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REGULAR PAPER

Effects of different elevated CO_2 concentrations on chlorophyll contents, gas exchange, water use efficiency, and PSII activity on C_3 and C_4 cereal crops in a closed artificial ecosystem

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Abstract Although terrestrial CO_2 concentrations [CO_2] are not expected to reach 1000 μmol mol^{-1} (or ppm) for many decades, CO_2 levels in closed systems such as growth chambers and greenhouses can easily exceed this concentration. CO_2 levels in life support systems (LSS) in space can exceed 10,000 ppm (1 %). In order to understand how photosynthesis in C_4 plants may respond to elevated CO_2, it is necessary to determine if leaves of closed artificial ecosystem grown plants have a fully developed C_4 photosynthetic apparatus, and whether or not photosynthesis in these leaves is more responsive to elevated [CO_2] than leaves of C_3 plants. To address this issue, we evaluated the response of gas exchange, water use efficiency, and photosynthetic efficiency of PSII by soybean (*Glycine max* (L.) Merr., 'Heihe35') of a typical C_3 plant and maize (*Zea mays* L., 'Susheng') of C_4 plant under four CO_2 concentrations (500, 1000, 3000, and 5000 ppm), which were grown under controlled environmental conditions of Lunar Palace 1. The results showed that photosynthetic pigment by the C_3 plants of soybean was more sensitive to elevated [CO_2] below 3000 ppm than the C_4 plants of maize. Elevated [CO_2] to 1000 ppm induced a higher initial photosynthetic rate, while super-elevated [CO_2] appeared to negate such initial growth promotion for C_3 plants. The C_4 plant had the highest ETR, φPSII, and qP under 500–3000 ppm [CO_2], but then decreased substantially at 5000 ppm [CO_2] for both species. Therefore, photosynthetic down-regulation and a decrease in photosynthetic electron transport occurred by both species in response to super-elevated [CO_2] at 3000 and 5000 ppm. Accordingly, plants can be selected for and adapt to the efficient use of elevated CO_2 concentration in LSS.

Keywords Elevated [CO_2] · Gas exchange · Water use efficiency · Chlorophyll fluorescence · C_3 and C_4 plants · Life support systems

Abbreviations

A	Net photosynthetic rate
LSS	Life support systems
Car	Carotenoid content
Chl *a*	Chlorophyll *a*
Chl *b*	Chlorophyll *b*
Chl *a*/*b*	Chlorophyll ratio
Chl tot	Total chlorophyll content
Ci	Intercellular carbon dioxide concentration
ETR	Photosynthetic electron transport
F_v/F_m	Optimal photochemical efficiency of PSII
F_v/F_o	Potential activity of PSII
Gs	Stomatal conductance
ICE	Instantaneous carboxylation efficiency
IWUE	Intrinsic water use efficiency
PTNDS	Porous-tube nutrient delivery system
qP	Photochemistry quenching
qN	Nonphotochemistry quenching
Tr	Transpiration rate
WUE	Water use efficiency
φPSII	Effective quantum yield of PSII
[CO_2]	Carbon dioxide concentration

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Introduction

According to the intergovernmental panel on climate change (IPCC), the preindustrial levels of carbon in the atmosphere rose from 285 µmol l^{-1} to the current level of 384 µmol l^{-1} and the predicted rise in the atmospheric CO_2 would approach 700 µmol l^{-1} by the year 2050 (IPCC 2007). Such an abnormal rise in the levels of atmospheric CO_2 would result in direct and indirect global climate changes. Although terrestrial CO_2 concentrations [CO_2] are not expected to reach 1000 µmol mol^{-1} (or ppm) for many decades, CO_2 levels in closed systems such as growth chambers and greenhouses can easily exceed this concentration. CO_2 levels in life support systems (LSS) in space can exceed 10,000 ppm (1 %). For several years, plant growth has been studying in closed, controlled environments typical of what might been countered in space. In particular, we have been interested in the effects of super-elevated CO_2 concentrations in these environments. In the space shuttle cabin atmosphere (~101 kPa total pressure), CO_2 concentrations typically range between 4000 and 6000 µmol mol^{-1}, but concentrations can exceed 10,000 µmol mol^{-1} with large crews (Wheeler et al. 1999). Thus, the CO_2 levels for advanced life support systems may be at super-elevated levels, and CO_2 may be used as a pressurizing gas for growing plants on Moon or Mars (Wheeler et al. 2001). Similar situations exist on the Russian Mir space station and will probably occur on the planned International Space Station (Mitchell et al. 1994). If plant research is to be carried out in these settings, and if plants are ultimately used to provide food, O_2, clean water, and CO_2 removal for long duration missions and bioregenerative life support system (BLSS) (Dong et al. 2014), the effects of super-elevated CO_2 on plants will need to be understood.

In general, these studies have shown that increases in CO_2 (e.g., a doubling from 350 to 700 µmol mol^{-1}) increase photosynthetic rates for C3 species and decrease water use per unit area of vegetation for C_3 and C_4 species (Högy and Fangmeier 2008; Allen et al. 2011). The present atmospheric [CO_2] limits growth of C_3 crop plants, which show responses to elevated [CO_2] via reduced photorespiration and enhanced photosynthetic rates, thereby increasing their growth and yield. The extensive literature on the impacts of elevated CO_2 demonstrates both positive and negative photosynthetic responses in different groups of higher plants, could be attributed to differences in experimental technologies, plant species used for the experiments, age of the plant as well as duration of the treatment (Sage 2002; Davey et al. 2006; Reddy et al. 2010). During photosynthesis, plants transpire large quantities of water to the atmosphere in exchange for CO_2 acquisition (Kocacinar 2014). Therefore, there should be a balance in plants for this exchange under each specific environmental niche (Tyree and Zimmermann 2002; Sperry 2003).

Though the C_3 pathway of photosynthesis dominates most terrestrial ecosystems, another pathway, C_4, is important in certain agricultural and natural systems. The C_4 pathway is common among species native to tropical and subtropical grasslands, and important crop species such as corn, sorghum, sugar cane, and pasture grasses possess C_4 photosynthesis. In general, these studies have shown that increases in CO_2 (e.g., a doubling from 350 to 700 µmol mol^{-1}) increase photosynthetic rates for C_3 species and decrease water use per unit area of vegetation for C_3 and C_4 species (Drake et al. 1997). C_4 differs from C_3 photosynthesis in several important biochemical and physiological properties. In both types, rubisco fixes CO_2 into the photosynthetic carbon reduction pathway common to all aerobic photosynthetic organisms, but the rubisco reaction is compartmented differently (Collatz et al. 1992). In C_3 photosynthesis, CO_2 fixed by rubisco is obtained directly from the intercellular spaces of the leaf by diffusion, whereas in C_4 plants, CO_2 is delivered to rubisco, which is located in the bundle sheath chloroplasts, by a metabolic pump that concentrates CO_2 (Terashima et al. 2001). The elevated CO_2 concentrations maintained in the bundle sheath cells at the cost of additional ATP have the benefit of inhibiting photorespiration. Consequently, C_4 plants lack several features of C_3 plants that are associated with photorespiration (Collatz et al. 1992). In addition, the kinetics of the photosynthetic CO_2 response also differ, because for C_4 metabolism, the initial CO_2 fixation is via a more efficient catalyst, PEP carboxylase (Drake et al. 1997). These differences in function and compartmentation result in differing sensitivities of net photosynthesis to environmental conditions such as temperature, CO_2 and O_2 concentrations, light intensity, and nitrogen availability. Generally, these differences tend to favor C_4 plants over C_3 plants at high temperatures where photorespiration is stimulated.

The Permanent Astrobase life-support artificial closed ecosystem ground-based integrative experimental Facility of Lunar Palace 1 is an ideal site for experiments with agricultural crops under different elevated CO_2, offering excellent environmental controls, and at the same time providing a growing environment that, because of the size of both its planting area and soil depth, is closer to mimicking field conditions than any other existing growth chamber. The system can produce water and fertilizer, process waste, and revitalize air. To develop decision support tools for growing plants on the Moon, Mars, or artificial closed ecosystem (Blüm et al. 1994), more studies on the effects of super-elevated [CO_2] on different species plants are required under the environmental conditions of human involved expected in space agriculture. However, to date, not much attention has been paid to the study about the mechanisms by which gas exchange and photosynthetic efficiency of PSII respond to long-term super-elevated [CO_2] between C_3 and C_4 plant in controlled environments for developing a safe, nutritious diet for BLSS. In particular, the effects of elevated CO_2 on the photosynthesis and transpiration rates between C_3 and C_4 plant in artificial closed ecosystem are unclear.

In this study, our objective was to investigate the photosynthetic pigment, gas changes, photosynthetic efficiency of PSII, and water use efficiency of a typical C_3 plant by soybean (*Glycine max* (L.) Merr., 'Heihe35') and maize (*Zea mays* L., 'Susheng') of C_4 plants under a range of CO_2 concentrations (500, 1000, 3000 and 5000 μmol mol^{-1}) in controlled environmental conditions of Lunar Palace 1 and reveal plausible underlying mechanisms for the differential physiological and developmental responses to elevated [CO_2] between C_3 and C_4 plants.

Materials and methods

Plant materials

Seeds of soybean (*Glycine max* (L.) Merr., 'Heihe35') and maize (*Zea mays* L., 'Susheng') provided by Heilongjiang Academy of Agricultural Sciences and China Agricultural University, respectively, were planted in stainless steel sinks containing a growth media of vermiculite. All seeds were screened for size, weight, uniformity, and lack of defects and subjected to the germination method by Fernandez-Orozco et al. (2008). The maize is a fast flowering mini variety. The height is only 55 ± 5.2 cm. Seeds were surface-sterilized by soaking in 80 mM hydrogen peroxide (H_2O_2) for 6 h, then rinsed five times in sterile distilled water (ddH_2O), and blot dried (He and Gao 2009). The sterilized seeds were preserved in the dark (Temp. 20 °C, R.H. 85 %). The planting density of was 120 and 80 plants per m^2 for soybean and maize, respectively.

Environmental conditions

Light systems were provided with mixtures of red plus white LEDs (R:W = 1:1) at a photosynthetic photon flux (PPF) of 500 ± 20 umol m^{-2} s^{-1} at the top of canopy, according to a light/dark regime of 12/12 h (light time from 8:00 a.m. to 8:00 p.m.) for both soybean and maize. Temperature (T) regime was established at 26/22 °C, (light/dark) and relative humidity (RH) in the light time was kept within the optimal range of 65–75 % for all treatments. A modified half-strength Hoagland solution (Table 1) was used as nutrient solution (Hoagland and Arnon 1950). The EC and pH were kept at 2.0 dS m^{-1} and 5.5 respectively (Wheeler et al. 2008). The air change and dehumidification were guaranteed by axial flow fans and dehumidifiers.

Experimental design and cultural procedures

Lunar Palace 1 could provide a largely closed environment for crop growth tests for BLSS and supply astronauts with basic living requirements. Oxygen, water, and food regenerate through biotechnology, making it possible for astronauts to live in space for long periods. Plants were cultivated in batches every 10 days in the stainless steel pots, which were equipped with the porous-tube nutrient delivery system (PTNDS) implementing water supply on demand (Hoehn et al. 2003) under controlled environmental conditions of Lunar Palace 1. At the flowering stages of plants, 500 ± 50, 1000 ± 50, 3000 ± 50, and 5000 ± 50 μmol mol^{-1} [CO_2] were used in the plant cabin at least 7 days. On the seventh day, all characteristics were analyzed. Both soybean and maize under different treatments of elevated [CO_2] (500–5000 ppm) were used in the cabin under 500 ppm [CO_2] before flowering stages of plants.

Photosynthetic pigment

The content of Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*), Carotenoid (Car), and the total Chlorophyll (Chl tot) were detected by ultraviolet spectrophotometer (SP-75, Shanghai spectrum instruments co., LTD, China) (Mackinney 1941). Chlorophyll ratio (Chl *a*/*b*) is the ratio of Chl *a*–Chl *b*. Samples were frozen in liquid nitrogen and stored at −80 °C until use.

Table 1 Nutrient compositions

Compositions	Concentration (mg/L)
$Ca(NO_3)_2 \cdot 4H_2O$	236
KNO_3	404
$(NH_4)_2HPO_4$	57
$MgSO_4 \cdot 7H_2O$	123
EDTA-2NaFe	20
H_3BO_3	2.86
$MnSO_4 \cdot 4H_2O$	2.13
$ZnSO_4 \cdot 7H_2O$	0.22
$CuSO_4 \cdot 5H_2O$	0.08
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.02

Gas exchange

Carbon dioxide gas exchange rates were measured on attached fully expanded third leaves after 7 days of elevated CO_2 treatment, using the LI-COR portable infrared CO_2 gas analyzer (LI-6400 XRT portable photosynthesis system, LI-COR Biosciences, Lincoln, NE, USA). Block temperature was kept at 25 °C during all measurements (registered leaf temperatures ranging 24–28 °C) and vapor pressure deficit (VPD) at around 1.5 kPa. Measurements were made at a photosynthetic photon flux density (PPFD) of 500 μmol m^{-2} s^{-1}, as provided by a Q-Beam (blue and red diode) light source. Given the small area (2 cm^2) of the leaf cuvette, the flow rate was adjusted to 150 mmol air min^{-1} to ensure that CO_2 differentials between the reference and the sample. IRGAs were >4 μmol mol^{-1} air. Leaf gas exchange parameters were performed to determine their net photosynthesis (A), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transient transpiration rate (Tr) under different elevated CO_2 concentrations. Water use efficiency (WUE) is the ratio of photosynthetic capacity to transpiration. The intrinsic water use efficiency (IWUE) was calculated by dividing A by Gs, and the instantaneous carboxylation efficiency (ICE) was calculated by dividing A by Ci (Pérez-López et al. 2013; Silva et al. 2010).

Chlorophyll fluorescence

After 7 days of elevated CO_2 treatment, Chlorophyll fluorescence measurements were collected using LI-6400 portable photosynthesis systems with 6400-40 Leaf Chamber Fluorometers (LI-COR Inc., Lincoln, NE, USA) on fully expanded leaves. All daytime measurements were carried out between 10:00 and 19:00 h. On the same night after the daytime measurements, fluorescence characteristics F_0 and F_m were measured between 02:00 and 04:00 h to obtain minimum and maximum fluorescence yields of fully relaxed samples. After the dark adaptation, minimal fluorescence (F_0) was measured with weak modulated irradiation (0.1 μmol m^{-2} s^{-1}), and a pulse (800 ms) of saturating light (7000 μmol photons m^{-2} s^{-1}) was applied to measure the maximum fluorescence yield (F_m) and to calculate F_v/F_m. The fluorescence parameters F_s and F_m' were determined as previously described (Baker and Rosenqvist 2004). F_s is the steady fluorescence under light-adapted state (red-blue actinic beams, 500 μmol m^{-2} s^{-1}). Revised parts are marked in yellow in the manuscript. The optimal photochemical efficiency of PSII (F_v/F_m) was calculated by dividing F_v by F_m, and the potential activity of PSII (F_v/F_o) was calculated by dividing F_v by F_o. The effective quantum yields of PSII (ΦPSII), photochemistry quenching (qP), nonphotochemistry quenching (qN), and photosynthetic electron transport (ETR) were calculated by the methods (Maxwell and Johnson 2000).

Statistical analysis

The measurements were replicated three times and randomly arranged in each block. Statistical analyses were conducted using Statistical Product and Service Solutions for Windows, version SPSS 20 (SPSS Inc., Chicago, Illinois). The data were analyzed using analysis of variance (ANOVA), and the differences between the means were tested using Duncan's multiple range test ($P < 0.05$).

Results and discussion

Photosynthetic pigment

The analysis of the chlorophyll content in different elevated [CO_2] showed that the chlorophyll patterns varied considerably between C_3 and C_4 plants. Elevated [CO_2] from 500 to 1000 ppm did have a significant effect on the photosynthetic pigment of the leaves by soybean, a typical C_3 plant, but no significance for maize, which is a typical C_4 plant (Fig. 1). The contents of Chl *a*, Chl *b*, Car, and Chl tot by the C_4 plants compared to that of the C_3 plants are very high at 500 ppm [CO_2], but no significance under 1000 and 3000 ppm [CO_2] except Chl *b* under 3000 ppm (Fig. 1a). A higher chlorophyll content in C_4 plant may be an energy utilization mechanism that would protect the photosynthetic apparatus against excess CO_2. Our results also suggest that high CO_2 levels may not always induce significant differences between C_3 and C_4 plant even when exposed to super-elevated CO_2 as shown in this study. The content of photosynthetic pigment including Chl a, Chl b, and Car and Chl tot in both soybean and maize leaves were reduced drastically under 5000 ppm [CO_2] (Fig. 1a–d), but the Chl *a*/*b* was not changed for C_3 species(Fig. 1e). The value reducing of Chl *b* of C_4 species was greater than that of chlorophyll *a* under 3000 and 5000 ppm [CO_2], thus the Chl *a*/*b* increased (Fig. 1a, b, e). The leaves of maize under 3000 ppm [CO_2] showed a progressively lower chlorophyll contents indicating a faster senescence of leaves that increased during the late growth period (Bindi et al. 2002). Photosynthetic pigment by the C_3 plants was more sensitive to elevated [CO_2] below 3000 μmol mol^{-1} than the C_4 plants (Fig. 1), indicating that higher CO_2 levels did not induced increased susceptibility of photosynthetic pigment composition for C_4 plants. Thus, senescence typically also involves cessation of photosynthesis and degeneration of cellular structures, with strong losses of chlorophyll under super-elevated [CO_2]. Our results show that super-elevated

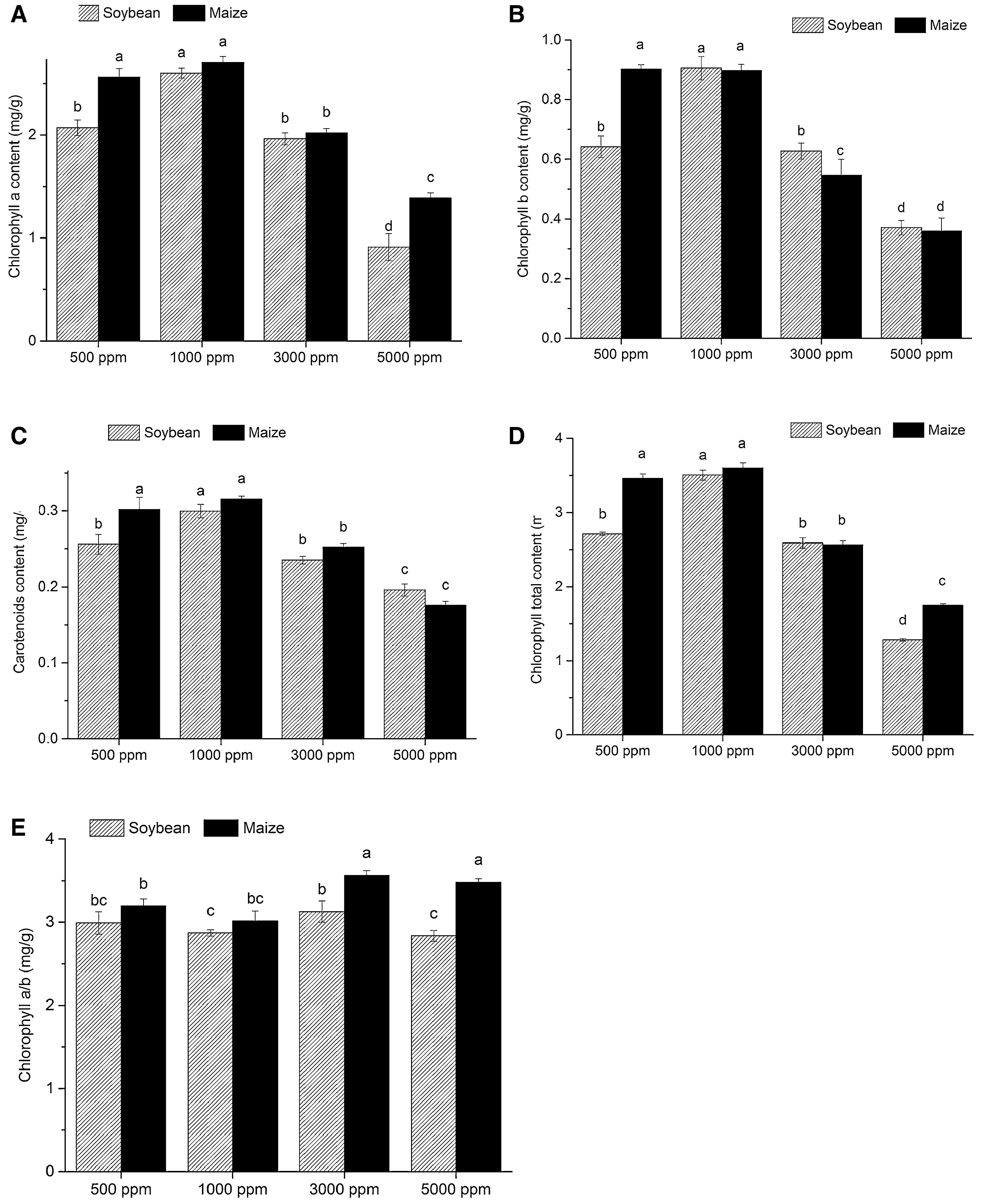


Fig. 1 Response of chlorophyll *a* (Chl *a*, **a**), chlorophyll *b* (Chl *b*, **b**), chlorophyll carotenoid (Car, **c**), total chlorophyll content (Chl tot, **d**), and chlorophyll ratio (Chl *a*/*b*, **e**) of C_3 and C_4 plants to different elevated [CO_2] conditions. *Vertical bars* are mean ± SD. Different letters in columns indicate statistically significant differences ($P < 0.05$)

CO_2 concentration of 5000 ppm may prompt loss of chlorophyll from the leaves for both soybean and maize which may accelerate the aging process. The negative CO_2 effect on chlorophyll content might be explained with the hypothesis that super-elevated [CO_2] induced a higher starch accumulation that disrupts chloroplasts and increases chlorophyll degradation or decreases chlorophyll synthesis (Delucia et al. 1985). Other hypotheses, however, cannot be excluded, such as that where CO_2 induced limitation of transpiration might cause decreased in N uptake (Conroy and Hocking 1993) which could contribute to chlorophyll loss. Shangguan et al. (2000) also reported reduction in total chlorophyll content due to nitrogen deficiency. During the process of chlorophyll degradation, Chl *b* is converted in Chl *a* (Fang et al. 1998) and this may explain the increase of the ratio Chl *a*/*b* in 3000–5000 ppm stressed maize leaves together with the depression of chlorophyll content.

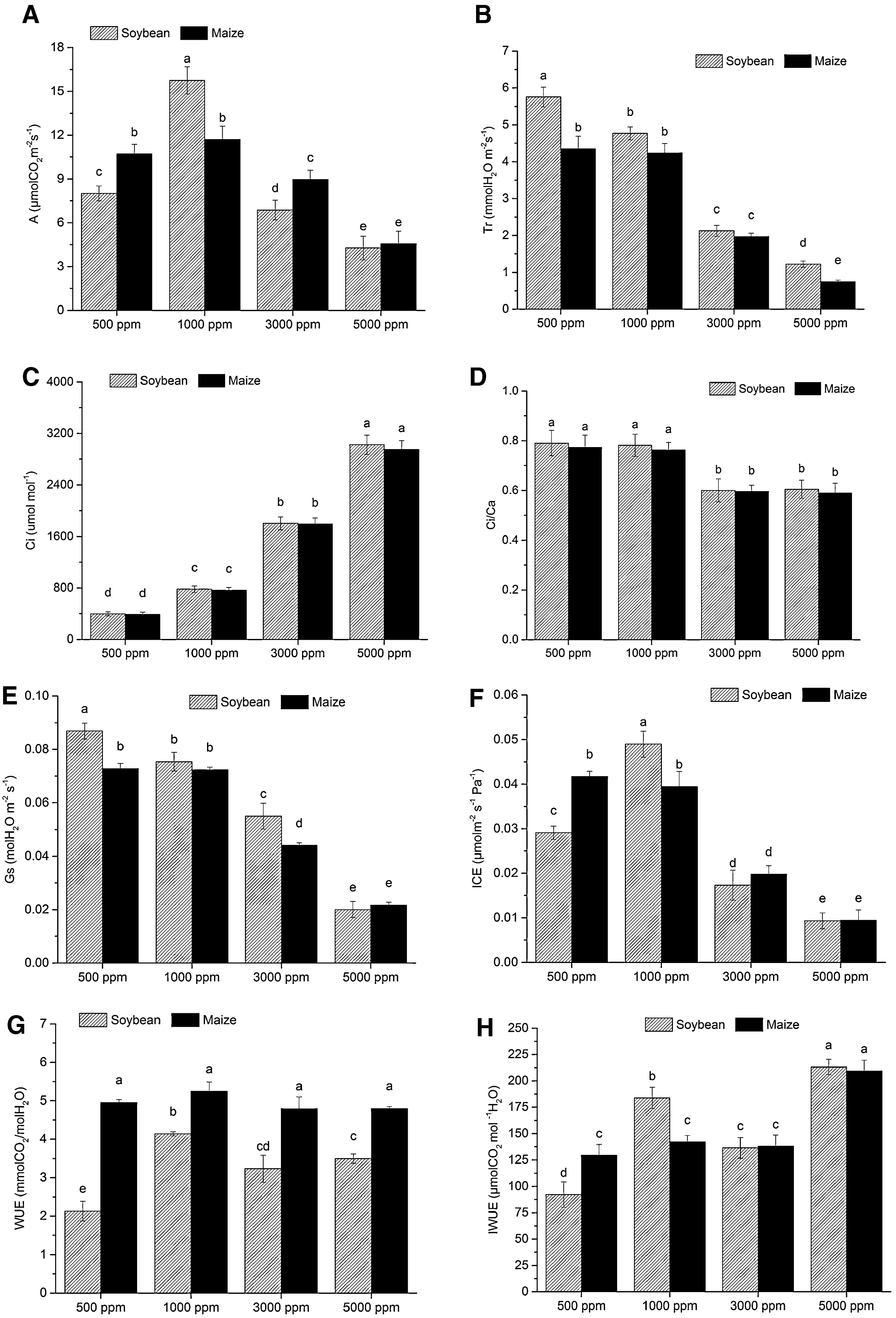
Gas exchange

The gas exchange parameters were measured in the various C_3 and C_4 species under the growth conditions at 7 days after the CO_2 enrichment for comparing their carbon assimilation efficiencies. As shown in Fig. 2, all gas exchange parameters except Ci/Ca showed significant differences between soybean and maize. The photosynthesis rate (A) of soybean which is typical of a C_3 plant showed a steep initial slope in response to varying intercellular CO_2 concentration (Ci) and saturated at high 1000 ppm CO_2 concentrations (Fig. 2a, b). A of C_3 plants increased by 96.8 % at 1000 ppm [CO_2], but decreased by 14.1 % at 3000 ppm and 46.7 % at 5000 ppm compared to the values detected under 500 ppm. While photosynthesis in C_3 species is stimulated by CO_2 concentrations above current ambient levels, it may exhibit photosynthetic acclimation when grown under long-term CO_2 enrichment, that is, reduced photosynthetic capacity under saturating light (Morgan et al. 1994). However, [CO_2] ranged from 500 to 1000 ppm, there was no significant change of A for C_4 plants of maize (Fig. 2a). The C_3 species had the highest A at 1000 ppm, but C_4 plants exhibited essentially no effect of A and Tr at 1000 ppm, presumably due to the CO_2 concentration mechanism of the C_4 pathway of photosynthesis. The effects of increased CO_2 have been attributed to the assumption that the inherent CO_2-concentrating mechanism in C_4 plants renders these plants insensitive to elevated CO_2 atmosphere (Reddy et al. 2010). Results from previous research with C_4 plants demonstrate that C_4 photosynthesis is a carbon-concentrating mechanism that evolved from C_3 progenitors to many times (Sage et al. 2012), due to a coordinated series of anatomical and biochemical adjustments, established the compartmentation and enzyme activities required to efficiently concentrate CO_2 around Rubisco (Monson and Rawsthorne 2000). Furthermore, the C_3 and C_4 showed approximately 1.6 and 2.7 times lower photosynthesis rate, respectively, at 5000 than 500 ppm (Fig. 2a). A decreased photosynthetic capacity or down-regulation of photosynthesis has been observed in super-elevated CO_2 enrichment studies and is termed acclimation (Levine et al. 2008). Based upon findings from elevated [CO_2], accelerated senescence has been proposed to explain this phenomenon (Miller et al. 1997; Ludewig and Sonnewald 2000).The mechanism underlying this acclimation is still very elusive. In addition, higher chlorophyll content is suggested to be an adaptation of the plants under elevated CO_2 to increase the photosynthetic activity (Bhatt et al. 2010). Besides, under super-elevated [CO_2] 5000 ppm by soybean, the CO_2 assimilation rate decreased about 40 % of that in 500 ppm [CO_2]. The results indicated that lower CO_2 assimilation rates could potentially be due either to biochemical photosynthetic capacity or diffusional limitations, the latter involving both stomatal and mesophyll conductance to CO_2 (Hanba et al. 2001; Grassi and Magnani 2005).

Fig. 2 Response of photosynthetic rate (A, **a**), transpiration rate (Tr, **B**), intercellular carbon dioxide concentration (Ci, **c**), Ci/Ca ratio (Ci/Ca, **d**), stomatal conductance (Gs, **e**), instantaneous carboxylation efficiency (ICE, **f**), water use efficiency (WUE, **g**), and intrinsic water use efficiency (IWUE, **h**) of C_3 and C_4 plants to different elevated [CO_2] conditions. Vertical bars are mean ± SD. *Different letters* in columns indicate statistically significant differences ($P < 0.05$) ▶

Elevated [CO_2] ranged from 1000 to 5000 ppm caused a larger increase of Ci and a greater reduction of transpiration rate (Tr) in both C_3 and C_4 species (Fig. 2b, c). In contrast, CO_2 enrichment of 3000 and 5000 ppm decreased Ci/Ca ratio in soybean and maize compared to the values under 500 and 1000 ppm (Fig. 2d). The decreased A and the increased Ci under elevated [CO_2] may indicate the non-stomatal limitation of photosynthesis. However,Meinzer et al. (1994) and Saliendra et al. (1996) found a Ci/Ca ratio relatively constant under adverse environmental conditions for sugarcane.

C_3 and C_4 plants exhibit significant differences in stomatal conductance (Gs) under 500 and 3000 ppm. There is no apparent inhibition of Gs by maize at 1000 ppm [CO_2], but Gs decreased greatly above 3000 ppm for both C_3 and C_4 species. C_4 plants in general have lower Gs than C_3 plants (Fig. 2e). The C_4 maize had significantly higher water use efficiencies (WUE) than the C_3 soybean under all elevated [CO_2]. At [CO_2] of 500 ppm, WUE was about 2.1 times greater in the C_4 than the C_3 species, but the tendency gradually decreased as [CO_2] increased up to 5000 ppm (Fig. 2g). While CO_2-induced stomatal closure


A
Soybean
Maize
A (μmolCO2m-2s-1)
0
3
6
9
12
15
18
c
b
a
b
d
c
e
e
500 ppm
1000 ppm
3000 ppm
5000 ppm
B
Soybean
Maize
Tr (mmolH2O m-2s-1)
0
1
2
3
4
5
6
7
a
b
b
b
c
c
d
e
500 ppm
1000 ppm
3000 ppm
5000 ppm
C
Soybean
Maize
Ci (μmol mol-1)
0
800
1600
2400
3200
4000
d
d
c
c
b
b
a
a
500 ppm
1000 ppm
3000 ppm
5000 ppm
D
Soybean
Maize
Ci/Ca
0.0
0.2
0.4
0.6
0.8
1.0
a
a
a
a
b
b
b
b
500 ppm
1000 ppm
3000 ppm
5000 ppm
E
Soybean
Maize
Gs (molH2O m-2 s-1)
0.00
0.02
0.04
0.06
0.08
0.10
a
b
b
b
c
d
e
e
500 ppm
1000 ppm
3000 ppm
5000 ppm
F
Soybean
Maize
ICE (μmolm-2 s-1 Pa-1)
0.00
0.01
0.02
0.03
0.04
0.05
0.06
c
b
a
b
d
d
e
e
500 ppm
1000 ppm
3000 ppm
5000 ppm
G
Soybean
Maize
WUE (mmolCO2/molH2O)
0
1
2
3
4
5
6
7
e
a
b
a
cd
a
c
a
500 ppm
1000 ppm
3000 ppm
5000 ppm
H
Soybean
Maize
IWUE (μmolCO2 mol-1H2O)
0
25
50
75
100
125
150
175
200
225
250
d
c
b
c
c
c
a
a
500 ppm
1000 ppm
3000 ppm
5000 ppm

and improved water relations, with no doubt, contribute significantly to the enhanced growth of C_4 species under CO_2 enrichment, the possible direct benefits on CO_2 fixation should not be discounted. This increased transpiration was found to be associated with increased Gs during both the light and dark cycles (Wheeler et al. 1999). WUE is known to be affected by atmospheric CO_2 concentration. Under elevated CO_2, Gs in most species would decrease, resulting in less transpiration per unit leaf area. Lower Gs coupled with higher photosynthetic capacity in leaves of C_4 plants under ambient $[CO_2]$ results in higher water use efficiencies in comparison to leaves of C_3 plants (Osborne and Sack 2012). Whereas photosynthesis and stomatal movement are physiological processes that occur within leaves but whose influence on CO_2, water vapor, and sensible heat fluxes extends to canopy (Collatz et al. 1992). Moreover, plants grown under 500 ppm had higher Tr than those grown under elevated CO_2 1000–5000 ppm, clearly the same of what would be expected (Jarvis and Davies 1998).

Instantaneous carboxylation efficiency (ICE) and intrinsic water use efficiency (IWUE) by soybean which is a typical C_3 plant showed a steep initial slope in response to 1000 ppm $[CO_2]$ (Fig. 2f, h). As a result of reduced Gs or enhanced A, an increase in IWUE was observed when plants were exposed to atmospheric CO_2 enrichment. Elevated CO_2 typically increases the rate of photosynthesis in many C_3 species by increasing the intercellular CO_2 concentration, enhancing the carboxylation efficiency, and reducing photorespiration (Robredo et al. 2007). In contrast, ICE and IWUE at 1000 ppm by C_4 maize responded slower to $[CO_2]$ compared to soybean (Fig. 2f, h). Additionally, ICEs in C_3 soybean and C_4 maize were declined fiercely by 200.53 and 89.4 %, respectively, at 3000 ppm compared to the values detected under 1000 ppm (Fig. 2f). When $[CO_2]$ was raised to 5000 ppm, IWUE increased in both C_3 and C_4 species, leading to approximately 131.4 and 62.1 % greater values at 500 ppm, respectively. However, $[CO_2]$ ranged from 500 to 3000 ppm, and IWUE had no significant change in maize (Fig. 2h). IWUE was higher in the C_3 and C_4 under 5000 ppm (Fig. 2h) that may be explained by the lowest Gs (Fig. 2e). Lower Gs is sometimes related to a drop in leaf water content (Robredo et al. 2007). In line with our findings, many articles have reported increases in IWUE ranging from 25 to 230 % as a consequence of both reduced stomatal conductance and enhanced photosynthesis (e.g., Drake et al. 1997; Saxe et al. 1998).

Chlorophyll fluorescence

Indeed, optimal photochemical efficiency of PSII (F_v/F_m) determined on the youngest fully expanded leaves was not changed. Elevated $[CO_2]$ did not significantly affected optimal photochemical efficiency of PSII (F_v/F_m) between soybean and maize ($P > 0.05$), whereas F_v/F_m was significantly higher in maize at lower 500 ppm $[CO_2]$ than in soybean. However, F_v/F_m decreased significantly with super-elevated $[CO_2]$ in both crops (Fig. 3a). The potential activity of PSII (F_v/F_o) for C_3 and C_4 plants leaves were significantly affected by elevated $[CO_2]$ (Fig. 3b). F_v/F_o was significantly lower at 500 than 1000 ppm $[CO_2]$ in both soybean and maize leaves, but declined fiercely at 3000 and 5000 ppm (Fig. 3b). The efficiency and stability of PS II, a major component of the photosynthetic apparatus, have been widely monitored through the measurement of fluorescence of PS II (F_v/F_m) in dark-adapted leaves (Singh and Agrawal 2014). In the present study, increase in F_v/F_m ratio under elevated $[CO_2]$ 1000 ppm by soybean indicates increased efficiency of PS II and also reduced risk of damage caused to PSII by oxidative stress. Zhao et al. (2010) also reported an increase in F_v/F_m ratio in *Betula platyphylla* exposed to 700 ppm CO_2.

The C_4 species *Z. mays* L. had the highest photosynthetic electron transport (ETR) and effective quantum yield of PSII (φPSII) under different $[CO_2]$ levels from 500 to 3000 ppm, and exhibited essentially no inhibition of ETR by 3000 ppm $[CO_2]$ (Fig. 3c). However, *Glycine max* (L.) had significantly higher ETR than C_4 *Z. mays* L. under 5000 ppm $[CO_2]$ (Fig. 3c). ETR and φPSII showed a steep initial slope in response to 1000 ppm $[CO_2]$ and a drastically decrease at 5000 ppm by both species $[CO_2]$ (Fig. 3c, d). These results suggest that photosynthesis was improved by elevated CO_2 concentration (1000 ppm) in both C_3 and C_4 plants, and such an improvement was associated with an improvement in stomatal conductance and the actual PSII efficiency. However, the results show that super-elevated $[CO_2]$ induced a decreasing φPSII for both C_3 and C_4 species, indicating that 5000 ppm $[CO_2]$ resulted in alterations in PSII photochemistry in the light-adapted leaves.

Under different elevated $[CO_2]$ levels, significant differences in photochemistry quenching (qP) and non-photochemical quenching (qN) were obvious for both C_3 and C_4 as compared with controls (500 ppm). The qP was similar under 3000 and 5000 ppm $[CO_2]$ between C_3 and C_4 species (Fig. 3e). Furthermore, the qP by both cereal crops increased significantly with increasing CO_2 up to 1000 ppm and then decreased substantially at 5000 ppm $[CO_2]$ (Fig. 3e). This suggests that Q_A, the primary electron acceptor of PS II, be more oxidized at super-elevated $[CO_2]$ despite the much lower rate of photosynthesis in this treatment. A ubiquitous type of regulation is the de-excitation of singlet-excited light-harvesting pigments, measured as qN of chlorophyll fluorescence, which results in the harmless thermal dissipation of absorbed light energy. The maize had high qP, whereas lower qN compared to

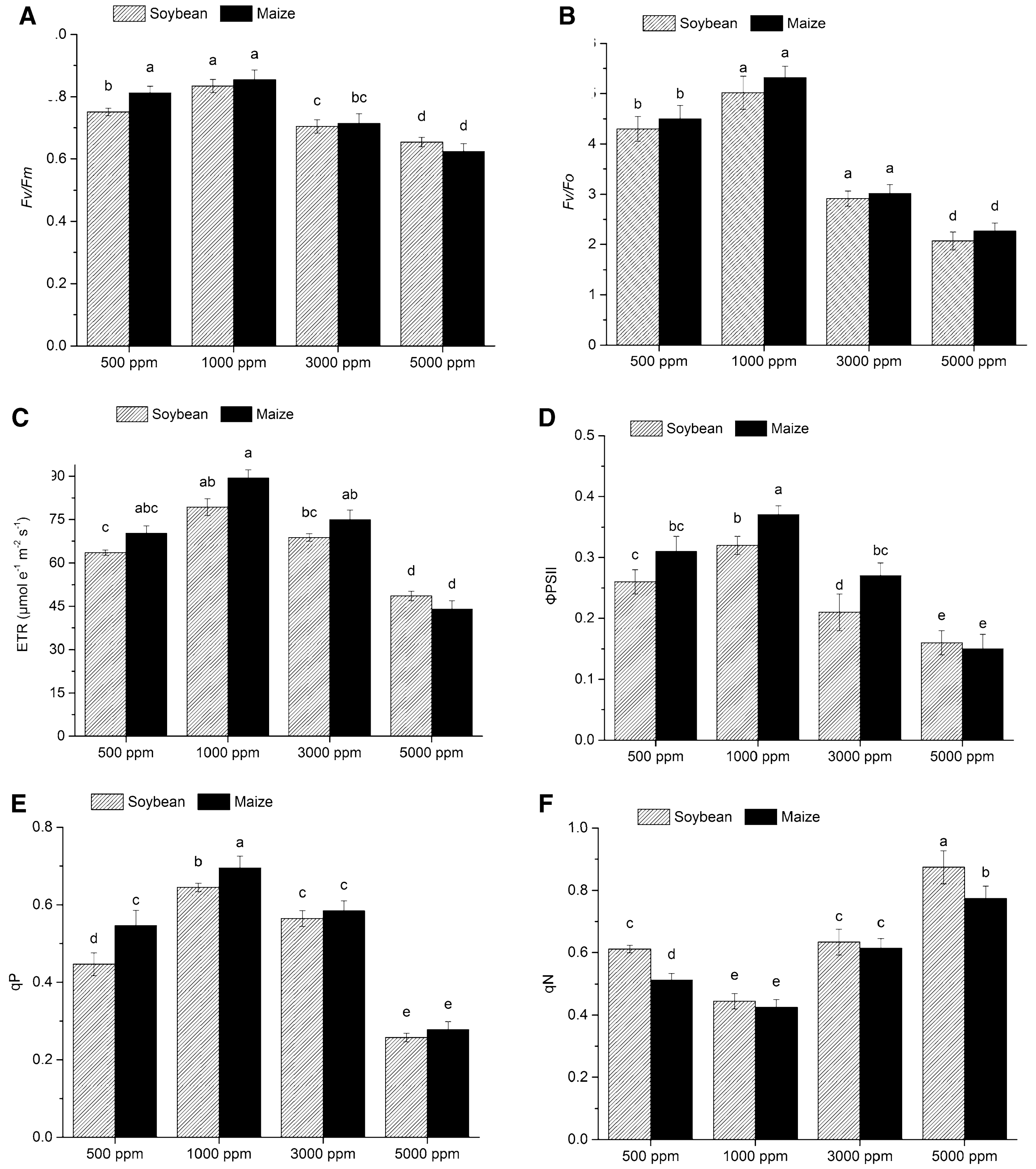


Fig. 3 Response of optimal photochemical efficiency of PSII (F_v/F_m, **a**), potential activity of PSII (F_v/F_o, **b**), photosynthetic electron transport (ETR, **c**), effective quantum yield of PSII (φPSII, **d**), photochemistry quenching (qP, **e**), and nonphotochemistry quenching (qN, **f**) of C_3 and C_4 plants to different elevated [CO_2] conditions. *Vertical bars* are mean ± SD. *Different letters* in columns indicate statistically significant differences ($P < 0.05$)

soybean under 500– 3000 ppm [CO_2]. *Glycine max* (L.) had significantly higher qN than C_4 *Z. mays* L. under 500 and 5000 ppm [CO_2]. (Figure 3e, f). CO_2 assimilation plays the role of a major sink for the reducing ATP and NADPH which generated by the primary photochemical reactions (Lu and Zhang 2000). When the rate of ATP and

NADPH synthesis exceeds the demand for CO_2 fixation, the regulation serves by a dissipation mechanism for excess excitation energy (Krause and Cornic 1987). Typically, qN is characterized by modulated pulse fluorometry and it is often assumed implicitly to be a good proxy for the actual physiological photoprotection capacity of the organism (Lambrev et al. 2012). We found that both C_3 and C_4 plants under 5000 ppm [CO_2] showed a lower capacity of photosynthesis and a lower rate of PSII photosynthetic electron transport compared with the plants under 500–3000 ppm [CO_2] as well as a corresponding increase in thermal energy of non-photochemical fluorescence quenching. The higher qN under higher [CO_2] indicates that a higher proportion of absorbed photons is lost as thermal energy instead of being used to drive photosynthesis (Shangguan et al. 2000) for regulating and protecting photosynthesis in the closed environments in which light energy absorption exceeds the capacity for light utilization.

In this study, measured F_v/F_m, ϕPSII, qP, and Pn values were somewhat lower in relation to values previously published for the same or closely related species probably due to differences in the growing conditions. We consider that the main reasons may be the following aspects: Firstly, the experiments were carried out in an artificial closed environment 'Lunar Palace 1.' Although it can offer environmental controls (including temperature, humility, light identity, etc.), the ventilation of plants cabin by air circulation fans was still not very good compared to the field. It influenced possibilities for crop production and product quality, and reduces the use of chemicals for plant protection (Mistriotis et al. 1997). Second, the traits of photosynthetic characters, nutrient uptake, and biomass yield in most plants in controlled environments were significantly different from the field plantings by the same cultivar (Dong et al. 2015; Collier and Huntington 1983; Patterson et al. 1977). The reasons for this difference in incidence between field and controlled environments are not completely understood, but it is believed to be related to plant growth rate and PSII efficiency (Collier and Huntington 1983). Besides, relative sensitivity of stomata to elevated [CO_2] has been described (Dong et al. 2015), and hence, the higher CO_2 endured during the seedling stage may have caused some influence on the Gs, leading to somewhat higher qN than expected.

Conclusion

Our results demonstrate that both soybean and maize were cultivated successfully in Lunar Palace 1, and they are the main food crop species which could provide vegetable protein, minerals, and fiber for the diet. In contrast to C_3 plants, chlorophyll content by the C_4 plants was not sensitive to [CO_2] under 1000 ppm, but highly sensitive to super-elevated [CO_2] (3000–5000 ppm) for both crops. Elevated [CO_2], ranging from 500 to 3000 ppm, in C_4 plants had the highest ETR, φPSII, and qP, but photosynthesis efficiency declined fiercely above 5000 ppm [CO_2] for both C_3 and C_4 plants. Therefore, photosynthetic down-regulation and a decrease in photosynthetic electron transport occurred by both species in response to super-elevated [CO_2] at 3000 and 5000 ppm. A major challenge would be to develop a whole plant for optimal acclimation responses for increasing atmospheric CO_2 concentrations and its interactions with various growth environments. Next we will conduct in-depth research on the high CO_2 on soybean and other seed crops by a complex combination of high CO_2 concentration and other environmental factors to optimize and use the space station source of high CO_2 and improve crop productivity and quality in BLSS. This study also provides technical personnel, a way to improve photosynthetic efficiency by increasing [CO_2], and thus to promote crop yields and quality for agricultural facilities (e.g., greenhouse and plant factory). Furthermore, it will play an essential reference to crop production and gas regulation of space technology in BLSS.

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