Cover page

Title: Effect of elevated carbon dioxide on growth and nitrogen fixation of two soybean cultivars in northern China

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Abstract

The effect of elevated carbon dioxide (CO_2) concentration on symbiotic nitrogen (N_2) fixation in soybean under open-air conditions has not been reported. Two soybean cultivars (*Glycine max* (L.) Merr. cv. Zhonghuang 13 and cv. Zhonghuang 35) were grown to maturity under ambient (415 ± 16 μmol mol^{-1}) and elevated (550 ± 17 μmol mol^{-1}) [CO_2] at the Free-Air Carbon dioxide Enrichment (FACE) experimental facility in northern China. Elevated [CO_2] increased above- and below-ground biomass by 16-18% and 11-20%, respectively, but had no significant effect on the tissue C/N ratio at maturity. Elevated [CO_2] increased the percentage of N derived from the atmosphere (%Ndfa, estimated by natural abundance) from 59 to 79% for Zhonghuang 13, and the amount of N fixed from 166 to 275 kg N ha^{-1}, but had no significant effect on either parameter for Zhonghuang 35. These results suggest that variation in N_2 fixation ability in response to elevated [CO_2] should be used as key trait for selecting cultivars for future climate with respect to meeting the higher N demand driven by a carbon rich atmosphere.

Keywords

Free-air carbon dioxide enrichment (FACE); symbiotic nitrogen fixation; natural abundance; soybean cultivar

Introduction

The atmospheric carbon dioxide (CO_2) concentration has increased from 280 μmol mol^{-1} in 1800 to 390 μmol mol^{-1} now, and is expected to reach about 550 μmol mol^{-1} in 2050 (Houghton et al. 2001). The response of symbiotic N_2 fixation in legumes to elevated [CO_2] is crucial to C sequestration in future CO_2 environments because continued C sequestration is contingent on additional N input (Hungate et al. 2003). A recent review suggests that symbiotic N_2 fixation at elevated [CO_2] could be enhanced as a result of [CO_2]-induced increase in nodule size, nodule number per plant, or specific nitrogenase activity (Rogers et al. 2009). Increased heterotrophic N_2 fixation in paddy soils under elevated [CO_2] has been attributed to enhanced nitrogenase activity (Das et al. 2011). Soybean (*Glycine max* (L.) Merr.) is an important oil seed crop legume and a major source of protein for human consumption and stockfeed. Soybean production and area harvested have increased by 7.6 and 3.8 times, respectively, during the past half century (Masuda and Goldsmith 2009). In closed environment studies, elevated [CO_2] increased soybean nodule dry mass and nitrogenase activity (Finn and Brun 1982; Matsunami et al. 2009; Miyagi et al. 2007; Serraj et al. 1998), but had no effect on the nodule number and mass under well-watered condition (Serraj et al. 1998). There are no reports on the effect of elevated [CO_2] on symbiotic N_2 fixation in soybean under fully open-air conditions. The present study was therefore conducted on a soybean field in a soybean-wheat rotation in Changping (40°10’N, 116°14’E), Beijing, China using Free-Air Carbon Dioxide (FACE) facility to investigate the effect of elevated [CO_2] on the growth and N_2 fixation of two soybean cultivars.

Materials and Methods

The FACE system consists of 12 octagonal experimental areas, six elevated (550 ± 17 μmol mol^{-1}) and six ambient (415 ± 16 μmol mol^{-1}), each with a diameter of 4 m. The experimental areas were separated by at least 14 m to minimize cross-contamination of CO_2 between the areas. The soil (0-0.20 m) is a clay loam with a pH (soil:water ratio of 1:5) of 8.4 and contained 1.06% organic C, 0.11% total N, 25.5 mg kg^{-1} mineral N (6.8 mg kg^{-1} ammonium-N and 18.7 mg kg^{-1} nitrate-N; 2M KCl extraction), 50 mg kg^{-1} available phosphorus (Bray 1) and 140 mg kg^{-1} ammonium acetate extractable potassium. The long term average rainfall and temperature during the soybean growing season are 475 mm and 21.3°C, respectively. The present experiment was conducted from mid June to October in 2009, under a randomized complete block design, with [CO_2] and cultivar as main effects with three replicates. The seeds of two cultivars of soybean (*Glycine max* (L.) Merr. cv. Zhonghuang 13 and cv. Zhonghuang 35) were sown on 17 June 2009 at row spacing of 0.5 m and with basal fertilizer application of diammonium phosphate and potassium chloride at 4.8 kg N ha^{-1}, 7.2 kg P ha^{-1} and 37.3 kg K ha^{-1}. Zhonghuang 13 is a high-protein cultivar while Zhonghuang 35 a high-oil cultivar. Soybean seeds were not artificially inoculated according to local farming practice. The plots were thinned after crop emergence to ensure uniform plant density. Irrigation of 37.5 mm equivalent rainfall was applied on 9 July to ease the drought stress. At full maturity (reproductive stage R8) on 6 October, 3 m^2 of soybean plants were harvested, and seven plants were randomly selected and the soil surrounding (10 cm radius) each plant was excavated to a depth of 25 cm to ensure all roots were recovered. Each of these seven plants was separated into above-ground and below-ground (including roots and nodules) parts, which were oven-dried at 70°C for 48 h, weighed, ground into powder and analyzed for total C (%), total N (%) and $\delta^{15}N$ values by isotope ratio mass spectrometry (IRMS) (Hydra 20-20, SerCon). All the above-ground parts harvested from the 3 m^2 were oven-dried at 70°C for 48 h to obtain yield and biomass in kg ha^{-1}. Below-ground biomass (in kg ha^{-1}) was calculated using the above/below-ground ratio of the seven plants. ^{15}N natural abundance technique (Unkovich et al. 1997) was adopted for the calculation of the percentage of soybean N derived from the atmosphere (%Ndfa). Wheat (*Triticum aestivum* L.) plants harvested from each treatment area were used as reference plant materials, and their $\delta^{15}N$ values determined by IRMS for the calculation of %Ndfa. The amount of N fixed in above-ground parts was calculated as the product of %Ndfa and total N content of the above-ground parts. Data were analysed with MINITAB 14 statistical package using a General Linear Model analysis of variance.

Results and Discussion

Plant biomass and tissue quality

Elevated [CO_2] significantly increased above-ground (16-18%) and below-ground biomass (11-20%) for both cultivars (Table 1), which is comparable to the corresponding increases (17 and 30%) reported from the SoyFACE experiment in the US (Morgan et al. 2005; Rodriguez 2004). Under elevated [CO_2], there was a significant ($p < 0.05$) increase in the yield of Zhonghuang 35 from 2172 to 2744 kg ha^{-1}, and a trend of increase for Zhonghuang 13 from 2006 and 2089 kg ha^{-1} ($p > 0.05$) (Hao et al. unpublished). The [CO_2]-induced increase in biomass has been attributed to a higher canopy C assimilation under elevated [CO_2] (Ainsworth et al. 2002). Elevated [CO_2] had no significant effect on the concentrations of total C and N of the above-ground and below-ground parts of both soybean cultivars at maturity and their C/N ratio (Table 1). Others also reported that the C/N ratio of soybean straw at maturity was unaffected by elevated [CO_2] (Prior et al. 2004; Torbert et al. 2004), although higher C/N ratio in fully expanded leaf was observed under elevated [CO_2] at vegetative and reproductive stages of soybean (Ainsworth et al. 2007). The increases in C accumulation (17%; $p < 0.05$) and N uptake (21%; $p < 0.05$) of the above-ground parts of soybean under CO_2 enrichment (Table 1) are consistent with Prévost et al. (2010). In the present study, the higher N acquisition of soybean due to the higher biomass accumulation under elevated [CO_2] was probably met by symbiotically fixed N and N from the soil.

(Insert Table 1)

Nitrogen fixation

The %Ndfa value calculated for Zhonghuang 13 (69%) was higher (p < 0.001) than Zhonghuang 35 (27%) when averaged across [CO_2] treatments, corresponding to 220 and 71 kg N ha^{-1} fixed from the atmosphere. The %Ndfa of the two cultivars is comparable to the upper (69%) and lower (36%) quartiles of a meta-analysis of N_2 fixation in soybean at ambient [CO_2] (Salvagiotti et al. 2008). The difference in %Ndfa between cultivars, which implies the variation in N_2 fixation ability among genotypes, was also reported in other soybean N_2 fixation studies at ambient [CO_2] (Danso et al. 1987; Houngnandan et al. 2008; Matsunami et al. 2009). Elevated [CO_2] increased %Ndfa for Zhonghuang 13 from 59 to 79% ($p = 0.07$) (Fig. 1a), and the amount of N fixed from 166 to 275 kg N ha^{-1} ($p < 0.05$) (Fig. 1b), but had no significant effect on either parameter for Zhonghuang 35 (Fig. 1). In the present study, the soybean plants were not artificially inoculated but exposed to a mixture of rhizobacterial strains in the field. Difference in the efficiency and the response to elevated [CO_2] of these strains associated with the two cultivars could be attributed to differences in the establishment and functioning of an effective symbiosis, which depends on the genetic background of determinants in both host plant and rhizobacteria species (Keyser and Li 1992).

The increase in %Ndfa for Zhonghuang 13 under elevated [CO_2] was unlikely attributed to the improved water-use efficiency at elevated [CO_2] (Leakey et al. 2009) nor the tolerance of N_2 fixation to drought stress under elevated [CO_2] for soybean (Serraj 2003; Serraj et al. 1998) because water supply was substantiated by irrigation to ease drought stress for all treatments. The lack of response to elevated [CO_2] of N_2 fixation in Zhonghuang 35 suggests that this cultivar relied more on soil mineral N to meet the higher N demand under elevated [CO_2] than Zhonghuang 13. This implies the rhizobacterium associated with Zhonghuang 35 was more sensitive to the soil nitrate level (25.5 mg N kg^{-1}) at the site, as high mineral N level was reported to inhibit the nitrogenase activity of soybean nodules (Arrese-lgor et al. 1997; Kanayama et al. 1990; Streeter and Wong 1988).

(Insert Fig. 1)

In summary, elevated [CO_2] increased the above- and below-ground biomass of the two soybean cultivars tested, but there is genetic variability in symbiotic N_2 fixation between these cultivars in response to elevated [CO_2]. The factors manipulating the variation in N_2 fixation ability under elevated [CO_2], such as nodule development, root exudation, nitrogenase activity and related gene expression, need to be assessed to explore the underlying mechanisms for the variation. This could be exploited to improve N supply in cropping systems in future high carbon dioxide atmospheres.

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Figure caption

Fig. 1 Effect of elevated [CO_2] on (a) %Ndfa and (b) amount of N fixed in the above-ground parts of two soybean cultivars. Each data point represents the mean of three replicates. Vertical bar indicates least significant difference (LSD) (p = 0.05)

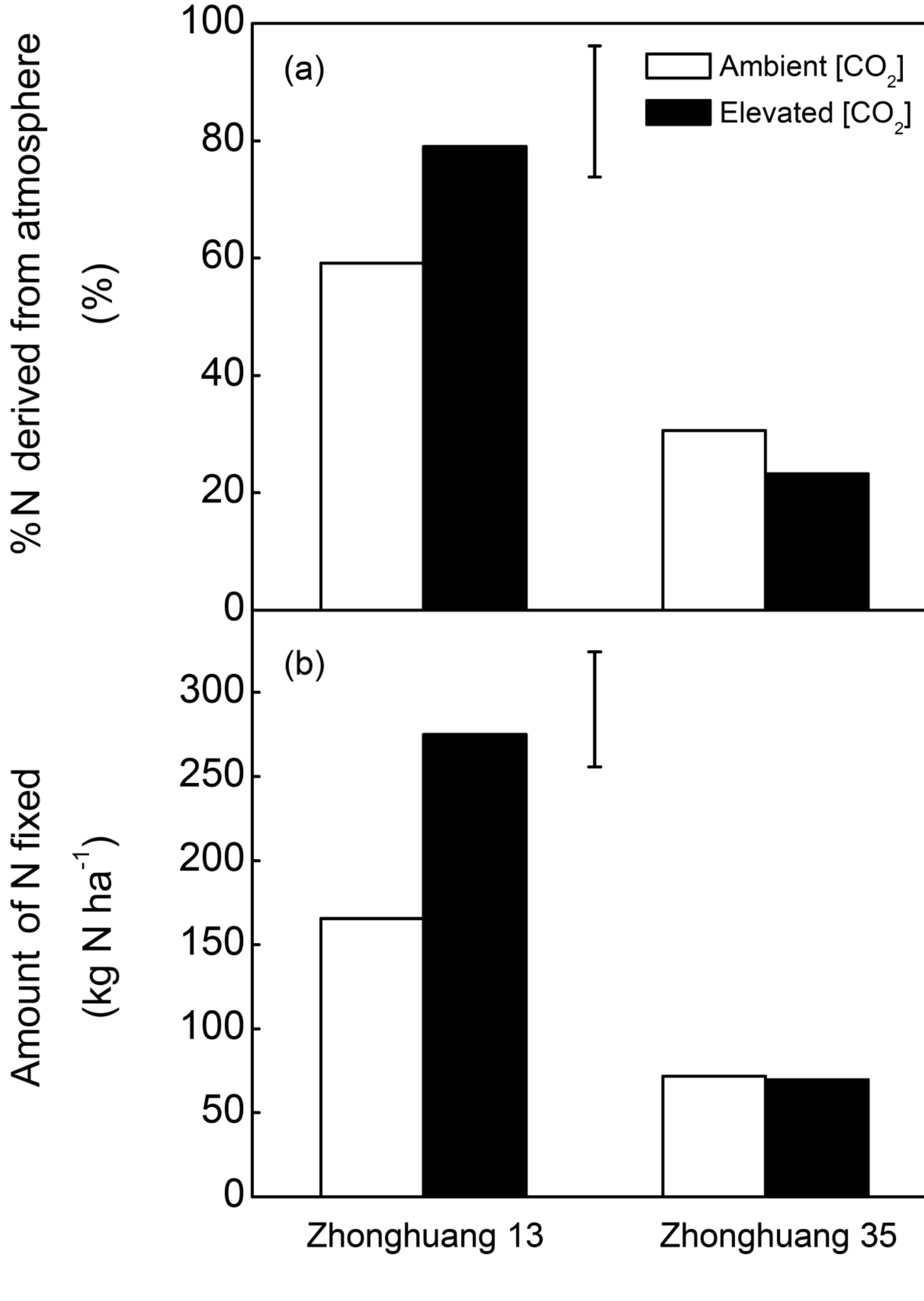
100
80
60
40
20
0
(a)
Ambient [CO2]
Elevated [CO2]
% N derived from atmosphere
(%)
300
250
200
150
100
50
0
(b)
Amount of N fixed
(kg N ha-1)
Zhonghuang 13
Zhonghuang 35

Fig. 1

Table

Table 1 Effect of elevated [CO_2] on the biomass, total C and N, C/N ratio, biomass C and N uptake of two soybean cultivars at full maturity.

Treatment	Biomass (kg ha^{-1})	Total C (%)	Total N (%)	C/N	Biomass C (kg ha^{-1})	N uptake (kg ha^{-1})
			Above-ground parts			
Zhonghuang 13						
Ambient [CO_2]	7684 (146)	46.2 (0.3)	3.69 (0.36)	12.7 (1.1)	3549 (51)	283 (22)
Elevated [CO_2]	9092 (255)	47.0 (1.0)	3.84 (0.13)	12.2 (0.2)	4269 (129)	349 (16)
Zhonghuang 35						
Ambient [CO_2]	7645 (834)	47.9 (0.2)	3.25 (0.11)	14.8 (0.5)	3659 (382)	249 (30)
Elevated [CO_2]	8840 (223)	47.5 (0.6)	3.34 (0.10)	14.2 (0.3)	4197 (147)	295 (16)
[CO_2]	*	NS	NS	NS	*	*
Cultivar	NS	NS	*	**	NS	NS
[CO_2] × Cultivar	NS	NS	NS	NS	NS	NS
			Below-ground parts			
Zhonghuang 13						
Ambient [CO_2]	792 (28)	44.8 (0.3)	0.64 (0.10)	74.5 (13.5)	355 (14)	5.08 (0.91)
Elevated [CO_2]	880 (55)	43.9 (0.0)	0.70 (0.05)	63.0 (4.4)	386 (24)	6.24 (0.76)
Zhonghuang 35						
Ambient [CO_2]	764 (32)	45.6 (0.2)	0.54 (0.03)	85.4 (4.6)	348 (16)	4.09 (0.26)
Elevated [CO_2]	917 (22)	44.8 (0.9)	0.53 (0.07)	86.7 (11.2)	410 (9)	4.90 (0.67)
[CO_2]	*	NS	NS	NS	*	NS
Cultivar	NS	NS	NS	NS	NS	NS
[CO_2] × Cultivar	NS	NS	NS	NS	NS	NS

Values are means of the three replicates (SE) for each treatment. Significant effects are indicated as *$p < 0.05$ and **$p < 0.01$. NS, not significant.

The values of biomass C and N uptake reported in this table were calculated before rounding of decimal place. They are not necessarily the perfect product of the biomass and %C and %N listed in this table.