Photosynthetic acclimation and leaf traits of *Stipa bungeana* in response to elevated CO₂ under five different watering conditions

H. WANG[*,**], G.S. ZHOU[***,+], Y.L. JIANG[*], Y.H. SHI[***], and Z.Z. XU[*]

State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China[*]
University of Chinese Academy of Sciences, Beijing 100049, China[**]
Chinese Academy of Meteorological Sciences, Beijing 100081, China[***]

Abstract

Although plant performance under elevated CO₂ (EC) and drought has been extensively studied, little is known about the leaf traits and photosynthetic performance of *Stipa bungeana* under EC and a water deficiency gradient. In order to investigate the effects of EC, watering, and their combination, *S. bungeana* seedlings were exposed to two CO₂ regimes (ambient, CA: 390 ppm; elevated, EC: 550 ppm) and five levels of watering (–30%, –15%, control, +15%, +30%) from 1 June to 31 August in 2011, where the control water level was 240 mm. Gas exchange and leaf traits were measured after 90-d treatments. Gas-exchange characteristics, measured at the growth CA, indicated that EC significantly decreased the net photosynthetic rate (P_N), water-use efficiency, nitrogen concentration based on mass, chlorophyll and malondialdehyde (MDA) content, while increased stomatal conductance (g_s), intercellular CO₂ concentration (C_i), dark respiration, photorespiration, carbon concentration based on mass, C/N ratio, and leaf water potential. Compared to the effect of EC, watering showed an opposite trend only in case of P_N. The combination of both factors showed little influence on these physiological indicators, except for g_s, C_i, and MDA content. Photosynthetic acclimation to EC was attributed to the N limitation, C sink/source imbalance, and the decline of photosynthetic activity. The watering regulated photosynthesis through both stomatal and nonstomatal mechanisms. Our study also revealed that the effects of EC on photosynthesis were larger than those on respiration and did not compensate for the adverse effects of drought, suggesting that a future warm and dry climate might be unfavorable to *S. bungeana*. However, the depression of the growth of *S. bungeana* caused by EC was time-dependent at a smaller temporal scale.

Additional key words: climate change; carbon balance; gas exchange; respiration; chlorophyll fluorescence.

Introduction

Carbon dioxide (CO₂) concentration has been supposed to increase from 280 ppm in the preindustrial period to 550 ppm by the middle of the century, and it is projected to surpass 750 ppm by the end of the century (IPCC 2014). Global redistributions in precipitation amounts and patterns and increases of heavy rain and drought events have been also observed (Huntington 2006). Water is recognized as one of the most important climatic factors determining plant growth and mortality (Edwards *et al.* 2012). Elevated CO₂ and changing precipitation have together produced profound influences from individual to ecosystem levels (Morgan *et al.* 2004, Bonan *et al.* 2008, Hooper *et al.* 2012, Peñuelas *et al.* 2013, Manea and Leishman 2015).

Many studies have reported the morphological and physiological responses of plants to CO₂ enhancement and their interactions with other environmental factors (such as temperature, watering, ozone, *etc.*) (Albert *et al.* 2011,

---

Biswas *et al.* 2013, Qaderi *et al.* 2006, Salazar-Parra *et al.* 2015). Generally, EC increases plant growth and biomass by increasing photosynthesis and water-use efficiency (WUE) and reducing g_s (Clifford *et al.* 2000, Kaminski *et al.* 2014, Yan *et al.* 2014). However, it has been reported that photosynthesis under prolonged EC declines, an effect described as photosynthetic acclimation or photosynthetic downregulation (Long *et al.* 2004, Ainsworth and Rogers, 2007). This phenomenon can be induced both by stomatal and nonstomatal limitations. Stomatal limitation is mainly attributed to stomata closure and, subsequently, to the decrement of C_i (Salazar-Parra *et al.* 2015). Nonstomatal limitation is consistent with the imbalance of carbon sinks and sources, N limitation (Ellsworth *et al.* 2004, Ainsworth and Long 2005), or reduction of photosynthetic capacity (Parry *et al.* 2003). Furthermore, the response of photosynthesis to EC largely depends on other environmental factors, such as water, temperature (Wertin *et al.* 2010), or ozone (Talhelm *et al.* 2014). Water availability is particularly important in driving individual or ecosystem functions, especially in water-limited grasslands (Hovenden *et al.* 2014). It has substantial and continuous effects on plant physiological processes, such as photosynthesis, WUE, and metabolite accumulation (Yin *et al.* 2005, Ohashi *et al.* 2006, Pizarro and Bisigato 2010, Sala *et al.* 2010, Edwards *et al.* 2012), and then changes the carbon partitioning at both leaf and whole plant levels (Chaves 1991). As mentioned above, numerous studies have illustrated the effects of CO$_2$ and watering independently; however, fewer studies have been focused on the interactive effects of EC and water status. A large number of studies have shown the fertilization effect of EC on plant growth, while the EC impact on plants related to drought stress is still under debate (Erice *et al.* 2006, Newingham *et al.* 2013, Duan *et al.* 2014). Some studies indicate that moderate water stress generally enhances the stimulation of photosynthesis induced by EC (Wullschleger *et al.* 2002, Xu *et al.* 2014), while over-watering may reverse this stimulation (Li *et al.* 2014).

Under stressful conditions, photosynthesis can vary with species, functional group, environmental conditions, and resource availability (Ainsworth and Rogers 2007). N availability can influence plant leaf biochemistry, as well as photosynthetic capacity, and ultimately, plant growth (Novriyanti *et al.* 2012). Furthermore, plant growth is rather sensitive to water availability, and the interactive effects of environmental factors are particularly important in order to ascribe their effects on water use (Zelikova *et al.* 2015).

Stipa bungeana, a dominant species of the warm-temperate steppe in semiarid areas and an important pasture species, is helpful in stabilization and conservation of grasslands (Yu *et al.* 2014). However, this species has been severely degraded due to climatic change and improper agricultural development and the region may suffer severely from climatic change in the future. Gao and Zhang (1997) found the plant biomass of *S. bungeana* responded more to EC than to an increase in the amount of water. Cheng *et al.* (2010) demonstrated that the growth of *S. bungeana* was influenced by different climatic factors and water was the major one. To date, studies on *S. bungeana* mainly focus on its community characteristics and functions, while there is limited information on its individual response to climate change. In the present study, we used annual seedlings of *S. bungeana* and we set a wide range for the watering gradient (five watering treatments: –30%, –15%, control, +15%, +30%, relative to the mean precipitation over the past 30 years in the same growing season) with ambient (CA) or EC concentrations (390 ppm and 550 ppm, respectively). The gas exchange and other leaf traits were measured after the completion of the 90-d experiment. Specifically, our aims were to test the hypotheses that (*1*) the elevated CO$_2$ effect on *S. bungeana* is determined by the watering status, and physiological traits in response to watering show higher sensitivity than to elevated CO$_2$; and (*2*) there might be significant relationships among these leaf physiological traits under different environmental conditions.

Materials and methods

Experimental design: *S. bungeana* seeds were collected the year before the experiment from the natural grassland in Ordos, Inner Mongolia, China. The soil type belongs to castanozem, the soil organic carbon, total nitrogen, and available nitrogen concentrations (± SE, n = 3) were 12.31 ± 0.19 g kg^{-1}, 1.45 ± 0.02 g kg^{-1}, and 81.61 ± 0.71 mg kg^{-1}, respectively. This region is characterized by a semiarid, north temperate continental monsoon climate with a frigid winter and hot summer.

The seeds were sown in plastic pots (10.9 cm in above diameter, 8.5 cm in below diameter, 9.5 cm in height). Each pot was filled with *ca*. 0.6 kg of dry soil and four plants were planted per pot. All pots were placed in a green-house [maximum light intensity: *ca*. 1,000 μmol(photon) m^{-2} s^{-1}; day/night temperature: 26–28°C/18–20°C] until

the third leaf completely emerged. Subsequently, the plants were transferred into open-top chambers (OTCs) located outdoors in the Institute of Botany, the Chinese Academy of Sciences. The OTCs were regular hexahedron in shape (85 cm in length of a side, 170 cm in diameter, and 150 cm in height), with a rain-shelter on the top; the shelter was larger than the top of the hexahedron and there was a space between it and the OTC. Pure CO$_2$ gas (99.999%) in a cylinder was used as the gas supply (*Chao Hong Ping Gas Co. Ltd,* Beijing, China), and a CO$_2$ gas sensor (*eSENSE-D, SenseAir,* Delsbo, Sweden) was used to regulate continuously the CO$_2$ concentration every minute by an electromagnetic valve; the CO$_2$ concentration was recorded for 24 h with a data logger (*DAM-3058RTU, Altai Sci TechDev Co. Ltd.,* Shanghai, China). The CO$_2$

concentrations had $\pm$ 30 μmol mol^{-1} variations relative to the set points. The air temperature was monitored using thermocouples (*HOBO S-TMB-M006, Onset Computer Co.,* Bourne, MA, USA) installed at a height of 75 cm inside and outside of the chambers. The data for the meteorological factors were recorded by a logger (*HOBO H21-002*) every 30 min automatically during the experiment. The relative humidity in the OTCs was *ca.* 60%. The monthly mean temperature inside the OTC was 27.7°C and 26.5°C in July and August, respectively; outside the OTC was 26.9°C and 25.7°C, the differences were approximately 0.8°C.

This experiment was designed to examine the effects of two CO_2 concentrations (CA of 390 ppm and EC of 550 ppm) and five watering treatments: (*1*) –30% (–30%W, reduced precipitation by 30% relative to local average precipitation over 30 years during 1978–2007), (*2*) –15% (–15%W, reduced precipitation by 15% relative to local average precipitation), (*3*) control (control, local average precipitation over 30 years during 1978–2007), (*4*) +15% (+15%W, increased precipitation by 15% relative to local average precipitation), (*5*) +30% (+30%W, increased precipitation by 30% relative to local average precipitation), roughly equalling 168, 204, 240, 275, and 311 mm precipitation during the growing season period, respectively. The two CO_2 concentrations were the main plots, while the five watering patterns were split plots (pots). Six OTCs were used for the two CO_2 treatments with three replicates for each one; every watering treatment was generated using two pots within each OTC. Thus, every watering treatment had six replicates at each CO_2 concentration. Water was added to the soil surface through a measuring flask at approximately 16:00 h every three days.

Gas exchange and chlorophyll (Chl) fluorescence: The net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) were measured by an open gas-exchange system (*LI-6400F; LI-COR, Inc.,* Lincoln, NE, USA) equipped with a leaf fluorometer chamber (*LI-6400-40, Li-Cor Inc.*). We measured at least three youngest and fully expanded leaves from different individuals (one plant per pot) for all replicates, at 9:00 to 16:30 h daily. All measurements were performed at a block temperature of 27°C, a chamber air-flow rate of 500 μmol s^{-1}, relative humidity of 50–70%, irradiance of 1,200 μmol(photon) m^{-2} s^{-1} (a saturated PPFD in the current experiment), and a chamber CO_2 concentration of either 390 ppm or 550 ppm. The water-use efficiency (WUE) was determined by the ratio of P_N to E. Dark respiration (R_D) was obtained from measuring the gas exchange of the dark-adapted leaves after one night in darkness.

The Chl fluorescence was determined after overnight dark adaptation. The minimal fluorescence yield (F_o) was measured under sufficiently low irradiance [1.0 μmol (photon) m^{-2} s^{-1}] in order to prevent the induction of any significant photosynthetic reaction, and the maximal

fluorescence yield (F_m) was determined using a 0.8-s saturating pulse at 8,000 μmol(photon) m^{-2} s^{-1} on dark-adapted leaves. The maximal efficiency of PSII photochemistry was expressed as $F_v/F_m = (F_m - F_o)/F_m$ (Maxwell and Johnson 2000). Meanwhile, the steady-state value of fluorescence (F_s) was determined, and the second saturating pulse at 8,000 μmol(photon) m^{-2} s^{-1} was imposed to determine the maximal light-adapted (F_m') fluorescence level. The actinic light was removed and the minimal fluorescence level in the light-adapted state (F_0') was determined after 3 s of far-red illumination. The parameters monitored were the actual PSII efficiency [$\Phi_{PSII} = (F_m' - F_s)/F_m'$], intrinsic PSII efficiency [$F_v'/F_m' = (F_m' - F_o')/F_m'$], and electron transport rate (ETR = $\Phi_{PSII} \times$ PPFD $\times f \times \alpha_{leaf}$), where PPFD is incident irradiation on the leaf, f is the fraction of incident radiation absorbed by the leaf sample (approximately 0.85), α_{leaf} is used as the fraction of excitation energy distributed to PSII (generally it is assumed to be equally distributed between PSI and PSII). The photorespiration (R_L) was estimated as 1/12 [ETR $- 4$ ($P_N + R_D$)] (Valentini *et al.* 1995, Salazar-Parra *et al.* 2015).

C and N content: The leaves used to measured gas exchange were harvested for the determination of C and N content based on mass (C_m, N_m), and area (C_a, N_a), respectively. The dry samples were previously ground to powder, and then 8 mg of samples were stored in tin capsules for *Vario EL III* elemental analyser (*Elementar Analysensysteme GmbH,* Hanau, Germany) to obtain C_m and N_m [% of DM].

$$C_a \,[\text{g m}^{-2}] = DM_{leaf} \times C_m/A_{leaf}$$
$$N_a \,[\text{g m}^{-2}] = DM_{leaf} \times N_m/A_{leaf}$$

where DM_{leaf} is leaf dry mass and A_{leaf} is total leaf area.

Chl content: The pigments of 0.2 g of fresh leaves were extracted in 8 ml of 95% acetone. Chl *a* and Chl *b* contents were determined spectrophotometrically at 663 and 645 nm with the use of a UV-visible spectrophotometer (*UV-2550 Shimadzu Corporation*, Japan). The total Chl content was calculated as the sum of Chl *a* and Chl *b* (Porra *et al.* 1989).

MDA content and leaf water potential (Ψ_w): The youngest fully expanded leaves (*ca.* 500 mg) was homogenized in 6 ml of 10% TCA solution. The homogenate was centrifuged at 4°C, 4,000 $\times$ *g* for 10 min. Then, the supernatant of 2 ml was obtained and mixed with 2 ml of 0.6% thiobarbituric acid (TBA). The mixture was incubated at 100°C in a shaking water bath for 15 min, and the reaction was terminated by placing the reaction tubes in an ice-water bath. The samples were then centrifuged at 4°C, 4,000 $\times$ *g* for 5 min. The absorbance of the supernatant was read at 450, 532, and 600 nm (Hernández and Almansa 2002) using a spectrophotometer (*UV-2550, Shimadzu Corporation*, Japan). The amount of MDA was

calculated according to Cakmak and Horst (1991). The leaf water potential (Ψ_w) was measured with *Dewpoint Potentiometer WP4-T* (*Decagon Devices, Inc.,* Pullman, WA, USA).

Statistical analyses were performed using *SPSS 16.0* statistical software (*SPSS Inc.*, Chicago, IL, USA). The data are presented as the mean with standard error (± SE) for each treatment. The differences between the treatments at the same CO₂ concentration or watering treatments were compared using one-way analysis of variance (*ANOVA*). The combined effects of the CO₂ concentration and watering were tested using two-way *ANOVA*, followed by *Duncan'*s multiple-range test. Statistical significance was determined to be 0.05 unless otherwise noted. The correlations of P_N and g_s, P_N and N_m, P_N and F_v/F_m, Chl and N_m, WUE and Ψ_w, P_N and R_D+R_L, N_m and Ψ_w, C_m and Ψ_w, MDA and Ψ_w, Chl and Ψ_w were tested with linear regression analysis. The significant differences of the slopes were analysed with analysis of covariance (*ANCOVA*).

Results

Significant differences in the photosynthetic responses of *S. bungeana* to EC and different watering treatments were detected. EC depressed P_N (Fig. 1*A*) and WUE (Fig. 1*D*) by an average of 16.2 and 34.9%, respectively, and promoted g_s and C_i by 59.9 and 60.7%, respectively (Fig. 1*B,C*). P_N, g_s, and C_i showed significantly positive correlations with the watering treatment, while WUE showed a negative correlation with watering only under the CA. EC and adding water both showed significant stimulation of the dark respiration (R_D) in *S. bungeana* (Fig. 1S, *supplement available online*), however, the effects on photorespiration (R_L) and F_v/F_m were not obvious (Figs. 1S, 2), except for F_v/F_m that was significantly lower under EC and the +30%W conditions. Overall, watering and CO₂ treatments in combination showed only significant influence on g_s and C_i, watering or CO₂ treatments alone showed significant influence on P_N, g_s, C_i, WUE, and R_D.

Compared to CA, the *S. bungeana* grown at EC had a lower nitrogen concentration based on mass (N_m) and slightly higher carbon concentration based on mass (C_m) by –11.6 and +1.55%, respectively (Fig. 3*A,C*). However,
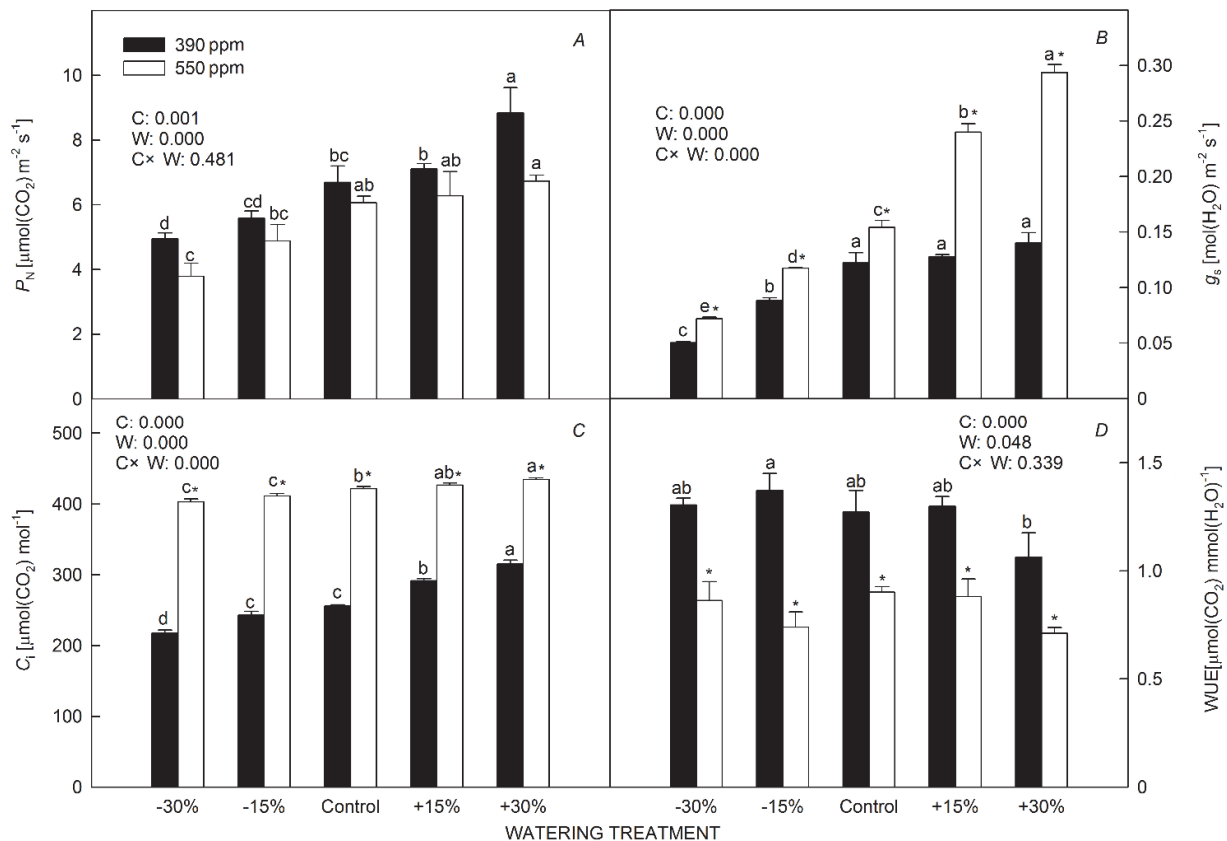
Fig. 1. Net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO₂ concentration (C_i), and water-use efficiency (WUE) of *Stipa bungeana* grown under ambient (390 ppm) and elevated (550 ppm) CO₂ concentrations with watering treatments. Values are mean ± SE (*n* = 3). *Different letters* above bars denote statistically significant differences between watering treatments at *P*<0.05 level according to *Duncan'*s multiple range test under the same CO₂ concentration. * – differences between CO₂ treatments under the same watering treatment. C – CO₂ effect; W – watering effect; C×W – CO₂ and watering effects in combination.

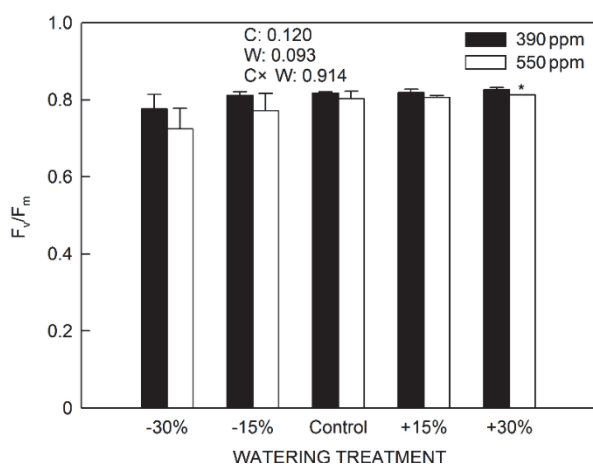Fig. 2. Maximal efficiency of PSII photochemistry (F_v/F_m) of *Stipa bungeana* grown under ambient (390 ppm) and elevated (550 ppm) CO_2 concentrations with watering treatments. Values are mean ± SE ($n = 3$). *Different letters* above bars denote statistically significant differences between watering treatments at $P<0.05$ level according to *Duncan's* multiple range test under the same CO_2 concentration. * – differences between CO_2 treatments under the same watering treatment. C – CO_2 effect; W – watering effect; C×W – CO_2 and watering effects in combination.

EC had a significant influence on N_m only under the control and the +30%W conditions, and on C_m only under the +15%W. Under EC, the nitrogen concentration based on area (N_a) and the carbon concentration based on area (C_a) both showed higher values under the water stress conditions and lower values in the well-irrigated plants, which were not significant (Fig. 3*B,D*). The carbon concentration based on mass (C_m) displayed a slight increase of 1.55% on average under EC, while the increase was significant only in the +15%W treatment (Fig. 3*C*).

Discussion

Climate change could affect the physiological behavior of *S. bungeana*. The objective of this experiment was to reveal the influence of climate change (CO_2 and watering) on *S. bungeana* physiological traits, its underlying mechanisms, carbon balance (carbon gain = P_N; carbon losses = respiration) and the adaptability to future climate conditions. Photosynthetic parameters and other physiological indicators, which were sensitive to one or two or neither factors to some extent, were used to detect these differences between plants and environmental stresses.

In general, the effect of elevated CO_2 concentration on P_N varies depending on the plant functional group and environment (Ainsworth and Rogers 2007). For C_3 plants, a CO_2 increase can result in higher P_N (Salazar-Parra *et al.* 2015). A meta-analysis of FACE measurements showed an increment *ca.* 37% in P_N for C_3 grass at 200 ppm above ambient CO_2 (Ainsworth and Rogers 2007). However, many previous studies have also reported that plants grown under long-term elevated CO_2 concentration can exhibit

Exposure to EC increased the C/N ratio by 16% on average (Fig. 2S, *supplement available online*); in the control and the +30%W conditions, the increase were significant. The Chl and MDA content both decreased *ca.* 10.5 and 13.4%, respectively, on average under EC (Figs. 3S, *supplement available online*; 4*A*). Nevertheless, EC showed only a significant effect on the MDA under the −15%W and control conditions. The Ψ_w showed an increase of 6.9% in response to EC, which was not significant (Fig. 4*B*). The C_m, C/N, and Ψ_w showed a significantly positive correlation with the watering treatment, while N_m, N_a, Chl, and MDA showed a significantly negative correlation with watering, while the watering effect on C_a was unclear. Overall, the watering and CO_2 treatments in combination showed the only significant influence on the MDA content. The watering or CO_2 treatments alone showed significant influence on N_m, C_m, C/N, Chl, MDA, and Ψ_w, while only the watering alone displayed an obvious influence on N_a.

Across the treatments, P_N significantly increased with increasing g_s and F_v/F_m (Fig. 5*A,C*), but it decreased with increasing N_m (Fig. 5*B*). In general, WUE was negatively correlated with Ψ_w under CA (Fig. 6). Overall the ratio of P_N/R was higher under CA than EC (Fig. 7), indicating the EC effects on respiration were larger than on photosynthesis. EC enhanced the influence of N_m on P_N compared to CA, while it reduced the g_s and F_v/F_m influence on P_N. The Chl content was significantly positively correlated with N_m (Fig. 4S, *supplement available online*), and EC lowered the correlation, but not significantly. N_m, MDA, and the Chl content were all significantly negatively correlated with Ψ_w (Fig. 8*A,C,D*), while C_m was positively correlated with Ψ_w only at CA (Fig. 8*B*). EC promoted the correlation between MDA and Chl with Ψ_w and declined the correlation between N_m and C_m with Ψ_w.

downregulation (a decreasing photosynthetic potential) (Long *et al.* 2004, Crous *et al.* 2008). We analyzed this phenomenon from the following perspectives. First, stomatal limitation mainly results from the closure of the stomata and the decline of C_i (Salazar-Parra *et al.* 2015). Our results of gas exchange showed that under EC during this growth stage, P_N showed average decline of 16.2%, while g_s and C_i had increments of 60% and 60.7%, on average, respectively, indicating the photosynthetic downregulation of *S. bungeana* did not result from stomatal limitation. Second, nonstomatal limitation is mainly ascribed to metabolic limitation, such as N limitation, an imbalance between carbon sinks and sources (Ellsworth *et al.* 2004) or a decrease in photosynthetic capacity (Irigoyen *et al.* 2014). Leaf nitrogen is usually a useful parameter for evaluating photosynthetic capacity (Aranjuelo *et al.* 2011) and photosynthetic downregulation under elevated CO_2 is often associated with a decrease in N_m (Ellsworth *et al.* 2004, Li *et al.* 2013). The
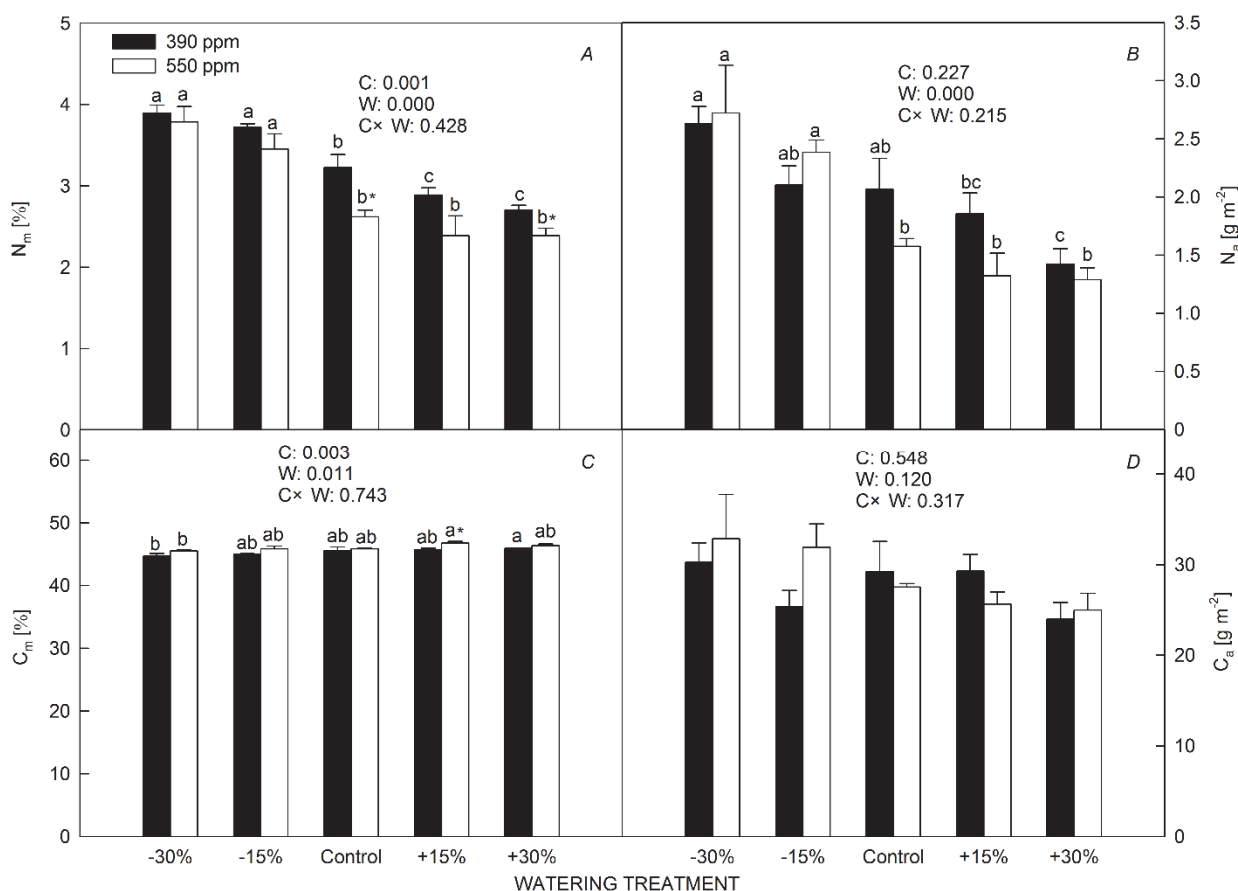
Fig. 3. Nitrogen and carbon concentration based on mass (N_m, C_m) and area (N_a, C_a) of the *Stipa bungeana* grown under ambient (390 ppm) and elevated (550 ppm) CO_2 concentrations with watering treatments. Values are mean ± SE (n = 3). *Different letters* above bars denote statistically significant differences between watering treatments at $P<0.05$ level according to *Duncan's* multiple range test under the same CO_2 concentration. [*] – differences between CO_2 treatments under the same watering treatment. C – CO_2 effect; W – watering effect; C×W – CO_2 and watering effects in combination.

significant decline of leaf N_m under EC in this experiment, which might be due to growth dilution and a direct negative effect on N assimilation (Shi *et al*. 2015), could result in a lower Rubisco protein content. Meanwhile, we found that the leaf Chl content declined under EC (Lewis *et al*. 2004). Thus, the photosynthetic downregulation in *S. bungeana* may be caused by N limitation, to some extent. Salazar-Parra *et al*. (2015) regarded the C/N ratio as an important indicator of plant source/sink balance, and the increase of C/N implies the decline of the plant's sink capacity for carbohydrate metabolism. In order to balance C source and sink, elevated CO_2 concentration reduces P_N and limits the strength of C sink increase (Aranjuelo *et al*. 2013). We detected *ca*. 16% increase in *S. bungeana* leaf C/N exposed to EC, indicating the imbalance of carbon source/sink and confirming the photosynthetic down-regulation phenomenon. In C_3 plants, the most significant effect of elevated CO_2 on leaf photosynthetic apparatus is a reduction of the Rubisco content and activity (Long *et al*. 2004, Vu and Allen Jr. 2009). In our experiment, F_v/F_m experienced a slight decline under EC, suggesting the change in photochemistry of PSII might be unfavourable

for photosynthesis, and photosynthetic downregulation might relate to the decline of photosynthetic activity. Furthermore, respiration can inhibit the potential photosynthetic activity of plants (Salazar-Parra *et al*. 2015). In this experiment, EC significantly increased leaf R_D, especially under restricted water conditions, suggesting the enhancement of *S. bungeana* respiration contributed to photosynthetic acclimation. Third, the previous studies on the effects of climate change on *S. bungeana* mainly involved perennial seedlings or communities. In our view, differences existed between annual and perennial seedlings of *S. bungeana* in terms of their growth habit and their responses to climatic change. The roots of perennial seedlings may be more fibrous and more widely distributed, and thus might influence the function of the aboveground plant parts and their sensitivity to climatic change. Nevertheless, this opinion needs to be confirmed. Moreover, the EC effects on the physiological traits of *S. bungeana* were closely related to the watering conditions.

In this study, the negative influence of water deficit on leaf photosynthesis was prominent in *S. bungeana*. The
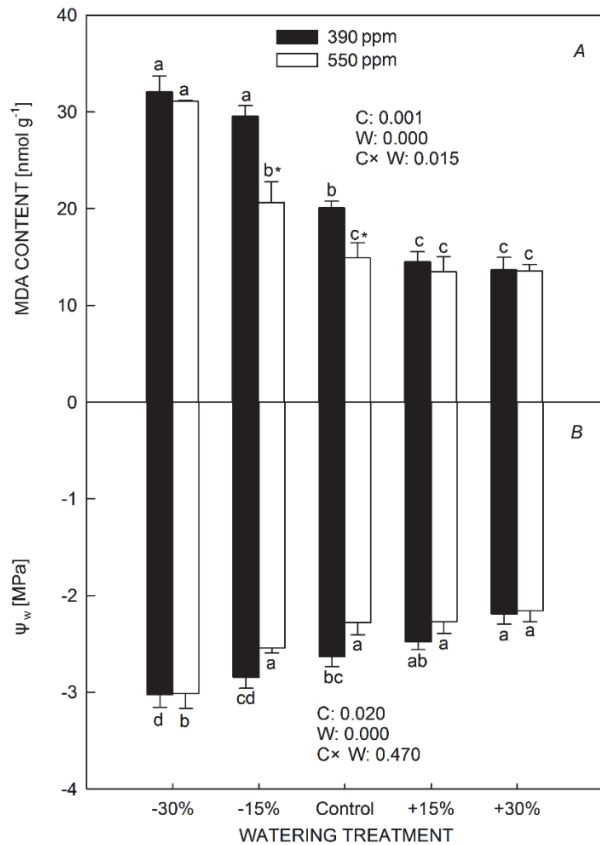
Fig. 4. Malondialdehyde (MDA) content and leaf water potential (Ψ_w) of *Stipa bungeana* grown under ambient (390 ppm) and elevated (550 ppm) CO_2 concentrations with watering treatments. Values are mean ± SE ($n = 3$). *Different letters* above bars denote statistically significant differences between watering treatments at $P<0.05$ level according to *Duncan's* multiple range test under the same CO_2 concentration. * – differences between CO_2 treatments under the same watering treatment. C – CO_2 effect; W – watering effect; C×W – CO_2 and watering effects in combination.

mechanism of leaf photosynthesis responding to watering is divided into stomatal and nonstomatal factors (Ghannoum 2009). The role of the two factors on photosynthesis was not conclusive, some studies indicated that stomatal limitation contributed more to the depression of photosynthesis under drought (Schulze 1986, Marques da Silva and Arrabaca 2004). von Caemmerer *et al.* (2004) suggested the stomata had no effect on photosynthesis change, and others thought the two factors existed simultaneously (Kaiser 1987, Flexas and Medrano 2002, Kauffman *et al.* 2007, Xu *et al.* 2011). In the current experiment, P_N and g_s (stomatal limitation) showed significant ($P<0.05$) positive correlation for both CA and EC as well as for the change in watering (Fig. 5*A*). Meanwhile, an extremely significant ($P<0.01$) positive correlation between P_N and F_v/F_m (PSII activity limitation) under EC was found (Fig. 5*C*). Moreover, there was a significant negative relationship between P_N and N_m (N limitation) under both CA ($P<0.05$) and EC ($P<0.01$) (Fig. 5*B*), and we also observed a significant positive correlation between N_m and Chl content (Fig. 4S), suggesting the direct negative influence of N_m on photosynthesis with an increased amount of water available. Additionally, the decrease of the C/N ratio under drought conditions confirmed that *S. bungeana* experienced problems with adjusting the sink/source balance. These results suggested that the stomatal and nonstomatal limitations to carbon assimilation of *S. bungeana* under different watering conditions were comparable. There was no significant interaction between EC and watering on *S. bungeana* photosynthesis, which might result from unfavourable CO_2 and water supplies for interaction effects. Generally, watering resulted in significant differences of the physiological traits in *S. bungeana* during this experiment. Gao and Zhang (1997) thought a 10% relative increase in watering was rather small for arid regions, thus, the effect of increased watering was limited. However, in the current experiment, we set a wider watering gradient (from –30% to +30%) that comprised both decreased and increased water supplies. The above conclusion confirmed our first hypothesis.

Most previous studies reported that elevated CO_2 could
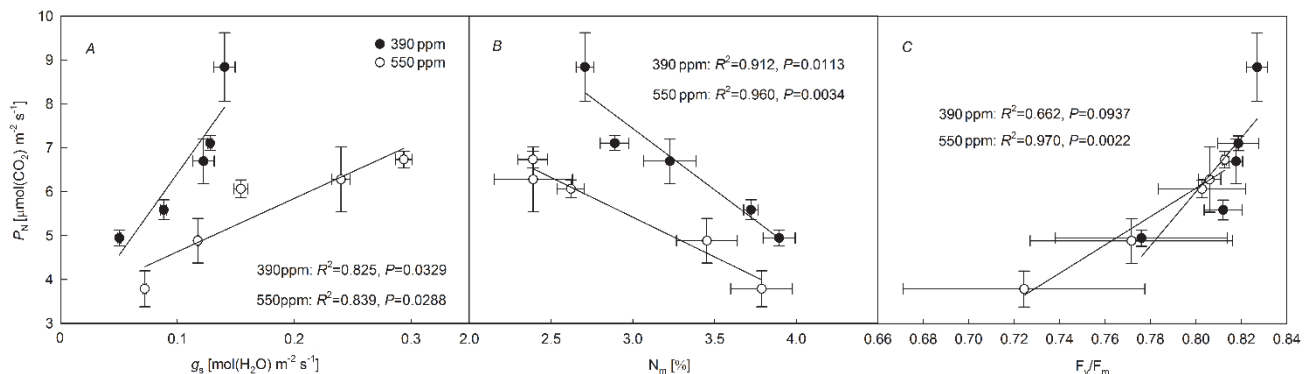

Fig. 5. Relationships between net photosynthetic rate (P_N) and stomatal conductance (g_s, *A*), nitrogen concentration based on mass (N_m, *B*), maximal efficiency of PSII photochemistry (F_v/F_m, *C*) in ambient (*full symbols*) and elevated (*open symbols*) CO_2 concentration under different watering treatments. Symbols represent mean ± SE ($n = 3$).

Fig. 6. Relationship between leaf water potential (Ψ_w) and water-use efficiency (WUE) in ambient (*full symbols*) and elevated (*open symbols*) CO₂ concentration under different watering treatments. Symbols represent mean ± SE ($n = 3$).

Fig. 7. Relationship between net photosynthetic rate (P_N) and leaf respiration (R_D+R_L) in ambient (*full symbols*) and elevated (*open symbols*) CO₂ concentration under different watering treatments. Symbols represent mean ± SE ($n = 3$). The dash line represents the line y = x.
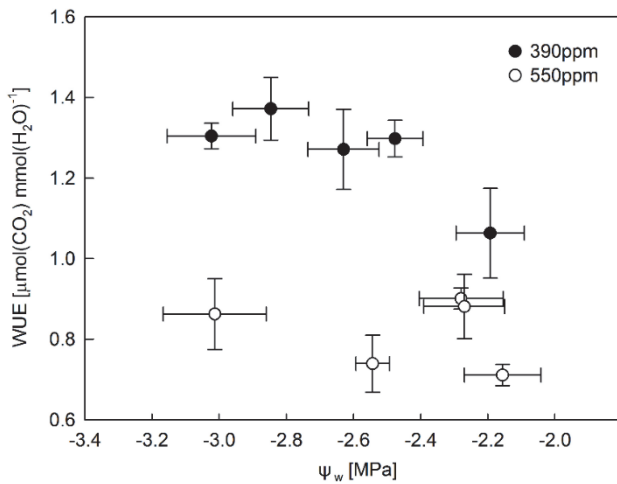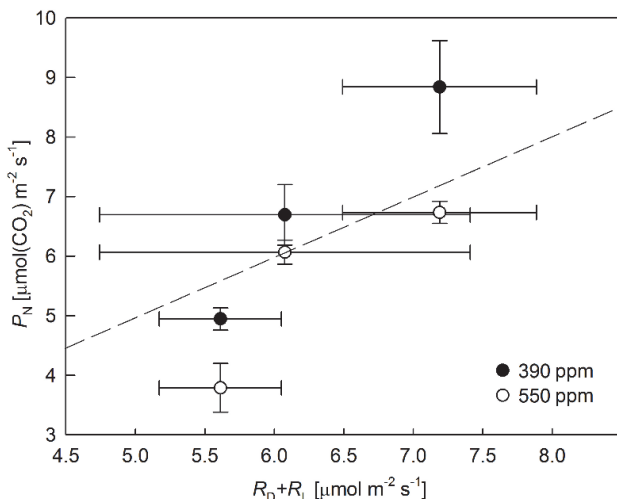
ameliorate the negative effect of drought and lead to an improvement in plant water status, and thus maintain photosynthesis (Vu and Allen Jr. 2009, Streit *et al.* 2014). However, Duan *et al.* (2014) did not observe this effect. Our results showed that under a water deficit condition (–30%W and –15%W), P_N declined by 21.3 and 28.5% on average, under CA and EC, respectively. Thus, the negative impact of a water deficit on *S. bungeana* photosynthesis was lesser under CA than under EC, indicating EC did not ameliorate the negative effect of the water deficit on photosynthesis in *S. bungeana*. In this experiment, EC significantly increased g_s, which suggested that

S. bungeana might suffer increased water loss at the leaf level or greater water stress in the future climate. The mechanism of increased g_s at elevated CO₂ concentrations remains unclear. Pataki *et al.* (2000) and LeCain *et al.* (2003) attributed this phenomenon to increased soil water availability under elevated CO₂ (the increases of the soil water content ranged from 4.5 to 10.7% in our experiment). The increased leaf area under elevated CO₂ could also potentially explain the increase in g_s (Zeppel *et al.* 2012) (data not published yet). We also observed that EC induced a significant decrease in WUE by *ca.* 34.9%, which likely resulted from the decrease in P_N and increase in g_s, or might result from the specific growth period of *S. bungeana*. However, given the high variance during the measurements and the lack of consistent results across the CO₂ treatments, we had no strong basis for this conclusion. Decreased watering caused a significant increase in WUE and enhanced the drought tolerance, which is in agreement with previous studies (Wullschleger *et al.* 2002, Ogaya and Peñuelas 2003). Simultaneously, under the CA, WUE was negatively correlated with Ψ_w until a lower Ψ_w threshold of –2.81 MPa (Fig. 6), which had been reported frequently (Limousin *et al.* 2010, 2015; Maseyk *et al.* 2011), while under EC there was no obvious relationship between WUE and Ψ_w. Moreover, we observed insignificant interactions with WUE, suggesting that EC was unfavourable to improve water use of *S. bungeana* and could not moderate the negative effects of a water deficit.

The mechanisms of respiration in response to environmental changes can be elaborated from molecular, biochemical, and physiological aspects (Ryan 1991, Ellsworth *et al.* 2004, Leakey *et al.* 2009, Crous *et al.* 2011). Meanwhile, the effect of drought on respiration was also related to the growth rate of plants (Galmés *et al.* 2007, Giméno *et al.* 2010): faster-growing species are often more restricted in respiration than slower-growing species. However, the responses of leaf respiration to elevated CO₂ and drought alone or in combination are highly variable (Galmés *et al.* 2007, Gimeno *et al.* 2010). This experiment found that R_D was significantly stimulated by *ca.* 35.2% under EC, which was consistent with a previous study (Leakey *et al.* 2009). Increased watering alone also had a significant positive impact on R_D; however, CO₂ and watering in combination showed no significant impact. R_D might be affected by increasing adenylate restriction, which has been attributed to decreased demand of respiratory products, such as ATP (Ellsworth *et al.* 2004, Atkin and Macherel 2009). In this experiment, there was the increase of MDA (an indicator of tissue cell damage) and decline in Ψ_w under water deficit conditions. Thus, we assumed that drought might cause some damage to cell structure and impact mitochondrial structure indirectly (Germ *et al.* 2007), which would then be reflected in lowered respiration. To some extent, leaf or plant carbon balance can be determined by
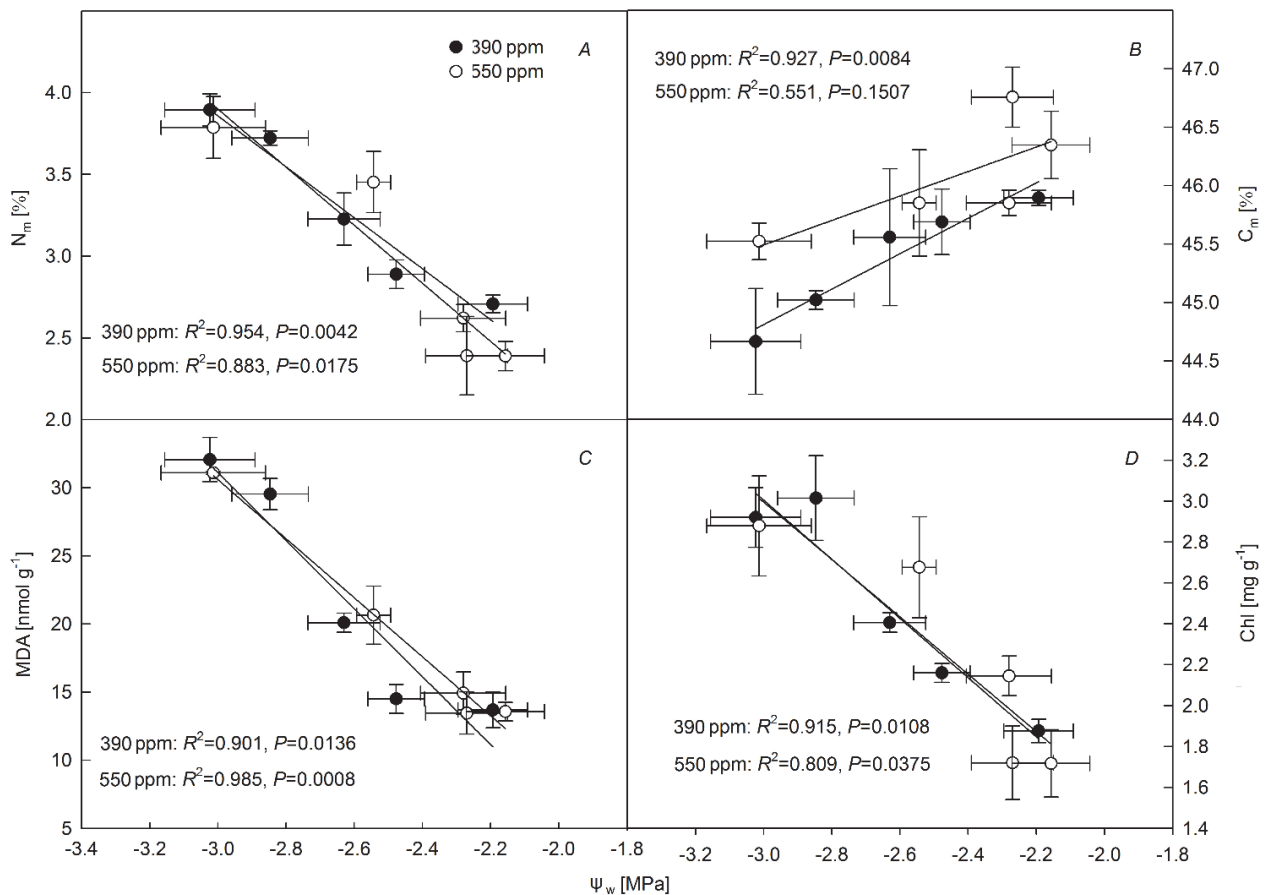
Fig. 8. Relationships between nitrogen concentration based on mass (N_m), carbon concentration based on mass (C_m), malondialdehyde content (MDA), chlorophyll (Chl) content, and leaf water potential (Ψ_w) in ambient (*full symbols*) and elevated (*open symbols*) CO_2 concentration under different watering treatments. Symbols represent mean ± SE (*n* = 3).

photosynthesis and respiration (Lawlor and Tezara 2009). Leaf respiration and photosynthesis responses to climate changes are crucial to predict the change of plant carbon exchange with the atmosphere (Crous *et al.* 2011) and the leaf carbon balance (Limousin *et al.* 2015). Previous studies indicated that, in general, P_N/R ratio remained in a steady state under climate change (Cox 2001). However, some researchers found that the responses of respiration and photosynthesis are not equal (Ziska and Bunce 1998, Sanhueza *et al.* 2015). Our results showed that watering alone or CO_2 and watering in combination had no significant effect on P_N/R (*P*=0.273 and 0.638, respectively), while EC decreased it markedly (*P*=0.028). From Fig. 7, the dots below the y = x line imply P_N/R was lower than 1, which means the leaf carbon balance was negative after the treatments. These results indicated that EC and drought effects on respiration were larger than on photosynthesis and might decrease the fraction of P_N converted to plant biomass (McDowell 2011), therefore carbon losses might be larger than carbon gains. Thus, the results of the present study suggest that EC might be unfavourable for the growth of *S. bungeana* to some extent. However, considering the promotion of P_N in June (data not published)

and the larger biomass at the harvest stage (Fig. 5S, *supplement available online*) under EC, the CO_2 effect on *S. bungeana* appears to be time-dependent at a smaller temporal scale (one growing season in this experiment).

Plant growth capacity has a close relationship with physiological traits, such as N_m, C_m, Chl content, MDA content, and Ψ_w. Plant Ψ_w is a direct indicator/parameter for estimating leaf water status and drought can reduce it (Vogt and Lösch 1999), while the effect of EC on Ψ_w depends on water relations (Valerio *et al.* 2011). In the present experiment, drought significantly reduced Ψ_w, and EC significantly increased Ψ_w, which is in agreement with the result of the prior study (Sicher and Barnaby 2012). Our previous analysis indicated that EC did not show the stimulation effect with differential watering treatments compared to CA, indicating the adverse effect of future EC on Ψ_w in *S. bungeana*. MDA content is an indicator for the extent of tissue cell damage under environmental stress (Munné-Bosch and Alegre 2003, Ogweno *et al.* 2008). In our experiment, drought significantly increased the MDA content, which is consistent with previous studies (Zhang *et al.* 2007), indicating that the water deficit under such growth condition might have disturbed the intercellular

redox balance and resulted in oxidative stress. EC led to a significant decrease in MDA, indicating that EC could prevent membrane damage to a certain extent. However, under reduced watering (–30%W, –15%W) conditions, the MDA content increased 53.3 and 73.4%, on average, with the CA and EC, respectively. These results indicated that the compensatory effect of EC to drought was not obvious and confirmed the conclusion that EC could weaken the adaptability of *S. bungeana* to a future warm and dry climate. Altered photosynthesis due to EC and watering is closely related to the N_m, C_m, and Chl contents (Luomala *et al.* 2005, Watanabe *et al.* 2011). Other physiological traits showed close relationships with Ψ_w (Fig. 8), which confirmed the second hypothesis. N_m, MDA, and Chl all showed significant negative relationships with plant Ψ_w along watering and CO₂ change, while C_m represented significant positive relationship with plant Ψ_w only under CA, indicating that photosynthesis may be closely related to water relations under CO₂ and watering in combination.

Conclusion: In conclusion, regardless of watering conditions, growth under elevated CO₂ concentration induced photosynthetic downregulation/acclimation in *S. bungeana*. This acclimation mainly came from N limitation, C source/sink imbalance, and decline in photosynthetic activity (such as decreased F_v/F_m, increased respiration). The effect of elevated CO₂ on photosynthesis was closely related to leaf water relations, and it could not ameliorate the negative effect of drought. However, the interactive effects of elevated CO₂ and watering on plant growth were rather complex, and not a simple super-position of CO₂ and watering effect (Li *et al.* 2013). Elevated CO₂ caused growth inhibition in *S. bungeana* which was time-dependent at a smaller temporal scale. Our results can be used only as references for the adaptability of *S. bungeana* to climate change, and more data are needed to illustrate the adaptive mechanism.

References

Ainsworth E.A., Long S.P.: What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. – New Phytol. **165**: 351-372, 2005.

Ainsworth E.A., Rogers A.: The response of photosynthesis and stomatal conductance to rising CO₂: mechanisms and environmental interactions. – Plant Cell Environ. **30**: 258-270, 2007.

Albert K.R., Ro-Poulsen H., Mikkelsen T.N. *et al.*: Interactive effects of elevated CO₂, warming, and drought on photosynthesis of *Deschampsia flexuosa* in a temperate heath ecosystem. – J. Exp. Bot. **62**: 4253-4266, 2011.

Aranjuelo I., Cabrera-Bosquet L., Morcuende R. *et al.*: Does ear C sink strength contribute to overcoming photosynthetic acclimation of wheat plants exposed to elevated CO₂? – J. Exp. Bot. **62**: 3957-3969, 2011.

Aranjuelo I., Sanz-Sáez A., Jauregui I. *et al.*: Harvest index, a parameter conditioning responsiveness of wheat plants to elevated CO₂. – J. Exp. Bot. **64**: 1879-1892, 2013.

Atkin O.K., Macherel D.: The crucial role of plant mitochondria in orchestrating drought tolerance. – Ann. Bot.-London **103**: 581-597, 2009.

Biswas D.K., Xu H., Li Y.G. *et al.*: Modification of photosynthesis and growth responses to elevated CO₂ by ozone in two cultivars of winter wheat with different years of release. – J. Exp. Bot. **64**: 1485-1496, 2013.

Bonan G.B.: Forests and climate change: forcings, feedbacks, and the climate benefits of forests. – Science **320**: 1444-1449, 2008.

Bunce J.A.: Short-term and long-term inhibition of respiratory carbon-dioxide efflux by elevated carbon-dioxide. – Ann. Bot. **65**: 637-642, 1990.

Cakmak I., Horst W.J.: Effect of aluminum on lipid-peroxidation, superoxide-dismutase, catalase and peroxidase-activities in root-tips of soybean (*Glycine max*). – Plant Physiol. **83**: 463-468. 1991.

Chaves M.M.: Effects of water deficits on carbon assimilation. – J. Exp. Bot. **42**: 1-16, 1991.

Cheng J., Hu T.M., Cheng J.M. *et al.*: Distribution of biomass

and diversity of *Stipa bungeana* community to climatic factors in the Loess Plateau of northwestern China. – Afr. J. Biotechnol. **9**: 6733-6739, 2010.

Cox P.M.: Description of the ''TRIFFID'' Dynamic Global Vegetation Model. Pp. 17. Hadley Technical Note 24 Bracknell 2001.

Clifford S.C., Stronach I.M., Black C.R. *et al.*: Effects of elevated CO₂, drought and temperature on the water relations and gas exchange of groundnut (*Arachis hypogaea*) stands grown in controlled environment glasshouses. – Physiol. Plantarum **110**: 78-88, 2000.

Crous K.Y., Walters M.B., Ellsworth D.S.: Elevated CO₂ concentration affects leaf photosynthesis-nitrogen relationships in *Pinus taeda* over nine years in FACE. – Tree Physiol. **28**: 607-614, 2008.

Crous K.Y., Zaragoza-Castells J., Löw M. *et al.*: Seasonal acclimation of leaf respiration in *Eucalyptus saligna* trees: impacts of elevated atmospheric CO₂ and summer drought. – Glob. Change Biol. **17**: 1560-1567, 2011.

da Silva J.M., Arrabaca M.C.: Photosynthesis in the water-stressed C4 grass *Setaria sphacelata* is mainly limited by stomata with both rapidly and slowly imposed water deficits. – Physiol. Plantarum **121**: 409-420, 2004.

Duan H., Duursma R.A., Huang G. *et al.*: Elevated CO₂ does not ameliorate the negative effects of elevated temperature on drought-induced mortality in *Eucalyptus radiata* seedlings. – Plant Cell Environ. **37**: 1598-1613, 2014.

Edwards C.E., Ewers B.E., McClung C.R. *et al.*: Quantitative variation in water-use efficiency across water regimes and its relationship with circadian, vegetative, reproductive, and leaf gas-exchange traits. – Mol. Plant **5**: 653-668, 2012.

Ellsworth D.S., Reich P.B., Naumburg E.S. *et al.*: Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO₂ across four free-air CO₂ enrichment experiments in forest, grassland and desert. – Glob. Change Biol. **10**: 2121-2138, 2004.

Erice G., Irigoyen J.J., Pérez P. *et al.*: Effect of elevated CO2, temperature and drought on dry matter partitioning and

photosynthesis before and after cutting of nodulated alfalfa. – Plant Sci. **170**: 1059-1067, 2006.

Flexas J., Medrano H.: Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. – Ann. Bot.-London **89**: 183-189, 2002.

Galmés J., Ribas-Carbó M., Medrano H. *et al.*: Response of leaf respiration to water stress in Mediterranean species with different growth forms. – J. Arid Environ. **68**: 206-222, 2007.

Gao Q., Zhang X.S.: A simulation study of responses of the Northeast China Transect to elevated CO_2 and climate change. – Ecol. Appl. **7**: 470-483, 1997.

Germ M., Kreft I., Stibilj V. *et al.*: Combined effects of selenium and drought on photosynthesis and mitochondrial respiration in potato. – Plant Physiol. Bioch. **45**: 162-167, 2007.

Ghannoum O.: C4 photosynthesis and water stress. – Ann. Bot.-London **103**: 635-644, 2009.

Giméno T.E., Sommerville K., Valladares F., Atkin O.K.: Homeostasis of respiration under drought and its important consequences for foliar carbon balance in a drier climate: insights from two contrasting *Acacia* species. – Funct. Plant Biol. **37**: 323-333, 2010.

Hernández J.A., Almansa M.S.: Short-term effects of salt stress on antioxidant systems and leaf water relations of leaves. – Physiol. Plantarum **115**: 251-257, 2002.

Hooper D.U., Adair E.C., Cardinale B.J. *et al.*: A global synthesis reveals biodiversity loss as a major driver of ecosystem change. – Nature **486**: 105-108, 2012.

Hovenden, M.J., Newton P.C.D., Wills K.E.: Seasonal not annual rainfall determines grassland biomass response to carbon dioxide. – Nature **511**: 583-586, 2014.

Huntington T.G.: Evidence for intensification of the global water cycle: review and synthesis. – J. Hydrol. **319**: 83-95, 2006.

IPCC: Summary for policymakers. – In: Edenhofer O, Pichs-Madruga R, Sokona Y *et al.* (ed.): Climate Change 2014. Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Pp. 25, 77-78. Cambridge University Press. Cambridge 2014.

Irigoyen J.J., Goicoechea N., Antolín M.C. *et al.*: Growth, photosynthetic acclimation and yield quality in legumes under climate change simulations: an updated survey. – Plant Sci. **226**: 22-29, 2014.

Kaiser W.M.: Effects of water deficit on photosynthetic capacity. – Physiol. Plantarum **71**: 142-149, 1987.

Kaminski K.P., Kørup K., Nielsen K.L. *et al.*: Gas-exchange, water use efficiency and yield responses of elite potato (*Solanum tuberosum* L.) cultivars to changes in atmospheric carbon dioxide concentration, temperature and relative humidity. – Agr. Forest Meteorol. **187**: 36-45, 2014.

Kauffman G.L., Kneivel D.P., Watschke T.L.: Effects of a bio-stimulant on the heat tolerance associated with photosynthetic capacity, membrane thermostability, and polyphenol pro-duction of perennial ryegrass. – Crop Sci. **47**: 261-267, 2007.

Lawlor D.W., Tezara W.: Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. – Ann. Bot.-London **103**: 561-579, 2009.

Leakey A.D.B., Ainsworth E.A., Bernacchi C.J. *et al.*: Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. – J. Exp. Bot. **60**: 2859-2876, 2009.

LeCain D.R., Morgan J.A., Mosier A.R., Nelson J.A.: Soil and plant water relations determine photosynthetic response of C3 and C4 grasses in a semi-arid ecosystem under elevated CO_2. – Ann. Bot.-London **92**: 41-52, 2003.

Lewis J.D., Lucash M., Olszyk D.M., Tingey D.T.: Relationships between needle nitrogen concentration and photosynthetic responses of Douglas-fir seedlings to elevated CO_2 and temperature. – New Phytol. **162**: 355-364, 2004.

Li D.X., Liu H.L., Qiao Y.Z. *et al.*: Physiological regulation of soybean (*Glycine max* L. Merr.) growth in response to drought under elevated CO_2. – J. Food Agric. Environ. **11**: 649-654, 2013.

Li Y., Xu J., Haq N.U. *et al.*: Was low CO_2 a driving force of C4 evolution: *Arabidopsis* responses to long-term low CO_2 stress. – J. Exp. Bot. **65**: 3657-3667, 2014.

Limousin J.M., Misson L., Lavoir A.V. *et al.*: Do photosynthetic limitations of evergreen *Quercus ilex* leaves change with long-term increased drought severity? – Plant Cell Environ. **33**: 863-875, 2010.

Limousin J.M., Yepez E.A., McDowell N.G., Pockman W.T.: Convergence in resource use efficiency across trees with differing hydraulic strategies in response to ecosystem precipitation manipulation. – Funct. Ecol. **29**: 1125-1136, 2015.

Long S.P., Ainsworth E.A., Rogers A., Ort D.R.: Rising atmospheric carbon dioxide: plants FACE the future. – Annu. Rev. Plant Biol. **55**: 591-628, 2004.

Luomala E.M., Laitinen K., Sutinen S. *et al.*: Stomatal density, anatomy and nutrient concentrations of Scots pine needles are affected by elevated CO_2 and temperature. – Plant Cell Environ. **28**: 733-749, 2005.

Manea A., Leishman M.R.: Competitive interactions between established grasses and woody plant seedlings under elevated CO_2 levels are mediated by soil water availability. – Oecologia **177**: 499-506, 2015.

Maseyk K., Hemming D., Angert A. *et al.*: Increase in water-use efficiency and underlying processes in pine forests across a precipitation gradient in the dry Mediterranean region over the past 30 years. – Oecologia **167**: 573-585, 2011.

Maxwell K., Johnson G.N.: Chlorophyll fluorescence - a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

McDowell N.G.: Mechanisms linking drought, hydraulics, carbon metabolism, and mortality. – Plant Physiol. **155**: 1051-1059, 2011.

Morgan J.A., Pataki D.E., Körner C. *et al.*: Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO_2. – Oecologia **140**: 11-25, 2004.

Munné-Bosch S., Alegre L.: Drought-induced changes in the redox state of alpha-tocopherol, ascorbate, and the diterpene carnosic acid in chloroplasts of Labiatae species differing in carnosic acid contents. – Plant Physiol. **131**: 1816-1825, 2003.

Newingham B.A., Vanier C.H., Charlet T.N. *et al.*: No cumulative effect of 10 years of elevated [CO_2] on perennial plant biomass components in the Mojave Desert. – Glob. Change Biol. **19**: 2168-2181, 2013.

Novriyanti E., Watanabe M., Kitao M. *et al.*: High nitrogen and elevated [CO_2] effects on the growth, defense and photosynthetic performance of two eucalypt species. – Environ. Pollut. **170**: 124-130, 2012.

Ogaya R., Peñuelas J.: Comparative field study of *Quercus ilex* and *Phillyrea latifolia*: photosynthetic response to experimental drought conditions. – Environ. Exp. Bot. **50**: 137-148, 2003.

Ogweno J.O., Song X.S., Shi K. *et al.*: Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carbo-

xylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*. – J. Plant Growth Regul. **27**: 49-57, 2008.

Ohashi Y., Nakayama N., Saneoka H., Fujita K.: Effects of drought stress on photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean plants. – Biol. Plantarum **50**: 138-141, 2006.

Parry M.A.J., Andralojc P.J., Mitchell R.A.C. *et al*.: Manipulation of Rubisco: the amount, activity, function and regulation. – J. Exp. Bot. **54**: 1321-1333, 2003.

Pataki D.E., Huxman T.E., Jordan D.N. *et al*.: Water use of two Mojave Desert shrubs under elevated CO_2. – Glob. Change Biol. **6**: 889-898, 2000.

Peñuelas J., Sardans J., Estiarte M. *et al*.: Evidence of current impact of climate change on life: a walk from genes to the biosphere. – Glob. Change Biol. **19**: 2303-2338, 2013.

Pizarro L.C., Bisigato A.J.: Allocation of biomass and photoassimilates in juvenile plants of six Patagonian species in response to five water supply regimes. – Ann. Bot.-London **106**: 297-307, 2010.

Porra R.J., Thompson W.A., Kriedemann P.E.: Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. – BBA-Bioenergetics **975**: 384-394, 1989.

Qaderi M.M., Kurepin L.V., Reid D.M.: Growth and physiological responses of canola (*Brassica napus*) to three components of global climate change: temperature, carbon dioxide and drought. – Physiol. Plantarum **128**: 710-721, 2006.

Ryan M.G.: Effects of climate change on plant respiration. – Ecol. Appl. **1**: 157-167, 1991.

Sala A., Piper F., Hoch G.: Physiological mechanisms of drought-induced tree mortality are far from being resolved. – New Phytol. **186**: 274-281, 2010.

Salazar-Parra C., Aranjuelo I., Pascual I. *et al*.: Carbon balance, partitioning and photosynthetic acclimation in fruit-bearing grapevine (*Vitis vinifera* L. cv. Tempranillo) grown under simulated climate change (elevated CO_2, elevated temperature and moderate drought) scenarios in temperature gradient greenhouses. – J. Plant Physiol. **174**: 97-109, 2015.

Sanhueza C., Bascunan-Godoy L., Turnbull MH., Corcuera L.J.: Response of photosynthesis and respiration to temperature under water deficit in two evergreen *Nothofagus* species. – Plant Spec. Biol. **30**: 163-175, 2015.

Schulze E.D.: Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. – Annu. Rev. Plant Physio. **37**: 247-274, 1986.

Shi Y.H., Zhou G.S., Jiang Y.L. *et al*.: Interactive effects of elevated CO_2 and precipitation change on leaf nitrogen of dominant *Stipa* L. species. – Ecol. Evol. **5**: 2956-2965, 2015.

Sicher R.C., Barnaby J.Y.: Impact of carbon dioxide enrichment on the responses of maize leaf transcripts and metabolites to water stress. – Physiol. Plantarum **144**: 238-253, 2012.

Streit K., Siegwolf R.T.W., Hagedorn F. *et al*.: Lack of photosynthetic or stomatal regulation after 9 years of elevated CO_2 and 4 years of soil warming in two conifer species at the alpine treeline. – Plant Cell Environ. **37**: 315-326, 2014.

Talhelm A.F., Pregitzer K.S., Kubiske M.E. *et al*.: Elevated carbon dioxide and ozone alter productivity and ecosystem carbon content in northern temperate forests. – Glob. Change Biol. **20**: 2492-2504, 2014.

Valentini R., Epron D., Angelis P. *et al*.: *In situ* estimation of net CO_2 assimilation, photosynthetic electron flow and photo-

respiration in Turkey oak (*Quercus cerris* L.) leaves: diurnal cycles under different levels of water supply. – Plant Cell Environ. **18**: 631-640, 1995.

Valerio M., Tomecek M.B., Lovelli S., Ziska L.H.: Quantifying the effect of drought on carbon dioxide-induced changes in competition between a C3 crop (tomato) and a C4 weed (*Amaranthus retroflexus*). – Weed Res. **51**: 591-600, 2011.

von Caemmerer S., Lawson T., Oxborough K. *et al*.: Stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco with reduced amounts of Rubisco. – J. Exp. Bot. **55**: 1157-1166, 2004.

Vogt U.K., Lösch R.: Stem water potential and leaf conductance: A comparison of *Sorbus aucuparia* and *Sambucus nigra*. – Phys. Chem. Earth Pt. B **24**: 121-123, 1999.

Vu J.C.V., Allen Jr L.H.: Growth at elevated CO_2 delays the adverse effects of drought stress on leaf photosynthesis of the C4 sugarcane. – J. Plant Physiol. **166**: 107-116, 2009.

Watanabe M., Watanabe Y., Kitaoka S. *et al*.: Growth and photosynthetic traits of hybrid larch F1 (*Larix gmelinii* var. *japonica* × *L. kaempferi*) under elevated CO_2 concentration with low nutrient availability. – Tree Physiol. **31**: 965-975, 2011.

Wertin T.M., McGuire M.A., Teskey R.O.: The influence of elevated temperature, elevated CO_2 concentration and water stress on net photosynthesis of loblolly pine (*Pinus taeda* L.) at northern, central and southern sites in its native range. – Glob. Change Biol. **16**: 2089-2103, 2010.

Wullschleger S.D., Tschaplinski T.J., Norby R.J.: Plant water relations at elevated CO_2 - implications for water-limited environments. – Plant Cell Environ. **25**: 319-331, 2002.

Xu Z.Z., Zhou G.S., Han G.X., Li Y.J.: Photosynthetic potential and its association with lipid peroxidation in response to high temperature at different leaf ages in maize. – J. Plant Growth Regul. **30**: 41-50, 2011.

Xu Z.Z., Shimizu H., Ito S. *et al*.: Effects of elevated CO_2, warming and precipitation change on plant growth, photosynthesis and peroxidation in dominant species from North China grassland. – Planta **239**: 421-435, 2014.

Yan J.H., Zhang D.Q., Liu J.X., Zhou G.Y.: Interactions between CO_2 enhancement and N addition on net primary productivity and water-use efficiency in a mesocosm with multiple subtropical tree species. – Glob. Change Biol. **20**: 2230-2239, 2014.

Yin C., Peng Y., Zang R. *et al*.: Adaptive responses of *Populus kangdingensis* to drought stress. – Physiol. Plantarum **123**: 445-451, 2005.

Yu J., Jing Z.B., Cheng J.M.: Genetic diversity and population structure of *Stipa bungeana*, an endemic species in Loess Plateau of China, revealed using combined ISSR and SRAP markers. – Genet. Mol. Res. **13**: 1097-1108, 2014.

Zelikova T.J., Williams D.G., Hoenigman R. *et al*.: Seasonality of soil moisture mediates responses of ecosystem phenology to elevated CO_2 and warming in a semi-arid grassland. – J. Ecol. **103**: 1119-1130, 2015.

Zeppel M.J.B., Lewis J.D., Chaszar B. *et al*.: Nocturnal stomatal conductance responses to rising CO_2, temperature and drought. – New Phytol. **193**: 929-938, 2012.

Zhang M., Duan L., Tian X. *et al*.: Uniconazole-induced tolerance of soybean to water deficit stress in relation to changes in photosynthesis, hormones and antioxidant system. – J. Plant Physiol. **164**: 709-717, 2007.

Ziska L.H., Bunce J.A.: The influence of increasing growth temperature and CO_2 concentration on the ratio of respiration to photosynthesis in soybean seedlings. – Glob. Change Biol. **4**: 637-643, 1998.