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The optimal CO_2 concentrations for the growth of three perennial grass species

Yunpu Zheng[1,2], Fei Li[1], Lihua Hao[1], Arshad Ali Shedayi[2,3], Lili Guo[1], Chao Ma[1], Bingru Huang[4*] and Ming Xu[5*]

Abstract

Background: Grasslands are one of the most representative vegetation types accounting for about 20% of the global land area and thus the response of grasslands to climate change plays a pivotal role in terrestrial carbon balance. However, many current climate change models, based on earlier results of the doubling-CO_2 experiments, may overestimate the CO_2 fertilization effect, and as a result underestimate the potentially effects of future climate change on global grasslands when the atmospheric CO_2 concentration goes beyond the optimal level. Here, we examined the optimal atmospheric CO_2 concentration effect on CO_2 fertilization and further on the growth of three perennial grasses in growth chambers with the CO_2 concentration at 400, 600, 800, 1000, and 1200 ppm, respectively.

Results: All three perennial grasses featured an apparent optimal CO_2 concentration for growth. Initial increases in atmospheric CO_2 concentration substantially enhanced the plant biomass of the three perennial grasses through the CO_2 fertilization effect, but this CO_2 fertilization effect was dramatically compromised with further rising atmospheric CO_2 concentration beyond the optimum. The optimal CO_2 concentration for the growth of tall fescue was lower than those of perennial ryegrass and Kentucky bluegrass, and thus the CO_2 fertilization effect on tall fescue disappeared earlier than the other two species. By contrast, the weaker CO_2 fertilization effect on the growth of perennial ryegrass and Kentucky bluegrass was sustained for a longer period due to their higher optimal CO_2 concentrations than tall fescue. The limiting effects of excessively high CO_2 concentrations may not only associate with changes in the biochemical and photochemical processes of photosynthesis, but also attribute to the declines in stomatal conductance and nitrogen availability.

Conclusions: In this study, we found apparent differences in the optimal CO_2 concentrations for the growth of three grasses. These results suggest that the growth of different types of grasses may respond differently to future elevated CO_2 concentrations through the CO_2 fertilization effect, and thus potentially alter the community composition and structure of grasslands. Meanwhile, our results may also be helpful for improving current process-based ecological models to more accurately predict the structure and function of grassland ecosystems under future rising atmospheric CO_2 concentration and climate change scenarios.

Background

It is widely evident that global atmospheric carbon dioxide (CO_2) concentration has dramatically increased since the nineteenth century industrial revolution, elevating by about 1.6 ppm/yr. during the past five decades [1, 2]. According to the most recent report released by the Inter-Governmental Panel on Climate Change (IPCC, 2013), global atmospheric CO_2 levels have increased from the pre-industrial level of 280 ppm to the present level of nearly 410 ppm and the growth rate of CO_2 concentration is projected to be accelerated with an unprecedented pace of ~1.0 ppm/yr. [2–4]. Moreover, the global atmospheric CO_2 concentration may even reach 1000 ppm by the end of this century and nearly 2000 ppm by the end of the next century if no effective control measures are implemented [4]. This elevated global atmospheric CO_2 concentration may not only cause climate warming, but also cause profound impacts on the net primary productivity of agricultural and natural ecosystems [5–9].

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* Correspondence: huang@aesop.rutgers.edu; mingxu@crssa.rutgers.edu
[4]Department of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ 08901, USA
[5]Center for Remote Sensing and Spatial Analysis, Department of Ecology, Evolution and Natural Resources, Rutgers University, 14 College Farm Road, New Brunswick, NJ 08901, USA
Full list of author information is available at the end of the article

It is well known that CO_2 is not only one of the most important greenhouse gases, but also a critical reactant for the biochemical processes of plant photosynthesis, and thus future elevated CO_2 concentrations may affect plant growth by altering metabolic rates [10–13]. Many studies have reported that most plants may benefit from enriched atmospheric CO_2 concentrations through the "CO_2 fertilization effect". Plant growth can be boosted by absorbing more CO_2 molecules for photosynthesis under elevated CO_2 concentrations [10, 14–17]. For example, Wand [18] reviewed the responses of wild grasses to elevated atmospheric CO_2 concentrations and found that elevated CO_2 increased the total biomass of C_3 grass species by about 50%. However, other studies have shown that the CO_2 fertilization effect on plant growth might decline or vanish beyond certain CO_2 concentrations [7, 19, 20], and even CO_2 enrichment induced adverse effects on some plants when the ambient CO_2 level was above 1000 ppm [21]. In addition, many previous studies also found that the CO_2 fertilization effect on plants had a large variation among different species. For example, Wang [18] reported a substantial increase of the biomass of young birch tree by 59% when CO_2 concentration was doubled from about 350 ppm to 700 ppm. By contrast, Körner et al. [22] showed that the growth and biomass of five tree species in a mature deciduous forest were barely affected by increasing CO_2 concentration to 530 ppm based on a four-year FACE experiment. These results indicate that different plant species may have different optimal CO_2 concentrations, and that plants with higher optimal CO_2 concentrations are likely to benefit the most from the CO_2 fertilization effect, and at the same time, suffer less negative impacts from future climate change, mainly due to higher nitrogen and water use efficiency [23, 24].

The CO_2 fertilization effect on plant growth was fundamentally mediated by leaf photosynthesis [19, 25, 26], which is highly correlated with plant carbon balance [27] and biochemical composition [28, 29]. Previous studies have demonstrated that elevated CO_2 could dramatically affect net photosynthetic rates through various processes including up-regulation or down-regulation when the growth CO_2 below or above the optimal CO_2 for plants. Elevated CO_2 levels generally stimulate net photosynthetic rate through directly enhancing carboxylation rates [13, 30] while competitively reducing photorespiration and dark respiration [19, 22, 31–33]. Nevertheless, the decline of net photosynthetic rate under high CO_2 levels may be related to changes in leaf biochemical composition associated with reductions in the amount and/or activity of Rubisco [22, 26], and increases in total non-structural carbohydrates [7, 34]. Moreover, the down-regulation of net photosynthetic rate is also associated with the availability of nutrients such as nitrogen (N), which exerts an important control over the response of plants and ecosystems in rising atmospheric CO_2 conditions [28, 35–37]. Previous studies showed that down-regulation of photosynthesis occurred in plants grown in elevated CO_2 and limited N indicated decreased leaf N concentration [38, 39] High N availability could alleviate the down-regulation of photosynthesis in plants under elevated CO_2 environments [19, 26, 29].

Grasslands are an important part of terrestrial ecosystems, and account for about 20% of the earth's land area [6, 40]. Perennial grasses are the dominant species in temperate grasslands and pastures [40], and are utilized as fine turf grass, which serves many important environmental functions including erosion control, surface water detoxification and control of allergens and diseases [41, 42]. A majority of the research investigating plant response to elevated CO_2 have been focused on crops [43–45] or trees [26, 29, 34, 46–48] and few studies have examined the effects of elevated CO_2 on perennial grasses [17, 19, 40]. In addition, most previous studies regarding the CO_2 fertilization effect have focused primarily on "doubling-CO_2 experiments" with twofold higher CO_2 concentration of about 700 or 800 ppm than the current global CO_2 concentration [40, 42, 45, 48]. Nevertheless, the CO_2 fertilization effect may sustain up to about 1000 ppm for leaf photosynthesis [46, 49] and 1800 ppm for grain yield of crops [50]. For example, Xu [23] examined the optimal atmospheric CO_2 concentration of the CO_2 fertilization effect on the growth of winter wheat and found that the optimal atmospheric CO_2 concentration was 894 and 968 ppm for total biomass and leaf photosynthesis. So far, few experimental studies have been conducted to examine the optimal CO_2 concentration for maximizing the CO_2 fertilization effect on perennial grasses, which are the most important grass species in both natural grasslands and managed turf grass. Moreover, most of the modeling projections are based on strong CO_2 fertilization according to the conclusions from earlier "doubling-CO_2 experiments" [29, 34]. However, it should be noted that in the future, continuously rising atmospheric CO_2 concentrations may substantially lower the CO_2 fertilization effect when the atmospheric CO_2 concentration rises beyond the optimal CO_2 level [23]. As a result, many current climate change models based on earlier results of the doubling-CO_2 experiments may overestimate the CO_2 fertilization effect and underestimate the potential risks that climate change poses on global grasslands when the atmospheric CO_2 concentration goes beyond the optimal CO_2 level. Therefore, identifying optimal CO_2 concentrations and understanding the mechanisms that determine these optima are not only critical to accurately estimating the impacts of climate change on global grassland production, but also have important significance for policy implementations under future climate change scenarios. Therefore, this study was conducted based on the following objectives: (1) investigate the effects of elevated CO_2 concentrations on the growth of three perennial grass species, (2) examine the optimal CO_2 concentration for maximizing the CO_2 fertilization effect of these grasses, and (3) explore potential mechanisms that determine the optimal CO_2 concentrations for the growth of perennial grasses.

Methods

Plant materials and growing conditions

Three grass species, tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), and Kentucky bluegrass (*Poa pratensis* L.), were collected using a golf-hole cutter (10 cm diameter × 20 cm long) to ensure the same aboveground and belowground biomass of each species from field plots in the research farm at Rutgers University (Adelphia, NJ, USA). These grasses were irrigated with groundwater once a week in the field research farm to maintain a 10-cm soil surface moisture of about 40% (% volume) during the growing 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 21/16 °C (day/night) and about 800 μmol photon m^{-2} s^{-1} Photosynthetic Active Radiation (PAR) in natural sun light, and 65% relative humidity for 70 d (May–June 2012) to establish canopy and root system. During the establishment period, grasses were irrigated daily to water-holding capacity and fertilized twice per week with half-strength Hoagland's solution [51]. We trimmed grasses once a week to maintain a canopy height of 5 cm during the canopy development and root establishment period. Then the plants were trimmed to a 2-cm canopy height and moved to growth chambers (Environmental Growth Chamber) with temperatures set at 21/18 °C (day/night), 60–70% Relative Humidity (RH), light level at grass canopy of 1000 μmol m^{-2} s^{-1} PAR, and a 12-h photoperiod for 2 weeks prior to the CO_2 treatment. During the eight weeks of the CO_2 treatment, these grasses were maintained under the same environmental factors as before the start of CO_2 treatment, such as chamber temperature of 21/18 °C (day/night), relative humidity of 60–70%, light level at the grass canopy of 1000 μmol m^{-2} s^{-1} PAR, and 12-h photoperiod (6:00–18:00). In addition, the grasses were also well-watered with daily irrigation and fertilized with half-strength Hoagland's solution twice a week.

Treatments and experimental design

We exposed grasses to five CO_2 treatments: ambient concentration (400 ± 10 ppm) or elevated concentrations (600, 800, 1000, and 1200 ± 10 ppm). 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 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 control system connected to a CO_2 source-tank containing 100% research-grade CO_2 (Airgas, Inc.). The CO_2 concentrations inside the chambers were continuously monitored through an infrared gas analyzer (LI-820; LICOR, Inc., Lincoln, NB, USA) connected to a computer logger maintaining the CO_2 concentration within 10 ppm of the ambient and elevated target levels.

Plant biomass measurements

We trimmed the plants to a 2-cm canopy height again at 14, 28, 42, and 56 days after the CO_2 treatments. The trimmed leaves were collected and oven dried at 80 °C for 7 days, and the dry weights were subsequently measured. The dry weights of leaves collected at 14, 28, 42, and 56 days of CO_2 treatment were put together for calculating shoot biomass during the CO_2 treatment period. At the end of the treatment period (56 days), all plant samples were destructively removed for an analysis of root biomass accumulation. The roots were severed from the shoots at the soil line and washed to make free of fritted clay medium. All of the washed roots were then oven dried at 80 °C for 3 days, and the dry weights were subsequently measured.

Leaf gas exchange measurements

Leaf gas exchange measurements were performed at the end of the CO_2 treatment period (56 days). Five fully expanded leaves were randomly selected and arranged in a 2 × 3 cm^2 cuvette chamber attached to a portable photosynthetic system (LI-6400; LICOR, Inc.). Before each measurement, leaves were equilibrated in the cuvette at saturating PPFD (1000 μmol photon m^{-2} s^{-1}), the growth CO_2 level, the target temperature and Vapor Pressure Deficit (VPD). CO_2 concentrations in the cuvette were controlled using an injector system (LI-6400, LI-COR Inc.), which utilizes a CO_2 mixer and compressed CO_2 cartridges sealed with plasticene to prevent leakage. Then, the 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 1400 ppm. 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 was used to obtain V_{cmax} and J_{max} for each observed A_n-C_i curve [52]. Meanwhile, stomatal conductance (g_s), and transpiration rate (T_r) were also determined with the portable photosynthesis system (LI-6400; LICOR, Inc.). 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 CO_2 treatment period (56 days), the leaves and roots for analyzing Total Non-structural Carbohydrates (TNC) were sampled at midday, immediately frozen in liquid nitrogen and stored at – 80 °C until freeze-drying. Freeze-dried tissues were then ground to fine powder with a ball mill (MM2, Fa. Retsch, Haan, Germany), applied desiccant and stored at 20 °C. Total carbon (C) and nitrogen (N) contents in leaves and roots were determined using an elemental analyzer (Vario Max CN, Elemnetar Corp., Germany). Glucose, fructose, sucrose and starch concentrations were determined spectrophotometrically (UV-1750, Shimadzu Corp., Tokyo, Japan), using a glucose kit (GAHK-20, Sigma, St Louis, MO, USA). Phospho-glucose isomerase (P5381–1 KU, Sigma) and invertase (I-4504, Sigma) were used to convert fructose to glucose and sucrose to glucose respectively. Biochemical analyses were repeated five times and expressed on a percentage dry matter basis for each.

Data analysis

The raw data from the leaf photosynthesis measurements was cleaned and processed in Excel spreadsheets where the non-linear A_n-C_i curve fitting was performed as in Sharkey et al. (2007) [52]. The net assimilation rate (A_n) versus intercellular CO_2 concentration (A_n-C_i curve), were fitted to estimate the maximum carboxylation rate (V_{cmax}), maximum electron transport rate (J_{max}) based on the measurements of A_n-C_i curves. In addition, linear and non-linear (quadratic equations) regressions were employed to examine relationships between CO_2 concentration and other variables.

Results

Elevated CO_2 effects on plant biomass

We found very strong CO_2 fertilization effects on the aboveground and total biomass of the three species. The optimal CO_2 levels for the aboveground biomass were 945, 915, and 1151 ppm, and for the total biomass were 915, 1178, and 1386 ppm for tall fescue, perennial ryegrass, and Kentucky bluegrass, respectively (Fig. 1). However, an optimal CO_2 of 895 ppm for the belowground was found only for the tall fescue, while no obviously optimal CO_2 of the belowground biomass for the other two species was detected. Beyond the optimum, further elevating the ambient CO_2 concentration significantly reduced the growth of perennial grasses, indicating the adverse impacts of high CO_2 concentration on the grass species. Quadratic models can be used to adequately quantify the CO_2 fertilization effect on the biomass of the three grasses (Fig. 1).

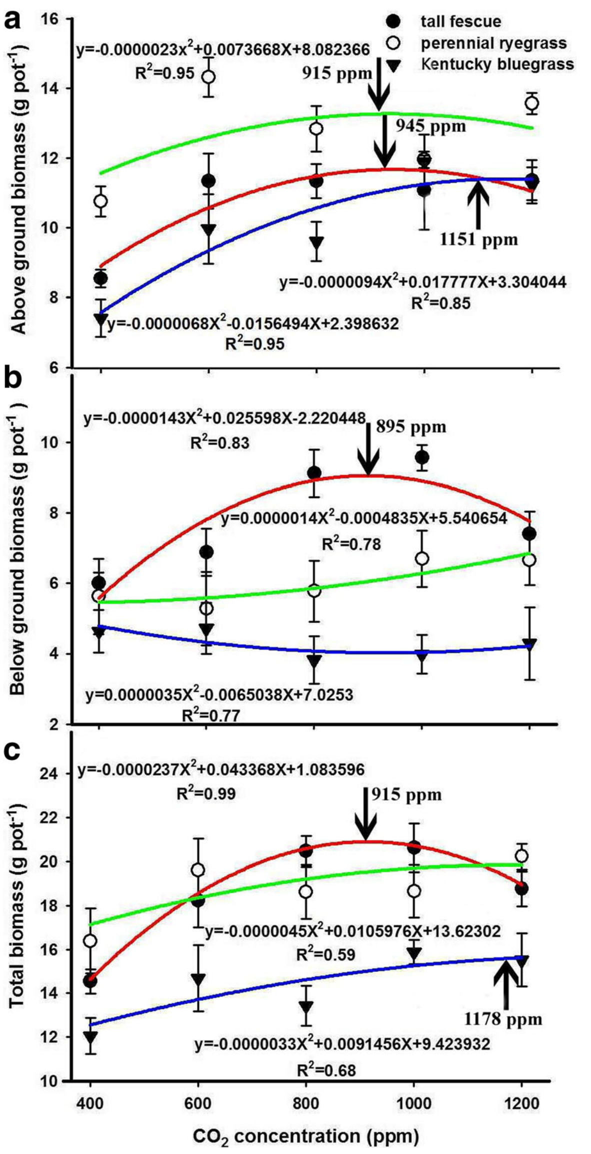

Fig. 1 Effects of elevated CO_2 concentrations on above ground biomass (**a**), below ground biomass (**b**), and total biomass (**c**) of the three grass species. Values given are mean ± standard deviation for $n = 4$ pots

Elevated CO_2 effects on leaf gas exchange

As with plant growth, the CO_2 fertilization effect was also evident in the leaf net photosynthetic rate (A_n) of Kentucky bluegrass, stimulating A_n by 75% when the

CO_2 increased from 400 ppm to 1000 ppm. The CO_2 stimulation effect on A_n reached a maximum at 959 ppm, at which point further increase in CO_2 resulted in a decline of A_n (Fig. 2). However, the response of A_n to elevated CO_2 also varied with grass species. The leaf net photosynthetic rates of the other two species (tall fescue and perennial ryegrass) consistently increased with increasing CO_2, which can also be described by quadratic relationships with optimal CO_2 beyond the maximum CO_2 treatment of this study. In contrast to A_n, the stomatal conductance (g_s) and transpiration rates (T_r) of the three grasses decreased non-linearly with the increase of CO_2 and the relationships of CO_2-g_s and CO_2-T_r also typically followed quadratic equations with maximum g_s and T_r occurring around 400 ppm, which was much lower than the optimal CO_2 for plant growth and leaf photosynthesis.

As a result, the WUE of tall fescue and Kentucky bluegrass also featured bell-shaped curves in relation to CO_2 concentration, with the maximum CO_2 fertilization effect occurring at approximately 1062 ppm and 910 ppm, respectively. However, the maximum WUE of perennial ryegrass was beyond the highest CO_2 concentration treatment of 1200 ppm. Thus, we quantified the relationship between CO_2 and WUE of perennial ryegrass through quadratic models and found that the optimal CO_2 for WUE would occur at about 2700 ppm, which was much higher than those of the other two species (Fig. 2).

The maximum carboxylation rate (V_{cmax}) of the three grasses demonstrated bell-shaped curves in relation to CO_2 concentration, peaking at 906 ppm, 863 ppm, and 743 ppm for tall fescue, perennial ryegrass, and Kentucky bluegrass, respectively (Fig. 3a). Similar to the V_{cmax}, the maximum electron transport rate (J_{max}) in response to increasing CO_2 concentrations also shared bell-shaped curves for all three grasses. The optimal CO_2 concentration of J_{max} was 877 ppm, 941 ppm, and 665 ppm for tall fescue, perennial ryegrass, and Kentucky bluegrass, respectively (Fig. 3b).

Elevated CO_2 effects on leaf dark respiration and non-structural carbohydrates

Our results showed that leaf dark respiration (R_d) of the three species substantially declined with increasing CO_2 (Fig. 4). The relationships between R_d and CO_2 of the three species were quantified through quadratic models with R^2 values of 0.99, 0.99 and 0.94 for tall fescue, perennial ryegrass and Kentucky bluegrass respectively (Fig. 4a). Similar to the R_d, the leaf total non-structural carbohydrate (TNC) of the three grasses also quadratically decreased with elevated CO_2 (Fig. 4b). Meanwhile, we estimated the relationships between R_d and TNC (Fig. 5) and found that R_d was increased linearly by the

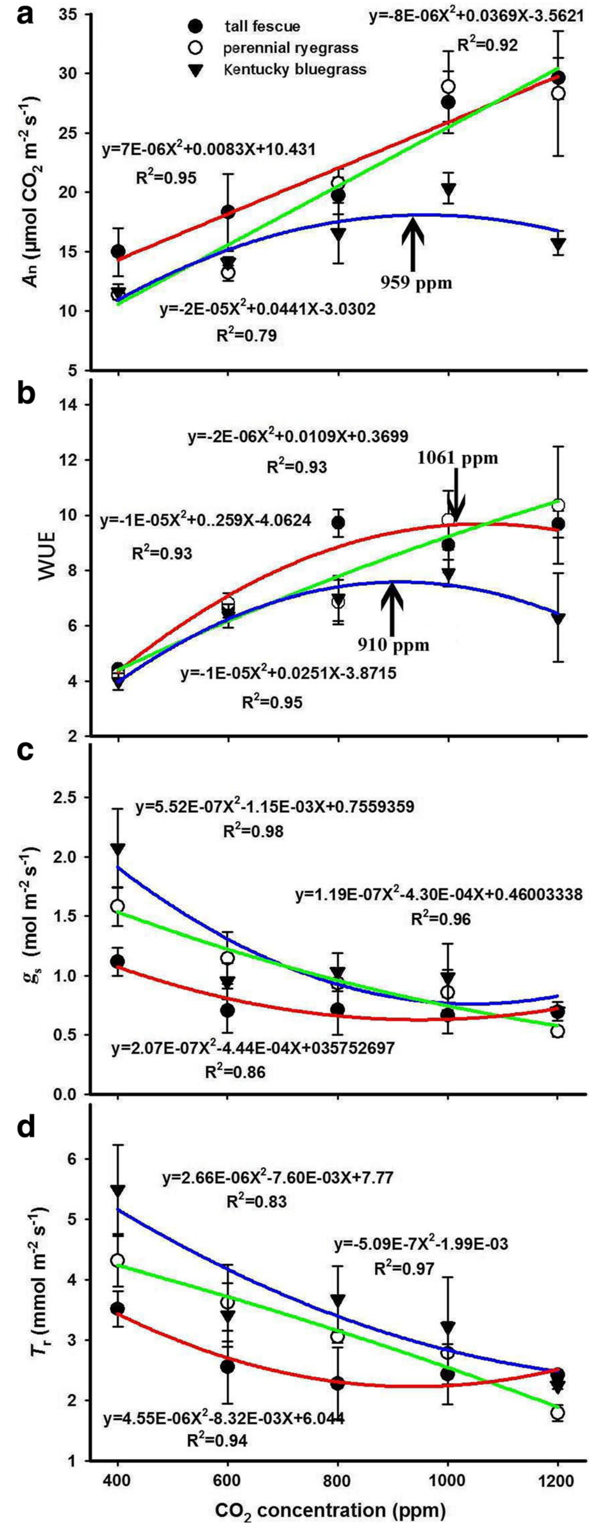

Fig. 2 Effects of elevated CO_2 concentrations on (**a**) leaf net photosynthesis rate (A_n), (**b**) water use efficiency (WUE), (**c**) stomatal conductance (g_s), and (**d**) transpiration rate (T_r) of the three grass species. Values given are mean ± standard deviation for $n = 4$ pots

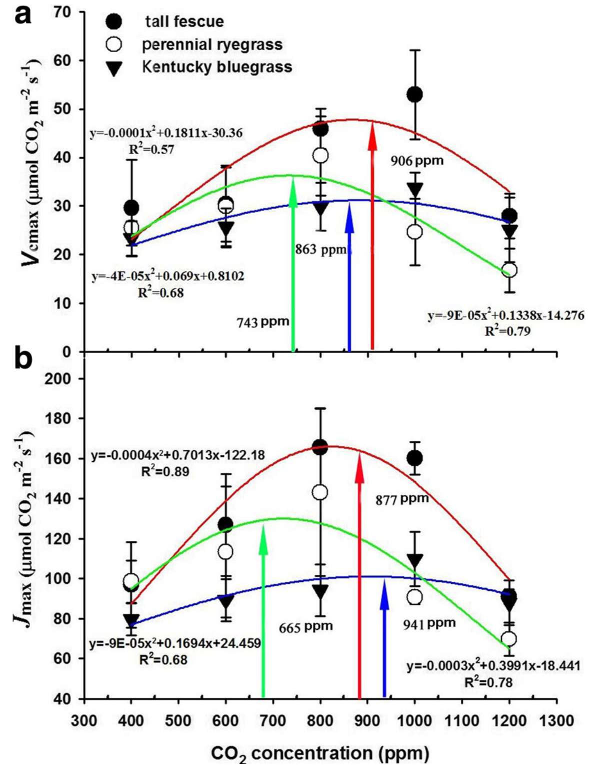

Fig. 3 Effects of elevated CO_2 concentrations on (**a**) the maximum carboxylation rate (V_{cmax}) and (**b**) the maximum capacity of electron transport RuBP regeneration (J_{max}) of the three grasses. Values given are mean ± standard deviation for $n = 4$ pots

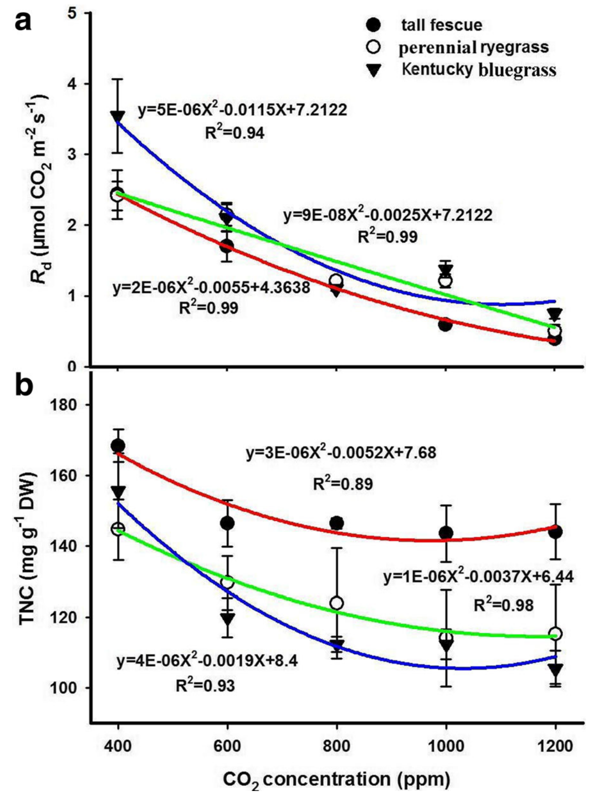

Fig. 4 Effects of elevated CO_2 concentrations on (**a**) the leaf dark respiration rates (R_d) and (**b**) total non-structural carbohydrates (TNC) of the three grass species

enhancement of TNC, with R^2 values such as 0.73, 0.78 and 0.95 for the tall fescue, perennial ryegrass, and Kentucky bluegrass, respectively.

Elevated CO_2 effects on tissue carbon (C) and nitrogen (N) contents and the relationships between leaf N and V_{cmax} or leaf N and J_{max}

We found optimal CO_2 concentrations in both the leaf and root of tall fescue and perennial ryegrass. The relationships between leaf carbon and CO_2 featured bell-shaped curves with maximum values occurring at approximately 1388 and 1600 ppm for tall fescue and perennial ryegrass with R^2 values 0.96 and 0.99 respectively (Fig. 6a). Interestingly, root carbon in response to elevated CO_2 was also characterized by similar curves with R^2 values 0.71 and 0.78 and optimal CO_2 levels of 1011 and 1200 ppm for tall fescue and perennial ryegrass, respectively. However, we obtained very weak relationships between CO_2 and tissue carbon with R^2 values 0.23 for leaf and 0.19 for root of Kentucky bluegrass (Fig. 6a). In contrast to tissue carbon, both the leaf and root nitrogen of the tall fescue and Kentucky bluegrass quadratically decreased with elevated CO_2 (Fig. 6b). By using the quadratic functions, we analyzed the relationships of leaf and root nitrogen with CO_2 and found the R^2 values to be 0.79 and 0.71, and 0.31 and 0.44 for the tall fescue and Kentucky bluegrass respectively (Fig. 6d). Our results also showed that elevated CO_2 barely affected the tissue nitrogen of perennial ryegrass, evidenced by the weak quadratic relationships between CO_2 and nitrogen with R^2 values 0.03 and 0.04 for leaf and root respectively (Fig. 6c-d).

We also evaluated the relationships between leaf N and V_{cmax} as well as leaf N and J_{max} of the three grass species (Fig. 7). Our results showed that the V_{cmax} values were linearly enhanced with the increases of leaf N for tall fescue ($R^2 = 0.70$), perennial ryegrass ($R^2 = 0.70$), and Kentucky bluegrass ($R^2 = 0.65$, Fig. 7a-c). Similarly, we also found linearly positive relationships between leaf N and J_{max} with R^2 values of 0.57, 0.55, and 0.62 for tall fescue, perennial ryegrass, and Kentucky bluegrass, respectively (Fig. 7d-f).

Discussion

Different optimal CO_2 fertilization concentrations for the growth of perennial grasses

Most plants generally benefit from elevated atmospheric CO_2 concentration through the "CO_2 fertilization effect",

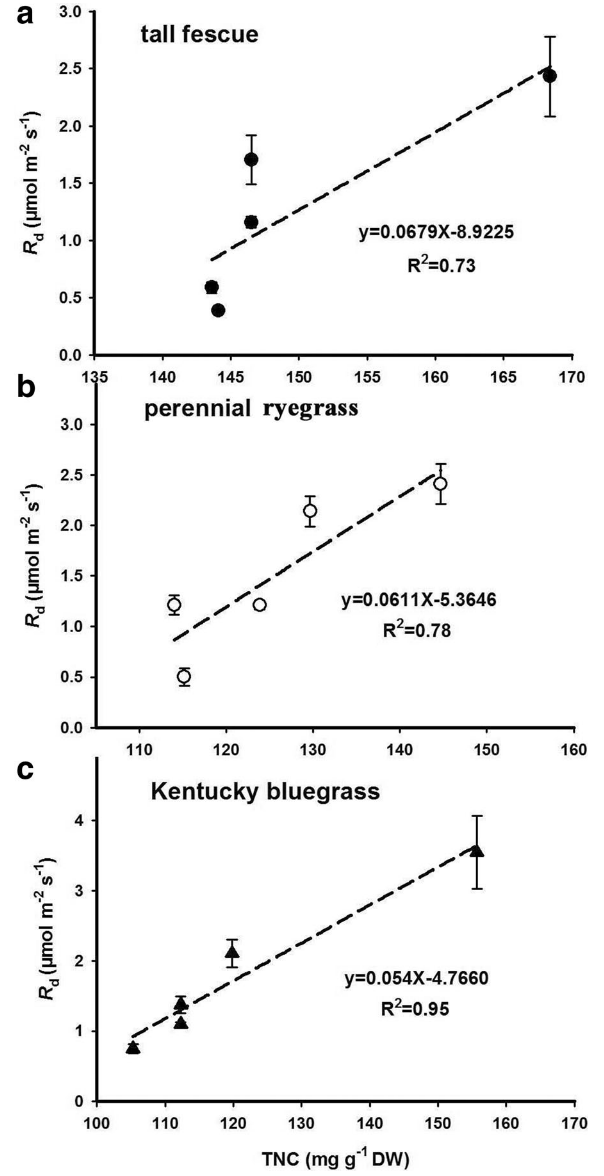

Fig. 5 Effects of elevated CO_2 concentrations on the linear relationship between TNC and R_d for tall fescue (**a**), perennial ryegrass (**b**), and Kentucky bluegrass (**c**)

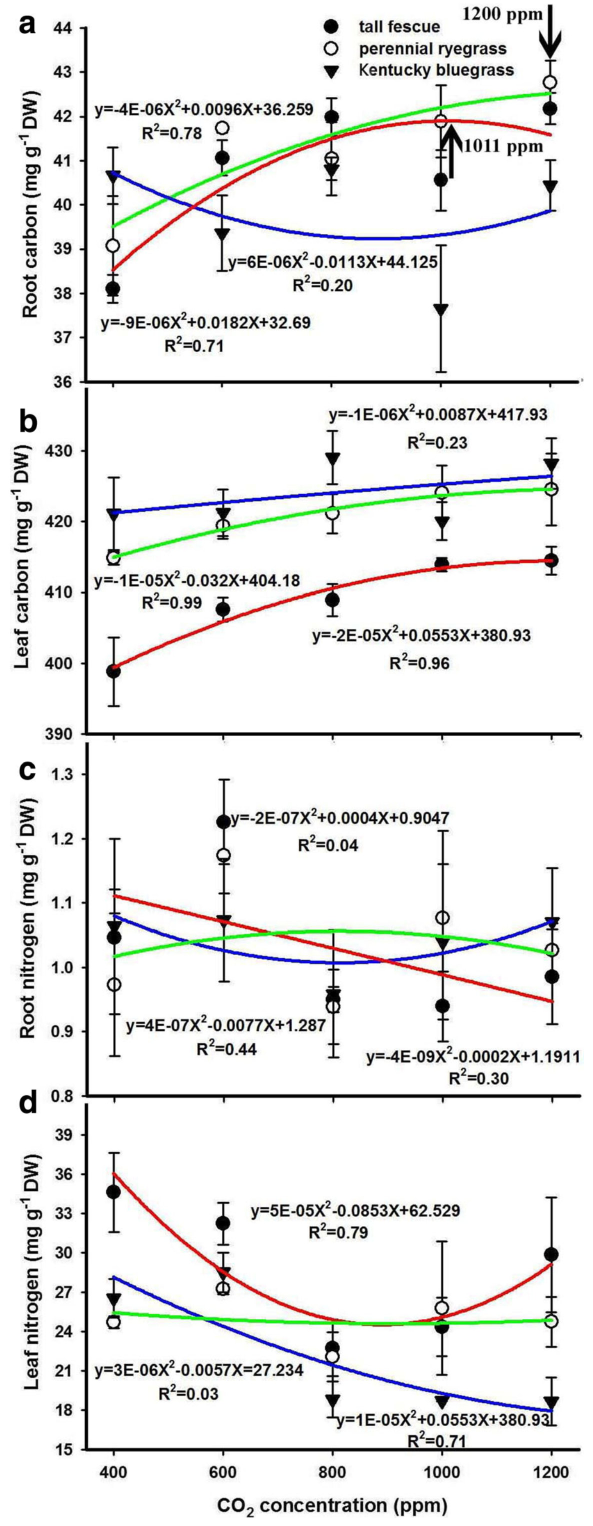

Fig. 6 Effects of elevated CO_2 concentrations on the carbon content of root (**a**) and leaf (**b**) as well as the nitrogen content of root (**c**) and leaf (**d**) of the three grass species. Values given are mean ± standard deviation for n = 4 pots

which boosts growth and yield [19, 23, 46, 52]. However, this positive CO_2 fertilization effect strongly depends on the plant functional groups and species [7, 22, 53–56]. Even within the same species of winter wheat, the results from previous studies are inconsistent [22, 50, 57–60]. These contradictory results suggest that different plants and/or species may have different optimal CO_2 concentrations for their growth. Our results showed that the optimal CO_2 concentrations occurred at 945, 915, and 1151 ppm for the aboveground biomass and at 915, 1178, and 1386 ppm for the total biomass of tall fescue, perennial ryegrass, and Kentucky bluegrass (Fig. 1),

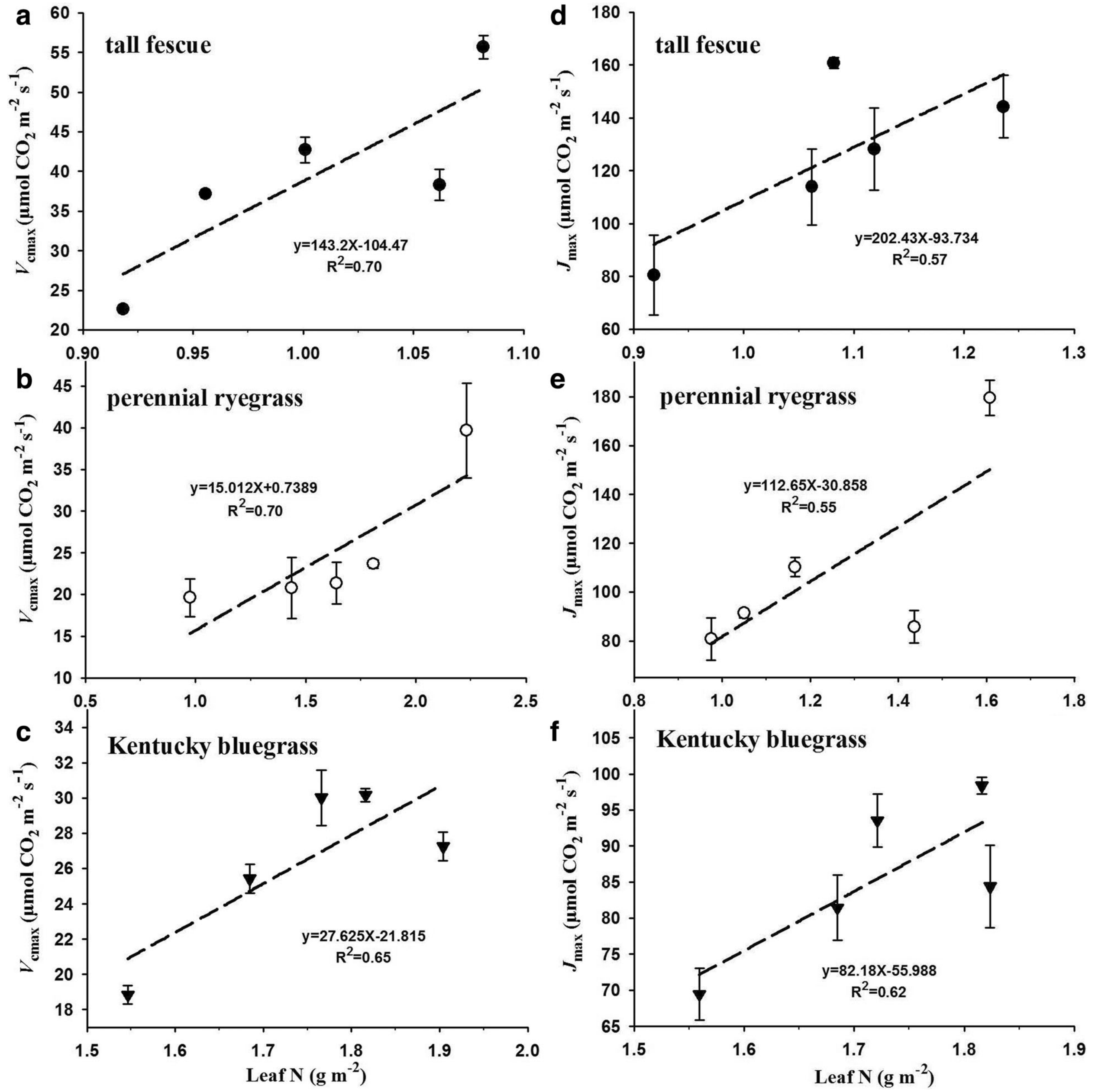

Fig. 7 Effects of elevated CO$_2$ concentrations on the relationships between V_{cmax} and leaf N (**a**-**c**) or J_{max} and leaf N (**d**-**f**) for the three grass species. Values given are mean ± standard deviation for $n = 4$ pots

suggesting that a strong CO$_2$ fertilization effect occurred at different optimal CO$_2$ concentrations for these three perennial grasses. This result also indicated that Kentucky bluegrass has the highest optimal CO$_2$ concentration among the three grasses, and thus may suffer less from future climate change than the other two grasses. In addition, by enhancing the atmospheric CO$_2$ concentration from 400 ppm to the optimum for each grass species, the maximum CO$_2$ fertilization effect substantially increased the total biomass of the by 60%, 15%, and 30% for tall fescue, perennial ryegrass, and Kentucky bluegrass respectively. Interestingly, biomass enhancements of 15% and 30% for perennial ryegrass and Kentucky bluegrass are very similar with the average of approximately 20% for C$_3$ plants as estimated in meta-analysis of Free-Air CO$_2$ Enrichment (FACE) studies [61, 62], and 32% of Open Top Chamber (OTC) and greenhouse experiments [63]. However, the increased rate of tall fescue (60%) is much higher than those of the other two species, indicating this specie will benefit the most from the positive fertilization effect among these three perennial grasses under future high CO_2 environmental conditions. It is noted that we found no obviously optimal CO_2 for the belowground biomass of two species (Kentucky bluegrass and perennial ryegrass), as evidenced by the upward quadratic relationships between belowground biomass and CO_2 concentrations. These results suggest that the carbon allocation between aboveground and belowground of the three grasses characterize different strategies, and tall fescue might select a more effective strategy to balance the carbon investment between aboveground and belowground than the other two species under high CO_2 concentrations.

The positive CO_2 fertilization effect on the growth of perennial grasses

Previous studies have well demonstrated that plant growth is highly correlated with biochemical and photochemical processes [64, 65] such as photosynthesis and respiration, through which the CO_2 fertilization effect is developed and regulated [22]. In the current study, the photosynthesis-CO_2 relationship followed a similar bell-shaped curve like the biomass-CO_2 relationship (Figs. 1 and 2), suggesting that the positive CO_2 fertilization effect might be attributed to the up-regulation of A_n, as evidenced by the increased leaf net photosynthetic rates (A_n), with the maximum CO_2 fertilization effect occurring at 959 ppm for Kentucky bluegrass, and 1200 ppm for both tall fescue and perennial ryegrass (Fig. 2a). Further analysis showed that leaf biochemical and photochemical processes played a key role in determining the positive CO_2 fertilization effect through directly increasing both carboxylation rates and electron transport rates of perennial grasses. Our results showed that both the maximum carboxylation rate of Rubisco (V_{cmax}) and the maximum capacity of electron transport RuBP regeneration (J_{max}) of the three grasses were dramatically stimulated by elevated CO_2 concentrations before reaching their optimums (Fig. 3), suggesting that the initial increase in CO_2 concentration may favor both the light and dark reactions of photosynthesis through boosting the Rubisco carboxylation and the RuBP regeneration processes. Also, a recent study has reported that the V_{cmax} of winter wheat was dramatically increased by elevating ambient CO_2 concentrations from 400 ppm to about 800 ppm [23].

In addition to leaf photosynthesis, the positive CO_2 fertilization effect on the growth of perennial grasses may also closely associate with the changes in leaf respiration and total non-structural carbohydrates (TNC) under high CO_2 concentrations. Our results showed that the leaf dark respiratory rates (R_d) and leaf TNC of the three grasses consistently decreased with elevated CO_2 concentrations. Meanwhile, we found a linear relationship between leaf R_d and TNC, suggesting that R_d reduction may partially attribute to decrease in leaf TNC, which is the most important substrate for leaf respiration [14, 66]. Overall, the up-regulation of A_n and the decline of R_d may both play pivotal roles in explaining the positive CO_2 fertilization effects on the growth of perennial grasses in the current study.

The diminishing returns of CO_2 fertilization effect on perennial grasses

Previous studies have found that beyond certain thresholds, high CO_2 concentration cause diminishing returns of CO_2 fertilization effect on plants [13, 22, 23]. Several studies found that the stimulation of A_n induced by elevated CO_2 decreased or even diminished if exposed for a longer time period, because plants acclimate to elevated CO_2 concentrations through a process known as down-regulation [19, 32]. We also found bell-shaped curves for biomass-CO_2 relationships for the three grasses similar to the A_n-CO_2, indicating a reduction in biomass due to a decline in the photosynthetic rate at high CO_2 concentrations. It is well demonstrated that the down-regulation of A_n is possibly attributed to the changes in carbohydrates [31], under high CO_2 environments. In the current study, elevated CO_2 concentrations beyond the optima of the three grasses consistently reduced leaf TNC, suggesting that the imbalance of carbohydrate concentration in the source and sink was not a limiting factor for the down-regulation of A_n. In addition, it is important to noted that hexokinase is a key functional enzyme for mediating sugar sensing [67] and may also decrease Rubisco content through inhibiting the expression of photosynthetic genes [68]. Previous studies have well demonstrated that the Rubisco content and activity of higher plants were dramatically decreased under high CO_2 concentrations [68, 69], because leaf N was prior to enzymes relating to the metabolic processes of starch and sucrose than invested in Rubisco when plants was subjected to high CO_2 concentrations [70]. Consequently, the changes in hexokinase with CO_2 concentrations may contribute to the bell-shaped relationship between A_n and CO_2 concentration, especially for the down-regulation of A_n under high CO_2 concentrations.

It is well documented that stomatal conductance (g_s) declines when exposed to elevated atmospheric CO_2 concentration, and a doubling of CO_2 from the present ambient concentration generally results in a reduction in g_s of 10–70% depending on species or functional groups [58]. In the current study, we also found that the g_s of all three grasses were dramatically decreased with elevated CO_2 concentrations, which may be partly due to the down-regulation of A_n caused by CO_2. Moreover, the reduced g_s under high CO_2 concentrations might result in a decline in leaf transpiration and thus reduced nutrient availability, as observed in many previous studies [22].

Previous studies have claimed that elevated CO_2 concentration increased plant C/N ratios mainly due to a decrease in N content [12, 26]. Similarly, we also found that the nitrogen contents of both tall fescue and Kentucky bluegrass were markedly decreased with increasing CO_2 concentrations, which may also be caused by the CO_2 effects on A_n, since nitrogen content is associated with photosynthetic enzymes such as Rubisco [35–37]. In addition, the linearly positive relationships between leaf N and V_{cmax} for the three grasses (Fig. 7) were directly supporting the above conclusion that the down-regulation of A_n was partly attributed to the decline of leaf N under high CO_2 concentrations.

It should be noted that the CO_2 fertilization effect on plant growth may be confounded by future climate change such as global warming, nitrogen deposition, and drought, which may reduce or cancel out the CO_2 fertilization effect [39]. For example, the global surface temperature may continue to increase and cause global precipitation to become unevenly distributed both temporally and spatially [2]. As a result, drought stress caused by the increased global surface temperature and the declined precipitation may also be a critical factor affecting leaf photosynthesis and respiration [17] and thus plant growth and biomass accumulation [49], and in turn the structure and function of ecosystems such as grasslands and pastures [37, 40]. Therefore, the fates of the three grasses cannot only be determined by elevated CO_2 concentrations because warming and drought may have interactive effects with CO_2 enhancement on the growth, physiological, and biological processes of the three grasses under future climate change [20]. Therefore, more controlled experiments with multiple factors such as temperature, drought, nutrition availability and CO_2 concentration are needed for predicting the fates of grass species and thus the community dynamics of grasslands under future global climate change [31]. However, it is important to note that this study was carried out under controlled conditions with sufficient nutrients and water for plants during the experiment, which is obviously different from actual field conditions. Therefore, many similar experiments should be carried out in natural conditions without fertilization and watering for predicting the fates of the three cool-season C_3 grasses in future climate change scenarios.

Conclusions

We found that the optimal CO_2 concentrations occurred at 945, 915, and 1151 ppm for the aboveground biomass of tall fescue, perennial ryegrass, and Kentucky bluegrass, respectively. Higher CO_2 concentrations had diminishing returns of CO_2 fertilization effect on plant growth, causing limiting effects on stomatal conductance, nitrogen availability and changes in the biochemical and photochemical processes of photosynthesis. Our results suggest that the continuously increasing atmospheric CO_2 concentration in the future may dramatically lower the CO_2 fertilization effect, and thus many current climate change models based on earlier results of "doubling–CO_2" experiments may overestimate the CO_2 fertilization effect on grasslands beyond the optimum CO_2 concentration. According to recent IPCC reports, if global CO_2 emissions are not effectively mitigated, the atmospheric CO_2 concentration might be over 900 ppm in the second half of this Century. Nevertheless, the optimal CO_2 concentrations found in this study can be used as an indicator in predicting the fates of the cool-season C_3 grasses under future rising atmospheric CO_2 concentration and climate change, because grasses with high optimal CO_2 concentrations may take full advantage of the CO_2 fertilization effect.

Abbreviations

A_n: net photosynthetic rates; g_s: stomatal conductance; R_d: dark respiration; TNC: total nonstructural carbohydrates; T_r: transpiration rates; VPD: vapor pressure deficit; WUE: water use efficiency

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YPZ, FL, MX and BH designed the experiments. FL, LHH, LLG and CM performed the experiments and analyzed the data. YPZ, MX, AAS and BH analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Author details

[1]School of Water Conservancy and Hydropower, Hebei University of Engineering, Handan 056038, China. [2]Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, 11A Datun Road, Beijing 100101, China. [3]Department of Biological Sciences, Karakoram International University Gilgit, Gilgit, Pakistan. [4]Department of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ 08901, USA. [5]Center for Remote Sensing and Spatial Analysis, Department of Ecology, Evolution and Natural Resources, Rutgers University, 14 College Farm Road, New Brunswick, NJ 08901, USA.

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References

1. Pearson PN, Palmer MR. Atmospheric carbon dioxide concentrations over the past 60 million years. Nature. 2000;406:695–9.
2. IPCC: Summary for Policy makers: Climate Change 2013: The Physical Science Basis. In: Church J, Clark P, Cazenave A, Gregory J, Jevrejeva S, Levermann A, Merrifield M, Milne G, Nerem SR, Nunn P, Payne A, Pfeffer WT, Stammer D, Alakkat U, editors. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. UK: Cambridge University Press; 2013.
3. NOAA: The history of atmospheric carbon dioxide on earth 2013. Available at: http://www. Planet for life. Com/CO_2 history/.
4. NASA: Global climate change: Vital signs of the planet 2014. Available at: http:// climate. nasa.
5. Bazzaz FA. The response of natural ecosystems to the leaf photosynthesis and water use of big bluestem under elevated carbon dioxide. Crop Sci. 1990;31:1589–94.
6. Steffen WL, Canadell JG. Carbon dioxide fertilization and climate change policy. Department of Environment and Heritage. AGO: Australian Greenhouse Office; 2005.
7. Wand SJE, Midgley GF, Jones MH, Curtis PS. Responses of wild C_4 and C_3 grass (*Poaceae*) species to elevated atmospheric CO_2 concentration: a meta-analytic test of current theories and perceptions. Glob Change Biol. 1999;5:723–41.
8. Arnone JA, Zaller JG, Spehn EM, Niklaus PA, Wells CE, Korner C. Dynamics of root systems in native grassland: effects of elevated atmospheric CO_2. New Phytol. 2000;147:73–85.
9. Kirkham MB. elevated carbon dioxide, impacts on soil and plant water relations. Boca Raton, FL: CRC Press. 2011;978:4398–5504.
10. Ainsworth EA. Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. Glob Change Biol. 2008;14:1642–50.
11. Lloyd J, Farquhar GD. The CO_2 dependence of photosynthesis, plant growth response to elevated atmospheric CO_2 concentrations and their interaction with soil nutrient status. I. General principles and forest ecosystems. Funct Ecol. 1996;10:4–32.
12. LeCain DR, Morgan JA. Growth, gas exchange, leaf nitrogen and carbohydrate concentrations in NAD-ME and NADP-ME C_4 grasses grown in elevated CO_2. Physiol Plantarum. 1998;102:2927–306.
13. Reddy AR, Rasineni GK, Raghavendra AS. the impact of global elevated CO_2 concentration on photosynthesis and plant productivity. Curr Sci. 2010;99:46–57.
14. Amthor JS. Effects of atmospheric CO_2 concentration on wheat yield: review of results from experiments using various approaches to control CO_2 concentration. Field Crops Res. 2001;73:1–34.
15. Jablonski LM, Wang X, Curtis PS. Plant reproduction under elevated CO_2conditions: a meta-analysis of reports on 79 crop and wild species. New Phytol. 2002;156:9–26.
16. Drewry DT, kumar P, Long S, Bernacchi C, Liang XZ, Sivapalan M. Ecohydrological responses of dense canopies to environmental variability: 2. Role of acclimation under elevated CO_2. J Geophys Res. 2010;155:2005–12.
17. Yu J, Chen L, Xu M, Huang B. Effects of elevated CO_2 on physiological responses of tall fescue to elevated temperature, drought stress, and the combined stress. Crop Sci. 2012;52:1848–58.
18. Wang X, Taub DR. Interactive effects of elevated carbon dioxide and environmental stress on root mass fraction in plants: a meta-analytical synthesis using Pairwise Techniques. Oecologia. 2010;163:1–11.
19. Lee TD, Tjoelker MG, Ellsworth DS, Reich PB. Leaf gas exchange responses of 13 prairie grassland species to elevated CO_2 and increased nitrogen supply. New Phytol. 2001;150:405–18.
20. Poorter H, Navas M. Plant growth and competition at elevated CO_2: on winners, losers and functional groups. New Phytol. 2003;157:175–98.
21. Yu J, Du H, Xu M. Metabolic responses to heat stress under elevated atmospheric CO_2 concentration in a cool-season grass species. J Am Soc Hortic Sci. 2012;137:221–8.
22. Körner C, Asshoff R, Bignucolo O, Hättenschwiler S, Keel SG, Peláez-Riedl S, Pepin S, Siegwolf RTW, Zotz G. Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2. Science. 2005;309:1360–2.
23. Xu M. The optimal atmospheric CO_2 concentration for the growth of winter wheat (*Triticum aestivum*). J Plant Physiol. 2015;184:89–97.
24. Long SP, Ainsworth EA, Rogers A, Ort DR. Rising atmospheric carbon dioxide: plants FACE the future. Annu Rev Plant Biol. 2004;55:591–628.
25. Morgan JA, Milchunas DG, LeCain DR. Carbon dioxide enrichment alters plant community structure and accelerates shrub growth in the shortgrass steppe. Proc Natl Acad Sci U S A. 2007;104:14724–9.
26. Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR. Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. J Exp Bot. 2009;60:2859–76.
27. Arp WJ. Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. Plant Cell Environ. 1991;14:869–75.
28. Lewis JD, Lucash M, Olszyk DM, Tingey DT. Relationships between needle nitrogen concentration and photosynthetic responses of Douglas-fir seedlings to elevated CO_2 and temperature. New Phytol. 2004;162:355–64.
29. Borjigidai A, Hikosak K, Hirose T. Carbon balance in a monospecific stand of an annual herb *Chenopodium album* at an elevated CO_2 concentration. Plant Ecol. 2009;203:33–44.
30. Jin VL, Evans RD. Elevated CO_2 increases plant uptake of organic and inorganic N in the desert shrub Larrea tridentate. Oecologia. 2010;163:257–66.
31. Zhang L, Yang Y, Zhan X, Zhan XY, Zhang CJ, Zhou SX, Wu DX. responses of a dominant temperate grass plant (Leymus chinensis) to elevated carbon dioxide and nitrogen addition in China. J Environ Qual. 2010;39:251–9.
32. Leakey ADB, Uribelarrea M, Ainsworth EA, Naidu SL, Rogers A, Ort DR, Long SP. Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. Plant Physiol. 2006;140:779–90.
33. Gunderson CA, Wullschleger SD. photosynthetic acclimation in trees to rising CO_2: a broader perspective. Photosynth Res. 1994;39:369–88.
34. Rey A, Jarvis PG. Long-term photosynthetic acclimation to increased atmospheric CO_2 concentration in young birch (*Betula pendula*) trees. Tree Physiol. 1998;18:441–50.
35. Cure JD, Acock B. Crop responses to carbon dioxide doubling: a literature survey. Agric For Meteorol. 1986;38:127–45.
36. Hendrix MS, Dumitru TA, Graham SA. Late oligocene-early miocene unroofing in the Chinese tian Shan; an early effect of the India-Asia collision. Geology. 1994;22:487–90.
37. Luo Y, Hui D, Zhang D. Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. Ecology. 2006;87:53–63.
38. Taub DR, Wang XZ. Why are nitrogen concentrations in plant tissue lower under elevated CO_2? A critical examination of the hypotheses. J Integr Plant Biol. 2008;50:1365–74.
39. Arndal MF, Schmidt IK, Kongstad J, Beier C, Michelsen A. Root growth and N dynamics in response to multi-year experimental warming, summer drought and elevated CO_2 in a mixed heathland-grass ecosystem. Funct Plant Biol. 2014;41:1–10.
40. Coleman JS, McConnaughay KDM, Bazzaz FA. Elevated CO_2 and plant nitrogen-use: is reduced tissue nitrogen concentration size-dependent? Oecologia. 1993;93:195–200.
41. Cotrufo MF, Ineson P, Scott A. Elevated CO_2 reduces the nitrogen concentration of plant tissue. Glob Change Biol. 1998;4:43–54.
42. Suter D, Frehner M, Fischer BU, Nösberger J, Lüscher A. Elevated CO_2 increases carbon allocation to the roots of *Lolium perenne* under free-air CO_2 enhancement but not in a controlled environment. New Phytol. 2002;154:65–75.
43. Beard JB, Green RL. The rule of turfgrasses in environmental protection and their benefits to humans. J Environ Qual. 1994;23:452–60.

44. Burgess P, Huang B. Growth and physiological responses of creeping bentgrass (*Agrostis stolonifera*) to elevated carbon dioxide concentrations. Hort Res. 2014;1:14021.
45. Luo Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience. 2004;54:731–9.
46. Li X, Zhang G, Sun B, Zhang S, Zhang Y, Liao Y, Zhou Y, Xia X, Shi K, Yu J. Stimulated leaf dark respiration in tomato in an elevated carbon dioxide atmosphere. Sci Rep. 2013;3:3433.
47. Tan K, Zhou GS, Ren SX. Responses of leaf dark respiration of winter wheat to changes in CO_2 concentration and temperature. Chin Sci Bull. 2013;58:1795–800.
48. Ziska LH, Hogan KP, Smith AP, Drake BG. Growth and photosynthetic response of nine tropical species with long-term exposure to elevated carbon dioxide. Oecologia. 1991;86:383–9.
49. Jump A, Hunt JM, Peñuelas J. Rapid climate change-related growth decline at the southern range edge of Fagus sylvatica. Glob Change Biol. 2006;12: 2163–74.
50. Crous KY, Zaragoz-Castells J, Löw M, Ellsworth D, Tissue DT, Tjoelker M, CVM B, Gimeno T, Atkin OK. Seasonal acclimation of leaf respiration in Eucalyptus saligna trees: impacts of elevated atmospheric CO_2 and summer drought. Glob Change Biol. 2011;17:1560–76.
51. Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. Calif. Agr Exp Stat. 1950;347:1–32.
52. Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL. Fitting photosynthetic carbon dioxide response curves for C_3 leaves. Plant Cell Envion. 2007;30:1035–40.
53. Reuveni J, Bugbee B. Very high CO_2 reduces photosynthesis dark respiration and yield in wheat. Ann Bot. 1997;80:539–46.
54. Graaff MA, Van Groenigen KJ, Six J, Hungate B, Van Kessel C. Elevated CO_2 increases nitrogen rhizodeposition and microbial immobilization of root-derived nitrogen. New Phytol. 2006, 173:778–86.
55. Zhang L, Wu D, Shi H, Zhang CJ, Zhan XY, Zhou SX. Effects of elevated CO_2 and N addition on growth and N_2 fixation of a legume subshrub (Caragana microphylla lam.) in temperate grassland in China. PLoS One. 2011;6:e26842.
56. Wu LG, Kimble HJ, Hail JL Wu HF. Generation of squeezed states by parametric down conversion. Phys Rev Lett. 1986;57:2520–3.
57. Pooter H. Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. Vegetatio. 1993;104:77–9.
58. Drennan PM, Nobel PS. Responses of CAM species to increasing atmospheric CO_2 concentrations. Plant Cell Environ. 2000;23:767–81.
59. Kerstiens G. Meta-analysis of the interaction between shade-tolerancw, light environment and growth response of woody species to elevated CO_2. Acta Oecol. 2001;22:61–9.
60. Rawson HM. Yield responses of two wheat genotypes to carbon dioxide and temperature in field studies using temperature gradient tunnels. Aust J Plant Physiol. 1995;22:23–32.
61. Grotenhuis TP, Bugbee B. Super optimal CO_2 reduces seed yield but not vegetative growth in wheat. Crop Sci. 1997;37:1215–22.
62. Pleijel H, Gelang J, Sild E, Danielsson H, Younis S, Karlsson PE, Wllin G, Skärby L, Selldén G. Effects of elevated carbon dioxide, ozone and water availability on spring wheat growth and yield. Physiol Plant. 2000;108:61–70.
63. Hogy P, Brunnbauer M, Koehler P, Schwadorf K, Breuer J, Franzaring J, Zhunusbayeva D, Fangmeier A. Grain quality characteristics of springwheat (*Triticum aestivum*) as affected by free-air CO_2 enrichment. Environ Exp Bot. 2013;88:11–8.
64. Long SP, Ainsworth EA, Leakey ADB, Nösberger J, Ort DR. Food for thought: lower-than-expected crop yield stimulation with rising CO_2 concentrations. Science. 2006;312:1918–21.
65. Wang L, Feng ZZ, Schjoerring JK. Effects of elevated atmospheric CO_2 on physiology and yield of wheat (*Triticum aestivum* L.): a meta-analytic test of current hypotheses. Agric Ecosyst Environ. 2013;178:57–63.
66. Curtis PS, Wang X. A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. Oecologia. 1998;113:299–313.
67. Kelly G, Sade N, Attia Z, Secchi F, Zwieniecki M, Holbrook NM, Asher L, Alchanatis V, Moshelion M, Granot D. Relationship between hexokinase and the aquaporin PIP1 in the regulation of photosynthesis and plant growth. PLoS One. 9(2):e87888. doi:10.1371/journal.pone.0087888.
68. Salvucci ME, Crafts-Brandner SJ. Inhibition of photosynthesis by heatstress: the activation state of rubisco as a limiting factor in photosynthesis. Physiol Plant. 2004;120:179–86.
69. Sicher RC, Bunce JA. Relationship of photosynthetic acclimation to changes of rubisco activity in field-grown winter wheat and barley during growth in elevated carbon dioxide. Photosynth Res. 1997;52:27–38.
70. Sharwood RE, Crous KY, WhitneySM EDS, Ghannoum O. Linking photosynthesis and leaf N allocation under future elevated CO_2 and climate warming in Eucalyptus globulus. J Exp Bot. 2017;68:1157–67.