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Comparison of photosynthetic acclimation to elevated CO_2 and limited nitrogen supply in soybean

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ABSTRACT

Plants grown at elevated CO_2 often acclimate such that their photosynthetic capacities are reduced relative to ambient CO_2-grown plants. Reductions in synthesis of photosynthetic enzymes could result either from reduced photosynthetic gene expression or from reduced availability of nitrogen-containing substrates for enzyme synthesis. Increased carbohydrate concentrations resulting from increased photosynthetic carbon fixation at elevated CO_2 concentrations have been suggested to reduce the expression of photosynthetic genes. However, recent studies have also suggested that nitrogen uptake may be depressed by elevated CO_2, or at least that it is not increased enough to keep pace with increased carbohydrate production. This response could induce a nitrogen limitation in elevated-CO_2 plants that might account for the reduction in photosynthetic enzyme synthesis. If CO_2 acclimation were a response to limited nitrogen uptake, the effects of elevated CO_2 and limiting nitrogen supply on photosynthesis and nitrogen allocation should be similar. To test this hypothesis we grew non-nodulating soybeans at two levels each of nitrogen and CO_2 concentration and measured leaf nitrogen contents, photosynthetic capacities and Rubisco contents. Both low nitrogen and elevated CO_2 reduced nitrogen as a percentage of total leaf dry mass but only low nitrogen supply produced significant decreases in nitrogen as a percentage of leaf structural dry mass. The primary effect of elevated CO_2 was to increase non-structural carbohydrate storage rather than to decrease nitrogen content. Both low nitrogen supply and elevated CO_2 also decreased carboxylation capacity (V_{cmax}) and Rubisco content per unit leaf area. However, when V_{cmax} and Rubisco content were expressed per unit nitrogen, low nitrogen supply generally caused them to increase whereas elevated CO_2 generally caused them to decrease. Finally, elevated CO_2 significantly increased the ratio of RuBP regeneration capacity to V_{cmax} whereas neither nitrogen supply nor plant age had a significant effect on this parameter. We conclude that reductions in photosynthetic enzyme synthesis in elevated CO_2 appear not to result from limited nitrogen supply but instead may result from feedback inhibition by increased carbohydrate contents.

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INTRODUCTION

In the short term (hours to days), elevated CO_2 increases the rate of photosynthesis in C_3 plants. However, over the longer term (days to weeks), growth in elevated CO_2 often decreases photosynthetic capacity because of reductions in the content of photosynthetic enzymes (Griffin & Seemann 1996). Reductions in the synthesis of photosynthetic enzymes has been proposed to result from sugar repression of photosynthetic gene transcription (Stitt *et al*. 1990; Jang & Sheen 1994). However, it is also possible that elevated CO_2 restricts the uptake of nitrogen from the soil and thus results in a limited supply of nitrogen for enzyme synthesis. Recent studies have suggested that nitrogen uptake is depressed by elevated CO_2, or at least that it is not increased sufficiently to match the increases in carbohydrate production (Conroy & Hocking 1993; Jackson & Reynolds 1996). However, studies reporting CO_2 effects on root uptake capacities and soil nitrogen availability are not entirely consistent. Jackson & Reynolds (1996) found that the nitrate uptake capacity of excised roots was decreased by high CO_2 but BassiriRad *et al*. (1997) found that nitrate uptake capacity of loblolly and ponderosa pine roots was increased by elevated CO_2, while ammonium uptake capacity declined. Diaz *et al*. (1993) found that increased root exudation from high CO_2 plants resulted in microbial immobilization of nutrients that limited plant uptake. However, Zak *et al*. (1993) reported the opposite response, namely that elevated CO_2 increased soil nitrogen availability.

Instead of attempting directly to measure soil nitrogen availability and root uptake rates, another approach to this question is to compare plant responses to elevated CO_2 with plant responses to low nitrogen supply. One of the superficial similarities between acclimation to high CO_2 and acclimation to low nitrogen is a decrease in leaf nitrogen concentration per unit dry mass. However, decreases in nitrogen concentration in elevated CO_2 grown plants often result from increases in total non-structural carbohydrate contents rather than a decrease in nitrogen per unit leaf structural mass (Kuehny *et al*. 1991; Wong 1979, 1990; Rogers *et al*. 1996a, &b). In contrast, limiting nitrogen nutrition almost always reduces leaf nitrogen on a structural dry mass basis. Elevated CO_2 also has distinctive effects

on photosynthesis. Carbon fixation by Rubisco is more efficient at elevated CO_2, and consequently less enzyme may be required (Sage 1990; Masle *et al*. 1993). Since Rubisco is the most abundant enzyme in plants, accounting for 15–30% of total leaf nitrogen (Evans 1989; Evans & Seemann 1989), reduced investment in Rubisco could have substantial effects on nitrogen allocation.

If CO_2 acclimation were a response to limited nitrogen uptake it would be expected to affect photosynthesis and nitrogen allocation in the same way as observed for a limiting nitrogen treatment. To test this hypothesis, we compared CO_2 and nitrogen acclimation by growing non-nodulating soybeans at two levels each of nitrogen and CO_2 concentration and measuring leaf nitrogen contents, photosynthetic capacities and Rubisco contents. The results suggest that CO_2 acclimation is distinct from the response to nitrogen limitation.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of a non-nodulating variety of soybean, *Glycine max*, Lee (Hartwig 1994), were planted in 8 L pots in a 50/50 (by volume) mixture of fine sand and sandy loam topsoil. The pots were placed in 6 naturally lit growth chambers inside a greenhouse at the Desert Research Institute in Reno, NV, USA. Temperatures were controlled to 28 ± 2 ºC in the daytime and 22 ± 1 ºC at night. Relative humidity at midday was 66 ± 7%. The experimental design was a randomized split block with three blocks and one replicate per block. Each block contained two plots (growth chambers) at either 350 ± 10 p.p.m. or 700 ± 10 p.p.m. CO_2. The CO_2 concentration in each chamber was measured once every 6 min by an infrared gas analyser (model 6262, LICOR Inc, Lincoln, NE, USA). A datalogger (model CR10, Campbell Scientific, Logan, UT, USA) collected the data and controlled the duration of CO_2 injection into the chambers on a 30 s cycle to maintain the CO_2 set-point. Because the ambient CO_2 concentration within the greenhouse was quite variable, CO_2 scrubbers were used in the 350 p.p.m. chambers to maintain a constant CO_2 concentration. The scrubber boxes measured 14 × 45 × 56 cm, and were constructed from Plexiglas with two fans in the top and an open grill covered by screening in the bottom. CO_2 in the air flowing through the boxes was absorbed by cooler pads ('Coolpad' brand, Research Products Corp., Avondale, AZ, USA) dipped in a slurry of hydrated lime (Chemical Lime Co., Scottsdale, AZ, USA) and water. These boxes were placed inside the chambers and the fans were operated continuously. Control to the 350 p.p.m. set-point was achieved through additions of CO_2.

Nutrient treatments were randomly arranged within the CO_2 treatments. After seedling emergence, half of the plants were watered with 1/2 strength Hoagland solution (7·5 mol m^{-3} NO_3, 0·5 mol m^{-3} PO_4, 3 mol m^{-3} K, 2·5 mol m^{-3} Ca, 1 mol m^{-3} Mg, 1 mol m^{-3} SO_4, 0·067 mol m^{-3} Fe-EDTA, plus micronutrients) and the other half received a modified 1/2 strength Hoagland solution with 1/10 of the normal nitrogen concentration (0·75 mol m^{-3} NO_3, 0·5 mol m^{-3} PO_4, 3 mol m^{-3} K, 2·5 mol m^{-3} Ca, 1 mol m^{-3} Mg, 2·1 mol m^{-3} SO_4, 4·5 mol m^{-3} Cl, 0·067 mol m^{-3} Fe-EDTA, plus micronutrients).

In addition to the nitrogen and CO_2 treatments, the plants were measured at two different ages, after two trifoliate leaves had expanded and just prior to flowering. The experiment was repeated three times over the course of the summer but each age group was measured only twice. Plants were measured at both ages in the second replication but only the older plants were measured in the first replication and only young plants were measured in the third replication. Consequently, for each nitrogen, CO_2 and age combination there were six replicates.

Gas exchange measurements

Photosynthesis of one leaf on each of six plants per treatment was measured after 4–5 weeks (young plants) or 6–8 weeks (older plants) of growth. On the young plants, measurements were made on the first trifoliate leaf when the second trifoliate had mostly expanded. On the older plants, measurements were made on fully expanded leaves two leaves down from the youngest expanding leaf greater than 1 cm long. This leaf was found to have the highest photosynthetic rates in preliminary measurements of all leaves on three high- and three low-CO_2-grown plants (data not shown). The older plants had 8–14 nodes and began to flower within 1–2 weeks following the gas exchange measurements.

Photosynthesis was measured in open flow gas exchange systems (model 6400, LICOR Inc., Lincoln, NE, USA and a modified system similar to the MPH-1000, Campbell Scientific Inc., Logan, UT, USA). The MPH-1000 system used a nickel-plated chamber with a glass window. CO_2 and O_2 concentrations in the air entering the chamber were controlled by mixing pure O_2 and N_2 with CO_2 in N_2 using mass flow controllers (model 825, Edwards High Vacuum International, Wilmington, MA, USA). The dew point of the air was controlled by a dew-point humidifier (model DPH02, Armstrong Enterprises, Palo Alto, CA, USA). When the LICOR 6400 was used, the air supply was first mixed by the MPH-1000 system. The light source for all measurements was a tungsten halogen projector lamp (model ENH, 120 V-250 W, Radiac Inc, Japan), light from which was reflected off a 45° cold mirror.

The response of assimilation to intercellular CO_2 concentration was measured at light saturation, 1400–1600 μmol m^{-2} s^{-1}. Leaf temperature was 28 °C and water vapour concentration was 30 ± 2 mmol mol^{-1}. Leaves were initially allowed to equilibrate for 30 min at 350 p.p.m. CO_2, then the CO_2 concentration was reduced to ≈80 p.p.m. and subsequently increased in eight steps to ≈1000 p.p.m. allowing 6–10 min for equilibration at each CO_2 concentration.

Carboxylation capacity (V_{cmax}) and electron transport rate (J) were calculated according to Farquhar, von Caemmerer

& Berry (1980). The kinetic constants used were: K_m for carboxylation = 327, K_m for oxygenation = 457 600, true CO_2 compensation point = 50 p.p.m. Since actual electron transport capacity may be substantially greater than the CO_2- and light-saturated rate of photosynthesis (Kirschbaum & Pearcy 1988; Laisk *et al*. 1992; Evans & von Caemmerer 1996) we calculated RuBP regeneration capacity as *J*/4 (since four electrons are utilized to regenerate one RuBP) and report the results as RuBP regeneration capacity.

Leaf characteristics

Leaf disks were collected from the same leaves as used in the gas exchange measurements. Prior to collection of the leaf disks, the plants were returned to the growth chambers for 1–2 d and then disks were collected in mid-afternoon. Collection at a consistent time of day reduced variation due to diurnal changes in carbohydrate contents. Dry mass (after drying for 48 h at 60 °C), total non-structural carbohydrates (using the technique of Hendrix 1993), and total nitrogen content (model 2400 CHN analyser, Perkin Elmer, Norwalk, CT, USA) were measured for all samples (six replicates per treatment). For half of the plants (three replicates per treatment) additional leaf samples, collected at the same time as those above, were frozen in liquid nitrogen and stored in a −80 °C freezer. These samples were used for measurements of total Rubisco content (as described by Evans & Seemann 1984).

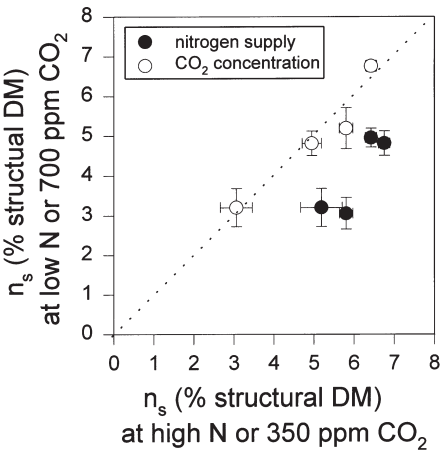

Figure 1. Comparison of leaf nitrogen as a percentage of leaf structural dry mass (n_s) for plants grown at high or low N (filled symbols) or CO_2 concentrations of 350 or 700 p.p.m. (open symbols). The dotted line marks the point of no difference between the treatments. Error bars are ± 1 SE.

Statistical analysis

Analysis of variance of the effects of nitrogen, CO_2 and plant age on leaf parameters was carried out with the general linear models routine in SAS (SAS Institute Inc. Cary, NC, USA) following log transformation of the data.

RESULTS

Nitrogen as a percentage of leaf structural dry mass (n_s) was reduced by low nitrogen but not by elevated CO_2 (Fig. 1). Elevated CO_2 did significantly reduce nitrogen as a percentage of total leaf dry mass (Tables 1 & 2) but this was entirely due to the increase in total non-structural carbohydrates in the elevated CO_2 leaves. Nitrogen limitation, as measured by the difference in nitrogen as a percentage of structural mass between the high- and low-nitrogen treatments, did not change significantly with plant age (i.e. no significant nitrogen by age interaction effect on n_s; Table 1).

In contrast, photosynthetic capacities increased with plant age in high nitrogen but decreased with plant age in low nitrogen, so that the difference between the nitrogen treatments was much greater in the older plants (Fig. 2). This resulted both from a greater increase in structural dry

Table 1. Significance levels for the effect of plant age, nitrogen and CO_2 treatments on nitrogen expressed as a percentage of total dry mass (n_m), as a percentage of TNC free dry mass (n_s), or per unit leaf area (n_a), total non-structural carbohydrates as a percentage of leaf dry mass (TNC), photosynthetic rate measured at 350 p.p.m. CO_2 (A_{350}) or 700 p.p.m. CO_2 (A_{700}) and expressed per unit leaf area (area) or per unit leaf nitrogen (N), carboxylation capacity (V_{cmax}) and RuBP regeneration capacity calculated from the measured A/c_i curves (using the equations of Farquhar, von Caemmerer & Berry 1980) and expressed per unit leaf area (area) or per unit leaf nitrogen (N), the ratio of V_{cmax} to RuBP regeneration capacity (*R*/*V*), and Rubisco protein content expressed per unit leaf area (area) or per unit leaf nitrogen (N). Significance levels: *** $P < 0{\cdot}001$, ** $P < 0{\cdot}01$, * $P < 0{\cdot}05$

					A_{350}		A_{700}		V_{cmax}		RuBP regen			Rubisco	
	n_m	n_s	n_a	TNC	area	N	area	N	area	N	area	N	*R*/*V*	area	N
Age	***	***	**	*				**		*		***		***	**
Nitrogen (N)	***	***	***	**	***	***	***	***	***	***	***	**		***	
CO_2 (C)	***			**	**	**			**	*			***	**	
N*age	**		***		***	***	***	***	***	***	***	*		***	***
C*age	**				*										
N*C															
N*C*age	**														*

Table 2. Mean (and SE) for nitrogen expressed as a percentage of total dry mass (n_m), as a percentage of TNC free dry mass (n_s), or per unit leaf area (n_a), total non-structural carbohydrates as a percentage of leaf dry mass (TNC), photosynthetic rate measured at 350 p.p.m. CO_2 (A_{350}) or 700 p.p.m. CO_2 (A_{700}), carboxylation capacity (V_{cmax}) and RuBP regeneration capacity calculated from the measured A/c_i curves (using the equations of Farquhar, von Caemmerer & Berry 1980), the ratio of V_{cmax} to RuBP regeneration capacity (R/V), and Rubisco protein content

	Young plants				Older plants			
	Low nitrogen		High nitrogen		Low nitrogen		High nitrogen	
p.p.m. CO_2:	350	700	350	700	350	700	350	700
n_m	3·55 (0·21)	2·56 (0·17)	5·12 (0·15)	4·81 (0·31)	1·72 (0·13)	1·19 (0·09)	4·81 (0·26)	2·34 (0·23)
n_s	4·95 (0·24)	4·81 (0·31)	6·43 (0·14)	6·76 (0·14)	3·06 (0·40)	3·21 (0·48)	5·81 (0·17)	5·19 (0·52)
n_a	1·00 (0·08)	0·920 (0·065)	1·34 (0·09)	1·46 (0·06)	0·677 (0·037)	0·667 (0·053)	1·65 (0·11)	1·29 (0·07)
TNC	28·5 (2·3)	44·4 (6·4)	21·2 (3·4)	28·4 (5·2)	41·8 (3·2)	58·3 (6·0)	18·8 (4·4)	57·5 (2·0)
A_{350}								
μmol m^{-2} s^{-1}	15·1 (0·9)	12·8 (1·0)	12·7 (0·8)	12·8 (0·9)	10·2 (0·7)	7·02 (0·73)	22·2 (1·8)	16·8 (1·4)
μmol gN^{-1} s^{-1}	15·2 (0·5)	14·1 (0·8)	9·8 (1·0)	9·0 (0·9)	14·8 (0·53)	10·7 (0·94)	13·5 (0·69)	13·0 (0·73)
A_{700}								
μmol m^{-2} s^{-1}	18·7 (1·3)	18·1 (2·0)	16·5 (0·6)	18·1 (0·7)	12·8 (0·9)	11·5 (1·1)	29·8 (3·0)	24·8 (2·3)
μmol gN^{-1} s^{-1}	18·8 (0·8)	19·7 (1·5)	12·6 (0·6)	12·5 (0·7)	18·6 (0·57)	17·3 (1·22)	18·0 (1·22)	19·0 (1·1)
V_{cmax}								
μmol m^{-2} s^{-1}	55·3 (4·9)	46·1 (5·3)	44·6 (2·6)	43·6 (2·4)	36·8 (2·5)	24·8 (1·8)	83·6 (11)	63·1 (8·2)
μmol gN^{-1} s^{-1}	55·0 (1·2)	50·1 (4·0)	34·4 (3·2)	30·3 (2·5)	54·8 (3·5)	38·2 (3·2)	50·6 (6·1)	48·1 (4·1)
RuBP regen								
μmol m^{-2} s^{-1}	24·7 (2·2)	29·8 (4·3)	43·7 (2·1)	28·8 (2·5)	19·9 (1·6)	20·4 (1·2)	46·2 (6·4)	41·8 (4·7)
μmol gN^{-1} s^{-1}	24·9 (1·6)	32 (3·5)	20 (0·3)	19·5 (1·3)	29·3 (1·6)	31·4 (2·2)	27·5 (2·9)	32·0 (2·4)
R/V	0·46 (0·03)	0·64 (0·03)	0·62 (0·06)	0·68 (0·08)	0·55 (0·04)	0·83 (0·02)	0·57 (0·07)	0·67 (0·02)
Rubisco								
g m^{-2}	0·910 (0·13)	0·876 (0·11)	1·02 (0·087)	0·778 (0·015)	0·365 (0·064)	0·210 (0·026)	1·07 (0·081)	0·956 (0·075)
g gN^{-1}	0·951 (0·092)	0·855 (0·069)	0·673 (0·069)	0·498 (0·018)	0·588 (0·098)	0·364 (0·047)	0·583 (0·031)	0·708 (0·092)

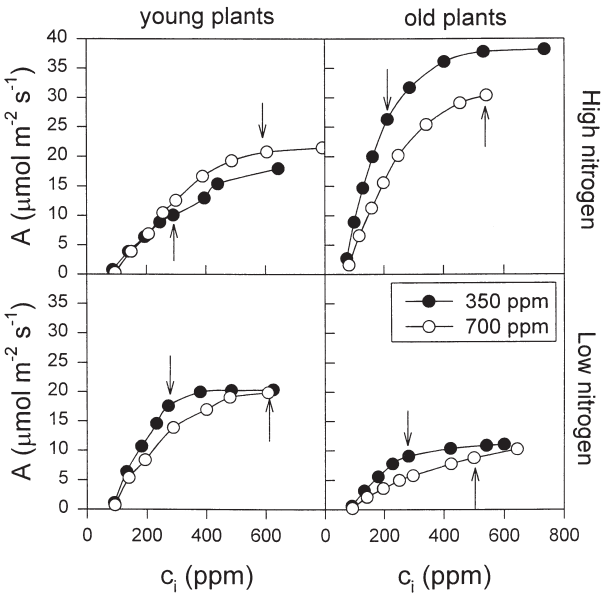

Figure 2. Representative curves of the response of assimilation (A) to intercellular CO_2 concentration (c_i) for young and old soybean plants supplied with high (7·5 mol m^3 nitrate in nutrient solution) or low (0·75 mol m^3 nitrate) nitrogen and grown at 350 p.p.m. CO_2 (filled symbols) or 700 p.p.m. CO_2 (open symbols). Measurements were made at light saturation and 28 °C leaf temperature.

mass per unit leaf area with plant age in the high- than in the low-nitrogen plants and from an increase in photosynthesis per unit nitrogen with plant age in the high-nitrogen plants but not in the low-nitrogen plants (Table 2). The effect of elevated CO_2 on photosynthetic capacities depended on the nitrogen supply and plant age. At high nitrogen, elevated CO_2 initially increased photosynthetic capacities but then depressed them as the plants aged. In contrast, elevated CO_2 reduced photosynthetic capacities to a similar extent in young and old plants in the low-nitrogen treatment.

Although both elevated CO_2 and low nitrogen reduced carboxylation capacity (V_{cmax}) per unit leaf area (Table 2), V_{cmax} per unit nitrogen was increased at low nitrogen supply but decreased by elevated CO_2 (Fig. 3) suggesting a different allocation of nitrogen in response to nitrogen supply and CO_2 concentration. V_{cmax} is a function of the quantity and activity of ribulose bisphosphate carboxylase-oxygenase (Rubisco), the primary carboxylating enzyme in plants. Changes in Rubisco content were qualitatively similar to those in V_{cmax} (Table 2) although the large increase in V_{cmax} for the high-nitrogen older plants cannot fully be explained by changes in Rubisco content.

Low nitrogen supply reduced RuBP regeneration capacity per unit leaf area in both young and old plants (Table 2), but elevated CO_2 did not have any consistent effect on RuBP regeneration capacity. Low nitrogen supply and increasing plant age significantly increased RuBP regeneration capacity per unit nitrogen but these effects were quite small. Elevated CO_2 had no significant effect on RuBP regeneration capacity per unit nitrogen. Since elevated CO_2 decreased V_{cmax} but had no consistent effect on RuBP regeneration capacity, there was a significant increase in the ratio of RuBP regeneration capacity to V_{cmax} for elevated CO_2 plants (Fig. 4). Nitrogen supply and plant age had no significant effects on this ratio (Table 1).

DISCUSSION

These results demonstrate that CO_2 acclimation in soybean is distinct from acclimation to limited nitrogen supply. Although both low nitrogen and elevated CO_2 reduced nitrogen as a percentage of total leaf dry mass, only low nitrogen supply resulted in significant decreases in nitrogen as a percentage of leaf structural dry mass, suggesting that the primary effect of elevated CO_2 was to increase non-structural carbohydrate storage rather than decrease nitrogen uptake. Both low nitrogen supply and elevated CO_2 decreased V_{cmax} and Rubisco content per unit leaf area, but when these were expressed per unit nitrogen, low nitrogen supply generally resulted in an increase whereas elevated CO_2 generally resulted in a decrease. Finally, elevated CO_2 significantly increased the ratio of RuBP regeneration capacity to V_{cmax} whereas neither nitrogen supply nor plant age had a significant effect on this parameter.

Although a limited nitrogen supply for photosynthetic enzyme synthesis did not appear to account for the CO_2 acclimation response, nitrogen limitation might have had an indirect effect by reducing sink strength and resulting in increased feedback limitation of photosynthesis (Wong 1979; Rogers *et al*. 1996b). However, we did not find any simple interactions between the CO_2 and nitrogen treatments. All the significant interactions included plant age as a factor, suggesting that plant size and developmental stage were important in these responses. This was not simply a

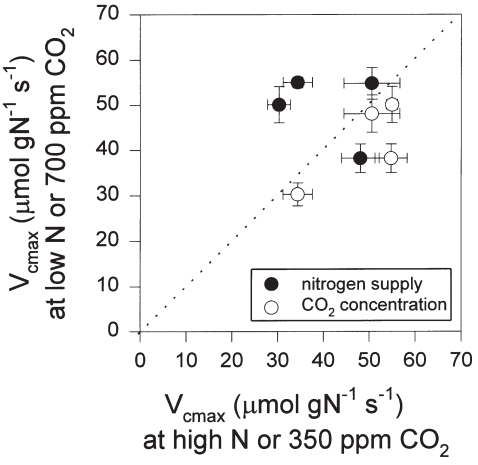

Figure 3. Comparison of carboxylation capacity (V_{cmax}) on a leaf nitrogen basis for plants grown at high or low N (filled symbols) or CO_2 concentrations of 350 or 700 p.p.m. (open symbols). The dotted line marks the point of no difference between the treatments. Error bars are ± 1 SE.

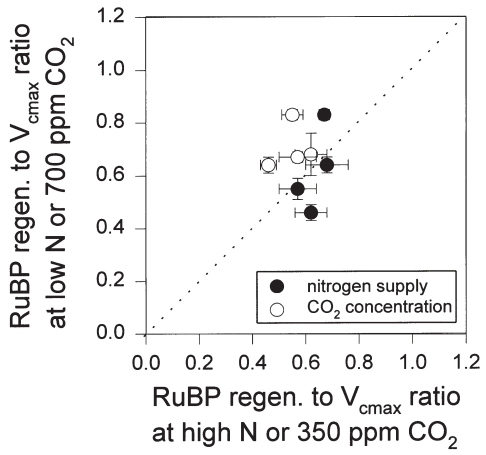

Figure 4. Comparison of the ratio of RuBP regeneration capacity to carboxylation capacity (V_{cmax}) for plants grown at high or low N (filled symbols) or CO_2 concentrations of 350 or 700 p.p.m. (open symbols). The dotted line marks the point of no difference between the treatments. Error bars ± 1 SE.

function of increasing nitrogen limitation as the plant size increased since the difference in nitrogen as a percentage of structural dry mass between the high- and low-nitrogen treatments was similar for the young and old plants. Neither does it appear likely that these interactions were a result of comparing plants of different sizes (Coleman *et al*. 1993) since the ambient- and elevated-CO_2 plants had the same number of nodes (data not shown). The elevated-CO_2 plants did have a greater total dry mass but this was largely due to increased carbohydrate storage rather than an acceleration of development. Changes in the CO_2 effect with plant age may have resulted from increased limitation of root growth by pot limitations (Thomas & Strain 1993) or changes in plant response during the vegetative to reproductive shift (Nie *et al*. 1995).

Our results suggest that reduction in photosynthetic capacity at elevated CO_2 is a direct response to accumulation of carbohydrates in leaves, rather than a limited supply of nitrogen for enzyme synthesis. Accumulation of carbohydrates in leaves at elevated CO_2 could result from a limitation in carbohydrate transport or utilization at many different points. It is possible that the increased downregulation of photosynthesis in the older, high-nitrogen, elevated-CO_2 plants resulted from a limitation in root growth and thus sink demand as the plants became larger (Thomas & Strain 1991). Sink strength might also be limited by other factors such as temperature (Hofstra & Hesketh 1975) or maximal rates of cell division and expansion (Kinsman *et al*. 1996). However, some studies suggest that the limitation is not at the sinks. In a study of carbohydrate production and utilization in soybean, Cure *et al*. (1991) concluded that rates of phloem loading and/or sucrose synthesis, rather than sink demand, limited carbohydrate export from source leaves. In studies currently underway in our laboratory, treatment of single soybean leaflets with high CO_2, while the rest of the plant remains at ambient CO_2, results in carbohydrate accumulation in the treated leaflet to levels similar to those of high-CO_2 plants, also suggesting that carbohydrate accumulation is not the result of sink limitation (D. A. Sims, unpublished results). Morin *et al*. (1992) found that the same increase in photosynthetic rate of clover had very different effects on carbon partitioning depending on whether it was induced by increased PFD or increased CO_2 concentration. Starch accumulated only in response to elevated CO_2 and this appeared to result from a Pi limitation. Consequently, increased accumulation of non-structural carbohydrates, and decreases in leaf nitrogen concentration, may represent a limitation in capacities for carbohydrate processing within leaves rather than a limitation in sink demand.

A number of studies have suggested a linkage between carbohydrate contents and the levels of photosynthetic enzymes (Krapp *et al*. 1991; Stitt 1991) and mRNAs (Krapp *et al*. 1993; Jang & Sheen 1994; Van Oosten & Besford 1994; Van Oosten *et al*. 1994). Specific sugars in specific cellular compartments are hypothesized to effect gene transcription via their interaction with hexokinase (Jang & Sheen 1994). However, many aspects of the mechanisms by which changes in carbohydrate status are transduced into changes in photosynthetic gene transcription remain unclear. Bulk leaf carbohydrates often do not correlate with photosynthetic response. Jacob *et al*. (1995) found little effect of growth at elevated CO_2 on photosynthetic capacity in spite of significant increases in leaf carbohydrates. Xu *et al*. (1994), using soybean and pea, and Nie *et al*. (1995), using wheat, both found that the relationship between leaf carbohydrate concentrations and reductions in photosynthetic capacity changed with plant developmental stage. Sugars in leaves are often highly partitioned between subcellular compartments and this partitioning can change dramatically between day and night (Moore *et al*. 1997). In addition, Rubisco mRNA levels fluctuate on a diurnal cycle and may be more susceptible to sugar signals at particular times of day (Pilgrim & McClung 1993; Cheng & Moore Bd Seemann 1998). Consequently, measurements of bulk leaf carbohydrates at one time of day may not adequately describe the temporal and spacial variation in sugars directly responsible for the photosynthetic response.

Regardless of the mechanisms involved, reductions in Rubisco content in elevated CO_2 plants are consistent with optimization predictions. Since high CO_2 increases the carboxylation to oxygenation ratio for Rubisco, the same photosynthetic rate can be maintained with relatively less Rubisco (Sage 1990). Consequently, predictions based on optimization of nitrogen use suggest that investment in Rubisco should be reduced relative to other photosynthetic components. Experimental tests of this prediction have yielded mixed results. Reductions in investment in Rubisco in response to growth at elevated CO_2 have been reported for several species (Sage *et al*. 1989; Tissue *et al*. 1993; Ghannoum *et al*. 1997). However, not all studies have found this response (Campbell *et al*. 1988) and in a

survey of the literature Sage (1994) concluded that the data were not consistent with a general effect of high CO_2 on investment in Rubisco.

Although we found a reduction in Rubisco, the increase in nitrogen use efficiency was modest. Assuming 16% nitrogen in Rubisco protein, Rubisco accounted for only 6–15% of total nitrogen and thus the reduction in Rubisco in elevated CO_2 plants would save only 1–2% of total leaf nitrogen. Similar conclusions can be drawn from the data of Ghannoum *et al*. (1997) where Rubisco accounted for 10–15% of total leaf nitrogen in *Panicum laxum* and the reduction in Rubisco resulting from acclimation to high CO_2 would allow reallocation of only 4% of total leaf nitrogen. Masle *et al*. (1993), working with Rubisco antisense plants, calculated that a 6% nitrogen savings could be achieved by reducing Rubisco at elevated CO_2 but did not actually observe this savings because increased nitrate concentrations in the antisense plants compensated for the reduction in Rubisco nitrogen. Another way to look at this question is from the perspective of photosynthetic nitrogen use efficiency under the elevated CO_2 conditions. Since photosynthesis was primarily limited by RuBP regeneration capacity at 700 p.p.m. CO_2 (based on the breakpoints of the A/ci responses), RuBP regeneration capacity per unit nitrogen gives an estimate of nitrogen use efficiency. Although there was a slight trend towards an increase in RuBP regeneration capacity per unit nitrogen in elevated CO_2 plants this was not significant. Consequently, we conclude that acclimation to elevated CO_2 has very little effect on nitrogen use efficiency.

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