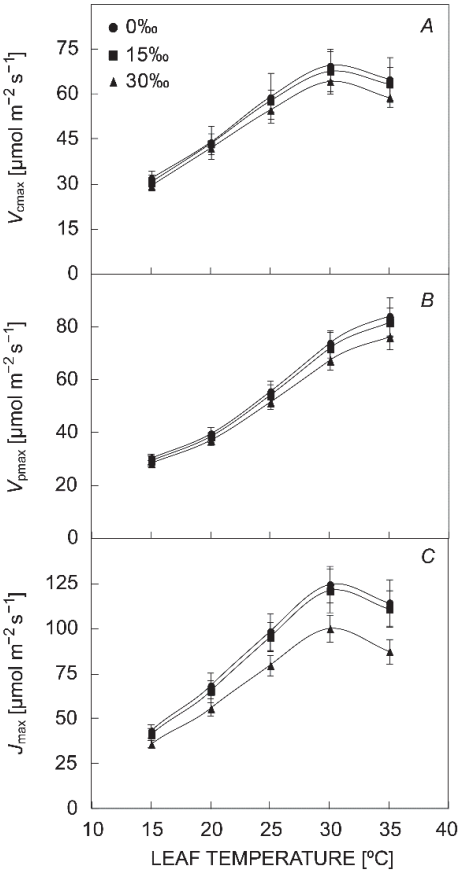

Fig. 4. Temperature responses of the maximum rate of Rubisco activity (V_{cmax}) (*A*), the maximum rate of PEP carboxylation (V_{pmax}) (*B*) and the potential rate of electron transport (J_{max}) (*C*) in *S. alterniflora* under different salinity treatments (0‰, 15‰, and 30‰).

not limited at MS, which revealed the greater salt tolerance of the non-native grass when compared with the native *P. australis*. Generally, C_4 plants have greater water use efficiency than C_3 plants because of the higher CO_2 assimilation rate. It contributes to their salt tolerance by reducing the amount of water and therefore salt that the roots must process to support growth (Flowers *et al*. 1977). As reported by Vasquez *et al*. (2006), *S. alterniflora* was able to use nitrate ions for osmotic adjustment in its shoots, thus, avoiding salinity stress. However, stomatal conductance of *S. alterniflora* was significantly reduced under both MS and HS. The imbalance between theresponses of P_{Nsat} and g_s indicated that stomata closure was not directly related to intercellular carbon under salt stress in the C_4 grass.

Currently, the responses of biochemical parameters (V_{cmax}, J_{max}, and V_{pmax}) to changes in growth temperatures are widely used to estimate the multi-enzyme kinetic properties in photosynthesis under environmental stresses (Centritto *et al*. 2003, Yin *et al*. 2011, Ge *et al*. 2012).

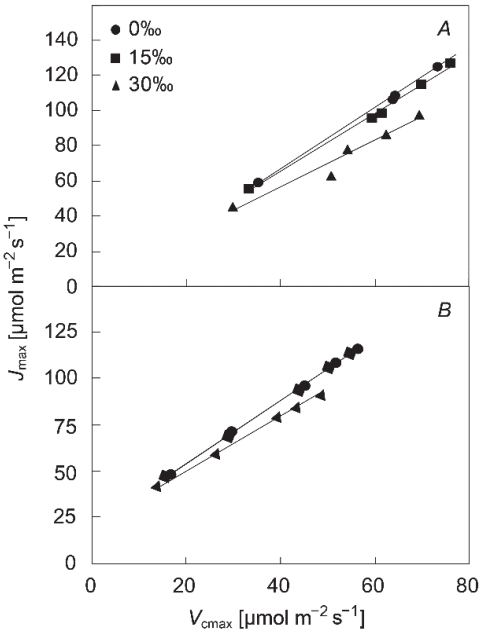

Fig. 5. Ratio of the potential rate of electron transport (J_{max}) to the maximum rate of Rubisco activity (V_{cmax}) for *P. australis* (*A*) and *S. alterniflora* (*B*) under different salinity treatments (0‰, 15‰, and 30‰).

Under our experimental conditions, the reductions in both V_{cmax} and J_{max} by about 10–20% were observed across the range of tested temperatures in *P. australis* even though it was treated only with MS. This suggested that the limitation in photosynthesis observed in *P. australis* grown under salt treatments was related to changes in Rubisco activity, which agrees with the findings of Agastian *et al*. (2000). He *et al*. (2002) noted that the transcription level of the small subunit of Rubisco was suppressed by salt stress in a C_3 crop. According to their most recent report (He *et al*. 2014), the formation of chloroplast protrusions under the salt stress resulted in a much greater increase in the production of Rubisco-containing bodies, which may be an important mechanism for the rapid degradation of Rubisco under salt stress.

In *P. australis*, the photosynthetic parameter, J_{max}, decreased further under salt stress compared with V_{cmax}, especially at high temperatures. It suggested that the rate of electron transport was limited more than the Rubisco activity. The declined ratio of J_{max} to V_{cmax} also implicated a lower rate of RuBP regeneration. In this paper, salt treatments shifted the optimum temperatures for photosynthetic parameters to lower temperatures. This might be attributed to the decreasing photosynthetic enzyme activity (He *et al*. 2002, Centritto *et al*. 2003), which was previously reported in terms of chlorophyll fluorescence parameters in *P. australis* (Deng *et al*. 2011) and other C_3 plants (Sudhir and Murthy 2004, Li *et al*. 2013, Wu *et al*. 2013). Lower photochemical efficiency in the salt-stressed plants indicates that RuBP regeneration, which needs adequate electron translocation from PSII to electron acceptors, might be disrupted by salinity (Desingh and Kanagaraj 2007, Zhang and Xing 2008, Dadkhah 2011).

Regarding the non-native species, *S. alterniflora*, V_{cmax} and V_{pmax} of salt-stressed plants appeared to be similar to plants without salt over the range of temperatures used here. The salt treatments had no effect on the optimum temperature for photosynthesis and the ratio of J_{max}/V_{cmax}. This indicated that Rubisco and PEPC enzyme activities in mesophyll cells of the non-native C_4 grass were not affected under both MS and HS. As already documented, the effect of salt-alkaline stress on C_4 enzymes varied in different species. Rubisco activity remained relatively constant under salt stress in *Atriplex lentiformis* (Meinzer and Zhu 1999). Wang *et al*. (2013) noted that PEPC activities could be regulated by soil salinity, depending on the source of the enzyme as well as on the concentration of the substrate (PEP). Nevertheless, some studies have reported that moderate salt stress had little effect on PEPC activity (Rout and Shaw 2001). Additionally, the redox potential encountered in salt marsh environments (Vasquez *et al*. 2006). Therefore, the C_4 type of carbon

Table 2. Mean (± SE, n = 4) estimates of model parameters (H_a, H_d, ΔS [kJ mol^{-1}]) used to describe the temperature-dependent photosynthetic parameters (V_{cmax}, V_{pmax}, and J_{max}) in *P. australis* (C_3) and *S. alterniflora* (C_4) over a range of temperatures (15–35°C) under different salinity treatments (0‰, 15‰, and 30‰). Different *uppercase letters* denote significant differences among means within each column (p<0.05, *Tukey*'s HSD test).

Salinity treatments	For V_{cmax}			For J_{max}			For V_{pmax}		
	H_a	H_d	ΔS	H_a	H_d	ΔS	H_a	H_d	ΔS
P. australis									
0‰	54.4 ± 3.5^{ab}	200.2 ± 15.4^{a}	0.67 ± 0.05^{a}	57.3 ± 2.3^{b}	201.3 ± 16.5^{a}	0.67 ± 0.04^{a}	-	-	-
15‰	60.4 ± 5.1^{a}	200.1 ± 18.6^{a}	0.67 ± 0.04^{a}	62.4 ± 3.8^{ab}	201.2 ± 13.4^{a}	0.67 ± 0.04^{a}	-	-	-
30‰	62.5 ± 4.9^{a}	199.2 ± 19.5^{a}	0.66 ± 0.05^{a}	72.5 ± 4.5^{a}	200.2 ± 17.4^{a}	0.66 ± 0.02^{a}	-	-	-
S. alterniflora									
0‰	48.5 ± 4.1^{a}	146.5 ± 10.7^{a}	0.49 ± 0.02^{a}	71.1 ± 6.8^{a}	192.8 ± 14.5^{a}	0.63 ± 0.06^{a}	59.2 ± 2.2^{a}	108.4 ± 7.4^{a}	0.38 ± 0.02^{a}
15‰	51.4 ± 2.5^{a}	146.4 ± 12.5^{a}	0.49 ± 0.02^{a}	71.2 ± 5.2^{a}	192.7 ± 11.4^{a}	0.63 ± 0.04^{a}	59.5 ± 4.6^{a}	108.2 ± 5.5^{a}	0.38 ± 0.01^{a}
30‰	51.5 ± 4.2^{a}	146.2 ± 9.4^{a}	0.49 ± 0.02^{a}	71.6 ± 5.4^{a}	192.8 ± 10.5^{a}	0.63 ± 0.01^{a}	60.2 ± 5.4^{a}	108.1 ± 8.1^{a}	0.38 ± 0.01^{a}

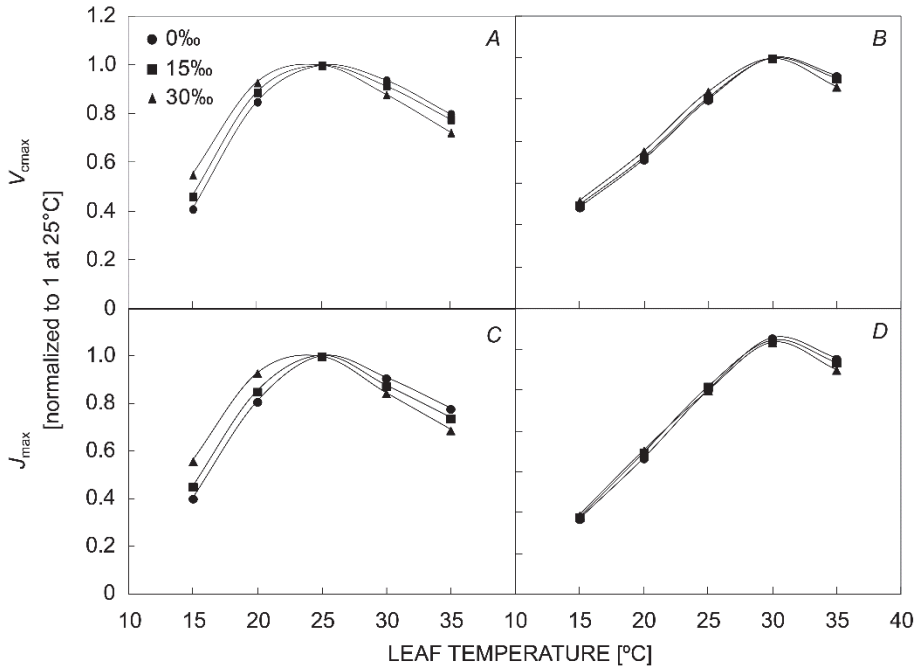

Fig. 6. Normalized (to 1 at 25°C) parameters of the maximum rate of Rubisco activity (V_{cmax}) and the potential rate of electron transport (J_{max}) for *P. australis* (*A*,*C*) and *S. alterniflora* (*B*,*D*) in response to analyzed temperatures (15–35°C), under different salinity treatments (0‰, 15‰, and 30‰).

metabolism might contribute to some extent to the saline tolerance in these plants.

As measured, the rate of photosynthesis in *S. alterniflora* declined significantly under HS, which could be probably attributed to the inhibition of J_{max}, as well as the lower g_s. Some studies on C_4 crops have demonstrated that high salinity in the presence of high light induced significant changes in photochemistry and increased the susceptibility of PSII to photoinhibition (Hichem *et al*. 2009). Maricle *et al*. (2007) also concluded that stomatal conductance, coupled with photoinhibition, was the main factor responsible for a reduction in gas exchange in *S. alterniflora*. However, the synergetic effect of stomatal behavior and enzyme activity on photosynthesis is not well understood, and more detailed investigations are still required.

In conclusion, this study compared the effects of salinity on photosynthesis, particularly, on the biochemical parameters, with variations in temperature in two grasses, a native C_3 and a non-native C_4 salt marsh grass. *S. alterniflora* showed the greater CO_2 assimilation rate than *P. australis*, across the range of temperatures analyzed here. The photosynthetic parameters in *P. australis* declined with increasing salinity; in addition, a great limitation to stomatal conductance occurred, resulting in significantly lower gas-exchange rates. Although moderate stress inhibited stomatal conductance, *S. alterniflora* exhibited a greater tolerance to salt than that of the native grass, with lesser sensitivity of its photosynthetic parameters. High salinity decreased significantly the photosynthetic efficiency and electron transport in the C_4 grass. The findings indicated that the combined factors of stomatal conductance, enzyme activity, and electron transport affected the photosynthetic performance of plants in response to salt treatments. On the moderately saline coastal marshes of the Yangtze Estuary, the success of *S. alterniflora* could be probably attributed to its C_4 photosynthetic pathway and salt tolerance. In this study, we suggested that a modified parameterization of the photosynthetic model could achieve an improved or reasonable photosynthetic simulation under salt stress.

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