



Interactive Effects of Elevated CO₂ Concentration and Irrigation on Photosynthetic Parameters and Yield of Maize in Northeast China

Fanchao Meng^{1,2}, Jiahua Zhang^{1,3*}, Fengmei Yao⁴, Cui Hao¹

1 Institute of Eco-Environment and Agro-Meteorology, Chinese Academy of Meteorological Sciences, Beijing, China, **2** College of Atmospheric Science, Nanjing University of Information Science & Technology, Nanjing, China, **3** Key Laboratory of Digital Earth Science, Institute of Remote Sensing and Digital Earth, Chinese Academy of Sciences, Beijing, China, **4** Key Laboratory of Computational Geodynamics, Chinese Academy of Sciences, Beijing, China

Abstract

Maize is one of the major cultivated crops of China, having a central role in ensuring the food security of the country. There has been a significant increase in studies of maize under interactive effects of elevated CO₂ concentration ([CO₂]) and other factors, yet the interactive effects of elevated [CO₂] and increasing precipitation on maize has remained unclear. In this study, a manipulative experiment in Jinzhou, Liaoning province, Northeast China was performed so as to obtain reliable results concerning the later effects. The Open Top Chambers (OTCs) experiment was designed to control contrasting [CO₂] i.e., 390, 450 and 550 μmol·mol⁻¹, and the experiment with 15% increasing precipitation levels was also set based on the average monthly precipitation of 5–9 month from 1981 to 2010 and controlled by irrigation. Thus, six treatments, i.e. C₅₅₀W_{+15%}, C₅₅₀W₀, C₄₅₀W_{+15%}, C₄₅₀W₀, C₃₉₀W_{+15%} and C₃₉₀W₀ were included in this study. The results showed that the irrigation under elevated [CO₂] levels increased the leaf net photosynthetic rate (*P_n*) and intercellular CO₂ concentration (*C_i*) of maize. Similarly, the stomatal conductance (*G_s*) and transpiration rate (*T_r*) decreased with elevated [CO₂], but irrigation have a positive effect on increased of them at each [CO₂] level, resulting in the water use efficiency (*WUE*) higher in natural precipitation treatment than irrigation treatment at elevated [CO₂] levels. Irradiance-response parameters, e.g., maximum net photosynthetic rate (*P_{nmax}*) and light saturation points (*LSP*) were increased under elevated [CO₂] and irrigation, and dark respiration (*R_d*) was increased as well. The growth characteristics, e.g., plant height, leaf area and aboveground biomass were enhanced, resulting in an improved of yield and ear characteristics except axle diameter. The study concluded by reporting that, future elevated [CO₂] may favor to maize when coupled with increasing amount of precipitation in Northeast China.

Citation: Meng F, Zhang J, Yao F, Hao C (2014) Interactive Effects of Elevated CO₂ Concentration and Irrigation on Photosynthetic Parameters and Yield of Maize in Northeast China. PLoS ONE 9(5): e98318. doi:10.1371/journal.pone.0098318

Editor: Gerrit T.S. Beemster, University of Antwerp, Belgium

Received: January 10, 2014; **Accepted:** May 1, 2014; **Published:** May 21, 2014

Copyright: © 2014 Meng et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by Global Change Research Key Project of MOST 973 Program (No. 2010CB951302), the Hundred Talents Program of CAS (Y24002101A), the Social Commonweal Meteorological Research Project (No. GYHY201106027), 1-3-5 Innovation Project of CAS-RADI (Y3ZZ15101A) and CAS-TWAS project for drought monitoring and assessment. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: jhzhang@ceode.ac.cn

Introduction

The CO₂ concentration ([CO₂]) in the atmosphere is about 390 μmol·mol⁻¹ as a consequence of fossil fuel combustion and deforestation, which is predicted to reach 550 μmol·mol⁻¹ by the middle of this century [1]. Elevated [CO₂] is an important abiotic factor, and has significant fertilization effects on crops. Extensive previous studies have reported that elevated [CO₂] significantly improved water use efficiency, lower transpiration rate, shorten maize growth period, and increased plant height, leaf number, leaf area, growth rate and yield [2–12]. In addition, the increasing of atmospheric [CO₂] affects precipitation balance, which can change the seasonal precipitation distribution [13]. It has been estimated that this effect would bring about a 10% increase or decrease in water resources at different areas [14]. The global annual average precipitation increase is about 2% since the beginning of the 20th century [15–16], and this rise over the area of 30°–85°N has shown a 7%–12% increase [17]. It has been

predicted that the rainfall decrease will be noticed in middle-and-lower regions of Yangtze River (24°N–34°N, 108°E–122°E), while the rain belts are likely to move towards north of China and precipitation would increase in Northeast China in the future [18]. The crop growth of Northeast China will likely be affected by both elevated [CO₂] and increasing precipitation, which are important abiotic factors that directly or indirectly affect crop growth, physiological processes and productivity. Thus, it is necessary to understand the interactive effects of elevated [CO₂] and increasing precipitation on crop growth in Northeast China under future climate change.

In fact, lots of studies have been focused on the interactive effects of elevated [CO₂] and other environmental factors on plant growth. The study of the interactive effects of elevated [CO₂] and temperature indicated that the effects on photosynthesis and growth in C₄ species are obvious [19–22]. FACE (Free Air Carbon-dioxide Enrichment) and chamber experiment have demonstrated that the interactive effects of elevated [CO₂] and

drought stress have an increase in the leaf water-use efficiency [23–24], and more recent evidence shows that maize will benefit from the increase in [CO₂] under drought condition [25–29]. Also, the studies of the interactive effects of elevated [CO₂] and light on plant found that high light have a great effect on net photosynthesis in condition of elevated [CO₂] [30–31]. Regarding the interactive effects of elevated [CO₂] and Ozone (O₃), studies showing that elevated [CO₂] inhibits adverse effects of O₃ and increased trees seedling stem diameters at low O₃ [32–35]. Moreover, the interactive effects of elevated [CO₂] and soil nutrition have been investigated. For example, the studies of the interactive effects of elevated [CO₂] and nitrogen (N) indicated that there is a positive CO₂ × N interaction for grain yield of rice [36–38], while the research on the interactive effects of elevated [CO₂] and potassium (K) found that plants grown under elevated [CO₂] are more sensitive to K deficiency with higher leaf critical K levels [39]. Further, there are lots of studies involving the interactive effects of elevated [CO₂] and other factors (e.g., NaCl-salinity, plant diversity) have been reported [40–41]. However, the interactive effects of elevated [CO₂] and increasing precipitation on photosynthesis and yield of maize are not well understood. In particular, there has been no detailed study evaluating the interactive effects of elevated [CO₂] and increasing precipitation on photosynthesis efficiency, water use efficiency and yield of maize in Northeast China.

Northeast China (38°N–56°N, 120°E–135°E) is located in the middle-high latitudes and east of the Eurasian continent, which has a cultivated land area of 21.53 million hm², accounted for 16.6% of the country's total cultivated areas [42]. The summer is warm and short, and the annual precipitation is 400–800 mm. The precipitation from July to September is accounting for 60% of the annual precipitation. Moreover, Northeast China has fertile black soil, belonging to one of the three pieces of black soil in the world [43], hence, which is the biggest commercial grain production base and provides 30–35 million tons of commercial grain to country every year [44]. Therefore, it plays an important role to stabilize the grain market and keep sustainable development of China's national economy. In Northeast China, maize (*Zea mays* L.) is the major cultivated crop, and its yield has accounted for about 1/3 of the national total maize yield [45]. The growth of maize requires more water, which yields tend to decrease if water deficit occurred during the key growing stages (e.g., silking stage) [46]. The precipitation in Northeast China can meet the water requirements of maize in most of the years, but slight drought has been discovered to occur in some of the past years. Therefore, for the rain-fed maize in Northeast China, precipitation is a very important climatic factor. If the water deficit occurred at silking stage of maize will cause disaccord flowering season, and then affect the pollination and seed formation, resulting in maize yield reduction.

To examine the interactive effects of elevated [CO₂] and increasing precipitation on maize in Northeast China, we conducted an Open Top Chambers (OTCs) experiment under the combined effects of elevated [CO₂] and precipitation in Jinzhou, Liaoning province during maize growing season (May to September) in 2013. Firstly, we tested the response of leaf gas exchange parameters (e.g., P_n , T_r) and irradiance-response parameters (e.g., P_{nmax} , LSP) to the combined elevated [CO₂] and increasing precipitation. Secondly, we examined the change in the growth parameters of maize (e.g., leaf area, aboveground biomass), yield and ear characters (e.g., ear length, ear diameter). The results of this study would be crucial for evaluating the possible consequences of climate change on crop photosynthetic

capacity and yield in Northeast China, and may help inform regulatory policies to cope with the future climate change.

Materials and Methods

Experimental site

This study site is located at Jinzhou Ecological and Agricultural Meteorological Experiment Center (41°09'N, 121°10'E, 27.4 m a.s.l.) in Liaoning province of China, which is a warm temperature monsoon climate zone. The mean annual precipitation is 568.8 mm, and the mean annual temperature is 9.1°C. The annual frost-free period is approximately 180 d in duration, with an annual accumulated activity temperature is 3700°C·d. The site has typical brown soil, and the soil pH is approximately 6.3. The soil organic matter and total N are 6.41–9.43 g/kg and 0.69 g/kg, respectively [47].

Open Top Chamber design

Three pairs of Open Top Chambers (OTCs), each 3.5 m high with an octagonal ground surface area of 11.73 m² were constructed. An inclined plane of 45° (inward on upper side of chambers) was provided for reducing gas escape from the top. The set up was completed in May 2011 (Fig. 1).

Additionally, the OTCs were constructed with a 5.5-m-wide buffer zone between them to prevent mutual shading. Carbon dioxide was supplied to the chambers through a pipe with pinholes connected to industrial carbon dioxide cylinders (liquid carbon dioxide, purity was 99.99%, supplied by Anjin Gas Corporation) outside the chambers. There was an exchange fan of each chamber, which mixed the entered carbon dioxide and fresh air from outside, then transported by pipe and well distributed in the entire chamber by the octagonal-pipe with holes, and the gas would discharge from the opening on top and put in air circulation. Carbon dioxide was supplied for 24 hours a day and the [CO₂] was monitored by taking constant measurements with an infrared gas analyzer.

Experimental design

The effects of elevated [CO₂] and precipitation on photosynthesis, growth, yield and ear characteristics of maize were examined in the chamber experiments. Considering the present ambient [CO₂] and projected increasing [CO₂] levels in next several decades from IPCC (2007) [1], three [CO₂] levels were conducted with the OTC experiments including 390 μmol·mol⁻¹ (C₃₉₀), 450 μmol·mol⁻¹ (C₄₅₀) and 550 μmol·mol⁻¹ (C₅₅₀).



Figure 1. View of Open Top Chambers on 21 July 2013 at Liaoning province, China.

doi:10.1371/journal.pone.0098318.g001

According to 5–9 month average monthly precipitation in Jinzhou during 1981–2010 (see Fig. 2) and the prediction of increasing rainfall in Northeast China [18,48–49], two levels of watering including natural precipitation (W_0) and precipitation increased by 15% (W_{+15}) were designed. With this, maize, grown from seeds, was subjected to 6 different combined treatments (Table 1).

Three pairs of OTCs were used, and within each pair one was randomly assigned to receive the control ($390 \mu\text{mol}\cdot\text{mol}^{-1}$) and the others as the elevated [CO₂] (450 and $550 \mu\text{mol}\cdot\text{mol}^{-1}$). Every chamber was designed with two watering gradient (0 and +15%), and each gradients with 7 pots (50.5 cm (diameter)×32.5 cm (height)), producing 14 plots per treatment with a total of 84 plots. Based on the local long-term (1981–2010) monthly mean precipitation, watering volumes of each pot were 20.43 L, 35.43 L, 32.21 L and 11.96 L in the $W_{+15\%}$ treatment plots from June to September, respectively. It was divided into everyday average irrigation, directed into the pots in the morning and evening daily, and covering the raining days too.

The maize cultivar used in this study was Danyu 39, and now it is widely planted in Northeast China. Three maize seeds were planted in the pots using field soil on 10 May 2013, with final seeding at four-leaf stage. On 1 June, the 84 pots containing plants were moved into the chambers randomly, and each chamber contained 14 pots. Meanwhile, the levels of [CO₂] and water supplied were under monitored and controlled until harvest time (15 September).

Rainfall date and relative soil moisture measurements

The rainfall data were obtained from the Jinzhou weather station. The relative soil moisture (RSM) was calculated by equation (1) given below [50]:

$$RSM(\%) = \frac{\text{soil moisture content}}{\text{soil field capacity}} \times 100\% \quad (1)$$

The soil moisture content was measured at a 0–20 cm soil depth. The soil samples of each treatment was collected and recorded as fresh weights and the samples were dried in an oven at 105°C for at least 48 hours. The soil moisture content was then measured using equation (2) given below:

$$\text{Soil moisture content}(\%) = \frac{\text{fresh weight of soil} - \text{dry weight of soil}}{\text{dry weight of soil}} \times 100\% \quad (2)$$

And the soil field capacity in this study was used the value of 21%, which according to the average value of soil field capacity in the last few years at this study site [51].

Gas exchange measurements of maize

Measurements were made between 8:30 a.m. and 11:30 a.m. (local time) from 24–26 July, 2013 (at the maize silking stage). Three representative plants were chosen for per treatment and the middle of the ear-leaves was measured, then the averages were taken. Gas exchange measurements were conducted using portable gas exchange systems (LI-6400, LI-Cor, Lincoln, NE, USA). The [CO₂] in the leaf chamber was controlled by the LI-Cor CO₂ injection system, and the built-in LED lamp (red/blue) supplied the irradiance. Temperature in leaf chamber was set at 30°C, and the actual temperature of the leaf chamber ranged from 29 to 33°C. The vapour pressure deficit on the leaf surface (VpdL) was between 2.9 and 3.4 kPa, and the flow control was at $500 \mu\text{mol}\cdot\text{s}^{-1}$. The lamp settings across the series of 2000, 1600, 1200, 1000, 800, 600, 400, 200, 150, 100, 80, 50, 20 and $0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the measurements were recorded after equilibrium was reached. Each individual curve took approximately 30 min to complete.

P_n curve fitting and analyzed of parameters were performed using the modified rectangular hyperbolic model by Ye and Yu [52–53], and its expression and correlative equations is as given below:

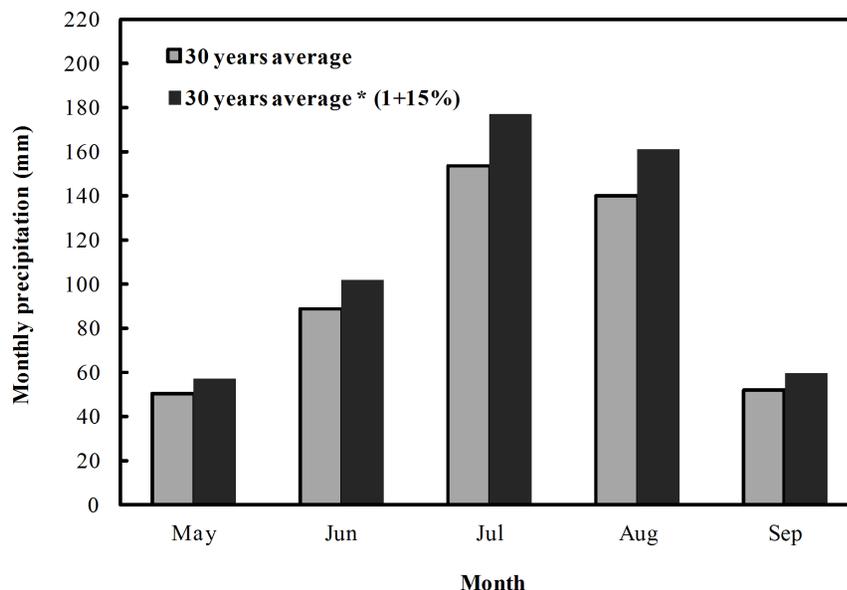


Figure 2. Monthly average precipitation during 1981–2010 years in the growing season of Maize in study area. Gray bars indicate the regional monthly averages precipitation from 1981 to 2010, and black bars indicate increased 15% precipitation based on gray bars. doi:10.1371/journal.pone.0098318.g002

Table 1. Treatments performed in OTCs.

Treatments	Description
C ₅₅₀ W _{+15%}	Elevated [CO ₂] concentration (550 μmol·mol ⁻¹) and Increased 15% of precipitation
C ₅₅₀ W ₀ (CK)	Elevated [CO ₂] concentration (550 μmol·mol ⁻¹) and Natural precipitation
C ₄₅₀ W _{+15%}	Elevated [CO ₂] concentration (450 μmol·mol ⁻¹) and Increased 15% of precipitation
C ₄₅₀ W ₀ (CK)	Elevated [CO ₂] concentration (450 μmol·mol ⁻¹) and Natural precipitation
C ₃₉₀ W _{+15%}	Ambient [CO ₂] concentration (390 μmol·mol ⁻¹) and Increased 15% of precipitation
C ₃₉₀ W ₀ (CK)	Ambient [CO ₂] concentration (390 μmol·mol ⁻¹) and Natural precipitation

doi:10.1371/journal.pone.0098318.t001

$$P_n(PAR) = \alpha \frac{1 - \beta PAR}{1 + \gamma PAR} PAR - R_d \quad (3)$$

where PAR is irradiance, α is the initial slope of irradiance-response curve of photosynthesis when irradiance approaches to zero, β and γ are coefficients which are independent of PAR , R_d is dark respiration (μmol·m⁻²·s⁻¹). From these parameters, we can calculate P_{nmax} (maximum net photosynthetic rate, μmol·m⁻²·s⁻¹), LSP (light saturation point, μmol·m⁻²·s⁻¹), LCP (light compensation point, μmol·m⁻²·s⁻¹) and ϕ_c (quantum efficiency of the light compensation point, mol·mol⁻¹) using equations (4)–(7) as described by Han et al. [54].

$$P_{nmax} = \alpha \left(\frac{\sqrt{\beta + \gamma} - \sqrt{\beta}}{\gamma} \right)^2 - R_d \quad (4)$$

$$\phi_c = \alpha \frac{1 - 2\beta LCP - \beta \gamma LCP^2}{(1 + \gamma LCP)^2} \quad (5)$$

$$LCP = \frac{\alpha - \gamma R_d - \sqrt{(\gamma R_d - \alpha)^2 - 4\alpha\beta R_d}}{2\alpha\beta} \quad (6)$$

$$LSP = \frac{\sqrt{(\beta + \gamma)/\beta} - 1}{\gamma} \quad (7)$$

T_r (transpiration rate, mol·m⁻²·s⁻¹), G_s (stomatal conductance, μmol·m⁻²·s⁻¹) and C_i (intercellular CO₂ concentration, μmol·mol⁻¹) were also measured at the same irradiance, temperature and vapour pressure when the measurements of P_n were conducted. Additionally, WUE (water use efficiency, μmol·mol⁻¹) was calculated as P_n/T_r .

Growth and harvesting of maize

Maize growth stages were recorded throughout the growing season. The plant height, ear height, stem diameter, leaf area and aboveground biomass were measured at the silking stage of the maize. Leaf area of each plant was determined with long-width coefficient method (length × width × 0.75). Aboveground biomass

was obtained by dry weight. Before weighing, three plants from each treatment were separated into stem, leaf and grain. This was as a result of the need to shivel it in oven at 105°C for 45 min and drying to constant weight at 85°C for at least 48 h.

At maturity stage, ten plants of each treatment were harvested for the yield components. The grains from each ear of maize were threshed by hand after air dried and weighed. The measured ear characteristics include: ear length, ear diameter, ear weight, 100-kernel weight, rows per ear, kernel number, shriveled kernels, bare-tip length and axle diameter. The yield of each plant was calculated by 14% moisture content of grain.

During sampling, three representative plants of each treatment were randomly selected for measurement.

Statistical analysis

In this study, statistical significance of growth and yield components was tested at 0.05 probability level ($P < 0.05$) following the DUNCAN test, performed using DPS 7.05 (Data-processing System, Zhejiang University, China). Irradiance-response curve fitting and parameter analysis based on modified rectangular hyperbolic model. The two-way analysis of variances (ANOVAS) were used to examine the interactive effects of elevated [CO₂] and irrigation on growth parameters and yield of maize, and statistical significance were set at $P < 0.05$ and $P < 0.01$. These statistical analyses were conducted with SPSS 17.0 software (SPSS Institute Incorporated, Chicago, Illinois, USA). The standard deviation (S.D.) was calculated to compare the treatment means.

Results

Rainfall data and relative soil moisture

The variations of 5–9 month average monthly precipitation in Jinzhou during 1981–2010 are as shown in this work (Fig. 2). The work indicated that the amount of rainfall was maximum in July (176.93 mm) and less in May (57.43 mm).

The relative soil moisture of irrigation treatment was higher than that of the natural precipitation treatment at each [CO₂] level (Fig. 3). The range of relative soil moisture in irrigation treatment was about 68.55%–69.84% and that of natural precipitation was about 56.68%–58.32%. According to the national standard of the Classification of Meteorological Drought (GB/T20481-2006) [50], we can see that the natural precipitation treatments are in-fact under slight drought.

Photosynthetic gas exchange parameters of maize

According to previous studies, the relationship between photosynthetic rate and irradiance could be well described by modified rectangular hyperbolic model [52]. This model was used to obtain the irradiance-response curve (Fig. 5 A), with $R^2 > 0.99$

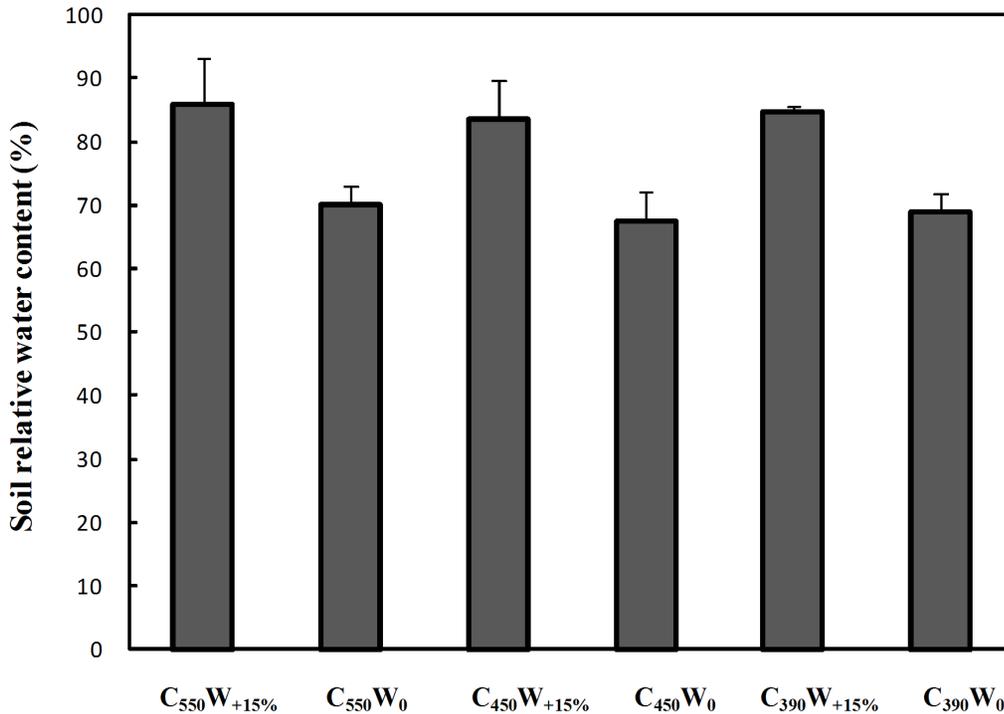


Figure 3. The relative soil moisture (means \pm SD) ($n=3$) in six treatments ($C_{550}W_{+15\%}$, $C_{550}W_0$, $C_{450}W_{+15\%}$, $C_{450}W_0$, $C_{390}W_{+15\%}$ and $C_{390}W_0$) in silking stage of maize under effects of elevated [CO₂] and irrigation.
doi:10.1371/journal.pone.0098318.g003

for all treatments, meanwhile the characteristic parameters were calculated from table 2. From the comparison between P_n of experimental value and model predicted value (Fig. 4), it could be seen that there was a good agreement between them.

Figure 5 showed the dynamic changes of P_n , T_r , WUE , G_s and G_i with PAR increased during silking stage of maize under interactive effects of elevated [CO₂] and irrigation. With increased of PAR , P_n curves of six treatments increased with elevated [CO₂] and irrigation. Similarly, when PAR is above 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, P_n

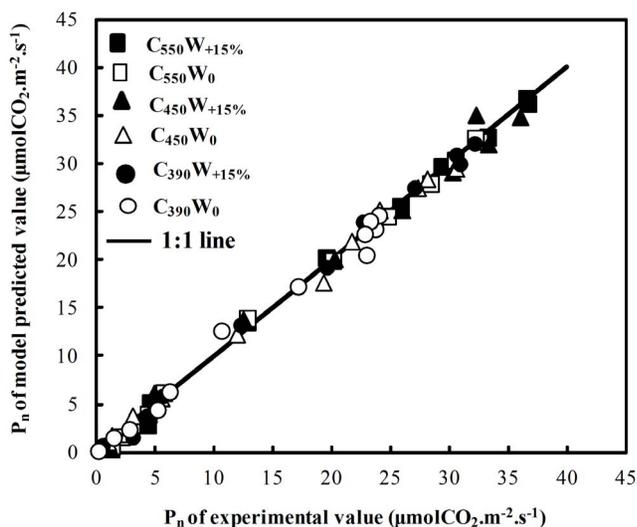


Figure 4. P_n of experimental value comparing with P_n of model predicted value. The 1:1 line indicates P_n of experimental value equals to the predicted value.
doi:10.1371/journal.pone.0098318.g004

curves closed to saturation and becomes stable. The order of six treatments were: $C_{550}W_{+15\%} > C_{450}W_{+15\%} > C_{550}W_0 > C_{390}W_{+15\%} > C_{450}W_0 > C_{390}W_0$ (Fig. 5A); Whereas all the T_r curves decreased with elevated [CO₂], and were much lower in natural precipitation than irrigation treatment at each [CO₂] level (Fig. 5B). WUE showed higher values with elevated [CO₂], and natural precipitation treatment showed higher than irrigation treatment at elevated [CO₂] levels (Fig. 5C). However, G_s curves showed opposite trend. The G_s curves were lower with elevated [CO₂] and rose with irrigation at the same [CO₂] levels (Fig. 5D). There were high trends of G_i under elevated [CO₂], and the irrigation treatments were higher with the increased amounts in PAR at each [CO₂] level (Fig. 5E).

The P_n curves were fitted and the parameters were generated by the modified rectangular hyperbolic model. The parameter $P_{n\text{max}}$ of the ear-leaves for maize was increased by 12.43%, 19.80% and 29.70% under irrigation conditions above the natural precipitation at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively. φ_c was not affected by irrigation and elevated [CO₂] (Table 2). Irrigation increased LSP by 8.25%, 7.60% and 9.97% at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively, but LCP increased by 38.73%, 15.81% and 42.92%, and R_d increased by 42.71%, 33.93% and 41.81% under the same conditions (Table 2).

Growth and development of maize

Plant height, ear height, stem diameter, leaf area and aboveground biomass of maize all had increasing trend under elevated [CO₂] and irrigation, while $C_{390}W_0$ always at its lowest value (Fig. 6). plant height of $C_{550}W_{+15\%}$, $C_{450}W_{+15\%}$ and $C_{390}W_{+15\%}$ were significantly higher than $C_{550}W_0$, $C_{450}W_0$ and $C_{390}W_0$ by 5.28%, 4.60% and 5.86%, respectively ($P < 0.05$; Fig. 6A), while ear height were higher by 5.69%, 3.34% and 3.50%, respectively, and the same trend as plant height ($P < 0.05$;

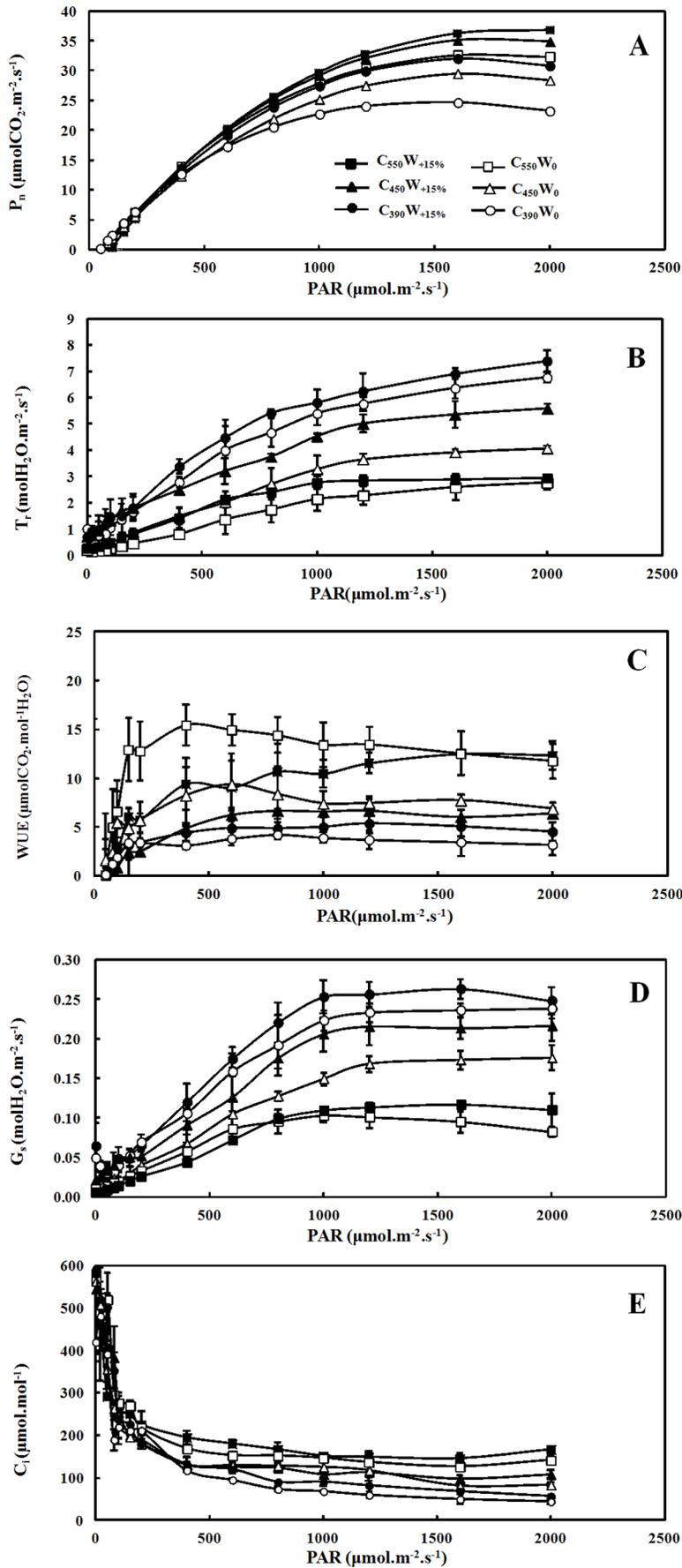


Figure 5. Dynamic curves of (A) net photosynthetic rate (P_n), (B) transpiration rate (T_r), (C) water use efficiency (WUE), (D) stomatal conductance (G_s) and (E) intercellular CO₂ concentration (G_i) (means \pm SD) ($n=3$) in six treatments ($C_{550}W_{+15\%}$, $C_{550}W_0$, $C_{450}W_{+15\%}$, $C_{450}W_0$, $C_{390}W_{+15\%}$ and $C_{390}W_0$) in silking stage of maize under effects of elevated [CO₂] and irrigation.
doi:10.1371/journal.pone.0098318.g005

Fig. 6B). There were no significant differences of stem diameter for all treatments at silking stage, but $C_{390}W_0$ was observed to be low ($P<0.05$; Fig. 6C). There was a significant interactive effects for elevated [CO₂] and irrigation in plant height ($P<0.05$; Table 4), but no significant interactive effects in ear height and stem diameter ($P<0.05$; Table 4). Irrigation significantly increased the leaf area by 12.04%, 9.90% and 7.75% at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively ($P<0.05$; Fig. 6D). In addition, irrigation significantly increased aboveground biomass by 16.66%, 10.75% and 7.65% at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively ($P<0.05$; Fig. 6E). The interactive effects of elevated [CO₂] and irrigation were highly significant in leaf area ($P<0.05$; Table 4) and aboveground biomass ($P<0.05$; Table 4).

Yield and ear characteristics of maize

The study also revealed that when irrigation was compared with the natural precipitation at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, significantly increased the seed yield by 17.48%, 14.91% and 10.59%, respectively ($P<0.05$; Table 3). There was also a significant increase in biological yield by 12.39%, 9.30% and 8.39%, respectively ($P<0.05$; Table 3). Following from the above, economic coefficient showed a significant increase that was higher in irrigation than natural precipitation at each [CO₂] level ($P<0.05$; Table 3). There were significant interactive effects of elevated [CO₂] and irrigation on maize seed yield ($P<0.05$; Table 4) and biological yield ($P<0.01$; Table 4).

The maize ear characteristics showed significant differences in six treatments under elevated [CO₂] and irrigation ($P<0.05$; Table 5). Irrigation increased 100-kernel weight by 10.59%, 8.20% and 5.19% at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] respectively, whereas that of shriveled kernels decreased by 70.33%, 74.70% and 73.68%, respectively. Kernels per row and kernel number of $C_{390}W_{+15\%}$, $C_{450}W_{+15\%}$ and $C_{550}W_{+15\%}$ were significantly increased more than $C_{390}W_0$, $C_{450}W_0$ and $C_{550}W_0$ ($P<0.05$). Also, rows per ear increased, but there was no difference among the six treatments. Maize ear length, ear diameter and ear weight were all significantly increased under irrigation, whereas axle diameter showed increasing trend at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels ($P<0.05$). However, there was no noteworthy difference for bare-tip length ($P<0.05$; Table 5).

Discussion

Interactive effects on photosynthetic parameters of maize

The response of plant photosynthesis to each of the environmental variables (e.g., water availability, temperature, nitrogen) associated with the elevated [CO₂] has not been sufficiently understood [55–58]. The present study indicated that leaf P_n of maize improved with both elevated [CO₂] and irrigation, and the curves of $C_{550}W_{+15\%}$, $C_{550}W_0$, $C_{450}W_{+15\%}$, $C_{450}W_0$ and $C_{390}W_{+15\%}$ showed higher values than $C_{390}W_0$ curve (Fig 5A). Similar results have been found in studies of *Stipa breviflora*, which reported a significant increase in P_n under elevated [CO₂] and increased precipitation of 15% [59], and wall *et al.* (2001) also observed that elevated [CO₂] increased P_n of sorghum by 9% in wet condition in FACE [60]. Leakey (2006) indicated that elevated [CO₂] can increase plant photosynthetic capacity and yield by adjusting its water state, so elevated [CO₂] will have positive effect in water deficit condition [61]. In this experimental site, the relative soil moisture of natural precipitation is lower as compared with irrigation (Fig. 3) and in-fact under slight drought, and it implied that water deficit is a key factor limiting maize growth. In contrast to P_n , T_r decreased with elevated [CO₂], and much lower in natural precipitation treatment than irrigation treatment at each [CO₂] level (Fig 5B). A number of other controlled environment studies [62–63] all observed the T_r decreased at elevated [CO₂]. It is worth mentioning, that the decrease of T_r was associated with decrease of G_s , when elevated [CO₂] decreased leaf G_s , and caused increasing resistance from intrinsic leaf to outside, resulting in the decrease of T_r [64]. Additionally, the studies of elevated [CO₂] and drought on plant reported that elevated [CO₂] declined T_r in low soil moisture than high soil moisture [25,62], which in accordance with our findings. This suggested that elevated [CO₂] may increase stomatal resistance and led to the reduce of T_r [25], but irrigation have a positive effect on stomatal opening and T_r have a increased trend in irrigation treatment compare with natural precipitation treatment at each [CO₂] level. Moreover, our data indicated that WUE increased with elevated [CO₂], and higher in natural precipitation treatment than irrigation treatment at elevated [CO₂] levels (Fig 5C). This is in agreement with the results of previous studies, which reported elevated [CO₂] have an increase trend in WUE under drought condition than well-watered condition [27,61,62,65]. Specifically, we found that the WUE have

Table 2. Parameters of irradiance-response curves of maize under effects of elevated [CO₂] and different irrigation.

Treatments	P_{nmax} / ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	LSP / ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	LCP / ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	ϕ_c / ($\text{mol}\cdot\text{mol}^{-1}$)	R_d / ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	R^2
$C_{550}W_{+15\%}$	36.8702	1884.3777	94.8387	0.0544	5.1268	0.997
$C_{550}W_0$ (CK)	32.7945	1740.8274	68.3083	0.0530	3.5925	0.998
$C_{450}W_{+15\%}$	35.3561	1768.8612	62.8339	0.0483	3.0519	0.994
$C_{450}W_0$ (CK)	29.5133	1650.8724	54.2541	0.0417	2.2788	0.997
$C_{390}W_{+15\%}$	32.0309	1643.9243	66.9802	0.0469	3.1668	0.993
$C_{390}W_0$ (CK)	24.6966	1501.2016	46.8651	0.0482	2.2332	0.992

Abbreviations are: P_{nmax} - maximum net photosynthetic rate, LSP - light saturation point, LCP - light compensation point, ϕ_c - quantum efficiency of the light compensation point, R_d - dark respiration.

doi:10.1371/journal.pone.0098318.t002

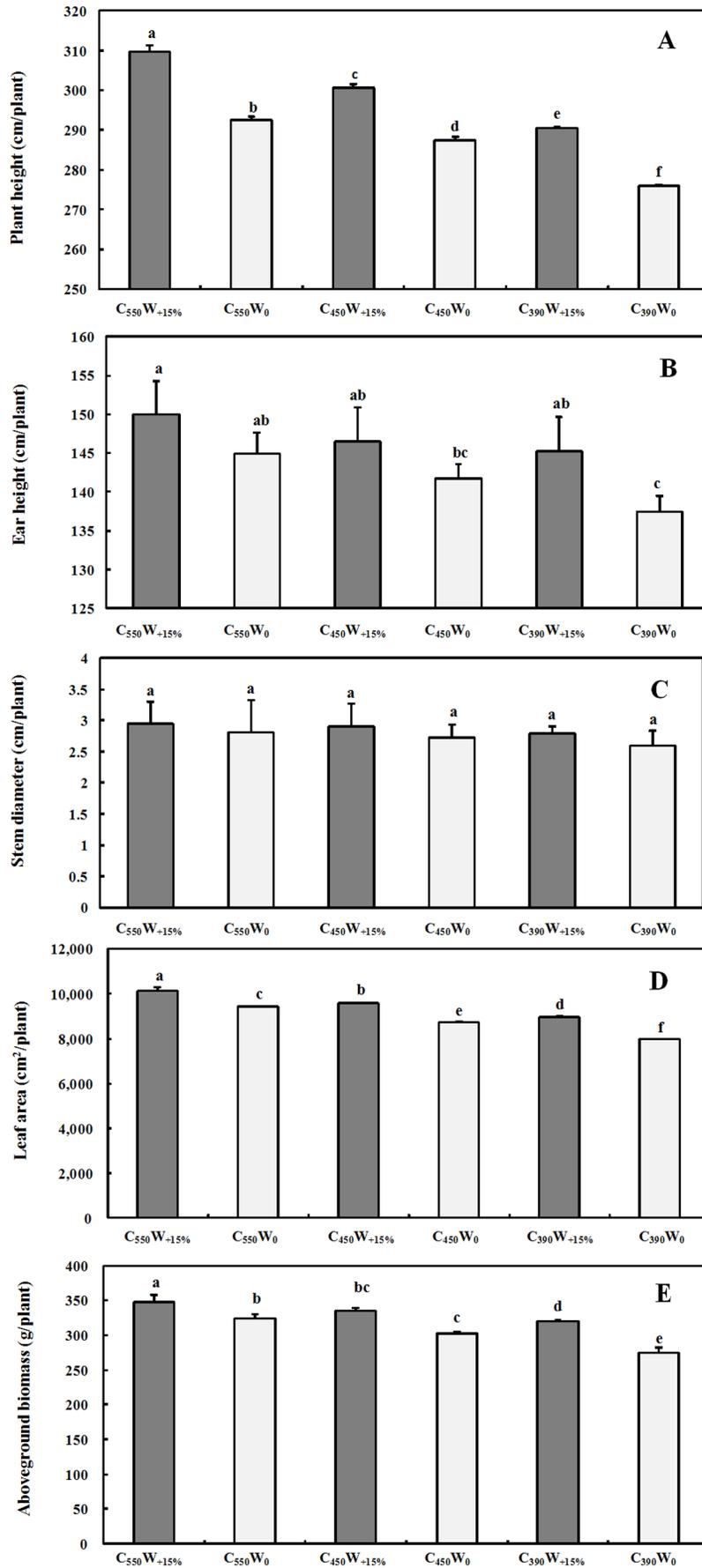


Figure 6. Changes of (A) plant height, (B) ear height, (C) stem diameter, (D) leaf area and (E) aboveground biomass (means \pm SD) (n=3) in six treatments (C₅₅₀W_{+15%}, C₅₅₀W₀, C₄₅₀W_{+15%}, C₄₅₀W₀, C₃₉₀W_{+15%} and C₃₉₀W₀) in silking stage of maize under effects of elevated [CO₂] and irrigation. Different lower cases letters indicated significant difference (P<0.05). doi:10.1371/journal.pone.0098318.g006

a decrease trend in irrigation treatment than natural precipitation treatment at elevated [CO₂] levels, whereas the yield of that increased. This is mainly due to the increase of leaf area and biomass of crop community under elevated [CO₂] and irrigation, resulting in the increase of *WUE* of crop community, at last performance increase in yield of maize [25,66–69]. In our study, *C_i* increased with elevated [CO₂], whereas irrigation caused a small increase of *C_i* at each [CO₂] level (Fig 5E). However, *G_s* decreased with elevated [CO₂], and irrigation increased it greatly at each [CO₂] level (Fig 5D). Some controlled environment studies [70–72] also showing that elevated [CO₂] could cause a decrease in plant stomatal conductance (*G_s*) and partly closing of the stomata. Curtis *et al.* (1998) found that doubling [CO₂] average reduced *G_s* by 11% [73]. Further, the decreased *G_s* might be associated with the increase in *C_i*, because a prior work showing that, *C_i* increased with rising [CO₂], wheat adjusted stomata opening width will bring about a decrease in *C_i* so as to keep the intercellular CO₂ partial pressure to be always lower than atmospheric CO₂ partial pressure [74].

Photosynthetic parameter represented photosynthetic capacity and efficiency [75], which was usually obtained from irradiance-response model. Ye and Yu (2008) indicated that the modified rectangular hyperbolic model fitting results were quite close to the real values compare with the other models by series verification test, and the main photosynthetic parameters can be directly generated without any assumption by the model [52–53]. The model mentioned above was used and the *P_n* curves were fitted, and producing the main parameters with *R*²>0.99 (Table 2). Irrigation increased *P_{nmax}* by 12.43%, 19.8% and 29.70% at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively (Table 2). The rise in *P_{nmax}* has shown an improvement concerning the photosynthetic electron transport rate and photophosphorylation activity. It has also shown an improvement in the photosynthetic capacity of maize. In addition, *LSP* increased by 9.51%, 7.15% and 8.25% under irrigation more than natural precipitation at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively (Table 2). The emergence of *LSP* is actually as a result of dark reactions which are able to keep up with light reactions under the intense radiation, thus restricting the increase of photosynthetic rate. However, additional irrigation under elevated [CO₂] might partly alleviate the negative effect and could promote photosynthetic capacity of maize under high light intensity. Normally, the

photosynthesis reaction in a plant is very weak when in *LCP*, thus there was no significant effect on maize photosynthesis, even after there was a change in *LCP* with elevated [CO₂] and irrigation (Table 2). In addition, irrigation increased *R_d* at each [CO₂] level, which is the limiting factor for maize photosynthesis. Generally, elevated [CO₂] can cause an increase in temperature, and make plant respiration quickened, leading to increase in consumption. Thus, the net effect is an increase in *R_d* [70]. Irrigation might enhance this effect, but further researches are needed to explore the reasons.

Interactive effects on growth of maize

The plant height increased by 5.28%, 4.60% and 5.86% under conditions of irrigation as compared with natural precipitation at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively (Fig. 6A). Similar results could be found for ear height (Fig. 6B), whereas stem diameter did not show any significant difference (Fig. 6C). Significant interactive effects of elevated [CO₂] and irrigation were observed in plant height, whereas there was no significant interactive effects of elevated [CO₂] and irrigation in ear height and stem diameter of maize (*P*<0.05; Table 4). Some studies reported that leaf area of maize significantly increased under elevated [CO₂] [76–77], and this research work is in agreement with those findings (Fig. 6D). In addition, irrigation increased leaf area by 12.04%, 9.90% and 7.75% at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively (Fig. 6D). A similar conclusion could be found in *Stipa breviflora*, in which, the leaf area increased to a maximum under conditions of elevated [CO₂] and increased precipitation of 15% [78]. Chamber studies showed that changing [CO₂] has no effect on biomass of maize and sorghum under adequate moisture conditions [79–81], and even the negative effects [82]. However, this study found that aboveground biomass of maize significantly increased by 16.66%, 10.75% and 7.65% under irrigation more than natural precipitation at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively (Fig. 6E). This result is consistent with Cure's findings [83], who reported that maize aboveground biomass increased by 7% under well-watered more than dry conditions at 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] in chamber (n = 4). Shi *et al.* (2013) observed that the aboveground biomass of *Stipa breviflora* significantly increased with elevated [CO₂] and increasing precipitation [78]. Moreover, There were significant interactive effects of elevated

Table 3. Multiple comparison on yield of maize under effects of elevated [CO₂] and irrigation.

Treatments	Seed yield /(g/plant)	Increase compare with its CK %	Biology yield /(g/plant)	Increase compare with its CK %	Economical coefficient
C ₅₅₀ W _{+15%}	336.76 \pm 2.75 ^a	10.59	617.34 \pm 5.23 ^a	8.39	0.55 \pm 0.01 ^a
C ₅₅₀ W ₀ (CK)	304.52 \pm 1.01 ^c		569.55 \pm 1.19 ^c		0.53 \pm 0.00 ^a
C ₄₅₀ W _{+15%}	314.34 \pm 4.22 ^b	14.91	585.81 \pm 3.84 ^b	9.30	0.54 \pm 0.00 ^a
C ₄₅₀ W ₀ (CK)	273.55 \pm 5.52 ^e		535.98 \pm 2.10 ^e		0.51 \pm 0.01 ^b
C ₃₉₀ W _{+15%}	281.53 \pm 6.35 ^d	17.48	554.18 \pm 3.78 ^d	12.39	0.51 \pm 0.01 ^b
C ₃₉₀ W ₀ (CK)	239.64 \pm 1.09 ^f		493.09 \pm 5.54 ^f		0.49 \pm 0.00 ^c

The data are means \pm SD (n=3).

Different lower cases letters (a,b,c,d,e,f) indicated significant difference (*P*<0.05).

doi:10.1371/journal.pone.0098318.t003

Table 4. The two-way analysis of variances on growth and yield of maize between different elevated [CO₂] and irrigation treatment.

Variable	CO ₂ concentration			Irrigation			Interaction		
	df	F	P	df	F	P	df	F	P
Plant height	2	536.048	0.000	1	1105.743	0.000	2	6.507	0.012*
Ear height	2	9.315	0.002	1	26.167	0.000	2	0.726	0.498
Stem diameter	2	0.988	0.392	1	2.454	0.135	2	0.020	0.980
Leaf area	2	622.766	0.000	1	777.613	0.000	2	4.821	0.029*
Aboveground biomass	2	58.838	0.000	1	137.036	0.000	2	4.339	0.038*
Seed yield	2	663.180	0.000	1	806.085	0.000	2	5.113	0.017*
Biology yield	2	945.868	0.000	1	1627.130	0.000	2	9.953	0.001**

* and ** indicated $P < 0.05$ and $P < 0.01$, respectively.
doi:10.1371/journal.pone.0098318.t004

Table 5. Change on ear characteristics of maize under effects of elevated [CO₂] and irrigation.

Treatments	Ear length (cm)		Ear diameter (cm)		Ear weight (g)		100-kernel weight (g)		Kernels per row (kernels/row)		Kernel number (No.)		Rows per ear (row/ear)		Shriveled kernels (kernels/ear)		Bare-tip length (cm)		Axle diameter (cm)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C ₅₅₀ W _{+15%}	24.17 ± 0.35 ^a		6.16 ± 0.04 ^a		362.45 ± 0.93 ^a		43.97 ± 0.21 ^a		44.67 ± 1.15 ^a		820.67 ± 2.31 ^a		18.67 ± 1.15 ^a		5.00 ± 1.00 ^e		4.51 ± 0.04 ^{bc}		3.80 ± 0.31 ^a	
C ₅₅₀ W ₀ (CK)	22.21 ± 0.05 ^c		5.97 ± 0.04 ^b		332.28 ± 16.86 ^b		41.80 ± 0.40 ^b		41.33 ± 0.58 ^b		757.67 ± 1.53 ^b		17.33 ± 1.15 ^a		19.00 ± 1.00 ^c		4.45 ± 0.04 ^c		3.72 ± 0.37 ^{ab}	
C ₄₅₀ W _{+15%}	23.05 ± 0.45 ^b		6.03 ± 0.04 ^b		344.69 ± 4.08 ^b		42.60 ± 0.50 ^b		42.33 ± 0.58 ^b		762.00 ± 1.39 ^b		18.00 ± 0.01 ^a		7.00 ± 1.00 ^d		4.42 ± 0.04 ^c		3.64 ± 0.28 ^{bc}	
C ₄₅₀ W ₀ (CK)	21.02 ± 0.12 ^d		5.57 ± 0.01 ^d		305.56 ± 4.07 ^c		39.37 ± 0.84 ^c		38.33 ± 0.58 ^c		614.67 ± 3.06 ^d		17.33 ± 1.15 ^a		27.67 ± 2.08 ^b		4.62 ± 0.01 ^b		3.61 ± 0.31 ^{bc}	
C ₃₉₀ W _{+15%}	21.25 ± 0.03 ^d		5.69 ± 0.17 ^c		312.01 ± 1.77 ^c		40.40 ± 0.75 ^c		39.00 ± 1.00 ^c		676.00 ± 1.58 ^c		17.33 ± 1.15 ^a		9.00 ± 1.00 ^d		4.44 ± 0.17 ^c		3.55 ± 0.26 ^c	
C ₃₉₀ W ₀ (CK)	19.28 ± 0.35 ^e		5.17 ± 0.04 ^e		254.25 ± 1.45 ^d		36.53 ± 1.31 ^d		35.67 ± 0.58 ^d		594.33 ± 2.89 ^e		16.67 ± 1.15 ^a		30.33 ± 1.15 ^a		4.87 ± 0.04 ^a		3.54 ± 0.28 ^c	

The data are means ± SD (n = 3).

Different lower cases letters (a,b,c,d,e) indicated significant difference ($P < 0.05$).

doi:10.1371/journal.pone.0098318.t005

[CO₂] and irrigation in leaf area ($P < 0.05$; Table 4) and aboveground biomass ($P < 0.05$; Table 4).

Interactive effects on yield and ear characteristics of maize

The results of that elevated [CO₂] increased maize yield have been indicated in previous studies. Cure *et al.* (1986) reported that maize average yield increased by 27% ($n = 3$) by doubling [CO₂] [83], while Guo (2003) indicated that maize yield might be increased by 22.88% when [CO₂] levels rise up to 700 $\mu\text{mol}\cdot\text{mol}^{-1}$ [84]. Long *et al.* (2006) used comprehensive observation data of chamber studies ($n = 14$) and found out that grain yield of maize and sorghum increased by an average of 18% when [CO₂] were elevated to 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [85]. This study has exhaustively shown that the maize yield of Northeast China increased by 14.15% and 27.07% with 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively (Table 3). Additionally, irrigation significantly increased maize yield by 10.59%, 14.91% and 17.48% as compared with natural precipitation at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively (Table 3). Similar results have been reported in previous studies [86–87], but others have reported decreases [82] or no significant change at all [79–80]. Allen (2011) predicted that management of irrigation water in a future high [CO₂] world could potentially increase overall C₄ crop yield (in water-limited areas) [62], we agree with this idea. Moreover, biological yield has also been increased by 8.39%, 9.30% and 12.39% under irrigation more than natural precipitation at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively (Table 3). The work also revealed that there has been significant interactive effects of elevated [CO₂] and irrigation on maize seed yield ($P < 0.05$; Table 4) and biological yield ($P < 0.01$; Table 4). Consequently, maize economic coefficient increased in irrigation more than natural precipitation. Economic coefficient reflects the transport and store ability of crop “source” to “sink” form photosynthetic products [88]. Kirschbaum (2010) reported that plant growth response to elevated [CO₂] increase with a plant’s sink capacity and nutrient status [89]. In present study, maize photosynthetic capacity was enhanced while the ear capacity was expanded under elevated [CO₂] and irrigation, resulting in a more dry matter accumulation and yield increase.

Maize ear characteristics significantly changed under the interactive effects of elevated [CO₂] and irrigation. 100-kernel weight and kernal number were all significantly increased under irrigation than natural precipitation at each [CO₂] level (Table 5). Additionally, ear length, ear diameter and ear weight were all increased in accordance with yield, and the increase of 100-kernel weight and ear length was consistent with the study done by Wang *et al.* (1996) [90]. There is a study reported that kernels per row is the main factor that will increase maize yield [91]. In this study, irrigation increased kernels per row by 9.34%, 10.44% and 8.08% at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels respectively,

whereas rows per ear change was not remarkable (Table 5). The decreased of shriveled kernels was beneficial to yield increase. In our study, irrigation decreased shriveled kernels by 70.33%, 74.70% and 73.68% at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels, respectively (Table 5). Optimization of the ear characteristics were reflected in an increase in yield under elevated [CO₂] and irrigation. Therefore, it is necessary to irrigate with additional water for the elevated [CO₂] plots of maize to compensate for photosynthesis resistance from lacking of water under elevated [CO₂] condition. Moreover, bare-tip length of maize made no significant difference in all treatments, but axle diameter increased within a narrow range. The increase was by 0.28%, 0.83% and 2.15% in irrigation more than natural precipitation at 390, 450 and 550 $\mu\text{mol}\cdot\text{mol}^{-1}$ [CO₂] levels (Table 5). This change implied that elevated [CO₂] and irrigation increased ear length, ear diameter as well as axle diameter. Thus, it can be estimated that maize yield could not be increased unlimitedly with elevated [CO₂] and irrigation, but increasing the axle diameter serves as a limiting factor.

Conclusions

Our results demonstrated the following: 1) With elevated [CO₂] and irrigation, leaf P_n and C_i of maize significantly increased, while elevated [CO₂] brought a notable decrease in G_s and T_r , but irrigation have a positive effect on them, thereby increasing WUE in natural precipitation treatment than irrigation treatment at elevated [CO₂] levels. 2) Irradiance-response parameters P_{max} and LSP increased more under irrigation conditions than natural precipitation at each [CO₂] level. R_d also increased under the same conditions, and this is a limiting factor. 3) Irrigation under elevated CO₂ increased plant height, ear height, stem diameter, leaf area and aboveground biomass, resulting in the increase of yield. In addition, ear characteristics of maize were all superior except axle diameter. However, further study should be taken to ensure the contribution rate of elevated [CO₂] and irrigation.

Acknowledgments

We would like to appreciate Drs. Zhenzhu Xu and Yueming Bai for their valuable advice on the experiment design, Jinzhou Ecological and Agricultural meteorological experiment center in Liaoning province of China for providing experiment facilities and field assistance, Zhengming Zhou, Pengcheng Qin, Hui Li, Xiliang Chen and Xiang Wu for their help with the fieldwork and laboratory analysis, and academic editor and additional editor for their valuable comments in the manuscript.

Author Contributions

Conceived and designed the experiments: JZ. Performed the experiments: FM CH. Analyzed the data: FM FY. Contributed reagents/materials/analysis tools: FM FY. Wrote the paper: FM JZ.

References

1. IPCC (2007) IPCC fourth assessment report: climate change 2007. Cambridge University Press.
2. Leadley PW, Drake BG (1993) Open top chambers for exposing plant canopies to elevated CO₂ concentration and for measuring net gas exchange. *Vegetatio* 104–105(1): 3–15.
3. Wang XL, Xu SH, Liang H (1998) The experimental study of the effects of CO₂ concentration enrichment on growth, development and yield of C₃ and C₄ crops. *Sci Agric Sin* 31(1): 55–61. (in Chinese).
4. Ziska LH, Sicher RC, Bunce JA (1999) The impact of elevated carbon dioxide on the growth and gas exchange of three C₄ species differing in CO₂ leak rates. *Physiol Plant* 105(1): 74–80.
5. Ghannoum O, Von Caemmerer S, Ziska LH, Conroy JP (2000) The growth response of C₄ plants to rising atmospheric CO₂ partial pressure: a reassessment. *Plant Cell Environ* 23(9): 931–942.
6. Shaw MR, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA, *et al.* (2002) Grassland responses to global environmental changes suppressed by elevated CO₂. *Science* 298(5600): 1987–1990.
7. Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annu Rev Plant Biol* 55: 591–628.
8. Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytol* 165(2): 351–372.

9. Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant Cell Environ* 30(3): 258–270.
10. Onoda Y, Hirose T, Hikosaka K (2007) Effect of elevated CO₂ levels on leaf starch, nitrogen and photosynthesis of plants growing at three natural CO₂ springs in Japan. *Ecol Res* 22(3): 475–484.
11. Wall GW, Garcia RL, Wechsung F, Kimball BA (2011) Elevated atmospheric CO₂ and drought effects on leaf gas exchange properties of barley. *Agr Ecosyst Environ* 144(1): 390–404.
12. Ziska L (2013) Observed changes in soybean growth and seed yield from *Abutilon theophrasti* competition as a function of carbon dioxide concentration. *Weed Research* 53(2): 140–145.
13. Easterling DR, Evans JL, Groisman PY, Karl TR, Kunkel KE, et al. (2000) Observed variability and trends in extreme climate events: a brief review. *Bull Amer Meteor Soc* 81: 417–425.
14. Wallace JS (2000) Increasing agricultural water use efficiency to meet future food production. *Agric Ecosyst Environ* 82(1): 105–119.
15. Jones PD, Hulme M (1996) Calculating regional climatic time series for temperature and precipitation: methods and illustrations. *Int J Climatol* 16: 361–377.
16. Hulme M, Osborn TJ, Johns TC (1998) Precipitation sensitivity to global warming: comparison of observations with HadCM2 simulations. *Geophys Res Lett* 25(17): 3379–3382.
17. Houghton RA (2001) Counting terrestrial sources and sinks of carbon. *Clim Change* 48(4): 525–534.
18. Wu JD, Wang SL, Zhang JM (2000) A numerical simulation of the impacts of climate change on water and thermal resources in northeast china. *Resources Sci* 22(6): 36–42. (in Chinese).
19. Kim SH, Gitz DC, Sicher RC, Baker JT, Timlin DJ, et al. (2007) Temperature dependence of growth, development, and photosynthesis in maize under elevated CO₂. *Environ Exp Bot* 61(3): 224–236.
20. Wang D, Heckathorn SA, Barua D, Joshi P, Hamilton EW, et al. (2008) Effects of elevated CO₂ on the tolerance of photosynthesis to acute heat stress in C₃, C₄, and CAM species. *Amer J Bot*, 95(2): 165–176.
21. Hamilton EW, Heckathorn SA, Joshi P, Wang D, Barua D (2008) Interactive effects of elevated CO₂ and growth temperature on the tolerance of photosynthesis to acute heat stress in C₃ and C₄ species. *J Integr Plant Biol* 50(11): 1375–1387.
22. Xu ZZ, Shimizu H, Ito S, Yagasaki Y, Zou CJ, et al. (2014) Effects of elevated CO₂, warming and precipitation change on plant growth, photosynthesis and peroxidation in dominant species from North China grassland. *Planta* 239(2): 421–435.
23. Liang N, Maruyama K (1995) Interactive effects of CO₂ enrichment and drought stress on gas exchange and water-use efficiency in *Alnus firma*. *Environ Exp Bot* 35(3): 353–361.
24. Burkart S, Manderscheid R, Weigel HJ (2004) Interactive effects of elevated atmospheric CO₂ concentrations and plant available soil water content on canopy evapotranspiration and conductance of spring wheat. *Eur J Agron* 21(4): 401–417.
25. Kang SZ, Zhang FC, Hu XT, Zhang JH (2002) Benefits of CO₂ enrichment on crop plants are modified by soil water status. *Plant Soil* 238(1): 69–77.
26. Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long S P, et al. (2009) Elevated CO₂ effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *J Exper Bot* 60(10): 2859–2876.
27. Manderscheid R, Erbs M, Weigel HJ (2014) Interactive effects of free-air CO₂ enrichment and drought stress on maize growth. *Eur J Agron* 52: 11–21.
28. Zong YZ, Wang WF, Xue QW, Shangquan ZP (2014) Interactive effects of elevated CO₂ and drought on photosynthetic capacity and PSII performance in maize. *Photosynthetica* 52(1):63–70.
29. Markelz RJC, Strellner RS, Leakey ADB (2011) Impairment of C₄ photosynthesis by drought is exacerbated by limiting nitrogen and ameliorated by elevated [CO₂] in maize. *J Exp Bot* 62(9): 3235–3246.
30. Allen SG, Idso SB, Kimball BA (1990) Interactive effects of CO₂ and environment on net photosynthesis of water-Lily. *Agric Ecosystems Environ* 30(1): 81–88.
31. Idso SB, Wall GW, Kimball BA (1993) Interactive effects of atmospheric CO₂ enrichment and light intensity reductions on net photosynthesis of sour orange tree leaves. *Environ Exp Bot* 33(3): 367–375.
32. Olszyk DM, Wise C (1997) Interactive effects of elevated CO₂ and O₃ on rice and *flacca* tomato. *Agric Ecosystems Environ* 66(1): 1–10.
33. Olszyk DM, Johnson MG, Phillips DL, Seidler RJ, Tingey DT, et al. (2001) Interactive effects of CO₂ and O₃ on a ponderosa pine plant/litter/soil mesocosm. *Environ Pollut* 115(3): 447–462.
34. Ainsworth EA (2008) Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentrations. *Global Change Biol* 14(7): 1642–1650.
35. Ainsworth EA, Rogers A, Leakey ADB (2008) Targets for crop biotechnology in a future high-CO₂ and high-O₃ world. *Plant Physiol* 147(1): 13–19.
36. Kim HY, Lieffering M, Kobayashi K, Okada M, Mitchell MW, et al. (2003) Effects of free-air CO₂ enrichment and nitrogen supply on the yield of temperate paddy rice crops. *Field Crop Res* 83(3): 261–270.
37. Kim HY, Lieffering M, Miura S, Kobayashi K, Okada M (2001) Growth and nitrogen uptake of CO₂-enriched rice under field conditions. *New Phytol* 150(2): 223–229.
38. Rogers A, Ainsworth EA, Leakey ADB (2009) Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? *Plant Physiol* 151(3): 1009–1016.
39. Reddy KR, Zhao D (2005) Interactive effects of elevated CO₂ and potassium deficiency on photosynthesis, growth, and biomass partitioning of cotton. *Field Crop Res* 94(2): 201–213.
40. Geissler N, Hussin S, Koyro HW (2009) Interactive effects of NaCl salinity and elevated atmospheric CO₂ concentration on growth, photosynthesis, water relations and chemical composition of the potential cash crop halophyte *Aster tripolium* L. *Environ Exp Bot* 65(2): 220–231.
41. Milcu A, Paul S, Lucac M (2011) Belowground interactive effects of elevated CO₂, plant diversity and earthworms in grassland microcosms. *Basic Appl Ecol* 12: 600–608.
42. Zhou HQ, Wang CJ (2006) Analysis on comprehensive capability of food production in Northeast area. *J Northeast Agric Univ* 4(1): 5–8. (in Chinese).
43. Hu G, Wu YQ, Liu BY, Yu ZT, You ZM, et al. (2007) Short-term gully retreat rates over rolling hill areas in black soil of Northeast China. *Catena*, 71(2): 321–329. (in Chinese).
44. Liu XT, Tong IJ, Wu ZJ, Liang WJ (1998) Analysis and prediction of grain production potential in Northeast region. *Sci Geogr Sin* 18(6): 501–509. (in Chinese).
45. Ma SQ, Wang Q, Luo XL (2008) Effect of climate change on maize (*Zea mays*) growth and yield based on stage sowing. *Acta Ecologica Sinica* 28(5): 2131–2139. (in Chinese).
46. Bai XL, Sun SX, Yang GH, Liu M, Zhang ZP, et al. (2009) Effect of water stress on maize yield during different growing stages. *J Maize Sci* 17(2): 60–63. (in Chinese).
47. Han GX, Zhou GS, Xu ZZ, Y Y, Liu JL, et al. (2007) Soil temperature and biotic factors drive the seasonal variation of soil respiration in a maize (*Zea mays* L.) agricultural ecosystem. *Plant Soil* 291(1): 15–26.
48. Panel on the Northeast Regional Climate Change Assessment Report (2013) Summary for policy-makers and implementation of Northeast Regional Climate Change Assessment Report 2012. Beijing: China Meteorological Press. (in Chinese).
49. Wigley TML, Raper SCB (2001) Interpretation of high projections for global-mean warming. *Science* 293: 451–454.
50. Zhang Q, Zou XK, Xiao FJ, Lu HQ, Liu HB, et al. (2006) GB/T20481-2006, Classification of meteorological drought. People's Republic of China National Standards (in Chinese). Beijing: Standardization Press of China.
51. Liu JL, Shi KQ, Liang T, Zhang LH (2008) Relationship between underground water level and precipitation, soil moisture in maize field in Jinzhou. *Anhui Agric Sci Bull* 14(20): 126–130. (in Chinese).
52. Ye ZP (2007) A new model for relationship between irradiance and the rate of photosynthesis in *Oryza sativa*. *Photosynthetica* 45(4): 637–640.
53. Ye ZP, Yu Q (2008) A coupled model of stomatal conductance and photosynthesis for winter wheat. *Photosynthetica* 46(4): 637–640.
54. Han RF, Li JM, Hu XH, Da HG, Bai RF (2012) Research on dynamic characteristics of photosynthesis in muskmelon seedling leaves. *Acta Ecologica Sinica* 32(5): 1471–1480. (in Chinese).
55. Lee JS (2011) Combined effect of elevated CO₂ and temperature on the growth and phenology of two annual C₃ and C₄ weedy species. *Agr Ecosyst Environ* 140(3): 484–491.
56. Reddy AR, Rasineni GK, Raghavendra AS (2010) The impact of global elevated CO₂ concentration on photosynthesis and plant productivity. *Current Science* 99(1): 46–57.
57. Hättenschwiler S, Zurburn T (2006) Hemiparasite abundance in an alpine treeline ecotone increases in response to atmospheric CO₂ enrichment. *Oecologia* 147: 47–52.
58. Temperton V, Lard PM, Jarvis P (2003) Does elevated atmospheric carbon dioxide affect internal nitrogen allocation in the temperate trees *Alnus glutinosa* and *Pinus sylvestris*? *Global Change Biol* 9: 286–294.
59. Wang H, Zhou GS, Jiang YL, Shi YH, Xu ZZ (2012) Interactive effects of changing precipitation and elevated CO₂ concentration on photosynthetic parameters of *Stipa breviflora*. *Chin J Plant Ecol* 36(7): 597–606. (in Chinese).
60. Wall GW, Brooks TJ, Adam NR, Cousins AB, Kimball BA (2001) Elevated atmospheric CO₂ improved *sorghum* plant water status by ameliorating the adverse effects of drought. *New Phytol*, 152(2): 231–248.
61. Leakey ADB, Uribealrrea M, Ainsworth EA, Naidu SL, Rogers A, et al. (2006) Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO₂ concentration in the absence of drought. *Plant Physiol* 140, 779–790.
62. Allen Jr LH, Kakani VG, Vu JCV, Boote KJ (2011) Elevated CO₂ increases water use efficiency by sustaining photosynthesis of water-limited maize and sorghum. *J Plant Physiol* 168(16): 1909–1918.
63. Bernacchi CJ, Kimball BA, Quarles DR, Long SP, Ort DR (2007) Decreases in stomatal conductance of soybean under open-air elevation of [CO₂] are closely coupled with decreases in ecosystem evapotranspiration. *Plant Physiol* 143(1): 134–144.
64. Xu L, Zhao TH, Hu YY, Shi Y (2008) Effects of CO₂ Enrichment on Photosynthesis and Grain Yield of Spring Wheat. *J Triticeae Crops* 28(5): 867–872. (in Chinese).
65. Kang SZ, Zhang FC, Liang YL, Ma QL, Hu XT (1999) Effects of soil water and the atmospheric CO₂ concentration increase on evapotranspiration, Photosynthesis, growth of Wheat, Maize and Cotton. *Acta Agron Sin* 25(1): 55–63.

66. Samarakoon AB, Müller WJ, Gifford RM (1995) Transpiration and leaf area under elevated CO₂: effects of soil water status and genotype in wheat. *Aust J Plant Physiol* 22: 33–44.
67. Roden JS, Ball MC (1996) The effect of elevated [CO₂] on growth and photosynthesis of two eucalyptus species exposed to high temperatures and water deficits. *Plant Physiol* 111(3): 909–919.
68. Newton PCD, Clark H, Bell CC, Glasgow EM (1996) Interaction of soil moisture and elevated CO₂ on the above-ground growth rate, root length density and gas exchange of turves from temperate pasture. *J Exp Bot* 47(299): 771–779.
69. Robredo A, Pérez-López U, de la Maza HS, González-Moro B, Lacuesta M, et al. (2007) Elevated CO₂ alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. *Environ Exp Bot* 59(3): 252–263.
70. Murray DR (1995) Plant responses to carbon dioxide. *Amer J Bot* 82(5): 690–697.
71. Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant Cell Environ* 30: 258–270.
72. Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytol* 165(2): 351–372.
73. Curtis PS, Wang XZ (1998) A meta-analysis of elevated CO₂ effects on woody plant mass, form and physiology. *Oecologia* 113: 299–313.
74. Wang RJ, Zhang XC, Gao SM, Yu XF, Ma YF (2010) Effects of atmospheric CO₂ enrichment and nitrogen application rate on photosynthetic parameters and water use efficiency of spring wheat. *Agric Res Arid Areas* 28(5): 32–37. (in Chinese).
75. Wang JL, Wen XF, Zhao FH, Fang QX, Yang XM (2012) Effects of doubled CO₂ concentration on leaf photosynthesis, transpiration and water use efficiency of eight crop species. *Chin J Plant Ecol* 36(5): 438–446. (in Chinese).
76. Samarakoon AB, Gifford RM (1995) Soil water content under plants at high CO₂ concentration and interactions with the direct CO₂ effects: a species comparison. *J Biogeogr* 22(2/3): 193–202.
77. Maroco JP, Edwards GE, Ku MSB (1999) Photosynthetic acclimation of maize to growth under elevated levels of carbon dioxide. *Planta* 210(1): 115–125.
78. Shi YH, Zhou GS, Jiang YL, Wang H, Xu ZZ (2013) Effects of interactive CO₂ concentration and precipitation on growth characteristics of *Stipa breviflora*. *Acta Ecologica Sinica* 33(14): 4478–4485. (in Chinese).
79. Marc J, Gifford RM (1984) Floral initiation in wheat, sunflower, and sorghum under carbon dioxide enrichment. *Can J Bot* 62(1): 9–14.
80. Mauney JR, Fry KE, Guinn G (1978) Relationship of photosynthetic rate to growth and fruiting of cotton, soybean, sorghum, and sunflower. *Crop Sci* 18(2): 259–263.
81. Ziska LH, Bunce JA (1997) Influence of increasing carbon dioxide concentration on the photosynthetic and growth stimulation of selected C₄ crops and weeds. *Photosynth Res* 54(3): 199–208.
82. Ellis RH, Craufurd PQ, Summerfield RJ, Roberts EH (1995) Linear relations between carbon dioxide concentration and rate of development towards flowering in sorghum, cowpea and soybean. *Ann Bot* 75(2): 193–198.
83. Cure JD, Acock B (1986) Crop responses to carbon dioxide doubling: a literature survey. *Agr Forest Meteorol* 38(1/3): 127–145.
84. Guo JP (2003) Responses of mainly plants in northern China to CO₂ enrichment and soil drought. Beijing: China Meteorological Press. (in Chinese).
85. Long SP, Ainsworth EA, Leakey ADB, Nösberger J, Ort DR (2006) Food for thought: lower-than-expected crop yield stimulation with rising CO₂ concentrations. *Science* 312(5782): 1918–1921.
86. Amthor JS, Mitchell RJ, Runion GB, Rogers HH, Prior SA, et al. (1994) Energy content, construction cost and phytomass accumulation of *Glycine max* (L.) Merr. and *Sorghum bicolor* (L.) Moench grown in elevated CO₂ in the field. *New Phytol* 128(3): 443–450.
87. Reeves DW, Rogers HH, Prior SA, Wood CW, Runion GB (1994) Elevated atmospheric carbon dioxide effects on sorghum and soybean nutrient status. *J Plant Nutr* 17(11): 1939–1954.
88. Mason TG, Maskell EJ (1928) Studies on the transport of carbohydrates in the cotton plant: II. The factors determining the rate and the direction of movement of sugars. *Ann Bot* 42(3): 571–636.
89. Kirschbaum MUF (2011) Does enhanced photosynthesis enhance growth? Lessons learned from CO₂ enrichment studies *Plant Physiol* 155(1): 117–124.
90. Wang CY, Bai YM, Wen M (1996) Effects of CO₂ concentration increase on yield and quality of corn. *Acta Sci circumst* 16(3): 331–336. (in Chinese).
91. Yang JH, Mao JC, Li FM, Ran LG, Liu J, et al. (2003) Correlation and path analysis on agronomic traits and kernal yield of maize hybrids. *Chin Agric Sci Bull* 19(4): 28–30. (in Chinese).