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GROWTH, PHYSIOLOGICAL, AND BIOCHEMICAL RESPONSES OF THREE GRASS SPECIES TO ELEVATED CARBON DIOXIDE CONCENTRATIONS

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Abstract

The increased atmospheric CO_2concentration may have profound impacts on the structure and function of grass land ecosystems, and the question that how C_3grasseswill respond to a wider range of higher CO_2 levels remains unanswered. Here, we exposed three widely distributed cool-season C_3 grass species Tall fescue (*Festuca arundinacea* Schreb.), Perennial ryegrass (*Lolium perenne* L.), and Kentucky bluegrass (*Poa pratensis* L.) to ambient (400 μmol mol^{-1}) or elevated CO_2 concentrations (600, 800, 1000, and 1200 μmol mol^{-1}) in growth chambers using an automatic CO_2 controlling system. We examined growth, physiological, and biochemical responses to elevated CO_2 with measurements on plant growth traits (relative growth rate and biomass), leaf gas exchange, and tissue biochemical composition (non-structural carbohydrates) of the three grass species during exposure to ambient or elevated CO_2 concentrations for eight weeks. Our results showed that elevated CO_2 concentrations significantly increased the averaged relative growth rates of Tall fescue and the total leaf area of Kentucky bluegrass. Meanwhile, CO_2 enhancement significantly stimulated the leaf net photosynthesis rate(A_n) of the three species. However, both the area-based and N-based leaf dark respiration rates (R_d) of the three species were sharply decreased, and thus increased the ratio of leaf net photosynthesis and dark respiration. These results suggested that Tall fescue might be more responsive to elevated CO_2in growth, physiological, and biochemical traits than the other two species, which has important implications for species composition, competition, and dynamics, and thus community structure and functionin natural and managed ecosystems under elevated CO_2levels.

Key words: CO_2 enhancement, Carbon balance, Non-structural carbohydrates, *Festuca arundinacea*, *Lolium perenne*, *Poa pratensis*.

Introduction

Global atmospheric concentration of carbon dioxide (CO_2) has increased by more than 100 μmolmol^{-1}since the beginning of industrialization period, and is projected to 936 μmol mol^{-1} by the end of the current century (Anon., 2013). Meanwhile, the elevated CO_2 is predicted to have profound impacts on the structure and function of individual plants, plant communities as well as natural and managed ecosystems such as grasslands (Patton *et al*., 1995; Wand *et al*., 1999; Arnone III *et al*., 2000; Suter *et al*., 2002; Morgan *et al*., 2007; Zhang *et al*., 2011). It is well documented that elevated atmospheric CO_2markedly affects multiple plant ecophysiological processes such as growth (Ziska *et al*., 1991), photosynthesis (Arp, 1991; Lee *et al*., 2001; Lewis *et al*., 2004; Zhang *et al*., 2010; Zong & Sangguan, 2014),respiration (Hamilton *et al*., 2001; Jahnke, 2001; Gonzàlez-Meler *et al*., 2009; Crous *et al*., 2011; Tan *et al*., 2013), biochemical processes (Hendrix *et al*., 1994; Taub &Wang, 2008; Yu *et al*., 2012a; Arndal *et al*., 2014), and biomass allocation (Suter *et al*., 2002; Wang & Taub, 2010), although these effects depend on plant species and functional groups (Lee *et al*., 2001) as well as nutrient availability (Poorter & Navas, 2003; Graaf *et al*., 2006; Hermans *et al*., 2006; Zhang *et al*., 2017).

Plant responses to elevated atmospheric $CO_2$are fundamentally mediated by leaf photosynthesis (Lee *et al*., 2001; Lewis *et al*., 2004), and can potentially lead to changes in growth (Ziska *et al*., 1991),chemical composition (Jin and Evans, 2010; Zhang *et al*., 2010), and carbon balance(Borjigidai *et al*., 2009).Earlier studies have shown that elevated CO_2stimulated short-term photosynthetic rate and growth in various plant species (Newton *et al*., 1996; Curtis and Wang, 1998; Long *et al*., 2004; Morgan *et al*.,2007), particularly C_3species (Wand *et al*., 1999; Lee *et al*., 2001; Poorter &Navas, 2003; Yu *et al*., 2012b). However, this view has been challenged in recent years with increasing evidences from larger-scale and longer-term observations and measurements (Ainsworth & Long, 2005; Ainsworth & Rogers, 2007). Several studies found a reduction in photosynthesis with increased CO_2, also terms as down-regulation or photosynthetic acclimation (Gunderson & Wullschleger, 1994; Rey &Jarvis, 1998; Lee *et al*., 2001). The decreased net photosynthetic rate in exposure to elevated CO_2 may result from the changes in leaf structure and chemical composition associated with decreases in the amount and/or activity of Rubisco (Long *et al*., 2004; Lewis *et al*., 2004), or increases in total nonstructural carbohydrate concentrations (Hendrix *et al*., 1994; Wand *et al*., 1999). Moreover, the net photosynthetic rates of plants may also be affected by the availability of nutrients such as nitrogen (N), which exerts an important control over the response of plants and ecosystems to rising atmospheric CO_2 (Luo *et al*., 2006; Taub &Wang, 2008; Jin &Evans, 2010; Arndal *et al*., 2014). For example, previous studies have reported that down-regulation of photosynthesis occurred in plants grown in

elevated [CO_2] with N limitation evidenced by the decreased leaf N concentration (Coleman *et al*., 1993; Cotrufo *et al*., 1998), and high N availability could alleviate the down-regulation of photosynthesis in plants under elevated CO_2 conditions (Lee *et al*., 2001; Lewis *et al*., 2004; Zhang *et al*., 2010, 2011).

In addition to photosynthesis, elevated atmosphere CO_2may also have significant effects on leaf dark respiration(Hamilton *et al*., 2001; Gonzàlez-Meler *et al*., 2009; Crous *et al*., 2011; Tan *et al*., 2013), and thus altered the carbon balance and allocation of plants and ecosystems (Borjigidai *et al*., 2009; Leakey *et al*., 2009). Leaf dark respiration is generally considered to be one of the most important determinants in the global carbon cycle because as much as 40-50% of the photosynthetically fixed carbon can be returned to the atmosphere through the dark respiration of plant leaves (Farrar, 1985; Amthor, 1995; Li *et al*., 2013). So far, however, the underlying effect and mechanism of elevated CO_2 on leaf dark respiration remain unclear (Gonzàlez-Meler *et al*., 2009; Li *et al*., 2013). Previous studies have found that elevated CO_2can influence leaf dark respiration directly by short-term effects and associated with the suppression of respiratory enzymes (Bunce, 1990;Gonzàlez-Meler *et al*., 1996) and indirectly by long-term effects through altering chemical composition such as non-structural carbohydrates and tissue nitrogen concentration (Saxe *et al*., 1998; Norby *et al*., 1999; Gonzàlez-Meler *et al*., 2009).Moreover, elevated CO_2 has been typically reported to cause an instaneous reduction in leaf dark respiration by inhibiting the activity of mitochondrial enzymes such as succinate dehydrogenase and cytochromec oxidase (Gonzàlez-Meler & Siedow, 1999). By contrast, several studies found that elevated CO_2may have little effects (Amthor, 2000), or even enhance the leaf dark respiration due to the increased growth and photosynthesis, which should result in higher respiration rate. For example, Amthor (2000) found that the direct inhibitory effect of CO_2 concentration on leaf dark respiration in nine temperate deciduous tree species was small with an average of 1.5% reduction in rate at 800μmol mol^{-1}compared with 400μmol $mol^{-1}CO_2$. However, Li *et al*. (2013) reported that elevated atmosphere CO_2 stimulated leaf dark respiratory rate of tomato plants due to the increased availability of carbohydrates and protein as well as energy status. In addition, the potentially systematic errors in influencing measured respiratory responses to CO_2, such as the diffusion of CO_2 into or out the measurement cuvette, remain unresolved and continued to be controversial (Drake *et al*., 1999; Jahnke, 2001).

Grassland accounts for about20% of the earth's land area and is considered to have a high CO_2 sink capacity (Patton *et al*., 1995; Suter *et al*., 2002), and thus plays a critical role in the global carbon cycling. The cool-season C_3 grasses such as Tall fescue (*Festuca arundinacea* Schreb.), Perennial ryegrass (*Lolium perenne* L.), and Kentucky bluegrass (*Poa pratensis* L.)are dominant species in temperate grasslands and pastures (Suter *et al*., 2002), which serve many important environmental functions including erosion control, surface water detoxification, and the control of allergens and disease (Beard & Green, 1994; Burgess & Huang, 2014). Therefore, understanding the responses of the three grass species to elevated CO_2may be of great importance for many aspects of environmental stewardship and turf grass management, and may help to explain the variations of species in response to elevated CO_2. However, most of the previous studies regarding plant response to elevated CO_2have been focused on crops (Hendrix *et al*., 1994; Jahnke, 2001; Li *et al*., 2013; Tan *et al*., 2013) or trees (Ziska *et al*., 1991; Lewis *et al*., 2004; Jump *et al*., 2006; Crous *et al*., 2011; Zhang *et al*., 2010, 2011), and few studies examined the effects of elevated CO_2on perennial grasses (Lee *et al*., 2001; Suter *et al*., 2002), especially concerning the growth and physiological traits of grass despite several recent studies investigated the changes in physiology, metabolism, and growth in response to elevated CO_2combined with heat and drought stresses (Yu *et al*., 2012a, 2012b; Burgess & Huang, 2014). It should be noted that most previous studies focus mainly on the effect of a twofold increase in the atmospheric CO_2 concentration (about 700 or 800μmol mol^{-1}), which isthe projected ambient CO_2 concentration at the end of the next century (Anon., 2013).Since elevated CO_2 reduces the oxygenase activity of RuBP carboxylase/oxygenase, the increases in photosynthesis and biomass can be expected up to a global CO_2 concentration of 1000μmol mol^{-1} (Pearcy & Bjorkman, 1983; Ziska *et al*., 1991). Meanwhile, concentrations of CO_2 have covered a much wider range throughout geological time scales with values estimated as high as 6000μmol mol^{-1}during the Paleozoicum (500 million years ago) (Long *et al*., 2004). However, few studies examined the plants response along a CO_2gradient, and thus the dynamic responses of plants to different elevated CO_2 concentrations are far from understood, especially for the growth, physiological, and biochemical responses of C_3perennial grasses to higher CO_2 concentrations than the twofold current ambient CO_2 concentration (800μmol mol^{-1}).

The objectives of this study are to examine the effects of elevated CO_2 concentrations on: (1) growth and biomass, (2) leaf gas exchange, and (3) biochemical composition of three widely used cool-season C_3grass species Tall fescue (*Festuca arundinacea* Schreb.), Perennial ryegrass (*Lolium perenne* L.), and Kentucky bluegrass (*Poapratensis* L.) through an automatic CO_2 controlling system with ambient (400μmol mol^{-1}) or elevated CO_2 concentrations (600, 800, 1000, and 1200μmol mol^{-1}).Given that most of previous studies focused on growth and physiological response of grasses to a twofold current atmospheric CO_2 concentration, this study aims to identify the optimal CO_2 concentration for perennial grasses and understand the potential mechanisms underlying grasslands response to elevated CO_2 concentrations under future global change.

Materials and Methods

Plant materials and growing conditions: We sampled three grass species, Tall fescue (*Festuca arundinacea* Schreb.), Perennial ryegrass (*Lolium perenne* L.), and Kentucky bluegrass (*Poa pratensis* L.), using a golf-hole cutter (10 cm diameter × 20 cm long) to ensure the same aboveground and belowground biomass of each species were collected from field plots in the research farm at Rutgers University in Adelphia, NJ, USA. These grasses were irrigated with groundwater once a week in the field research farm to maintain the 10-cm soil surface moisture of about40% (% volume) during the growth season. Then the collected plants were transplanted into pots (10 cm diameter × 40 cm long)

filled with fritted clay and maintained in a greenhouse with an average temperature of 21/16ºC (day/night) and about 800 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) in natural sun light, and 65% relative humidity for 70 d (May-June 2012) to establish canopy and roots. During the establishment period, plants were irrigated to water-holding capacity daily and fertilized twice per week with half-strength Hoagland's solution (Hoagland & Arnon, 1950). Plants were trimmed once per week to maintain a canopy height of 5 cm. Then the plants were trimmed to a 2-cm canopy height and moved to growth chambers (Environmental Growth Chamber) with the temperature set at 21/18ºC (day/night), 60-70% relative humidity, 1000 μmol m^{-2} s^{-1}PPFD, and a 12-h photoperiod for 2 weeks prior to CO_2 treatment. During the 8-week CO_2 treatment, these plants were maintained under well-watered conditions with daily irrigation and fertilized with half-strength Hoagland's solution twice per week.

Treatments and experimental design: Plants were exposed to five CO_2treatments: ambient concentration (400±10μmol mol^{-1}) or elevated concentrations (600, 800, 1000, and 1200±10μmol mol^{-1}). In order to minimize confounding effects of environmental variation between different chambers, we randomly changed the CO_2 concentration of each growth chamber every three days, and then we relocated the CO_2 treated grasses to the growth chambers with corresponding CO_2 concentrations. The experiment was arranged in a randomized complete block design with four replicates (pots) per treatment. The ambient and elevated CO_2 concentrations within the chambers were maintained through an automatic CO_2 controlling system connected to theCO_2 source-tank containing 100% research-grade CO_2(Airgas, Inc.). The CO_2concentrations inside the chambers were continuously monitored through an infrared gas analyzer (LI-820; LICOR, Inc., Lincoln, NB, USA) connected to a computer logger. The CO_2concentration was maintained using an automatic controlling system consisting of a programmable logic controller unit, solenoid valves, and a laptop computer with monitoring software capable of monitoring and maintaining the CO_2 concentration within 10μmol $mol^{-1}$of the ambient and elevated target levels.

Growth and biomass measurements: Shoot growth rate (expressed as millimeters per day) was calculated as the difference in average canopy height at3-d intervals and in which canopy height was measured with a floating disk ruler method as the vertical distance from a paper disk placed on the turf canopy and the base of the shoot (Yu *et al*., 2012a). The values of plant growth rates were averaged together within each replicate. After measuring the canopy height, we trimmed the plants to a 2-cm canopy height again at 14, 28, 42, and 56 days of the CO_2 treatments. The trimmed leaves were collected and the leaf area was measured with an area meter (LI-3100; LICOR, Inc.) and then dried in an oven set to 80ºC for 7 days, and the dry weight was subsequently measured. The dry weight of leaves collected at 14, 28, 42, and 56 days of CO_2 treatment were put together for calculating shoot biomass during the 56 days CO_2 treatment. All plants were destructively sampled at 56 days of CO_2 treatment for an analysis of root biomass accumulation. The roots were severed from the shoots at the soil surface and washed free of fritted clay medium. All the washed roots were then dried in an oven at 80ºC for 3 days, and the dry weight was subsequently measured.

Leaf gas exchange measurements: Measurements on leaf gas exchange were performed after 56 days of CO_2 treatments. Five fully expanded leaves were randomly selected and arranged in a 2 cm ×3 cm cuvette chamber attached to a portable photosynthesis system (LI-6400; LICOR, Inc.). Before each measurement, leaves were equilibrated in the cuvette at saturating PPFD (1000 μmol m^{-2} s^{-1}), the growth CO_2, and the growth temperature (21ºC). The vapor pressure deficit (VPD)in the foliar was controlled by the Licor 6400 system, and most of the measurements were conducted with VPD lower than 1.5 kPa, which means moisture was not a limiting factor. CO_2 concentrations in the cuvette were controlled using an injector system (LI-6400, LI-COR Inc.), which functions with a CO_2 mixer and compressed CO_2 cartridges. Cuvette sealed with plasticene to prevent leakage. Potential leakage of CO_2 out and into the empty cuvette was determined for each concentration and used to correct the measured foliar fluxes with the equations provided by von Caemmerer & Farquhar (1981) and Galmés *et al*.(2007). Photosynthesis *vs* intercellular CO_2 (A_n-C_i) curves were measured at cuvette chamber CO_2 of 50, 100, 150, 200, 300, 400, 600, 800, 1000, 1200, and 1400μmol mol^{-1}. Data from A_n-C_i curves were used to compare treatment effects on the light-saturated net photosynthetic rates at ambient or elevated CO_2 (A_n), the maximum carboxylation rate of Rubisco (V_{cmax}), and the maximum capacity of electron transport mediated ribulose bisphosphate (RuBP) regeneration (J_{max}).An estimation method of Sharkey *et al*. (2007) was used to obtain V_{cmax} and J_{max} for each observed A_n-C_i curve. Meanwhile, stomatal conductance (G_s), and transpiration rate (T_r) were also determined with the portable photosynthesis system (LI-6400; LICOR, Inc.).

After the measurement of each A_n-C_i curve, the red and blue light source was turned off at least 10 minutes, and then measured the leaf dark respiration rates (R_d) with the portable photosynthesis system (LI-6400; LICOR, Inc.). All other conditions were the same as A_n-C_i curve measurements. The leaf area was determined using a hand-held digital scanner immediately following leaf removal from the cuvette. Water use efficiency (*WUE*) was determined by the values of the net photosynthetic rate (A_n) and transpiration rate (T_r) according to the formula $WUE = A_n/T_r$.

Biochemical analysis: After the 56 days CO_2 treatment, the leaves and roots for analyzing total non-structural carbohydrates (TNC) were sampled at midday and immediately frozen in liquid nitrogen and stored at −80ºC until freeze-drying. Freeze-dried tissues were then ground to fine powder with a ball mill (MM2, Fa. Retsch, Haan, Germany) and stored at 20ºCwith desiccant. Total carbon (C) and nitrogen (N) contents in leaves and roots were determined using an elemental analyzer (Vario Max CN; Elemnetar Corp., Germany). Leaf samples were assayed for non-structural carbohydrates according to Hendrix (1994).

Glucose, fructose, sucrose, and starch concentrations were determined spectrophometrically (UV-1750, Shimadzu Corp., Tokyo, Japan), using a glucose kit (GAHK-20, Sigma, St Louis, MO, USA). Phosphoglucose isomerase (P5381-1 KU, Sigma) was used to convert fructose to glucose, and invertase (I-4504, Sigma) was used to convert sucrose to glucose. All the biochemical analyses were repeated five times and expressed on a percentage dry matter basis.

Statistical analysis: The main effects of the CO_2 treatment were tested using one-way analysis of variation (ANOVA) followed by Duncan's multiple range test (p<0.05).We also used two-way ANOVA (p<0.05) to estimate the interactive effects of species and [CO_2]. All statistical analyses were performed using the *SPSS* 13.0 software (Chicago, IL, USA).

Results

Relative growth rate and total leaf area: Both the relative growth rate and total leaf area in response to elevated [CO_2] were species-specific (all p<0.001; Table 1).The increase in the relative growth rate of Tall fescue and the increase in total leaf area of Kentucky bluegrass were only significant at 1200 μmol mol^{-1}.Elevated [CO_2] significantly increased the total leaf area of Kentucky bluegrass (p<0.05; Fig. 1b), while had little effect on those of Tall fescue and Perennial ryegrass (all p>0.05; Fig. 1b). Moreover, we did not detect any interactive effect of species and [CO_2]on the relative growth rate and total leaf area of grasses during the 56 days treatment (all p>0.05; Table 1).

Biomass: Our two-way ANOVA results showed that the grass biomass and root/shoot ratio were species specific (all p<0.001; Table 1), and elevated [CO_2] significantly enhanced the total biomass of Tall fescue due to the increased shoot biomass (p<0.001; Fig. 2), but had little effect on the root/shoot ratio of the grass species (p>0.05; Table 1 and Fig. 2). Species and [CO_2] had no interactive effect on the biomass of the three grasses (p>0.05; Table 1).

Leaf net photosynthetic rate, dark respiration rate, and A_n/R_d ratio: We found significant differences in the mean area-based photosynthetic rate (A_n) among the three grass species (p<0.001; Table 2), and meanwhile elevated [CO_2] increased the area-based A_n of these grasses (p<0.001; Fig. 3a). Interestingly, when A_n was expressed by per unit leaf N (μmol CO_2 g N^{-1} s^{-1}), A_n of Tall fescue did not show any difference between the ambient and elevated [CO_2] (p>0.05; Fig. 3b), and significant differences of the N-based A_n of Perennial ryegrass and Kentucky bluegrass were detected under elevated [CO_2] (all p<0.05; Fig. 3b). Elevated [CO_2] barely affected the V_{cmax} and J_{max} (p>0.05), although significantly different in V_{cmax} and J_{max} were detected among the three species (p<0.001; Table 2). Moreover, we also found that the area-based leaf dark respiration rate (R_d) was significantly different among the three grass species, and decreased linearly by elevated [CO_2] (Table 2; Fig. 4a). Similarly, when R_d was expressed by per unit leaf N, R_d (μmol CO_2 g N^{-1} s^{-1}) of the three species still showed sharply deceases with elevated [CO_2] (all p<0.01) compared with the R_d of plants grown at ambient [CO_2] (Fig. 4b).As a result, elevated [CO_2]substantially increased the area-based A_n/R_d ratio due to the increased A_n and the decreased R_d of the three grass species (Fig. 4c).Moreover, we also found significantly interactive effect of species and [CO_2] on the A_n/R_d ratio of the three grasses (p<0.05; Table 2).

Table 1. Effects of species and [CO_2] on growth and biomass of the three grass species.

Parameters	Species	[CO_2]	Species×[CO_2]
Relative growth rate	P<0.001	P=0.019	P=0.750
Total leaf area	P<0.001	P=0.197	P=0.577
Shoot biomass	P<0.001	P<0.001	P=0.103
Root biomass	P<0.001	P=0.274	P=0.108
Total biomass	P<0.001	P<0.001	P=0.407
Root/shoot ratio	P<0.001	P=0.120	P=0.034

Note: Mean values are compared by the two-way analysis of variance (ANOVA) at p<0.05

Table 2. Effects of species and [CO_2] on leaf gas exchange parameters of the three grass species.

Parameters	Species	[CO_2]	Species×[CO_2]
$A_{n\,(area\text{-}based)}$	P<0.001	P<0.001	P=0.084
$A_{n\,(N\text{-}based)}$	P=0.021	P=0.008	P=0.626
V_{cmax}	P<0.001	P=0.701	P=0.431
J_{max}	P<0.001	P=0.361	P=0.182
$R_{d\,(area\text{-}based)}$	P=0.001	P<0.001	P=0.059
$R_{d\,(N\text{-}based)}$	P=0.009	P<0.001	P=0.289
$A_n/R_{d\,(area\text{-}based)}$	P=0.001	P<0.001	P=0.010
G_s	P=0.006	P<0.001	P=0.441
T_r	P=0.025	P<0.001	P=0.588
WUE	P=0.001	P<0.001	P=0.010

Note: Values given are mean ± standard errors for four pots. Mean values are compared by the two-way analysis of variance (ANOVA) at p<0.05. Abbreviations: $A_{n\,(area\text{-}based)}$, the area based net CO_2 assimilation rate; $A_{n\,(N\text{-}based)}$, the nitrogen content based net CO_2 assimilation rate; V_{cmax}, the maximum carboxylation activity; J_{max}, the maximum electron transport capacity; $R_{d\,(area\text{-}based)}$, the area based leaf dark respiratory rate; $R_{d\,(N\text{-}based)}$, the nitrogen content based dark respiratory rate; G_s, stomatal conductance; T_r, transpiration rate; *WUE*, water use efficiency

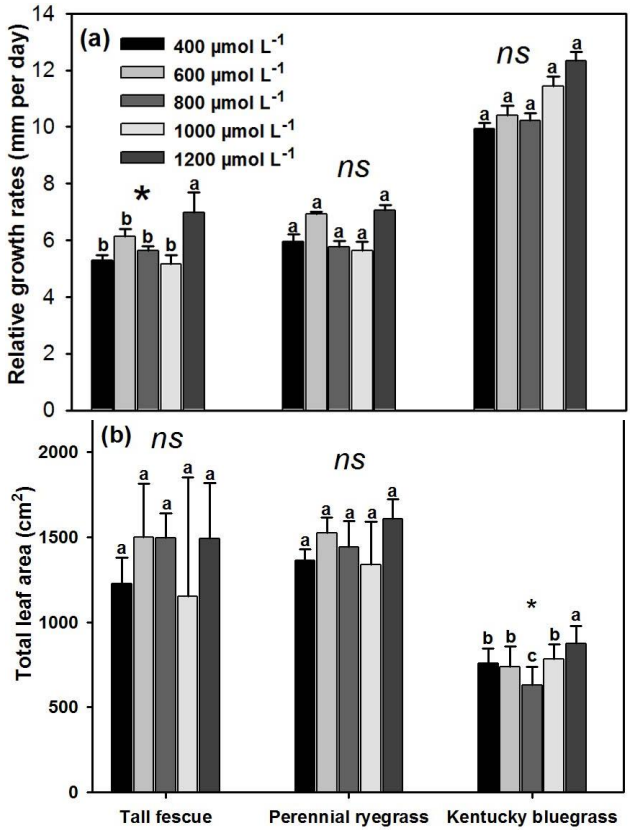

Fig. 1. Effects of elevated CO_2 concentrations on the mean relative growth rates (a) and total leaf area (b) of Tall fescue, Perennial ryegrass, and Kentucky blue grass during the 56 days treatments. Values given are mean± standard deviation for n=4 pots. Mean values were compared by the one-way ANOVA followed by Duncan's multiple range test, and the different letters represent statistical differences at $p<0.05$. *** indicates $p<0.001$; ** indicates $p<0.01$; * indicates $p<0.05$; *ns* indicates $p>0.05$.

Stomatal conductance, transpiration rates, and water use efficiency: Our results showed that elevated [CO_2] lead to a decline in both stomatal conductance (G_s) and transpiration rates (T_r), whereas sharply enhanced water use efficiency (*WUE*), although the responses of G_s, T_r, and *WUE* to elevated [CO_2] were highly species dependant ($p<0.05$; Table 2). We found no significant difference in G_s of Tall fescue among the ambient and elevated [CO_2] ($p>0.05$), whereas the elevated [CO_2] significantly decreased the G_s of Perennial ryegrass and Kentucky bluegrass (all $p<0.01$; Fig. 5a). Moreover, elevated [CO_2] also had different effects on T_r of the three species. Our results showed that the T_r of Tall fescue decreased with the increases of [CO_2], while no significant difference was detected among the ambient and elevated [CO_2] ($p>0.05$; Fig. 5b). However, elevated [CO_2] significantly decreased the T_r of Perennial ryegrass ($p<0.01$) and Kentucky bluegrass ($p<0.05$; Fig. 5b). In addition, elevated [CO_2] substantially enhanced the water use efficiency (*WUE*) of the three species mainly due to the increased net photosynthesis rates and the decreased transpiration rates under elevated CO_2 conditions (Fig. 5c).

Leaf non-structural carbohydrates and tissue C and N: Elevated [CO_2] profoundly decreased the content of leaf total non-structural carbohydrates (TNC) due to the decreases of soluble sugar (glucose, fructose, and sucrose) and starch (Table 3). However, the content of TNC in response to elevated CO_2 concentration was also species dependent ($p<0.001$; Table 4) as evidenced by the significantly decreased content of soluble sugar and starch in leaves of Tall fescue and Kentucky bluegrass under elevated CO_2environment, while it barely affected the content of TNC in leaves of Perennial ryegrass (Table 3).

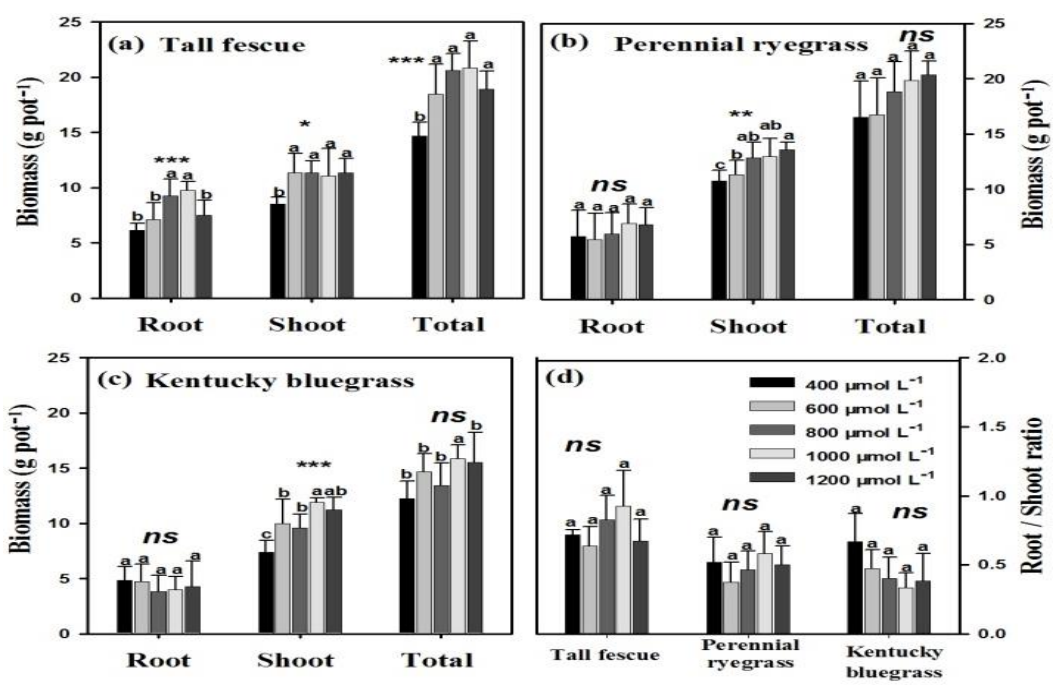

Fig. 2. Effects of elevated CO_2 concentrations on the biomass of Tall fescue (a), Perennial ryegrass (b), Kentucky bluegrass (c), and the root/shoot ratio of the three grass species (d). Values given are mean± standard deviation for n=4 pots. Mean values were compared by the one-way ANOVA followed by Duncan's multiple range test, and the different letters represent statistical differences at $p<0.05$. *** indicates $p<0.001$; ** indicates $p<0.01$; * indicates $p<0.05$; *ns* indicates $p>0.05$.

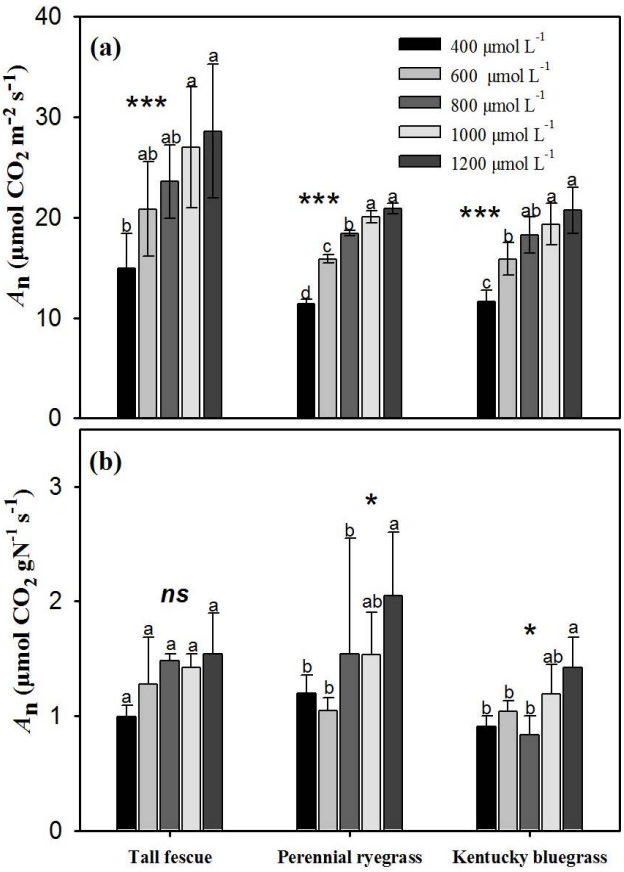

Fig. 3. Effects of elevated CO_2 concentrations on the area-based (a) and the N-based (b)net photosynthetic rates (A_n) of the three grass species. Values given are mean ± standard deviation for n=4 pots. Mean values were compared by the one-way ANOVA followed by Duncan's multiple range test, and the different letters represent statistical differences at p<0.05. *** indicates p<0.001; ** indicates p<0.01; * indicates p<0.05; *ns* indicates p>0.05.

In contrast to leaf TNC, elevated CO_2 concentration marginally increased the tissue C/N ratio due mainly to the increased carbon (C) and the decreased nitrogen (N) in both leaves and roots of Tall fescue and Kentucky bluegrass (Table 5). Leaf N and leaf C/N ratio were species-specific (p<0.001) and significantly affected by elevated [CO_2], whereas both species and [CO_2] had little effects on the root N (Table 4), indicated that leaf might be more sensitive than root in response to elevated [CO_2].

Discussion

CO_2 effects on growth and biomass: It is well demonstrated that most plants may benefit from elevated atmospheric CO_2concentrations through the "CO_2 fertilization effect", whereby enhanced atmospheric CO_2concentration in the ambient atmosphere induces plants to intake more CO_2 for stimulating plant growth (Ziska *et al*., 1991; Poorter & Navas, 2003; Graaff *et al*., 2006; Morgan *et al*., 2007; Zhang *et al*., 2011). However, other studies also reported that beyond certain CO_2 concentration thresholds, high CO_2 concentration may have adverse impact on plant growth (Bowler & Press, 1996; Wand *et al*., 1999; Long *et al*., 2004; Xu, 2015). In the current study, we found that the shoot and total biomass of Tall fescue were dramatically stimulated by the CO_2 concentration of 600μmol mol^{-1}, but for the CO_2 concentration higher than 600μmol mol^{-1}the growth is over (Fig. 2a). Similarly, increasing CO_2 concentration from 400 to 600μmol mol^{-1}also significantly decreased the starch and TNC contents of Tall fescue and Kentucky bluegrass, whereas no differences were found for these plants grown at higher CO_2concentrations than 600μmol mol^{-1} (Table 3).These results suggested that the optimal atmospheric CO_2 concentration for the growth of the grasses may be around 600μmol mol^{-1}. However, it should be noted that there is usually a CO_2 concentration threshold for each plant species, and thus exceeding their growth CO_2 concentration threshold may lead to adverse effects on the growth of higher plants including grasses as observed in this study. Meanwhile, a recent study also examined the optimal atmospheric CO_2 concentration of the CO_2 fertilization effect on the growth of winter wheat and found that the optimal CO_2 concentration is around 900 μmol mol^{-1} and high CO_2 concentration exceeding the optima resulted in negative effects on the growth of winter wheat (Xu, 2015).

Many experimental studies have shown that enhanced CO_2 usually resulted in a higher Root/Shoot ratio (R/S)in grass plants due mainly to an increase of root biomass (Arnone III *et al*., 2000; Poorter & Navas, 2003; Wang & Taub, 2010; Zhang *et al*., 2010; Arndal *et al*., 2014). However, several studies also reported that biomass allocation to roots under elevated CO_2 is dependent strongly on the experimental conditions (Hebeisen *et al*., 1997; Schapendonk *et al*., 1997; Suter *et al*., 2001). For example, Suter *et al*. (2002) found that elevated CO_2 increased root dry matter by 109% and thus enhanced R/S ratio by 44% of Perennial ryegrass (*Lolium perenne*) in field, whereas the CO_2 effects on the biomass allocation to roots were disappeared when the plants grown in pots under controlled conditions. In this study, we found that elevated CO_2 concentrations had little effect on both the root biomass and R/S ratio of the Perennial ryegrass and Kentucky bluegrass grown in culture pots (Fig. 2) supported the previous conclusions that elevated CO_2 did not affect the carbon allocation of plants grown in pots. The difference between the CO_2-inducedchanges in the field and the unchanged R/S ratio in pots, as observed in the current study, may be caused by the different supply of nutrients. Usually, plants are vulnerable to nutrient deficits under high CO_2 conditions, especially in natural ecosystems (Reich *et al*., 2006). It is reasonable to speculate that plants grown under a high CO_2 condition in the field may invest more carbon assimilates to the belowground tissues to form bigger and stronger root systems for enough nutrients supply (Rogers *et al*., 2008; Zhang *et al*., 2010). By contrast, in the pot experiments, the nutrient supply to plants was steady due mainly to the regular application of nutrient solution, and thus these plants under high CO_2 conditions might meet their additional nutrient demand, which was caused by an increase in the supply of carbon. Our results that the carbon contents in roots of the Perennial ryegrass and Kentucky bluegrass were irrespective to elevated CO_2 concentrations (Table 5),

directly demonstrated that carbon investment may be limited to roots when plants grown in pots without nutrient stress under elevated CO_2.Meanwhile, it should be noted that the growth of the root system might be constrained by pots, and thus the root response to elevated CO_2 was partly depressed when plants were grown in pots as reported by several previous studies (Arp, 1991; Mcconnaughay *et al*., 1993;Suter *et al*., 2002). Interestingly, our results that the elevated CO_2 concentrations have a significant positive effect on the root biomass of Tall fescue grown in the same pots in size (10 cm diameter × 40 cm long) as those of the Perennial ryegrass and Kentucky bluegrass, suggesting that the drought tolerance of Tall fescue may be enhanced due to the increased root biomass under future elevated CO_2 concentrations. The different responses of root biomass to elevated CO_2 between Tall fescue and the other two species may result from the different growth patterns in roots of the three grass species, because the roots of Perennial ryegrass and Kentucky bluegrass are mainly radial expansion (Jiang and Huang, 2000, 2001), which is more likely to be constrained by pots than the root growth of Tall fescue featured a longitudinal expansion pattern (Yu *et al*., 2012b).

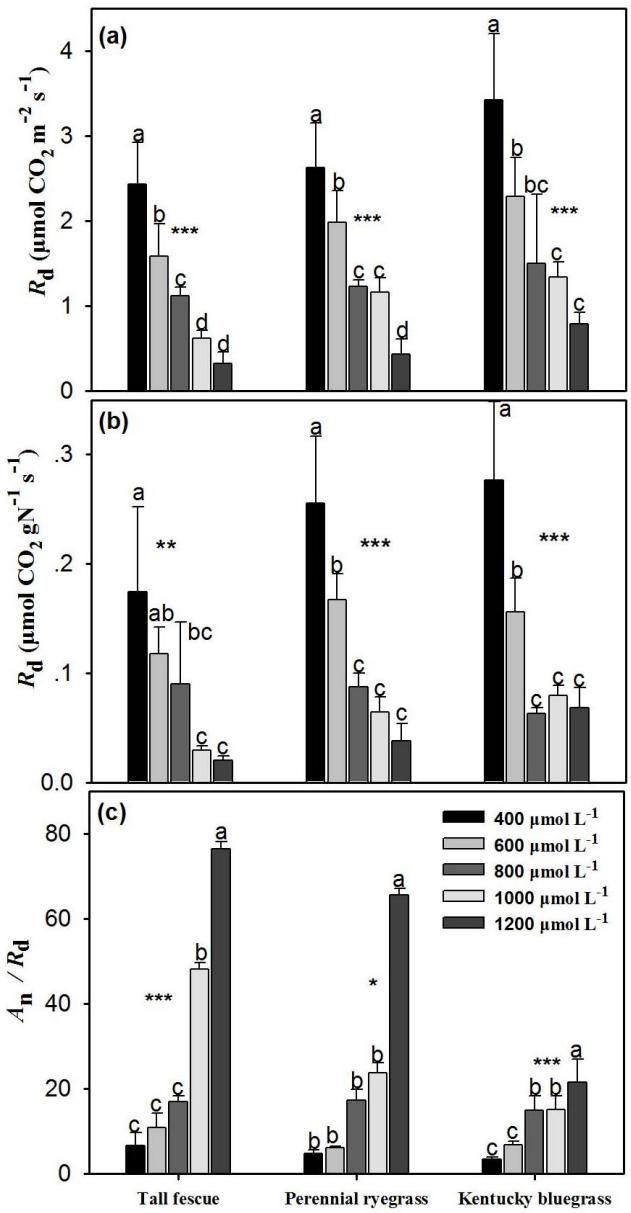

Fig. 4. Effects of elevated CO_2 concentrations on the area-based (a) and N-based (b) leaf dark respiration rates (R_d) and the area-basedA_n/R_d ratio (c) of the three grass species. Values given are mean± standard deviation for n=4 pots. Mean values were compared by the one-way ANOVA followed by Duncan's multiple range test, and the different letters represent statistical differences at p<0.05. *** indicates p<0.001; ** indicates p<0.01; * indicates p<0.05; *ns* indicates p>0.05.

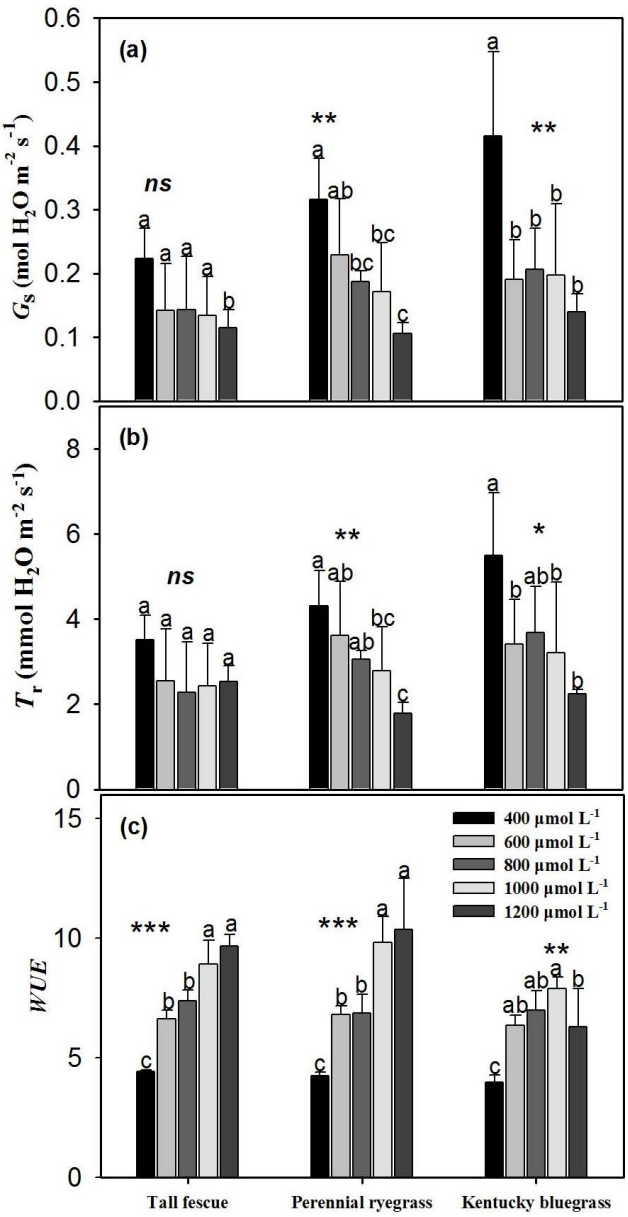

Fig. 5. Effects of elevated CO_2 concentrations on leaf stomatal conductance (a), transpiration rates (b), and water use efficiency (c) of the three grass species. Values given are mean± standard deviation for n=4 pots. Mean values were compared by the one-way ANOVA followed by Duncan's multiple range test, and the different letters represent statistical differences at p<0.05. *** indicates p<0.001; ** indicates p<0.01; * indicates p<0.05; *ns* indicates p>0.05.Abbreviations: G_s, stomatal conductance; T_r, transpiration rate; *WUE*, water use efficiency.

Table 3. Effects of elevated CO_2 concentrations on non-structural carbohydrate contents in the leaves of the three grass species.

Leaf non-structural carbohydrates (mg g $^{-1}$ DW)		CO_2 concentrations (μmol L^{-1})					*p*-value
		400	600	800	1000	1200	
Festuca arundinacea	Glucose	6.1 ± 0.2a	5.3 ± 0.2b	5.3 ± 0.2b	5.2 ± 0.3b	5.2 ± 0.1b	*
	Fructose	6.0 ± 0.3a	5.2 ± 0.2b	5.2 ± 0.1b	5.1 ± 0.1b	5.1 ± 0.3b	*
	Sucrose	5.8 ± 0.2a	5.1 ± 0.2b	5.0 ± 0.3b	4.9 ± 0.1b	4.9 ± 0.1b	*
	Starch	151 ± 4a	131 ± 5b	131 ± 1b	128 ± 7b	129 ± 6b	*
	TNC	168 ± 4a	147 ± 6b	147 ± 2b	144 ± 7b	144 ± 7b	*
Lolium perenne	Glucose	5.2 ± 0.3a	4.7 ± 0.3a	4.5 ± 0.5a	4.1 ± 0.5a	4.1 ± 0.5a	*ns*
	Fructose	5.2 ± 0.3a	4.6 ± 0.1a	4.4 ± 0.4a	4.0 ± 0.1a	4.1 ± 0.3a	*ns*
	Sucrose	5.0 ± 0.2a	4.5 ± 0.3a	4.3 ± 0.5a	3.9 ± 0.4a	4.0 ± 0.4a	*ns*
	Starch	129 ± 7a	116 ± 6a	111 ± 12a	102 ± 11a	103 ± 11a	*ns*
	TNC	145 ± 8a	130 ± 7a	124 ± 14a	114 ± 12a	115 ± 12a	*
Poapratensis	Glucose	5.6 ± 0.4a	4.3 ± 0.2b	4.0 ± 0.1b	4.0 ± 0.1b	3.8 ± 0.2b	***
	Fructose	5.5 ± 0.1a	4.1 ± 0.2b	3.9 ± 0.1b	4.0 ± 0.2b	3.9 ± 0.2b	***
	Sucrose	5.4± 0.3a	4.1 ± 0.1b	3.9 ± 0.1b	3.9 ± 0.3b	3.6 ± 0.1b	***
	Starch	139 ± 9a	107.1 ± 5b	100 ± 2b	100 ± 4b	94 ± 4b	***
	TNC	156 ± 9a	120 ± 5b	112 ± 2b	112 ± 4b	105 ± 5b	***

Note: Values given are mean ± standard errors for four pots. Mean values are compared by the one-way analysis of variation (ANOVA) followed by Duncan's multiple range test (p<0.05). *** indicates p<0.001; ** indicates p<0.01; * indicates p<0.05; *ns* indicates p>0.05

Table 4. Effects of specie sand [CO_2] on total non-structural carbohydrates and contents of carbon and nitrogen in leaves of the three grass species.

Parameters	Species	[CO_2]	Species×[CO_2]
Glucose	P<0.001	P<0.001	P=0.867
Fructose	P<0.001	P<0.001	P=0.867
Sucrose	P<0.001	P<0.001	P=0.866
Soluble sugars	P<0.001	P<0.001	P=0.867
Starch	P<0.001	P<0.001	P=0.867
TNC	P<0.001	P<0.001	P=0.867
Leaf C	P<0.001	P<0.001	P=0.680
Leaf N	P<0.001	P<0.001	P=0.001
Leaf C:N	P<0.001	P<0.001	P=0.001
Root C	P=0.001	P<0.001	P=0.007
Root N	P=0.977	P=0.095	P=0.856
Root C:N	P=0.748	P=0.043	P=0.832

Note: Values given are mean ± standard errors for four pots. Mean values are compared by the two-way analysis of variance (ANOVA) at p<0.05

Table 5. Effects of elevated CO_2 concentrations on carbon and nitrogen contents in the leaves of the three grass species.

Leaf carbon and nitrogen (mgg^{-1} DW)		CO_2 concentrations (μmol L^{-1})					*p*-value
		400	600	800	1000	1200	
		Leaf data					
Festuca arundinacea	C	399 ± 3c	408 ± 1b	409 ± 1b	414 ± 1a	415 ± 1a	***
	N	34.6 ± 1.5a	32.2 ± 0.8a	22.8 ± 1.1b	24.3 ± 1.1b	24.5 ± 0.7b	***
	C:N	11.6 ± 0.5b	12.7 ± 0.3b	18.1 ± 0.9a	17.1 ± 0.9a	17.0 ± 0.5a	***
Lolium perenne	C	415 ± 1b	419 ± 1ab	421 ± 2a	424 ± 2a	425 ± 3a	*
	N	27.2 ± 0.2a	24.7 ± 0.2ab	22.1 ± 1.0b	25.8 ± 2.5ab	24.7 ± 1.0ab	*ns*
	C:N	15.4 ± 0.2a	16.8 ± 0.2ab	19.2 ± 0.8b	16.9 ± 1.5ab	17.2 ± 0.6ab	*ns*
Poapratensis	C	421 ± 3b	425 ± 2ab	429 ± 2ab	430 ± 4a	428 ± 2ab	*ns*
	N	28.5 ± 0.8a	26.5 ± 0.8a	18.8 ± 0.7b	18.7 ± 0.3b	18.7 ± 0.9b	***
	C:N	14.8 ± 0.5a	15.9 ± 0.4a	22.9 ± 0.7b	22.5 ± 0.1b	23.1 ± 1.2b	***
		Root data					
Festuca arundinacea	C	381 ± 3c	411 ± 4ab	420 ± 4ab	406 ± 6b	422 ± 3a	***
	N	12.3 ± 0.6a	10.5 ± 0.7ab	9.5 ± 0.2b	9.4 ± 0.5b	9.9 ± 0.7b	*
	C:N	33.7 ± 1.3b	36.7 ± 2.0b	44.2 ± 0.5a	43.4 ± 1.7a	43.3 ± 2.6a	*
Lolium perenne	C	391 ± 1b	417 ± 1a	418 ± 2ab	419 ± 7a	428 ± 5a	*
	N	9.7 ± 1.0ab	11.7 ± 0.5a	9.4 ± 0.5ab	10.8 ± 1.2a	10.3 ± 0.4a	*ns*
	C:N	41.0 ± 3a	35.7 ± 1.5a	44.1 ± 3.2a	39.9 ± 3.6a	41.8 ± 1.5a	*ns*
Poapratensis	C	407 ± 6a	394 ± 8ab	408 ± 2a	377 ± 13b	404 ± 5a	*ns*
	N	10.6 ± 1.2a	10.7 ± 0.9a	9.6 ± 0.9a	10.4 ± 1.0a	10.7 ± 0.8a	*ns*
	C:N	39.6 ± 4.5a	37.3 ± 3.0a	43.5 ± 4.0a	37.1 ± 3.5a	38. 3 ± 3.0a	*ns*

Note: Values given are mean ± standard errors for four pots. Mean values are compared by the one-way analysis of variation (ANOVA) followed by Duncan's multiple range test (p<0.05). *** indicates p<0.001; ** indicates p<0.01; * indicates p<0.05; *ns* indicates p>0.05

CO_2effects on photosynthetic capacity and leaf dark respiration: Photosynthetic rates are well known to increase in C_3 plants in response to elevated CO_2 concentrations (Bowes, 1993; Lee *et al*., 2001; Leakey *et al*., 2009). Meanwhile, many studies found that the stimulation of photosynthetic rates induced by elevated CO_2 may decrease or even diminish over longer time as plants acclimate to elevated CO_2 concentrations through a process known as down-regulation (Schimel, 1995; Rogers *et al*., 1998; Rey & Jarvis, 1998; Lee *et al*., 2001; Liu *et al*., 2012). Our results showed that the area-based net photosynthetic rates were significantly increased with elevated CO_2 concentration, and even the down-regulation of photosynthesis did not occur at the CO_2 concentration of 1200μmol mol^{-1} for Tall fescue and Perennial ryegrass (Fig. 3a). This result may be attributed to the CO_2 treatment duration of eight weeks, which is possibly just a short-term response to elevated CO_2 concentration, and thus the leaf photosynthesis may be decreased with a longer CO_2 treatment duration. Moreover, in accordance with the tolerance law that organism would not survive when an ecological factor such as CO_2concentration is insufficient or in excess, the net photosynthetic rates should be declined with higher CO_2concentrations. In this study, we observed that the maximum photosynthetic rates of Kentucky bluegrass occurred at the CO_2 concentration of 1000μmol mol^{-1}, and beyond this peak further increasing the CO_2 concentration to 1200μmol mol^{-1} lead to a decline in net photosynthetic rates, suggesting that the CO_2concentration of 1200μmol mol^{-1}may be not high enough for decreasing the net photosynthetic rates of Tall fescue and Perennial ryegrass. Similarly, Xu (2015) also found that the leaf net photosynthetic rate of winter wheat was declined when the CO_2concentration beyond 1000μmol mol^{-1}.

In contrast to leaf net photosynthetic rates, previous studies have not drawn consistent conclusions on leaf dark respiration in response to elevated CO_2 (Gonzàlez-Meler &Siedow, 1999; Jahnke, 2001; Hamilton *et al*., 2001; Gonzàlez-Meler *et al*., 2009; Crous *et al*., 2011; Li *et al*., 2013; Tan *et al*., 2013). Most studies reported that doubling of atmospheric CO_2 concentration caused a reduction of respiration rate by 15-20% indirectly through the effects on the reduction of leaf N (Gonzàlez-Meler & Siedow, 1999), whereas others found absence of any effect (Hamilton *et al*., 2001),and even an enhancement of respiration due to the increased availability of carbohydrates and proteins (Li *et al*., 2013). Our results showed that the area-based R_d of the three species was significantly reduced by the elevated CO_2 concentrations (Fig. 4a). Meanwhile, we found that elevated CO_2concentrations also substantially decreased the N-based R_d (Fig. 4b), although the leaf N also decreased with elevated CO_2 concentrations (Table 5). These results suggested that the reduction of R_d under elevated CO_2 concentrations may not be due to the decrease of leaf N as evidenced by the decreased N-based R_d under elevated CO_2. Similarly, our results showed that elevated CO_2 concentrations decreased the leaf TNC across the three species (Table 3), suggesting that the reduction of R_d may partially be attributed to the decreases of leaf TNC, which is the most important substrate for leaf respiration. However, it should be noted that fructansis an important fraction of TNC, while fructans has not been taken into account carbohydrates in this study, which may affect the results of TNC content and thus relate to the reduction of leaf R_d. In addition, several previous studies have pointed that the chamber based gas exchange measurements of dark CO_2 efflux may lead to experimental artifacts such as systematic errors and gas leakage due to adsorption and absorption of CO_2 as well as leakages of CO_2both via chamber seals and the intercellular air spaces of leaves (Gonzàlez-Meler & Siedow, 1999; Amthor, 2000; McDermitt *et al*., 2001). Therefore, the potential role of systematic errors or gas leakage may also be involved in the measurements on the three grass species in the current study, although we took the measurements carefully and the cuvette of the gas exchange system was sealed with plasticine to prevent leakage.

CO_2effects on carbon and water balances: Changes in the balances of carbon and water in plant canopies have important implications for understanding the effects of elevated global atmospheric CO_2 levels on plant growth and primary productivity (Pooter & Navas, 2003; Gifford, 2004; Borjigidai *et al*., 2009), and thus predicting plant community dynamics and development in future higher CO_2 world (Morgan *et al*., 2007). Our results suggested that elevated CO_2 levels enhanced the ratio of A_n/R_d (Fig. 4c)mainly due to the increased A_n and decreased R_d (Figs. 3a and 4a). Similarly, elevated CO_2 levels also improved the canopy *WUE* of the three species (Fig. 5c) through reducing T_r and enhancing A_n (Fig. 5b and Fig. 3a). However, the three grass species exhibited different response sensitivity to elevated CO_2 levels in growth and physiology, and thus may benefit differently from the increased growth rates, enhanced A_n, and improved *WUE*, which may be responsible for the species composition and community dynamics as well as structure and function of grasslands under future higher CO_2 levels (Wand *et al*., 1999; Lee *et al*., 2001; Long *et al*., 2004; Morgan *et al*., 2007; Leakey *et al*., 2009).

In addition to elevated CO_2, the global surface temperature may continue to increase and the global precipitation may become unevenly distributed both temporally and spatially under future climate change (Anon., 2013). As a result, drought stress caused by the increased global surface temperature and the declined precipitation may also be a critical factor for affecting leaf photosynthesis and respiration (Jiang &Huang, 2000; Rachmilevitch *et al*., 2006), and thus the plant growth and biomass accumulation (Ballizany *et al*., 2012), and in turn the structure and function of ecosystems such as grasslands and pastures (Newton *et al*., 1996; Suter *et al*., 2002; Arndal *et al*., 2014).Therefore, the fates of the three grasses cannot be determined by elevated CO_2 concentration because warming and drought may have interactive effects with CO_2enhancement on the growth, physiological, and biological processes of the three grasses under future climate change (Jiang &Huang, 2001; Rachmilevitch *et al*., 2006; Wang & Taub, 2010; Chun *et al*., 2011; Yu *et al*., 2012b).More controlled experiments with multiple factors including temperature, water content, and CO_2 concentration are needed to conduct for predicting the fates of grass species, and thus the community dynamics of

grasslands or pastures under future global change featured with climate warming, drought stress, and CO_2 enhancements. However, it is important to note that this study is carried out under controlled conditions with sufficient nutrients and water for plants during the experiment, which is far away from the conclusion under field conditions. Therefore, several similar experiments should be carried out at natural conditions without fertilization and watering for predicting the fates of the three important cool-season C_3 grasses in future climate change scenarios.

Conclusions

We found that the growth of the three perennial grasses was apparently stimulated by initial increase in CO_2 concentration through the CO_2 fertilization effect. However, this CO_2 fertilization effect was substantially compromised with further increase in CO_2 concentration, suggesting optimal atmospheric CO_2 concentrations for the growth of perennial grasses. The negative effects of higher CO_2 concentration beyond the optimum on plant growth can be contributed to the changes of photochemical and biochemical processes with leaf photosynthesis and respiration. Overall, our results demonstrate that perennial grass species with high optimal CO_2 concentrations such as Tall fescue may suffer less from future climate change due to higher water and nitrogen use efficiency and meanwhile benefit the most from the CO_2 fertilization effect. Nevertheless, the optimal CO_2 concentrations for plants were substantially different, even for the three perennial grass species as found in the current study, indicating that rising future atmospheric CO_2 concentration and climate change may impact the species composition and community dynamics as well as structure and function of grasslands.

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