DOI: 10.1007/s11099-012-0043-5 PHOTOSYNTHETICA 50 (3): 362-370, 2012

Effects of fully open-air [CO_2] elevation on leaf ultrastructure, photosynthesis, and yield of two soybean cultivars

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Abstract

The objective of this study was to investigate the effect of elevated (550 ± 17 μmol mol^{-1}) CO_2 concentration ([CO_2]) on leaf ultrastructure, leaf photosynthesis and seed yield of two soybean cultivars [*Glycine max* (L.) Merr. cv. Zhonghuang 13 and cv. Zhonghuang 35] at the Free-Air Carbon dioxide Enrichment (FACE) experimental facility in North China. Photosynthetic acclimation occurred in soybean plants exposed to long-term elevated [CO_2] and varied with cultivars and developmental stages. Photosynthetic acclimation occurred at the beginning bloom (R1) stage for both cultivars, but at the beginning seed (R5) stage only for Zhonghuang 13. No photosynthetic acclimation occurred at the beginning pod (R3) stage for either cultivar. Elevated [CO_2] increased the number and size of starch grains in chloroplasts of the two cultivars. Soybean leaf senescence was accelerated under elevated [CO_2], determined by unclear chloroplast membrane and blurred grana layer at the beginning bloom (R1) stage. The different photosynthesis response to elevated [CO_2] between cultivars at the beginning seed (R5) contributed to the yield difference under elevated [CO_2]. Elevated [CO_2] significantly increased the yield of Zhonghuang 35 by 26% with the increased pod number of 31%, but not for Zhonghuang 13 without changes of pod number. We conclude that the occurrence of photosynthetic acclimation at the beginning seed (R5) stage for Zhonghuang 13 restricted the development of extra C sink under elevated [CO_2], thereby limiting the response to elevated [CO_2] for the seed yield of this cultivar.

Additional key words: free-air carbon dioxide enrichment (FACE); leaf ultrastructure; photosynthetic pigments; soybean cultivar; yield.

Introduction

Since around 1800, the atmospheric [CO_2] has risen from 280 μmol mol^{-1} to the current level of around 392 μmol mol^{-1}. It is predicted that the concentration will reach 550 μmol mol^{-1} by the middle of this century (Prentice *et al.* 2001). Elevated [CO_2] improves the photosynthetic efficiency of plants, which increases the supply of photoassimilates, dry mass and yield production (Drake *et al.* 1997, Long *et al.* 2004). Higher [CO_2] increases the carboxylation rate of Rubisco, and competitively inhibits the oxygenation of ribulose-1,5-bisphosphate (RuBP) (Drake *et al.* 1997) but these changes vary with crops, cultivars and their developmental stages.

This initial enhancement of photosysnthesis diminishes over time (days to years), termed as photosynthetic

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Received 6 August 2011, *accepted* 17 April 2012.

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Abbreviations: Chl – chlorophyll; C_i – intercellular CO_2 concentration; [CO_2] – atmospheric CO_2 concentration; FACE – free-air carbon dioxide enrichment; g_s – stomatal conductance; J_{max} – maximum rate of electron transport; P_N – net photosynthetic rate; V_{cmax} – maximum rate of carboxylation; Z13 – Zhonghuang 13; Z35 – Zhonghuang 35.

Acknowledgements: This work was supported by The China-UK SAIN fund project: Addressing vulnerabilities and building capacity for adaptation of agriculture to climate change in China , the National Basic Research Program of China (973 Program) (No.2012 CB955904), China CDM Fund project: The impact of climate change on Chinese agriculture and eco-systems under diferent RCP and Sino-Australia Cooperation on Climate Change: Impacts on wheat and soybean under mini-FACE foundation. The authors wish to thank Ji Gao , Xiaogang Dong and Zongpeng Yang for field assistance.

The first two authors contributed equally to this work.

acclimation (Long *et al.* 2004). As a consequence of acclimation, the CO_2 fertilization effect may be smaller than that would be predicted from instantaneous photosynthetic measurements (Ainsworth *et al.* 2005), although the response varies with plant functional groups and in response to environmental conditions (Ainsworth *et al.* 2005).

Soybean [*Glycine max* (L.) Merr.] is the world's most important seed legume, and a major source of protein and oil for human and livestock. The responses of soybean to elevated [CO_2] have been investigated extensively using enclosure studies. A meta-analysis of 111 of these studies showed that elevated [CO_2] (from 450 to 1,250 μmol mol^{-1}) increased the soybean leaf CO_2 assimilation rate by 39%, but decreased Rubisco activity by 11% (Ainsworth *et al.* 2002). However, these studies were all conducted in protected environments, either in laboratory or using field enclosures or open-top chambers (OTC). Above conditions could modify the microclimate and amplify the elevated [CO_2] effects on plant growth and development (McLeod and Long 1999, Long *et al.* 2004). The Soybean Free-Air CO_2 Enrichment (SoyFACE) facility (Rogers *et al.* 2004) was the first experimental setup to investigate the response of soybean to elevated [CO_2] under open-air conditions. FACE systems enable plants to be grown under natural conditions for long periods and have been used internationally at more than 30 sites (Ainsworth *et al.* 2008). Trees, crops or pastures are grown within rings with regulated supply of CO_2 fed into the experimental area, and the amount and position of CO_2 release controlled by sensors to maintain targeted CO_2 levels.

The leaf ultrastructure of plants could be improved under elevated [CO_2], and the amount of starch in chloroplasts and cytoplasmic lipids increased (Vu *et al.* 1989; Oksanen *et al.* 2001, Zuo *et al.* 2002) , which is another way to evaluate the changes of leaf carbonhydrate under elevated [CO_2]. Within the photosynthetically active radiation (PAR) region of the electromagnetic spectrum (400–700 nm) the reflectance of vegetation is controlled by the absorption characteristics of Chl *a* and *b*. These pigments provide energy for the reactions of photosynthesis, and the carotenoids, which protect the reaction centres from excess light and help them to intercept PAR as auxiliary pigments of Chl *a*. Therefore, changes in pigment concentrations relate strongly to the physiological status and productivity of a plant (Blackburn 1998, Strogonov 1973). Jiang *et al.* (2006), and Zhao *et al.* (2003) investigated the photosynthetic pigments basis for changes in soybeans leaf development at elevated [CO_2] in OTC, and showed that Chl *a*, Chl *b*, total Chl contents, and carotenoid contents in leaves were increased at elevated [CO_2]. This may improve photosynthetic capacity.

Photosynthetic acclimation is frequently associated with an accumulation of leaf nonstructural carbohydrates and a decrease in N concentration in the leaf and plant (Stitt and Krapp 1999, Nowak *et al.* 2004). In the SoyFACE experiment, non-nodulating soybeans showed downregulation of $V_{c,max}$ under elevated [CO_2], while nodulating varieties maintained the same photosynthetic capacity under ambient and elevated [CO_2] (Ainsworth *et al.* 2004).

The present research is the first study to report the effect of [CO_2] on leaf mesophyll cells ultrastructure and photosynthetic pigments of soybean under open-air conditions in a semiarid cropping system. Plant species and genotypes differ greatly in the response to elevated [CO_2] and environmental conditions. This study aims to address the following questions for response of soybean cultivars grown under elevated [CO_2]: (*1*) Is there any difference between the photosynthetic efficiency of cultivars in response to elevated [CO_2] in northern China? (*2*) Will the leaf mesophyll cells ultrastructure and photosynthetic pigments of soybean leaf be altered under elevated [CO_2] and correlated with changes in photosynthetic ability? (*3*) Will down-regulation of photosynthesis occur in soybean under elevated [CO_2] and what is its implication on soybean yield?

Materials and methods

Site description: The study was conducted at the China Mini-FACE facility located at an experimental station (winter wheat/soybean rotation) of Chinese Academy of Agricultural Sciences at Changping, Beijing (40.13°N, 116.14°E). The Mini-FACE facility consisted of 12 experiment areas, six elevated [CO_2] and six ambient [CO_2], each with a 4-m diameter octagonal plot. The carbon dioxide concentrations of six elevated and two of the six ambient [CO_2] rings were measured by sensors (*Vaisala*, Finland) at the centre of each octagonal plot and averaged 550 ± 17 μmol mol^{-1} and 415 ± 16 μmol mol^{-1}, respectively, during the experimental period. CO_2 exposure was given during day time only from 6:00 to 19:00 h. The plots were separated by at least 15 m, which was shown by monitoring ever and again to be sufficient to minimize the amount of cross-contamination of CO_2 between experimental areas. The soil is a clay loam with a pH (1:5, soil:water) of 8.4 and contained 1.1% of organic carbon (C) and 0.1% of total N. Fertilizers were applied at planting at rates of 4.8 kg(N) ha^{-1}, 7.2 kg(P) ha^{-1} and 37.3 kg(K) ha^{-1} which is standard regional agronomic practice for annual winter wheat/soybean rotation. The long-term average rainfall and temperature during the soybean growing season are 475 mm and 21.3°C, respectively.

Soybean cultivation, fertilization and irrigation: Two high-yielding cultivars of soybean [*Glycine max* (L.) Merr.] Zhonghuang 13 (Z13) and Zhonghuang 35 (Z35) bred by the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, were sown on 17 June 2009 in rows at 0.45 m spacing at a sowing density of 16 and 20 plants per m^2 for Z13 and Z35 respectively, under a randomized complete block design, with [CO_2] and cultivar as main effects and three replicates. Irrigation equivalent to 37.5 mm of rainfall was applied on 8 July 2009. Z13 is a semideterminate and high-protein cultivar while Z35 is more determinate and higher oil cultivar. The two cultivars were chosen because of their high yielding property and difference in seed quality.

Transmission electron microscopy: For anatomical and ultrastructural investigations, samples from the upper most fully-expanded sun leaves were collected from three replicate plants per exposure field on 30 July 2009 (between 10:00 and 12:00 h, at the R1 stage). Segments (5 mm) were cut 2 cm from the leaf apex, and placed immediately in a 5% (v/v) glutaraldehyde fixative (in 0.1 M phosphate buffer, pH 7.0). In the laboratory, 2 mm^2 pieces were cut from the strips under fixative solution using a razor blade. Leaf samples were post-fixed in 1% buffered OsO_4 solution, dehydrated with an ethanol series followed by a propylene oxide treatment, and then embedded in LX 122 Epon. Thin sections for electron microscopy were stained with lead citrate and uranyl acetate and were examined using an electron microscope (*JEM-2100F*, JEOL, Japan) operating at 80 kV (Oksanen *et al.* 2001).

Photosynthetic pigment content: Chl *a*, Chl *b*, and carotenoid content from the upper most fully-expanded leaves at 38 and 64 days after sowing (R1 and R3 stages) were determined according to the methods of Arnon (1949).

Gas-exchange measurements: Measurements of P_N *vs.* C_i were conducted at 44, 67, and 86 d after sowing for Z13 and 40, 66 and 85 d after sowing for Z35, corresponding to reproductive stages R1, R3, and R5, respectively. The plant height were about 28, 60, and 70 cm for Z13 and 32, 68, and 95 cm for Z35 at the corresponding reproductive stages, respectively. The number of leaves was 11, 18, and 20 for Z13 and 10, 15, and 19 for Z35, respectively. Gas-exchange measurements were made using portable gas-exchange systems (*LI-COR 6400; LI-COR*, Lincoln, NE, USA). One of the upper most fully-expanded leaves was randomly selected within the crop canopy. The [CO_2] in the leaf chamber was controlled by the *LI-COR* CO_2 injection system, and an irradiance of 1,600 μmol(photon) m^{-2} s^{-1} was supplied using an in-built LED lamp (red/blue). Temperature in the 6 cm^2 leaf chamber was set at 25ºC, and the actual temperature ranged from 25 to 28ºC. The vapour pressure deficit at the leaf surface was between 1.9 and 2.1 kPa. The [CO_2] surrounding the leaf for all control and treatment leaves was controlled across the series of 550, 390, 300, 200, 100, 50, 390, 550, 600, 700, 800; 1,000; 1,200; and 550 μmol mol^{-1}, and measurements were recorded after equilibrium was reached. Measurements were made between 9:00 and 14:00 h of the local time. Each individual curve took approximately 35 min to complete. Values for P_N and C_i were used to calculate V_{cmax} and J_{max} values using the model and software provided by Sharkey *et al.* (2007). P_N and g_s were also measured at the same irradiance, temperature and the vapour pressure when measurements of P_N *vs.* C_i were conducted. [CO_2] in the leaf chamber was set to 390 μmol mol^{-1} and 550 μmol mol^{-1} for each treatment, and three upper most fully-expanded leaves were measured per plot.

Harvesting: At maturity, soybean plants were hand-harvested on 6 October 2009 (111 d after sowing) from an area of 3 m^2 within each plot. Random subsamples of 10 plants from each subplot were taken to assess the number of seeds per plant and the mass of 100 seeds. After drying, the subsamples were mechanically threshed to separate seed from all other shoot components. The seeds were redried for 1–2 d and seed mass was determined. The mass and number of threshed seed was measured to calculate the number of seeds per plant and the mass of 100 seeds. Other plants were also threshed mechanically to separate seed from all other shoot components. Yield was determined for all the plants from the 3 m^2 patch in each plot.

Statistical analysis: All the experiment data presented were examined statistically by analysis of variance. Means of three replicates were subjected to the two-way analysis of variance at 0.05 probability level using *SAS System 8.1* (*SAS Institute Inc.*, USA).

Results

P_N and gas-exchange parameters: When photosynthesis was measured at the respective growth [CO_2], P_N of upper most fully-expanded leaves for Z13 was changed by –3.3, 23.0, and –9.1% under elevated [CO_2] at the R1, R3, and R5 stages, while P_N for Z35 was enhanced by 3.6, 17.3, and 18.9% under elevated [CO_2] at the R1, R3, and R5 stages, respectively (Table 1). The interactive effect between [CO_2], cultivar and growth stage on P_N was significant (Table 1). Meanwhile, g_s of the up most expanded leaves were reduced for both cultivars under elevated [CO_2] irrespective of developmental stage. Elevated [CO_2] reduced g_s of Z13 by 30.6, 30.0, and 32.7% at the R1, R3, and R5 stages, respectively, while reduced by 23.4 and 42.6% for Z35 at the R1 and R5 stages, respectively (Table 1).

However, when measurement of P_N in all treatments was taken at 390 μmol mol^{-1} [CO_2] (Fig. 1*A*,*C*), it was found that plants grown in elevated [CO_2] had lower

Table 1. P_N and g_s in the last fully-expanded leaves of soybean grown in ambient and FACE plots. Measurements were taken on their own growth [CO_2].Values are means ± SE of variables across the three replicates, 3 plants were taken in each plot, and the statistical significance level *P* values for the effects of [CO_2] treatment, cultivar, growth stage and their interaction. P_N – net photosynthetic rate; g_s – stomatal conductance. Z13 – Zhonghuang 13; Z35 – Zhonghuang 35. R1, R3, R5 – beginning bloom stage, beginning pod stage and beginning seed stage, respectively.

Growth stage	Cultivar	Growth [CO_2]	P_N [µmol(CO_2) m^{-2} s^{-1}]	g_s [mmol(H_2O) m^{-2} s^{-1}]
R1	Z13	ambient	17.75 ± 1.62	495.7 ± 6.8
		FACE	17.16 ± 1.30	344.6 ± 14.2
	Z35	ambient	18.40 ± 0.80	466.5 ± 17.8
		FACE	19.07 ± 0.50	361.0 ± 44.5
R3	Z13	ambient	14.80 ± 1.75	398.8 ± 15.5
		FACE	18.21 ± 1.17	392.7 ± 35.0
	Z35	ambient	15.83 ± 0.70	512.8 ± 13.8
		FACE	18.57 ± 1.30	298.4 ± 3.8
R5	Z13	ambient	21.40 ± 0.47	550.2 ± 22.6
		FACE	19.45 ± 0.26	366.3 ± 21.7
	Z35	ambient	18.50 ± 2.00	607.5 ± 29.8
		FACE	22.00 ± 0.16	346.3 ± 13.7
P values	CO_2		0.06	0.00
	Variety		0.31	0.91
	Growth stage		0.00	0.00
	CO_2 × variety		0.02	0.02
	CO_2 × growth stage		0.03	0.01
	Variety × growth stage		0.88	0.49
	CO_2 × variety × growth stage		0.04	0.00

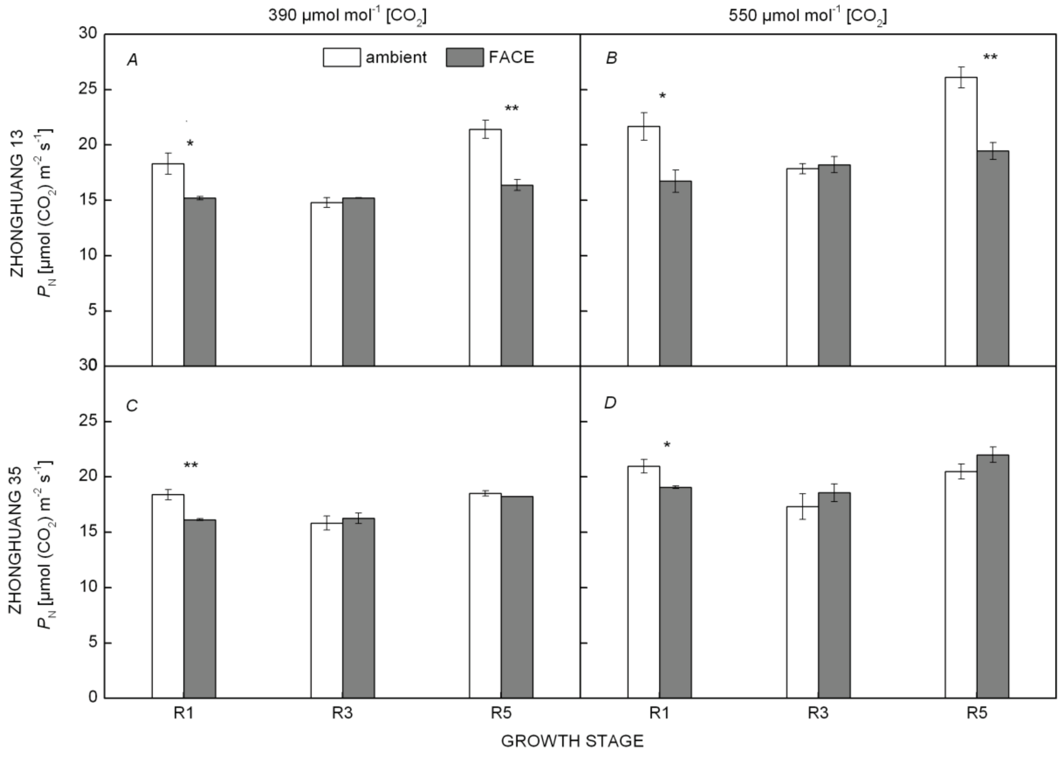

Fig. 1. P_N of up most fully expanded leaves of soybean grown in ambient and FACE plots. Measurements of Zhonghuang 13 and Zhonghuang 35 were made under 390 (*A*,*C*) and 550 µmol mol^{-1} [CO_2] (*B*,*D*). Values are the means of three plants for each of two [CO_2] treatments. Each bar represents the standard error of the difference between treatments, and is based on three replicates. Asterisks indicate significant differences between CO_2 treatments. * – $p \leq 0.05$; ** – $p \leq 0.01$. P_N – net photosynthetic rate. R1, R3, R5 – beginning bloom stage, beginning pod stage and beginning seed stage, respectively.

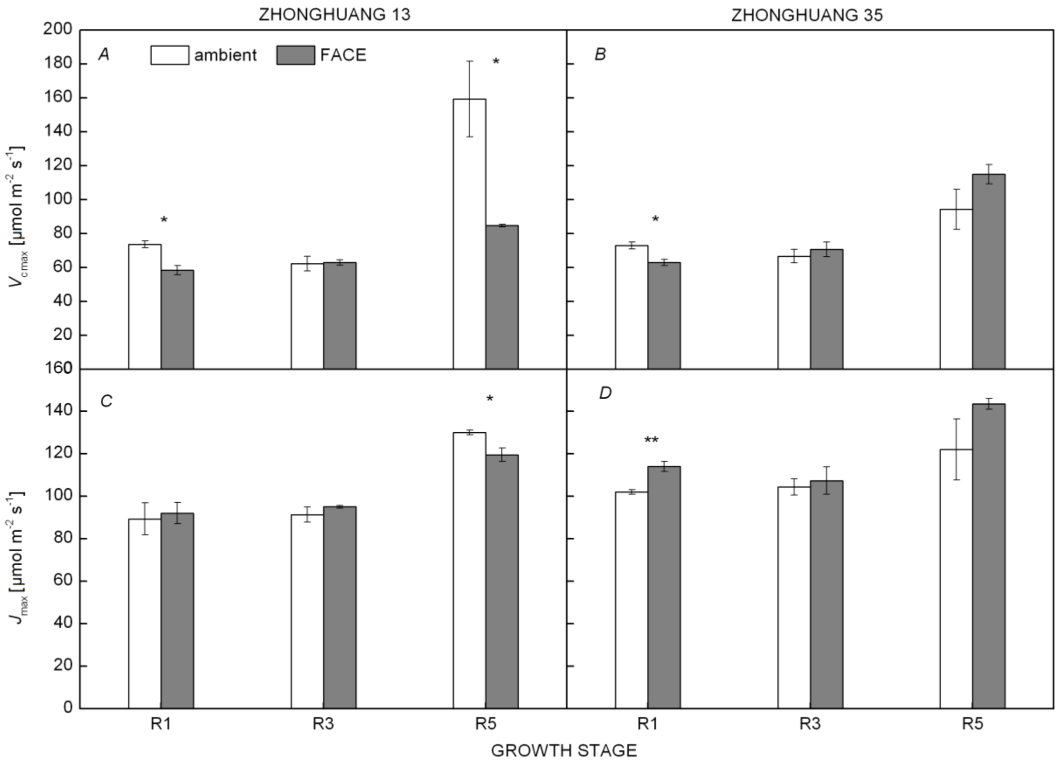

Fig. 2. CO_2 assimilation parameters in the last fully-expanded leaves of soybean grown in ambient and FACE plots. Values are means of three replicates. Each bar represents the standard error of the difference between treatment. Asterisks indicate significant differences between CO_2 treatments. * – $p \le 0.05$; ** – $p \le 0.01$. R1, R3, R5 – beginning bloom stage, beginning pod stage and beginning seed stage, respectively. J_{max} – maximum rate of electron transport; V_{cmax} – maximum rate of carboxylation.

photosynthetic activity than those grown in ambient [CO_2] irrespective of developmental stage. Elevated [CO_2] reduced the P_N by 12.57% for Z13 ($P<0.05$), while showed no significant effect on Z35 irrespective of developmental stage. When the photosynthetic activity was determined at 550 µmol mol^{-1} [CO_2] (Fig. 1*B*,*D*), elevated [CO_2] decreased P_N by 15.42% for Z13, while increased by 1.84% for Z35 irrespective of developmental stage ($P_{CO_2 \times \text{cultivar}} = 0.5$). The down regulation of photosynthetic activity under elevated [CO_2] varied with growth stages, the scale of changes interpreted as follows.

At the R1 stage: P_N of upper most fully-expanded leaves under elevated [CO_2] was significantly lower than those under ambient [CO_2] by 14.6% and 16.0%, when measured at the 390 µmol mol^{-1} and 550 µmol mol^{-1} for the two soybean cultivars, respectively (Fig. 1). Elevated [CO_2] signigicantly reduced V_{cmax} of the two soybean cultivars by 17.0% at the R1 stage (Fig. 2). The J_{max} values of the leaves for Z35 were significantly higher under elevated [CO_2] than ambient [CO_2] by 12% (Fig. 2). For Z13, the J_{max} values of leaves did not differ between ambient and elevated [CO_2] (Fig. 2). These changes were related to photosynthetic acclimation of the two cultivars soybean at the R1 stage.

At the R3 stage: elevated [CO_2] had no significant effect on leaves P_N when measured at 390 µmol mol^{-1} and 550 µmol mol^{-1}[CO_2] for both cultivars (Fig. 1). In addition, there was no significant difference in V_{cmax} and J_{max} of leaves between elevated and ambient [CO_2] (Fig. 2). No acclimation was observed in FACE leaves for the two soybean cultivars at the R3 stage.

At the R5 stage: when measured at 390 µmol mol^{-1} and 550 µmol mol^{-1} [CO_2], P_N of the upper most fully-expanded leaves remained unchanged for Z35 under elevated [CO_2] , but was significantly reduced by 23.5% and 25.5% for Z13 under elevated [CO_2], respectively (Fig. 1). V_{cmax} and J_{max} of leaves under elevated [CO_2] were significantly lower than ambient [CO_2] by 47.0% and 8.0%, respectively, for Z13 at the R5 stage, but was not affected for Z35 (Fig. 2). Photosynthetic acclimation occurred for Z13, but not for Z35 at the R5 stage.

Leaf mesophyll cells transmission electron microscopy: Elevated [CO_2] increased the number and size of starch grains in chloroplasts of the two cultivars (Fig. 3*A*,*B*,*D*,*E*. Fig. 3*G*,*H*,*J*,*K*). The structural chloroplast membrane and grana layer changed under elevated [CO_2], especially for Z35. Under elevated [CO_2], it was difficult to differentiate the chloroplast membrane and grana layer for Z35 (Fig. 3*H*,*I*,*K*,*L*). The structural chloroplast membrane and grana layer was intact for Z13 under elevated [CO_2], but not as clear as under ambient [CO_2] (Fig. 3*B*,*C*,*E*,*F*).

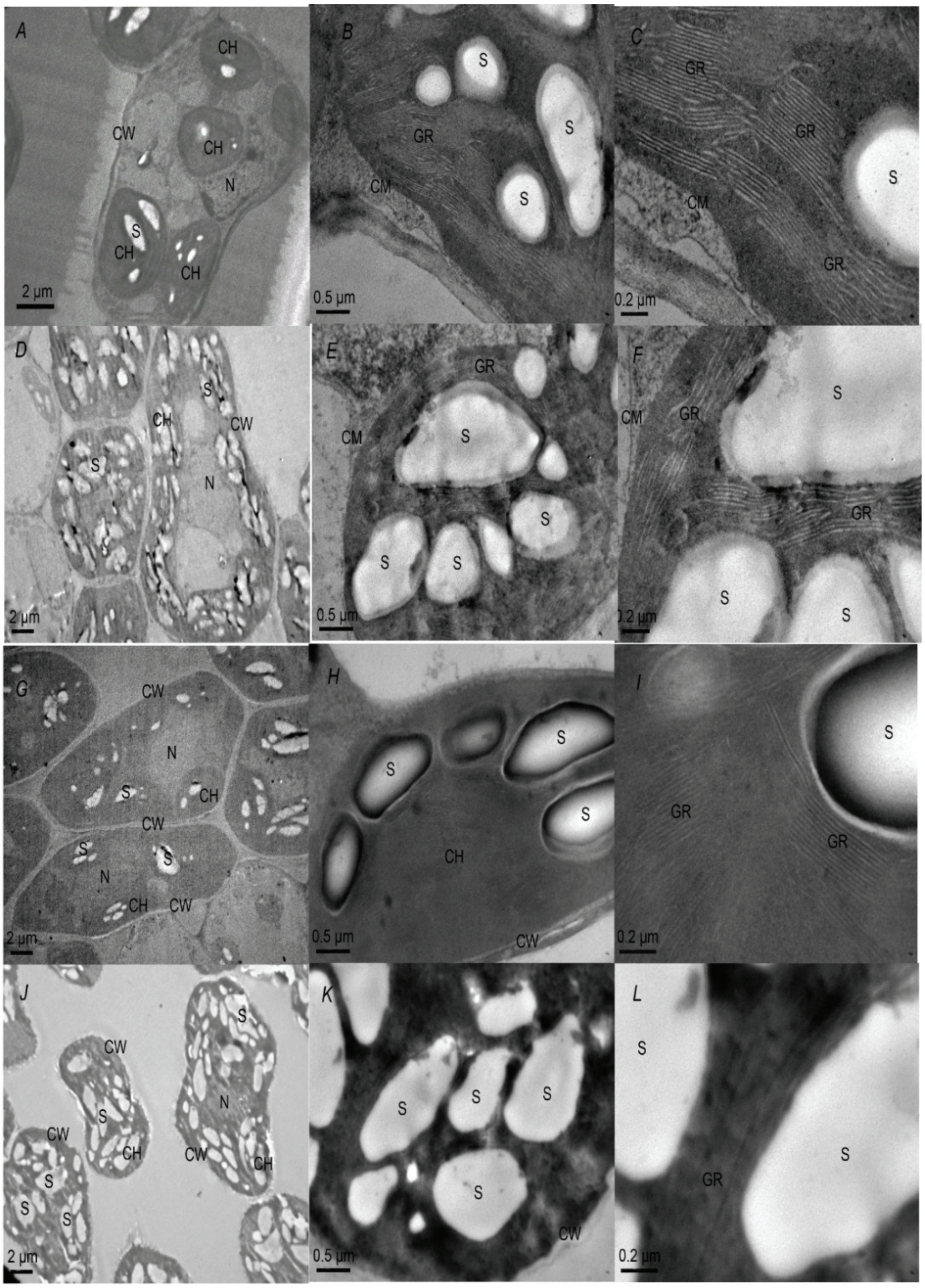

Fig. 3. Chloroplast ultrastructure in mesophyll cells of soybean leaf grown in ambient and FACE plots at R1 stage. *A–F*: Zhonghuang 13. *G–L*: Zhonghuang 35. *A–C*, *G–I*: chloroplast ultrastructure of soybean leaf grown in ambient (× 5,000; × 30,000; × 60,000). *D–F*, *J–L*: Chloroplast ultrastructure of soybean leaf grown under elevated [CO_2] (× 5,000; × 30,000; × 80,000). S – starch grain; GR – grana layer; O – osmophore; CM – chloroplast membrane; CH – chloroplast; CW – cell wall; N – nucleus. R1 –beginning bloom stage.

Photosynthetic pigment contents: The content of Chl *a*, Chl *b*, carotenoid, total Chl, and Chl *a*/*b* were not affected by elevated [CO_2] at the R1 and R3 stages. The contents of Chl *a*, Chl *b*, carotenoid, and total Chl of Z35 were less than those of Z13 by 13.6, 13.7, 12.8, and 13.6%, respectively, at the R1 stage. But the contents of four pigments of Z35 were greater than those in Z13 by 25.9, 32.8, 30.6, and 27.7% respectively, at the R3 stage. When averaged across the two cultivars, the contents of Chl *a*, Chl *b*, carotenoid and total Chl at the R3 stage were greater than those at the R1 stage by 90.8, 119.1, 93.6, and 97.9%, respectively. Chl *a*/*b* at the R3 stage was 12.5% lower than that at the R1 stage (Table 2).

Table 2. Photosynthetic pigment contents of soybean leaf grown in ambient and FACE plots. Values are means ± SE of variables across the three replicates, and the statistical significance level *P* values for the effects of [CO_2] treatment, cultivar, growth stage and their interaction. Chl – chlorophyll. Z13 – Zhonghuang 13; Z35 – Zhonghuang 35. R1, R3 – beginning bloom stage and beginning pod stage, respectively. FM – fresh mass.

Growth stage	Cultivar	Growth [CO_2]	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Carotenoid [μg g^{-1}(FM)]	Chl *a* and *b* [mg g^{-1}(FM)]	Chl *a*/*b*
R1	Z13	ambient	1.01 ± 0.05	0.32 ± 0.01	0.35 ± 0.01	1.34 ± 0.05	3.16 ± 0.09
		FACE	1.15 ± 0.02	0.40 ± 0.016	0.41 ± 0.03	1.55 ± 0.03	2.88 ± 0.07
	Z35	ambient	0.92 ± 0.11	0.32 ± 0.05	0.33 ± 0.04	1.24 ± 0.15	2.93 ± 0.14
		FACE	0.95 ± 0.01	0.30 ± 0.01	0.33 ± 0.01	1.25 ± 0.02	3.15 ± 0.15
R3	Z13	ambient	1.79 ± 0.04	0.67 ± 0.01	0.62 ± 0.01	2.46 ± 0.05	2.75 ± 0.05
		FACE	1.63 ± 0.01	0.60 ± 0.02	0.56 ± 0.02	2.22 ± 0.03	2.72 ± 0.05
	Z35	ambient	2.15 ± 0.22	0.84 ± 0.09	0.77 ± 0.08	2.99 ± 0.32	2.58 ± 0.06
		FACE	2.14 ± 0.17	0.84 ± 0.08	0.78 ± 0.07	2.99 ± 0.25	2.56 ± 0.10
P values	CO_2		0.98	0.99	0.93	0.99	0.91
	Cultivar		0.07	0.04	0.04	0.06	0.41
	Growth stage		0.00	0.00	0.00	0.00	0.00
	CO_2 × cultivar		0.89	0.88	0.99	0.96	0.13
	CO_2 × growth stage		0.28	0.37	0.35	0.30	0.81

Yield component and yield: Elevated [CO_2] significantly increased the pod number per plant of Z35 by 31.0% ($P<0.05$), but not for Z13. Elevated [CO_2] had no significant effect on seed number per pod and the mass of 100 seeds of either cultivar. Yield was significantly increased by 26% ($P<0.05$) only for Z35, but not for Z13 (Fig. 4).

Discussion

When photosynthesis was measured at the respective growth conditions, elevated [CO_2] significantly increased the photosynthesis by 13.1% and 3.53% for Z13 and Z35, respectively. However, the magnitude of the stimulation of photosynthesis response to long-term elevated [CO_2] decreased, referred to as photosynthetic acclimation (Sicher and Kremer 1995). Photosynthetic acclimation has been well documented in C_3 plants, *e.g.* rice (Huang *et al.* 2003, Seneweera *et al.* 2011), soybean (Sicher and Kremer 1995) and wheat (Aranjuelo *et al.* 2011), and the scale of down-regulation varies with genetic and environmental factors. Our results showed that cultivars grown under elevated [CO_2] had lower photosynthetic capacity than under ambient [CO_2] at the R1 stage (Figs. 1, 2). However, photosynthetic acclimation occurred for Z13, but not for Z35 at the R5 stage. This indicated that the scale of photosynthesis down-regulation under elevated [CO_2] varies with soybean cultivars and developmental stages.

The different photosynthesis responses to elevated [CO_2] between cultivars at the R5 stage contributed to the yield difference under elevated [CO_2]. Elevated [CO_2] significantly increased the yield of Z35 by 26%, but not for Z13 (Fig. 4). The yield of soybean on an area basis is determined by plants number per square meter, pod number per plant, seed number per pod and individual grain mass. In general, pod number per plants varies greatly with environmental factors, while less for the seed number per pod and individual grain mass (Yu 2003). The [CO_2]-induced changes in yield components indicated that increased yield of Z35 under elevated [CO_2] was due to the enhancement of pod number per plant (Fig. 4). Pod number per plant was determined by photosynthetic production and translocation of photosynthate to sink between beginning bloom (R1) and full seed (R6) (Yu 2003). However, photosynthetic acclimation occurred at the R1 and R5 stages for Z13, but only at the R1 stage for Z35. Therefore, the lack of yield response to elevated [CO_2] for Z13 was attributed to photosynthetic acclimation of this cultivar at the R5 stage.

The increase of starch grain in chloroplast indicates that more photosynthates were produced, but more photosynthates will restrain photosynthesis if no new sink is available to accommodate superfluous assimilation (Rogers *et al.* 1998, Isopp *et al.* 2000). This explained the occurrence of photosynthetic acclimation of the two soybean cultivars at the R1 stage. At the R5 stage, photosynthetic acclimation occurred for Z13, but not for Z35. This indicated the development of new C sinks was restricted under elevated [CO_2] for Z13 and its inability to utilise the extra assimilates synthesized. However, the pod sink strength was enhanced for Z35 under elevated [CO_2]. Therefore there was no photosynthetic acclimation for Z35. These results suggest that photosynthetic acclimation to high [CO_2] was correlated with changes in relationships between C source activity and sink capacity in the plant. There was no acclimation to high [CO_2] when the ratio of sink to source activity was increased,

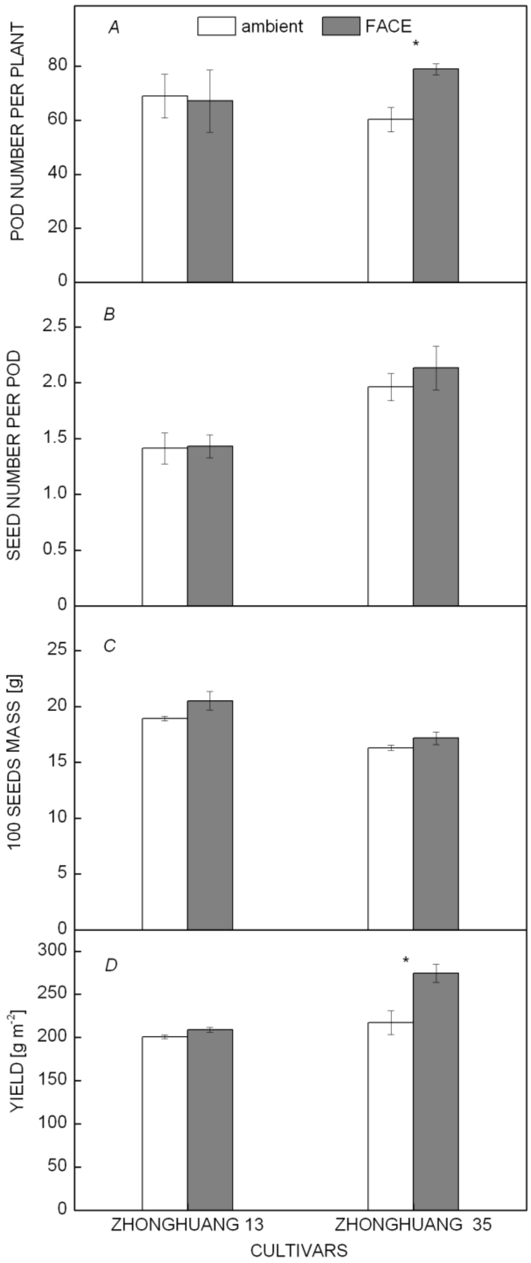

Fig. 4. Yield component and seed yield of soybean grown in ambient and FACE plots. Each bar represents the standard error of the difference between treatments, and is based on three replicates. Asterisks indicate significant differences between CO_2 treatments. * – $p \leq 0.05$.

but it was observed when the ratio was reduced (Rogers *et al*. 1998).

In addition, changes in grana layer and the membrane structure under elevated [CO_2] were detected, which was not as clear as those under ambient [CO_2] at the R1 stage (Fig. 3). Grana layer became unclear in senescent leaves, and the structural chloroplast membrane disintegrated gradually (Wang *et al*. 2012). Thus the senescence of the last fully-expanded leaves was accelerated by elevated [CO_2] at the R1 stage, which partly contributed to photosynthetic acclimation (Fangmeier *et al*. 2000, Ludewig and Sonnewald 2000, Seneweera *et al*. 2011).

In summary, photosynthetic acclimation occurred in soybean plants exposed to long-term elevated [CO_2] and varied with cultivars and developmental stages. At the R1 stage, there were more and larger starch grains in the chloroplast of both cultivars under elevated [CO_2], which indicated that the extra C supply from the source exceeded the demand for C from sink organs. At the R5 stage, new sinks were developed for Z35 through increased pod number per plant under elevated [CO_2], but this was not apparent for Z13. Therefore, photosynthetic acclimation occurred at the R1 stage for both cultivars, but at the R5 stage only for Z13. In addition, photosynthetic acclimation was partly due to accelerated leaf senescence under elevated [CO_2]. The difference in photosynthesis response to elevated [CO_2] between cultivars at the R5 stage contributed to the contrasting yield response to elevated [CO_2]. Soybean cultivars with the ability to develop new C sinks are able to overcome photosynthetic acclimation. Soybean cultivation technologies should focus on how to enhance the reproductive sink capacity by increasing the pod number per plant and the remobilization of stored photosynthates to grain during reproductive stage. Soybean yield could be improved through selecting and breeding high yield cultivars that do not acclimate under future elevated [CO_2].

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