The optimal CO₂ concentrations for the growth of three perennial grass species

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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-CO₂ experiments, may overestimate the CO₂ fertilization effect, and as a result underestimate the potentially effects of future climate change on global grasslands when the atmospheric CO₂ concentration goes beyond the optimal level. Here, we examined the optimal atmospheric CO₂ concentration effect on CO₂ fertilization and further on the growth of three perennial grasses in growth chambers with the CO₂ concentration at 400, 600, 800, 1000, and 1200 ppm, respectively.

Results: All three perennial grasses featured an apparent optimal CO₂ concentration for growth. Initial increases in atmospheric CO₂ concentration substantially enhanced the plant biomass of the three perennial grasses through the CO₂ fertilization effect, but this CO₂ fertilization effect was dramatically compromised with further rising atmospheric CO₂ concentration beyond the optimum. The optimal CO₂ concentration for the growth of tall fescue was lower than those of perennial ryegrass and Kentucky bluegrass, and thus the CO₂ fertilization effect on tall fescue disappeared earlier than the other two species. By contrast, the weaker CO₂ fertilization effect on the growth of perennial ryegrass and Kentucky bluegrass was sustained for a longer period due to their higher optimal CO₂ concentrations than tall fescue. The limiting effects of excessively high CO₂ 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 CO₂ concentrations for the growth of three grasses. These results suggest that the growth of different types of grasses may respond differently to future elevated CO₂ concentrations through the CO₂ 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 CO₂ concentration and climate change scenarios.

Background

It is widely evident that global atmospheric carbon dioxide (CO₂) 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 CO₂ levels have increased from the pre-industrial level of 280 ppm to the present level of nearly 410 ppm and the growth rate of CO₂ concentration is projected to be accelerated with an unprecedented pace of ~1.0 ppm/yr. [2–4]. Moreover, the global atmospheric CO₂ 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 CO₂ concentration may not only cause climate warming, but also cause profound impacts on the net primary productivity of agricultural and natural ecosystems [5–9].
It is well known that CO₂ 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 CO₂ concentrations may affect plant growth by altering metabolic rates [10–13]. Many studies have reported that most plants may benefit from enriched atmospheric CO₂ concentrations through the “CO₂ fertilization effect”. Plant growth can be boosted by absorbing more CO₂ molecules for photosynthesis under elevated CO₂ concentrations [10, 14–17]. For example, Wand [18] reviewed the responses of wild grasses to elevated atmospheric CO₂ concentrations and found that elevated CO₂ increased the total biomass of C₃ grass species by about 50%. However, other studies have shown that the CO₂ fertilization effect on plant growth might decline or vanish beyond certain CO₂ concentrations [7, 19, 20], and even CO₂ enrichment induced adverse effects on some plants when the ambient CO₂ level was above 1000 ppm [21]. In addition, many previous studies also found that the CO₂ 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 CO₂ 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 CO₂ concentration to 530 ppm based on a four-year FACE experiment. These results indicate that different plant species may have different optimal CO₂ concentrations, and that plants with higher optimal CO₂ concentrations are likely to benefit the most from the CO₂ 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 CO₂ 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 CO₂ could dramatically affect net photosynthetic rates through various processes including up-regulation or down-regulation when the growth CO₂ below or above the optimal CO₂ for plants. Elevated CO₂ 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 CO₂ 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 CO₂ conditions [28, 35–37]. Previous studies showed that down-regulation of photosynthesis occurred in plants grown in elevated CO₂ and limited N indicated decreased leaf N concentration [38, 39] High N availability could alleviate the down-regulation of photosynthesis in plants under elevated CO₂ 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 CO₂ have been focused on crops [43–45] or trees [26, 29, 34, 46–48] and few studies have examined the effects of elevated CO₂ on perennial grasses [17, 19, 40]. In addition, most previous studies regarding the CO₂ fertilization effect have focused primarily on “doubling-CO₂ experiments” with twofold higher CO₂ concentration of about 700 or 800 ppm than the current global CO₂ concentration [40, 42, 45, 48]. Nevertheless, the CO₂ 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 CO₂ concentration of the CO₂ fertilization effect on the growth of winter wheat and found that the optimal atmospheric CO₂ concentration was 894 and 968 ppm for total biomass and leaf photosynthesis. So far, few experimental studies have been conducted to examine the optimal CO₂ concentration for maximizing the CO₂ 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 CO₂ fertilization according to the conclusions from earlier “doubling-CO₂ experiments” [29, 34]. However, it should be noted that in the future, continuously rising atmospheric CO₂ concentrations may substantially lower the CO₂ fertilization effect when the atmospheric CO₂ concentration rises beyond the optimal CO₂ level [23]. As a result, many current climate change models based on earlier results of the doubling-CO₂ experiments may overestimate the CO₂ fertilization effect and underestimate the potential risks that climate change poses on global grasslands when the atmospheric CO₂ concentration goes beyond the optimal CO₂ level. Therefore, identifying optimal CO₂ 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 CO₂ concentrations on the growth of three perennial grass species, (2) examine the optimal CO₂ concentration for maximizing the CO₂ fertilization effect of these grasses, and (3) explore potential mechanisms that determine the optimal CO₂ 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 CO₂ treatment. During the eight weeks of the CO₂ treatment, these grasses were maintained under the same environmental factors as before the start of CO₂ 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 CO₂ 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 CO₂ concentration of each growth chamber every three days, and then relocated the CO₂ treated grasses to the growth chambers with corresponding CO₂ concentrations. The experiment was arranged in a randomized complete block design with four replicates (pots) per treatment. The ambient and elevated CO₂ concentrations within the chambers were maintained through an automatic CO₂ control system connected to a CO₂ source-tank containing 100% research-grade CO₂ (Airgas, Inc.). The CO₂ 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 CO₂ 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 CO₂ 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 CO₂ treatment were put together for calculating shoot biomass during the CO₂ 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 CO₂ treatment period (56 days). Five fully expanded leaves were randomly selected and arranged in a 2 × 3 cm² 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 CO₂ level, the target temperature and Vapor Pressure Deficit (VPD). CO₂ concentrations in the cuvette were controlled using an injector system (LI-6400, LI-COR Inc.), which utilizes a CO₂ mixer and compressed CO₂ cartridges sealed with plasticene to prevent leakage. Then, the photosynthesis vs intercellular CO₂ (A_p-C_i) curves were measured at cuvette chamber CO₂ of 50, 100, 150, 200, 300, 400, 600, 800, 1000, 1200, and 1400 ppm. Data from A_p-C_i curves were used to compare treatment effects on the light-saturated net photosynthetic rates at ambient or elevated CO₂ (A_p), the maximum
carboxylation rate of Rubisco ($V_{\text{cmax}}$), and the maximum capacity of electron transport mediated ribulose bisphosphate (RuBP) regeneration ($J_{\text{max}}$). An estimation method was used to obtain $V_{\text{cmax}}$ and $J_{\text{max}}$ 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 $\text{WUE} = A_n / T_r$.

**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 $\sim 80 ^\circ\text{C}$ 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 ($V_{\text{cmax}}$), maximum electron transport rate ($J_{\text{max}}$) 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).

**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
CO₂ increased from 400 ppm to 1000 ppm. The CO₂ stimulation effect on \( A_n \) reached a maximum at 959 ppm, at which point further increase in CO₂ resulted in a decline of \( A_n \) (Fig. 2). However, the response of \( A_n \) to elevated CO₂ also varied with grass species. The leaf net photosynthetic rates of the other two species (tall fescue and perennial ryegrass) consistently increased with increasing CO₂, which can also be described by quadratic relationships with optimal CO₂ beyond the maximum CO₂ 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 CO₂ and the relationships of CO₂-\( g_s \) and CO₂-\( T_r \) also typically followed quadratic equations with maximum \( g_s \) and \( T_r \) occurring around 400 ppm, which was much lower than the optimal CO₂ 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 CO₂ concentration, with the maximum CO₂ fertilization effect occurring at approximately 1062 ppm and 910 ppm, respectively. However, the maximum WUE of perennial ryegrass was beyond the highest CO₂ concentration treatment of 1200 ppm. Thus, we quantified the relationship between CO₂ and WUE of perennial ryegrass through quadratic models and found that the optimal CO₂ 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 (\( V_{\text{cmax}} \)) of the three grasses demonstrated bell-shaped curves in relation to CO₂ concentration, peaking at 906 ppm, 863 ppm, and 743 ppm for tall fescue, perennial ryegrass, and Kentucky bluegrass, respectively (Fig. 3a). Similar to the \( V_{\text{cmax}} \), the maximum electron transport rate (\( I_{\text{max}} \)) in response to increasing CO₂ concentrations also shared bell-shaped curves for all three grasses. The optimal CO₂ concentration of \( I_{\text{max}} \) was 877 ppm, 941 ppm, and 665 ppm for tall fescue, perennial ryegrass, and Kentucky bluegrass, respectively (Fig. 3b).

**Elevated CO₂ 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 CO₂ (Fig. 4). The relationships between \( R_d \) and CO₂ 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 CO₂ (Fig. 4b). Meanwhile, we estimated the relationships between \( R_d \) and TNC (Fig. 5) and found that \( R_d \) was increased linearly by the
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 $V_{\text{cmax}}$ or leaf N and $J_{\text{max}}$

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 $V_{\text{cmax}}$ as well as leaf N and $J_{\text{max}}$ of the three grass species (Fig. 7). Our results showed that the $V_{\text{cmax}}$ 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 $J_{\text{max}}$ 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”,
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. 5** Effects of elevated CO$_2$ concentrations on the linear relationship between TNC and $R_o$ 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.
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 CO₂ environmental conditions. It is noted that we found no obviously optimal CO₂ for the belowground biomass of two species (Kentucky bluegrass and perennial ryegrass), as evidenced by the upward quadratic relationships between belowground biomass and CO₂ 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 CO₂ concentrations.

The positive CO₂ 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 CO₂ fertilization effect is developed and regulated [22]. In the current study, the photosynthesis-CO₂ relationship followed a similar bell-shaped curve like the biomass-CO₂ relationship (Figs. 1 and 2), suggesting that the positive CO₂ fertilization effect might be attributed to the up-regulation of Aₙ, as evidenced by the increased leaf net photosynthetic rates (Aₙ), with the maximum CO₂ 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 CO₂ 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 CO₂ concentrations before reaching their optimums (Fig. 3), suggesting that the initial increase in CO₂ 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 CO₂ concentrations from 400 ppm to about 800 ppm [23].

In addition to leaf photosynthesis, the positive CO₂ 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 CO₂ concentrations. Our results showed that the leaf dark respiratory rates (R_d) and leaf TNC of the three grasses consistently decreased with elevated CO₂ 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 Aₙ and the decline of R_d may both play pivotal roles in explaining the positive CO₂ fertilization effects on the growth of perennial grasses in the current study.

The diminishing returns of CO₂ fertilization effect on perennial grasses

Previous studies have found that beyond certain thresholds, high CO₂ concentration cause diminishing returns of CO₂ fertilization effect on plants [13, 22, 23]. Several studies found that the stimulation of Aₙ induced by elevated CO₂ decreased or even diminished if exposed for a longer time period, because plants acclimate to elevated CO₂ concentrations through a process known as down-regulation [19, 32]. We also found bell-shaped curves for biomass-CO₂ relationships for the three grasses similar to the Aₙ-CO₂, indicating a reduction in biomass due to a decline in the photosynthetic rate at high CO₂ concentrations. It is well demonstrated that the down-regulation of Aₙ is possibly attributed to the changes in carbohydrates [31], under high CO₂ environments. In the current study, elevated CO₂ 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 Aₙ. 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 CO₂ 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 CO₂ concentrations [70]. Consequently, the changes in hexokinase with CO₂ concentrations may contribute to the bell-shaped relationship between Aₙ and CO₂ concentration, especially for the down-regulation of Aₙ under high CO₂ concentrations.

It is well documented that stomatal conductance (gₛ) declines when exposed to elevated atmospheric CO₂ concentration, and a doubling of CO₂ from the present ambient concentration generally results in a reduction in gₛ of 10–70% depending on species or functional groups [58]. In the current study, we also found that the gₛ of all three grasses were dramatically decreased with elevated CO₂ concentrations, which may be partly due to the down-regulation of Aₙ caused by CO₂. Moreover, the reduced gₛ under high CO₂ 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 CO₂ concentrations 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 CO₂ concentrations, which may also be caused by the CO₂ effects on An, since nitrogen content is associated with photosynthetic enzymes such as Rubisco [35–37]. In addition, the linearly positive relationships between leaf N and Vc₉₉₉₉ for the three grasses (Fig. 7) were directly supporting the above conclusion that the down-regulation of An was partly attributed to the decline of leaf N under high CO₂ concentrations.

It should be noted that the CO₂ 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 CO₂ 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 CO₂ concentrations because warming and drought may have interactive effects with CO₂ 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 CO₂ 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 C₃ grasses in future climate change scenarios.

Conclusions
We found that the optimal CO₂ concentrations occurred at 945, 915, and 1151 ppm for the aboveground biomass of tall fescue, perennial ryegrass, and Kentucky bluegrass, respectively. Higher CO₂ concentrations had diminishing returns of CO₂ 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 CO₂ concentration in the future may dramatically lower the CO₂ fertilization effect, and thus many current climate change models based on earlier results of “doubling–CO₂” experiments may overestimate the CO₂ fertilization effect on grasslands beyond the optimum CO₂ concentration. According to recent IPCC reports, if global CO₂ emissions are not effectively mitigated, the atmospheric CO₂ concentration might be over 900 ppm in the second half of this Century. Nevertheless, the optimal CO₂ concentrations found in this study can be used as an indicator in predicting the fates of the cool-season C₃ grasses under future rising atmospheric CO₂ concentration and climate change, because grasses with high optimal CO₂ concentrations may take full advantage of the CO₂ fertilization effect.
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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/CO2 history/.
4. NASA: Global climate change: Vital signs of the planet 2014. Available at: http://climate.nasa.gov.
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;30:1589–94.
6. Steffen WL, Canadell JG. Carbon dioxide fertilization and climate change policy. Department of Environment and Heritage. AGO: Australian Greenhouse Office; 2005.
7. Wand SE, Midgley GF, Jones MH, Curtis PS. Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO2 concentration: a meta-analytic test of current theories and perceptions. Glob Change Biol. 1999;5:723–41.
8. Amone JA, Zaller JG, Spehn BM, Niklaus PA, Wells CE, Körner C. Dynamics of root systems in native grassland: effects of elevated atmospheric CO2. 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;978:4398–504.
10. Ainsworth EA. Rice production in a changing climate: a meta-analysis of results from experiments using various approaches to control CO2 concentration. Field Crops Res. 2008;104:1642–50.
11. Lloyd J, Farquhar GD. The CO2 dependence of photosynthesis, plant growth response to elevated atmospheric CO2 concentrations and their interaction with soil nutrient status. I. General principles and forest ecosystems. Funct Ecol. 1996;10:34–2.
12. LeCain DR, Morgan JA. Growth, gas exchange, leaf nitrogen and carbohydrate concentrations in NAD-ME and NADP-ME C4 grasses grown in elevated CO2. Physiol Plant. 1998;102:2927–306.
13. Reddy AR, Rasineni GK, Raghavendra AS. The impact of global elevated CO2 concentration on photosynthesis and plant productivity.Curr Sci. 2010;99:467–5.
14. Amthor JS. Effects of atmospheric CO2 concentration on wheat yield: review of results from experiments using various approaches to control CO2 concentration. Field Crops Res. 2001;73:31–34.
15. Jablonksi LM, Wang X, Curtis PS. Plant reproduction under elevated CO2conditions: a meta-analysis of results on 79 crop and wild species. New Phytol. 2002;150:9–26.
16. Drewry DT, Kumar P, Long S, Bernacchi C, Liang XZ, Sivapalan M. Ecophysiological responses of dense canopies to environmental variability: 2. Role of acclimation under elevated CO2. J Geophys Res. 2010;115:2005–12.
17. Yu J, Du H, Xu M. Metabolic responses to heat stress under elevated atmospheric CO2 concentration in a cool-season grass species. J Am Soc Hortic Sci. 2012;137:221–8.
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 Pairedwise 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 CO2 and increased nitrogen supply. New Phytol. 2001;150:405–18.
20. Poorter H, Navas M. Plant growth and competition at elevated CO2 on winners, losers and functional groups. New Phytol. 2003;157:717–98.
21. Yu J, Du H, Xu M. Metabolic responses to heat stress under elevated atmospheric CO2 concentration in a cool-season grass species. J Am Soc Hortic Sci. 2012;137:221–8.
22. Kömer C, Ashhoff R, Bignucolo O, Hättenschwiler S, Keel SG, Peléz-Riedl S, Peip S, Siegwolf RTW, Zott G. Carbon flux and growth in mature deciduous forest trees exposed to elevated CO2. Science. 2005;309:1360–2.
23. Xu M. The optimal atmospheric CO2 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 CO2 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 CO2. Plant Cell Environ. 1991;14:869–75.
28. Lewis JD, Lucas M, Olszyk DM, Tingley DT. Relationships between needle nitrogen concentration and photosynthetic responses of Douglas-fir seedlings to elevated CO2 and temperature. New Phytol. 2004;162:355–64.
29. Borjigidai A, Hiroak S, Hirose T. Carbon balance in a monospecific stand of an annual herb Chenopodium album at an elevated CO2 concentration. Plant Ecol. 2009;203:33–44.
30. Jin VL, Evans RD. Elevated CO2 increases plant uptake of organic and inorganic N in the desert shrub Larrea tridentata. 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, Uribelarea 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 CO2 concentration in the absence of drought. Plant Physiol. 2006;140:779–90.
33. Gunderson CA, Wullschleger SD. Photosynthetic acclimation in trees to rising CO2: a broader perspective. Photosynth Res. 1994;59:369–88.
34. Rey A, Jarvis PG. Long-term photosynthetic acclimation to increased atmospheric CO2 concentration in young birch (Betula pendula) trees. Tree Physiol. 1998;18:641–50.
35. Cui JD, Accob 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 olivecane-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 CO2 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 CO2? A critical examination of the hypotheses. J Integr Plant Biol. 2008;50:1365–74.
39. Amdal 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 CO2 in a mixed heathland-grass ecosystem. Funct Ecol. 2014;1:11–10.
40. Coleman JS, McConnaughay KDM, Bazzaz FA. Elevated CO2 and plant nitrogen-use: is reduced tissue nitrogen concentration size-dependent? Oecologia. 1993;93:195–200.
41. Cotrufo MF, Ineson P, Scott A. Elevated CO2 reduces the nitrogen concentration of plant tissue. Glob Change Biol. 1998;4:43–54.
42. Suter D, Frehner M, Fischer BJ, Nösberger J, Lüscher A. Elevated CO2 increases carbon allocation to the roots of Lolium perenne under free-air CO2 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;23:3452–60.
44. Burgess P, Huang B. Growth and physiological responses of creeping bentgrass (Agrostis stolonifera) to elevated carbon dioxide concentrations. Hort Res. 2014;11:4021.

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 CO2 concentration and temperature. Chin Sci Bull. 2013;59:1795–800.

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. Crours KY, Zaragoza-Castells J, Löw M, Ellsworth D, Tissue DT, Tjeleller M, OIM B, Gimeno T, Atkin OK. Seasonal acclimation of leaf respiration in Eucalyptus saligna trees: impacts of elevated atmospheric CO2 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 C3 leaves. Plant Cell Environ. 2007;30:1035–40.

53. Reuveni J, Bugbee B. Very high CO2 reduces photosynthesis dark respiration and yield in wheat. Ann Bot. 1997;80:359–46.

54. Gnaaff MA, Van Groenigen KJ, Six J, Hungate B, Van Kessel C. Elevated CO2 increases nitrogen rhizodeposition and microbial immobilization of root-derived nitrogen. New Phytol. 2006;173:778–86.

55. Zhang L, Wu Q, Shi H, Zhang CI, Zhan XY, Zhou SX. Effects of elevated CO2 and N addition on growth and N2 fixation of a legume subshrub (Caragana microphylla lam.) in temperate grassland in China. PLoS One. 2011;6:e26842.

56. Wu LG, Kimble HJ, Hall JL, Wu HF. Generation of squeezed states by parametric down conversion. Phys Rev Lett. 1986;57:2520–3.

57. Poorter H. Interspecific variation in the growth response of plants to an elevated ambient CO2 concentration. Vegetatio. 1993;104:77–9.

58. Drennan PM, Nobel PS. Responses of CAM species to increasing atmospheric CO2 concentrations. Plant Cell Environ. 2000;23:767–81.

59. Kertstiens G. Meta-analysis of the interaction between shade-tolerance, light availability on spring wheat growth and yield. Physiol Plant. 2000;108:61–6.

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. 1990;17:23–33.

61. Grotenhuis TP, Bugbee B. Super optimal CO2 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, Willin 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. Zhang L, Wu Q, Shi H, Zhang CI, Zhan XY, Zhou SX. Effects of elevated CO2 and N addition on growth and N2 fixation of a legume subshrub (Caragana microphylla lam.) in temperate grassland in China. PLoS One. 2011;6:e26842.

64. Wu LG, Kimble HJ, Hall JL, Wu HF. Generation of squeezed states by parametric down conversion. Phys Rev Lett. 1986;57:2520–3.

65. Poorter H. Interspecific variation in the growth response of plants to an elevated ambient CO2 concentration. Vegetatio. 1993;104:77–9.

66. Drennan PM, Nobel PS. Responses of CAM species to increasing atmospheric CO2 concentrations. Plant Cell Environ. 2000;23:767–81.

67. Kertstiens G. Meta-analysis of the interaction between shade-tolerance, light availability on spring wheat growth and yield. Physiol Plant. 2000;108:61–6.

68. Rawson HM. Yield responses of two wheat genotypes to carbon dioxide and temperature in field studies using temperature gradient tunnels. Aust J Plant Physiol. 1990;17:23–33.

69. Scher 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. Shawrook RE, Crous KY, Whitney SM ED, Ghannoum O. Linking photosynthesis and leaf N allocation under future elevated CO2 and climate warming in Eucalyptus globulus. J Exp Bot. 2017;68:1157–67.