Sensitivity and acclimation of *Glycine max* (L.) Merr. leaf gas exchange to CO_2 partial pressure

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Abstract

Theoretical studies suggest that partitioning leaf photosynthetic responses to CO_2 partial pressures into two components, sensitivity and acclimation, facilitates both scaling-up photosynthetic responses and predicting global terrestrial carbon influx. Here, we experimentally examine these two components by growing soybean (*Glycine max*) two CO_2 partial pressures, 35 and 70 Pa, and making a suite of ecophysiological measurements on expanding and lly expanded leaves. These CO_2 treatments resulted in a variety of acclimation responses, including changes in net notosynthetic rate and capacity, stomatal conductance, transpiration, and respiration. These responses were strongly pendent on leaf age. Despite the wide variety of acclimation responses, the experimentally derived photosynthetic nsitivity did not vary with CO_2 treatments or leaf age. In addition, the photosynthetic sensitivity to ambient CO_2 rtial pressure was consistent with the sensitivity to intercellular CO_2 partial pressure, indicating little effect of matal conductance on photosynthetic sensitivity. This study supports the theoretical conclusion that photosyn- tic sensitivity is independent of growth environment and leaf age, as well as photosynthetic acclimation, even ugh the latter varies with both environmental and developmental factors. Accordingly, photosynthetic sensitivity y be directly extrapolated from leaf to globe to predict the increment in terrestrial carbon influx stimulated by the rly increase in atmospheric CO_2, whereas the acclimation component must be used to adjust the overall global mate. © 1999 Elsevier Science B.V. All rights reserved.

words: Global change; CO_2 acclimation; *Glycine max*; Respiration; Stomatal conductance

ntroduction

.eaf-level studies have shown that photosyn- ic responses to CO_2 partial pressure vary tly with species, growth environments, and plant developmental stages (DeLucia et al., 1985; Gunderson and Wullschleger, 1994; Luo et al., 1994; Sage, 1994; Curtis, 1996). This large varia- tion poses a great challenge for plant biologists wishing to extrapolate from leaf-level studies to predict the stimulation in global terrestrial carbon influx resulting from rising atmospheric CO_2 par- tial pressure. Luo et al. (1996) have proposed that

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separating leaf photosynthetic responses to long-term growth in elevated CO_2 into two components, sensitivity and acclimation, can simplify this problem. The sensitivity component is a marginal increment in the gross photosynthetic rate of vascular plant leaves caused by a marginal increment in CO_2 partial pressure. By contrast, the acclimation component is the long-term, CO_2-induced changes in photosynthetic capacity. Theoretically the first component is not affected by growth environments or developmental stages, and therefore separating this component from acclimation leaves it directly scaleable from the leaf level to the globe.

Biochemically, the sensitivity of photosynthesis to CO_2 describes changes in the ratio of carboxylation to oxygenation of ribulose 1,5-bisphosphate (RuBP). In C_3 plants both reactions (carboxylation and oxygenation of RuBP) are catalyzed by a single enzyme: ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). Two features of this enzyme result in the biochemical sensitivity of photosynthesis to CO_2 partial pressure. First, under current atmospheric CO_2 partial pressures, Rubisco operates well below its $K_m[CO_2]$ at about 25% of its maximum capacity, and therefore elevated CO_2 substantially stimulates the rate of carboxylation in the very short-term (minutes) (Sage et al., 1987; Woodrow and Berry, 1988). Second, photorespiration (the oxygenation of RuBP) can reduce potential net carbon gain by as much as 40% (20), and accordingly as CO_2 partial pressure increases, the ratio of carboxylation to oxygenation increases, further contributing to the photosynthetic CO_2 sensitivity.

By contrast the acclimation component of photosynthetic responses to elevated CO_2 can be defined as physiological adjustments in photosynthetic capacity resulting from long-term growth in different CO_2 partial pressures. While short-term (minutes to hours) increases in ambient CO_2 partial pressure from 35 to 70 Pa typically increase photosynthesis 30–70% due to photosynthetic *sensitivity* (Stitt, 1991; Sage, 1994; Luo and Mooney, 1996), many species will not maintain this stimulation of photosynthesis when grown in elevated CO_2 for weeks or longer as the result of photosynthetic *acclimation* (Mauney et al., 1979;

Wong, 1979; Sionit et al., 1981; von Caemmerer and Farquhar, 1984; DeLucia et al., 1985; Sasek et al., 1985; Tissue and Oechel, 1987; Sage et al., 1988; Bunce, 1992). For example, the 52% average initial stimulation of photosynthesis in crop species reported by Cure and Acock (1986) decreased to an average of 29% after long-term exposure to elevated CO_2, as the result of changes in photosynthetic enzymes and/or leaf mesophyll structure. These acclimation responses can vary with species, source–sink relations, nitrogen and water availability, phenological stage, reproductive status, rooting volume, or combinations of these (and other) factors.

Both components, sensitivity and acclimation, are reflected in a biochemically based model of photosynthesis (Farquhar et al., 1980). In this model two parameters, J_{max} (maximum electron transport rate) and V_{cmax} (the maximum carboxylation rate), are used to describe the regulation of leaf photosynthetic rates by light, species, CO_2 and nutrient availability. Luo and Mooney (1996) have demonstrated that when only sensitivity is concerned, both parameters (J_{max} and V_{cmax}) can be eliminated mathematically from this model, resulting in a leaf-level function (L, a mathematical description of the photosynthetic sensitivity) that is independent of environmental and species variation. Furthermore, acclimation induced by growth in elevated CO_2 can be described by varying the values of these variables (J_{max} and V_{cmax}) in relation to leaf nitrogen concentration (Luo et al., 1994, 1996). As such, the L function is a potentially powerful tool for quantifying photosynthetic carbon influx into terrestrial ecosystems. Although a similar function was described by Polglass and Wang (1992) (termed the CO_2 fertilization factor), it was used to focus on the temperature dependence of photosynthetic sensitivity in different biomes and only more recently have Luo and Mooney (1996) suggested that the sensitivity can be used directly to estimate global photosynthetic influx, making this relationship applicable to quantification of global carbon cycling.

While these facts suggest that leaf photosynthetic sensitivity can be scaled to predict global photosynthetic carbon influx as stimulated by a

small increase in CO_2 partial pressure, acclimation is extremely variable with species and growth environments, and therefore is difficult to predict and may not be as easily scaled. Further application of the L function model requires that the interactions between the L function (photosynthetic sensitivity) and photosynthetic acclimation are experimentally examined. Similarly, several important scaling issues need to be addressed. For example, are leaf-level responses similar to the plant level responses and are the effects of stomatal and non-stomatal control on $C_i(C_i/C_a)$ predictable? Although Luo et al. (1996) have tested the L function with existing CO_2 response data from the literature, their analysis was based on an assumption that leaf non-photorespiratory respiration is simply proportional to photosynthetic rate. Sensitivity analysis indicates that this assumption has a considerable influence on the significance test (Luo et al., 1996) and thus a more careful, empirical consideration is required.

This experimental study was designed to provide a complete data set for a rigorous test of the L function. First, we make measurements of respiration and photosynthesis of the same leaves so that the L function can be more fundamentally tested. Second, this study relates the L function directly to ambient, in addition to intercellular, CO_2 partial pressure because the former is much more relevant to the global extrapolation. As a consequence, the stomatal influence on the L function is quantified. Third, we examine acclimation of not only photosynthesis but also respiration, stomatal conductance, leaf nitrogen concentration, and other leaf properties at two stages of leaf development. Thus the hypothesis that the L function is independent of leaf photosynthetic acclimation is tested in a much broader context.

Materials and methods

1. Plant propagation and environmental conditions

Seeds of *Glycine max* (L.) Merr. (cv Williams) were planted in 5-l pots filled with potting soil (SuperSoil, Rod Mclellun Co., San Francisco, CA) on 12 April 1995. Five pots, each with five seeds, were placed in each of two environmentally controlled greenhouses (Griffin et al., 1996) in the Great Basin Environmental Research Laboratory of the Desert Research Institute, Reno, NV (39.5°N latitude and 1524 m elevation). All pots were watered twice weekly with deionized water. One week after planting, all pots were thinned to a single plant, leaving a total of five replicates per treatment. Two weeks after planting, all pots were given 500 ml of half strength Hoagland's solution to assure adequate micro-nutrient availability.

Two greenhouses were maintained under a day/ night temperature regime of 28/20°C. Relative humidity was maintained at a constant 50% during the duration of the experiment. Solar transmission through the greenhouse was greater than 70%, and typically exceeded 1300 µmol m^{-2} per s on sunny days. Atmospheric CO_2 within the low CO_2 greenhouse tracked outside ambient (36–38 Pa) while the CO_2 partial pressure in the elevated CO_2 greenhouse was maintained at 70 Pa, 24 h a day for the duration of the experiment (36 days). CO_2 control was maintained by flowing ambient air through the greenhouse at a rate of 500–5000 mol min^{-1}; then adding the desired amount of CO_2 through a three-stage system that included a needle valve, a 100 l min^{-1} mass flow controller, and a 15 l min^{-1} mass flow controller as described in Griffin et al. (1996). Using this three-stage approach we were able to obtain CO_2 concentrations well within 2% of the desired setpoint. CO_2 and H_2O partial pressures were continuously monitored and logged with an infrared gas analyzer (Li-6262, Li-Cor, Lincoln, NE) and automated datalogger network (CR10T and RTMS, Campbell Scientific, Logan, UT).

2.2. Gas-exchange measurements

Twenty-four days after seedling emergence, steady state measurements of leaf-level CO_2 and H_2O fluxes were made with an open-flow infrared gas analysis system (Li-6400, Li-Cor, Lincoln, NE). Two leaves, one expanding and one fully expanded, from three to five individual plants per treatment were measured. Expanding leaves were

identified as the leaves nearest the growing meristem that were large enough to fill the gas-exchange cuvette (6 cm²). Fully expanded leaves were at least two tri-foliates further down the main stem and were judged visually to be the most recently produced, but fully expanded leaves. The center leaflet of the measurement tri-foliate was placed in the gas exchange cuvette and allowed to equilibrate for a minimum of 15 min. Gases of the desired CO_2, O_2 and N_2 partial pressures were mixed from three mass flow controllers (Type 831, Edwards High Vacuum International, Wilmington, MA), and humidified with a dew point generator to a humidity of 50% (Li-610, Li-Cor, Lincoln, NE), prior to entering the system. All measurements were made at a constant air temperature of 25°C and light intensity of 1500 µmol m^{-2} per s. Atmospheric pressure was automatically measured and recorded by the gas-exchange system and appropriate corrections were made to the gas-exchange calculations. The cuvette was illuminated with a 50 W tungsten halogen lamp. The photosynthetic response to intercellular CO_2 partial pressure (A/C_i) was measured by varying the CO_2 partial pressure of the air entering the leaf cuvette from 10 to 150 Pa in 15 roughly equal steps.

Photosynthetic parameters, V_{cmax} and J_{max}, were calculated from the measured responses of assimilation to intercellular CO_2 partial pressure (A/C_i curves) by fitting data to the Farquhar et al. (1980) model using linearized equations:

$$(A_1 + R)(C_i + K) = V_{cmax}(C_i - \Gamma) \tag{1}$$

$$(A_2 + R)(4.5C_i + 10.5\Gamma) = J(C_i - \Gamma) \tag{2}$$

where A_1 is the net photosynthetic rate for $C_i <$ 25 Pa, A_2 is the net photosynthetic rate over the entire range of C_i, R is the respiration rate, Γ is the photorespiratory CO_2 compensation point, K is a kinetic coefficient for the photosynthetic enzymes, and J_{max} is calculated from J as:

$$J_{max} \quad \sqrt{\left(\frac{\overline{a^2 I^2}}{J^2}\right)} \tag{3}$$

where a is the leaf absorptance, set to 0.85, and is the measured PFD (photon flux density).

Following the gas-exchange measurements (days after planting), all plants were harvested a separated into leaves, stems and roots. The le area of each plant was measured in a belt-driv leaf area meter (Li-3000A, Li-Cor, Lincoln, NI The leaves were then dried to a constant mass 60°C in a convection oven and subsequent weighed. Dried leaves were ground to fine powd in a ball mill (Model 2601, Cianflone Scienti Instrument Company, Pittsburgh, PA) and an lyzed in a CHN analy. for total carbon ar nitrogen (Model 2400, Perkin-Elmer, Norwal CT) (Horneck and Miller, 1998).

2.3. Model parameterization and calculation of photosynthetic parameters

A full description of the model developmer and parameterization can be found elsewher (Luo and Mooney, 1996) but briefly, the function describing the relative change in photo synthesis for a small change in CO_2 partial pres sure is defined as:

$$L = \frac{1}{P} \cdot \frac{dP}{d[CO_2]} \tag{4}$$

and is derived mathematically from the photo synthesis model of Farquhar et al. (1980) basec on gross photosynthetic response to intercellular CO_2 partial pressure (A/C_i) as:

$$L_1(A/C_i) \quad \frac{15\Gamma}{(C_i - \Gamma)(4.5C_i + 10.5\Gamma)}$$

and

$$L_2(A/C_i) = \frac{K + \Gamma}{(C_i - \Gamma)(C_i + K)} \tag{6}$$

where C_i is the intercellular CO_2 partial pressure (Pa), L_1 and L_2 define the upper and lower limits of the theoretical L function and represent the relative response generated either from the electron transport-limited or Rubisco-limited portions of the photosynthetic CO_2 response curve (Eqs. (1) and (2)).

In order to examine the effects of stomatal conductance on the L function, we define $L(A/C_a)$

from the photosynthetic response to ambient CO_2 as:

$$L_1(A/C_a) = \frac{15\alpha\Gamma}{(\alpha C_a - \Gamma)(4.5\alpha C_a + 10.5\Gamma)} \quad (7)$$

and

$$L_2(A/C_a) = \frac{\alpha(K + \Gamma)}{(\alpha C_a - \Gamma)(\alpha C_a + K)} \quad (8)$$

where $\alpha = C_i/C_a$. Theoretically:

$$L(A/C_i) = \alpha L(A/C_a) \quad (9)$$

2.4. Statistical analysis

Leaf respiration was added to the net photosynthetic rate to obtain the gross photosynthetic rate (A) and the responses to intercellular CO_2 partial pressure (C_i) or ambient CO_2 partial pressure (C_a) were used to derive experimental L values (L_e) with a difference equation:

$$L_e = \left(\frac{1}{\frac{(P_j + P_{j-1})}{2}}\right)\left(\frac{P_j - P_{j-1}}{C_{x,j} - C_{x,j-1}}\right) j = 2, 3, \cdot, n \quad (10)$$

where C_x denotes C_i or C_a, subscript j denotes the sequential number of observed data in one A/C_i or A/C_a response curve. We used two quantitative measures to indicate statistical fitness of the theoretical L function to the experimental data, L_e (Luo et al., 1996). The first statistic quantifies the portion of the variation in the experimental L_e values that can be explained by the theretical curves of the L function (equivalent to conventional determinant coefficient, r^2). When computing this r^2, L_e values of $C_i < 19$ Pa ($= 0.7 \times 27$; the former is a common value of C_i/C_a ratio and the latter is a preindustrial level of atmospheric CO_2 partial pressure) were considered irrelevant and thus were excluded. In addition, we used a t-test for paired comparisons to describe the probability that experimental data (L_e) are significantly different (either above or below) from the predicted range of the L function. To accomplish this, the predicted L function corresponding to each experimental L_e value was calculated at each

C_i and both the predicted and experimental L values were logarithmically transformed before the differences between them were used to compute t-values and probability.

The effect of growth CO_2 partial pressure and leaf age on measured physiological parameters and calculated use efficiencies were tested by ANOVA (Data Desk 4.1 statistical software, Data Description Inc., Ithaca, NY). Means separation based on planned comparisons were accomplished with a protected LSD test. Treatment effects and means separation were considered significant only when $P \leq 0.05$.

3. Results

Net photosynthetic rates measured under growth CO_2 partial pressures were affected significantly by both age and CO_2, increasing significantly under elevated CO_2 in the fully expanded leaves ($P = 0.042$), and increasing with leaf age in the 70 Pa CO_2 treatment ($P = 0.043$, Table 1). V_{cmax}, the maximum rate of carboxylation, was affected significantly by age, with expanding leaves having lower rates than fully expanded leaves, but was not affected by CO_2. Similar trends were seen in J_{max} which was more variable than V_{cmax}, yet no statistically significant differences were found. J_{max} and V_{cmax} were correlated linearly with each other (Fig. 1).

Respiration rates were also affected significantly by leaf age but not CO_2 (Table 1). Respiration rates of expanding leaves were about 60% higher than the rate in fully expanded leaves. The ratio of carbon gain to carbon loss (photosynthesis/respiration, or A/R) was quite consistent among leaves of the same age class, regardless of CO_2 partial pressure. Fully expanded leaves had a twofold higher A/R ratio than expanding leaves.

The growth conditions also affected leaf water vapor exchange. Both stomatal conductance and transpiration were reduced significantly in leaves of both age classes when plants were grown in elevated CO_2 (Table 1). The transpiration rate was also influenced by leaf age class, with fully expanded leaves having higher transpiration rates than expanding leaves. These changes in stomatal

Table 1

Carbon exchange characteristics of soybean leaves grown under two CO_2 partial pressures (35 or 70 Pa)[a]

	n	A (μmol m per s)	V_{cmax} (μmol m^{-2} per s)	J_{max} (μmol m^{-2} per s)	R (μmol m per s)	A/R	g_s (mol m^{-2} per s)	E (mmol m$^-$ per s)	C_i/C_a	WUE (A/E) (μmol mmol^{-1})
35 Pa exp.	3	11.1 ± 1.32	39.9 ± 3.5	109.9 ± 13.2	−2.45 ± 0.79	5.4	0.216 ± 0.060	3.18 ± 0.50	0.70 ± 0.02	3.59 ± 0.57
35 Pa full	5	13.4 ± 0.58	48.7 ± 6.0	131.3 ± 21.8	−1.33 ± 0.12	10.4	0.204 ± 0.034	5.09 ± 0.59	0.61 ± 0.01	2.72 ± 0.22
70 Pa exp.	3	12.8 ± 1.16	32.7 ± 3.1	105.3 ± 11.7	−2.53 ± 0.12	5.1	0.099 ± 0.013	2.38 ± 0.26	0.71 ± 0.02	5.42 ± 0.29
70 Pa full	5	17.1 ± 1.74	49.2 ± 6.1	139.6 ± 37.2	−1.76 ± 0.41	10.7	0.102 ± 0.013	2.91 ± 0.33	0.64 ± 0.04	5.90 ± 0.08
ANOVA (probability)										
CO_2		0.064	0.283	0.910	0.547	0.975	0.005	0.018	0.051	0.000
Age		0.029	0.001	0.080	0.041	0.002	0.897	0.044	0.000	0.500
$CO_2 \times$ age		0.475	0.038	0.615	0.678	0.815	0.804	0.226	1.000	0.034

[a] Gas exchange characteristics were measured on either fully expanded (full) or rapidly expanding (exp.) leaflets; A, net assimilation rate measured at the growth CO_2 partial pressure; V_{cmax}, maximum rate of carboxylation of RuBP by Rubisco; J_{max}, maximum rate of photosynthesis as limited by the capacity to regenerate RuBP; R, predawn respiration rate measured at the growth CO_2 partial pressure; A/R, ratio of photosynthesis to respiration; g_s, stomatal conductance; E, transpiration; C_i/C_a, calculated ratio of intercellular to ambient CO_2 partial pressures and WUE, photosynthetic water-use efficiency (A/E).
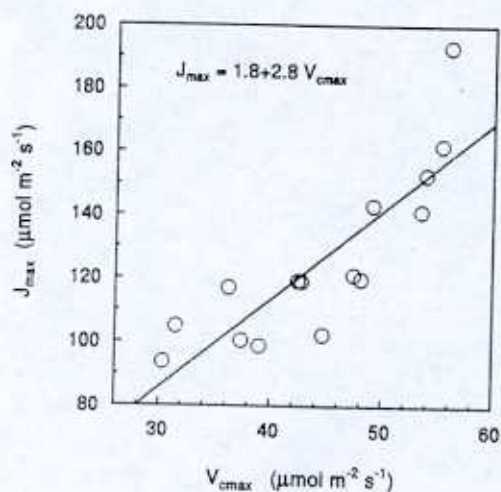
Fig. 1. Correlation between the two primary limitations to photosynthesis, carboxylation of RuBP by Rubisco (V_{cmax}) and regeneration of RuBP (J_{max}). Primary limitations calculated from CO_2 response curves of expanding and fully expanded leaves of soybean grown under two CO_2 partial pressures (35 or 70 Pa); $n = 3$–5 leaves per treatment; r^2 of the regression, 0.71.

conductance result in a lower C_i/C_a ratio for plants grown at 35 Pa CO_2 as compared to 70 Pa CO_2 and in fully expanded compared to expanding leaves. The amount of carbon gained per unit water lost (photosynthetic water use efficiency, WUE, μmol CO_2 mmol^{-1} H_2O) was increased significantly in elevated CO_2 and in fully expanded compared to expanding leaves of plants grown in 35 Pa CO_2.

Leaf nitrogen concentrations were all quite high and were not affected significantly by either age class or CO_2 (Table 2). Leaf carbon concentrations were significantly lower in fully expanded leaves compared to expanding leaves, but not influenced by CO_2. C/N ratio and specific leaf mass (g m^{-2}) were both unaffected by either leaf age class or CO_2. Photosynthetic nitrogen use efficiency (PNUE, μmol C mmol N^{-1}) was influenced significantly by leaf age class, with 70 Pa fully expanded leaves gaining 36% more carbon per unit nitrogen than expanding leaves from the same CO_2 treatment. There were no CO_2 by age interactions in PNUE.

The CO_2 treatments and leaf age provide substantial variation in leaf gas-exchange and tissue chemical composition so that the relationship between photosynthetic acclimation and sensitivity can be examined. The L function (photosynthetic sensitivity), is derived from photosynthetic responses to either intercellular (A/C_i) or ambient (A/C_a) CO_2 partial pressure for expanding and expanded leaves (Figs. 2 and 3). Photosynthetic response to intercellular CO_2 partial pressure varies considerably with CO_2 treatments for expanded leaves (Fig. 2A). Derived L_v values from the response curves are consistent with the theoretical range of the L function (Fig. 2C). Quantitatively, the L function accounted for over 90% of

Table 2

specific leaf mass (g leaf dry mass per m^2 of leaf area, SLM), leaf carbon (C% dry mass) and nitrogen (N% dry mass) concentrations, carbon/nitrogen ratio (C/N) and instantaneous photosynthetic nitrogen use efficiency (PNUE, μmol C gained per μmol of leaf N) of soybean grown under two CO_2 partial pressures (35 or 70 Pa)[a]

	n	SLM (g dry mass m^{-2})	C (% of dry mass)	N (% of dry mass)	C/N	PNUE (μmol C mmol^{-1} N)
35 Pa exp.	3	28.9 ± 2.3	42.8 ± 0.1	4.7 ± 0.5	9.1 ± 0.9	0.115 ± 0.014
35 Pa full	5	24.0 ± 1.2	40.9 ± 0.6	5.1 ± 0.2	8.1 ± 0.3	0.157 ± 0.013
70 Pa exp.	3	30.6 ± 3.9	42.3 ± 1.0	5.3 ± 0.4	8.1 ± 0.5	0.115 ± 0.012
70 Pa full	5	27.1 ± 2.6	41.2 ± 0.4	5.2 ± 0.2	8.0 ± 0.3	0.179 ± 0.028
ANOVA (probability)						
CO_2		0.484	0.885	0.275	0.186	0.632
age		0.235	0.064	0.685	0.192	0.031
CO_2 × age		0.840	0.603	0.488	0.304	0.611

[a] Measurements were made on either fully expanded (full) or rapidly expanding (exp.) leaflets.
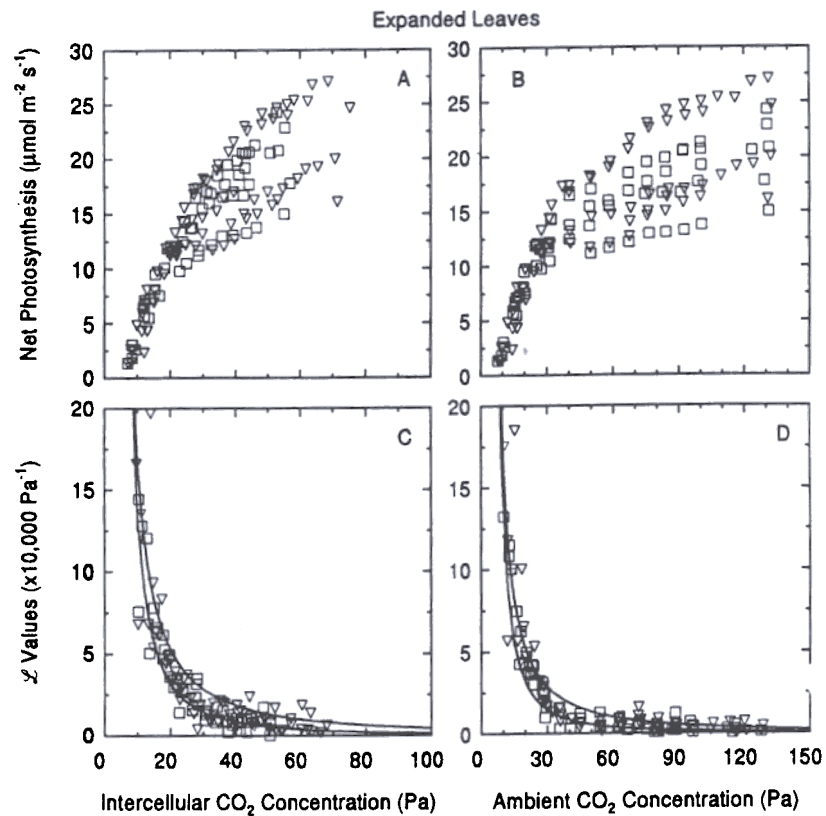
Fig. 2. Photosynthetic responses and calculated CO_2 sensitivity for expanded leaves of soybean grown under two CO_2 partial pressures (35 or 70 Pa). Panel (A) net photosynthetic response to intercellular CO_2 partial pressure; panel (B) net photosynthetic response to ambient CO_2 partial pressure; panel (C) L function (calculated CO_2 sensitivity) vs. intercellular CO_2 partial pressure, and panel (D) L function vs. ambient CO_2 partial pressure. Squares, plants grown in 35 Pa CO_2; triangles, plants grown in 70 Pa CO_2; $n = 3–5$ leaves per treatment.

the variation in the experimentally derived L_e values. The paired t-test indicates that the experimental L_e values are not significantly different from the predicted range of the L function ($P = 0.873$ and 0.831, respectively, for 35 and 70 Pa CO_2 treatments) (Table 3). Similarly, photosynthetic responses to ambient CO_2 partial pressure varied with CO_2 treatments for expanded leaves (Fig. 2B). Derived L_e values from the A/C_a curves are statistically consistent with the theoretical range of the L function (Fig. 2C). Quantitatively, the L function accounted for at least 86% of the variation in the calculated L_e values from the A/C_i curves and at least 82% of the variation in calculated L_e values from the A/C_a curves (Table 3). For expanding leaves, photosynthetic rate is

lower than that of expanded leaves as is the variation in A/C_i or A/C_a response curves (Fig. 3A,B). The experimentally derived L_e values are also statistically consistent with the theoretical L function (Fig. 3C,D Table 3). In addition, plotting L_e values derived from the A/C_i response curves against αL_e values derived from the A/C_a curves demonstrates all data points are very close to the 1:1 line over the entire range of derived L_e values (Fig. 4).

4. Discussion

The experimental data provided in this study validate that the L function was independent of

acclimation and little affected by stomatal conductance. The experimental treatments produced a wide variety of responses in leaf carbon flux, water balance, and tissue chemical composition. For example, stomatal conductance is reduced by, on average, 52% under elevated CO_2 in comparison to ambient CO_2. Leaf respiration rate differs by nearly twofold between the two age groups of leaves; and photosynthetic rate differs by 15–28% between the two CO_2 treatments. Despite the myriad responses, the L function was consistent across all treatment combinations. The value of determinant coefficient r^2, which measures the predictability of the variation in experimental L_e values by the theoretical L function, ranges from 0.82 to 0.93 for sample sizes of 28–63. The value

of probability (P) that experimental L_e values are not significantly different from the predicted range of the L function is 0.51 or greater for both A/C_i and A/C_a response curves.

The L function was used originally to describe the marginal change in the ratio of carboxylation to oxygenation of RuBP caused by a marginal change in intercellular CO_2 partial pressure (Polglass and Wang, 1992; Luo and Mooney, 1996). The independence of L function from many environmental and biological factors results from the independence of the carboxylation oxygenation ratio of Rubisco activity from the light-driven regeneration of RuBP and plant-specific Rubisco content (varying only with the CO_2 O_2 ratio and measurement temperature). In light of these bio-
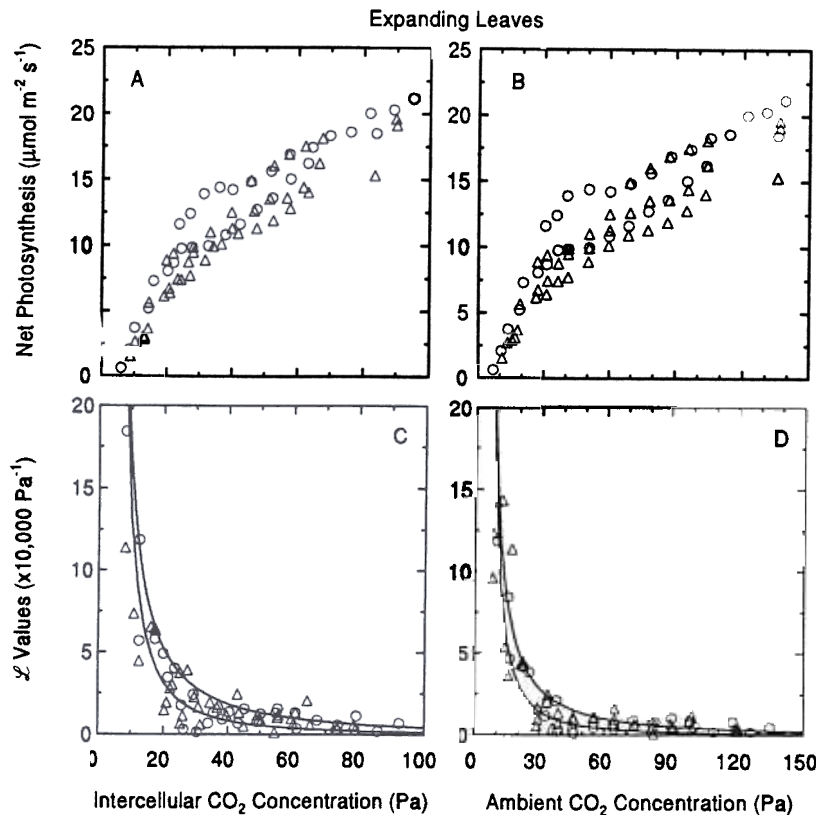

Fig. 3. Photosynthetic responses and calculated CO_2 sensitivity for expanding leaves of soybean grown under two CO_2 partial pressures (35 or 70 Pa). Panel (A) net photosynthetic response to intercellular CO_2 partial pressure; panel (B) net photosynthetic response to ambient CO_2 partial pressure; panel (C) L function (calculated CO_2 sensitivity) vs. intercellular CO_2 partial pressure, and panel (D) L function vs. ambient CO_2 partial pressure. Squares, plants grown in 35 Pa CO_2; inverse triangles, plants grown in 70 CO_2; $n = 3–5$ leaves per treatment.

Table 3

Quantitative measures of the variation in the experimental L_e values that can be explained by theoretical prediction of the L func (equivalent to determinant coefficient, r^2; L values at $C_i < 19$ Pa were excluded from the analysis, see text for explanation) probability (P) of the paired t-test that experimental L_e values are not significantly different from the predicted range of tl function for both A/C_i and A/C_a curves (Figs. 1 and 2) of soybean grown under two CO_2 partial pressures (35 or 70 Pa)[a]

Treatment	Age		A/C_i curves		A/C_a curves	
CO_2 (Pa)				P-value		P-value
35	Expanding	63	0.89	0.962	0.87	0.663
35	Expanded	54	0.92	0.873	0.93	0.510
70	Expanding	43	0.86	0.889	0.89	0.690
70	Expanded	28	0.91	0.831	0.82	0.799

The $/C_i$ and A/C_a response curves were measured on either fully expanded or rapidly expanding leaflets = sample size.

chemical properties, the L function becomes useful for photosynthetic scaling studies only if we can link the function with the CO_2 partial pressure in the atmosphere. This requires the introduction of another variable, $x(= C_i/C_a)$ (as in Eqs. (7) and (8)), representing stomatal effects on the L function. Stomatal conductance is regulated by many environmental and biological factors (Farquhar and Sharkey, 1982) including atmospheric CO_2 partial pressure (Table 1). Fortunately stomatal conductance has been found to co-vary with photosynthesis (Ball and Berry, 1982). Furthermore, the C_i/C_a ratio (α) has been found to be constant for various species grown in different environments of light, nitrogen, phosphorus, soil water availability, and CO_2 (Wong et al., 1985a,b,c). The C_i/C_a ratios vary however, with leaf age as shown in this study (Table 1), and for plants grown in different water vapor pressure deficits (V. Gustchick, pers. commun.). Despite the considerable variation in the C_i/C_a ratio between the two age groups, the experimentally derived L values from the A/C_a curves are well correlated with those from the A/C_i curves (Fig. 4). This correlation supports the use of the annual increments in atmospheric CO_2 partial pressure, a tractable value, for predicting incremental increases in photosynthetic carbon influx into global terrestrial ecosystems. Obviously further testing with experimental data that include varying degrees of drought stress or levels of vapor pressure deficit are needed. Nonetheless, the L function still predicted the incremental carbon gain result-

ing from a small change in either ambient o intercellular CO_2 partial pressure. Furthermore we find it encouraging that leaves of different age classes respond similarly, suggesting that whole plant photosynthetic carbon gains in response to a small change in CO_2 partial pressure may also be predictable.

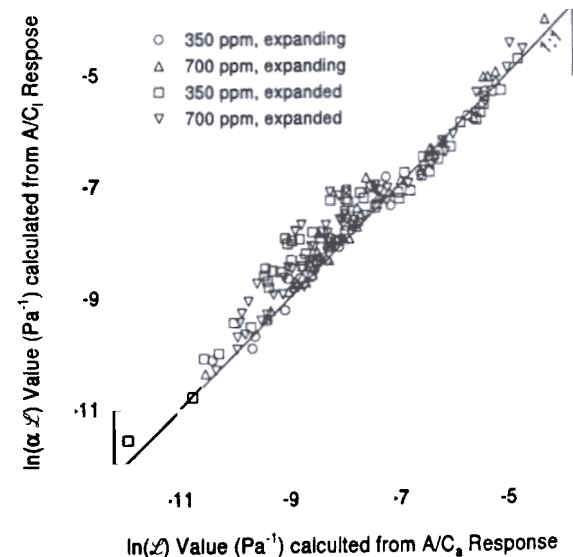Photosynthetic acclimation to growth in elevated CO_2 is another impor⁻t component of

Fig. 4. Relationship between L values derived from photosynthetic response to intercellular CO_2 partial pressure, vs. L values derived from photosynthetic response to ambient CO_2 partial pressure. Symbols are as in Figs. 2 and 3. Solid line represents a 1:1 relationship; $n = 185$ points (3–5 leaves per treatment).

predicting photosynthetic carbon influx. In this study, adjustments in the photosynthetic response curves (acclimation) of soybean grown under elevated CO_2 were age class-dependent. Similar results were reported by Xu et al. (1994) where photosynthetic adjustment occurred in young (12- to 14-day-old) but not old (21- to 26-day-old) soybean leaves. We found that younger, expanding leaves did have a lower V_{cmax}, indicating a smaller number of catalytically competent active sites of Rubisco as compared to fully expanding leaves (36.3 vs 49.0 μmol m^{-2} per s). Additionally, elevated CO_2 reduced V_{cmax} by 18% in these expanding leaves. Leaf expansion often is correlated with respiration rates (Amthor, 1989), and this study was no exception. However, unlike other CO_2 studies with soybean (e.g. Thomas and G, n, 1994), we did not find CO_2 effects on respiration in either age class; thus leaf carbon efficiency, the ratio of carbon gain to carbon loss, was nearly twice as high in expanded leaves compared to expanding leaves. This study suggests that including measurements of leaf respiration and photosynthesis on the same leaves improved the statistical fit between the theoretical L function and experimentally derived L values.

Although the L function also varies with measurement temperature (Polglass and Wang, 992), we did not investigate experimentally hese effects here. The kinetic effects of temperature on carboxylation verses oxygenation (the iochemical basis for the L function) have been ell studied (Kirschbaum and Farquhar, 1984; rooks and Farquhar, 1985) and can be used to llculate the temperature effects on J_{max} and $_{max}$ (Harley et al., 1992), and therefore L. For ample, when the temperature varies by ± 5°C, varies by 19% and L_2 varies by 4% (Luo and ooney, 1996). This temperature dependence s strong implications for global scaling, suggting that vegetation responses to rising CO_2 l be regionally specific (Polglass and Wang,)2; Kirschbaum, 1994). Simulation of global bon influx using a spatially explicit model inates that this temperature sensitivity results a higher global average carbon influx, roximating a mean temperature of 20°C, ipproximately 4°C above the current average

earth surface temperature (Y. Luo, unpublished data).

The lack of a strong CO_2 acclimation found in other treatments of this study may be a consequence of non-limiting nutrient supply (particularly nitrogen) and soil rooting volume (Thomas and Strain, 1991), Sage et al. (1989) suggest that plants that exhibit perfect acclimation reallocate N and other resources away from the down-regulated, non-limiting processes in order to keep them balanced with limiting processes. The lack of reallocation in the expanded leaves (e.g. away from Rubisco in elevated CO_2 as would be evidenced by a reduced initial slope of the A/C_i response curve and a lower V_{cmax}) suggests the lack of limiting processes, an inability to biochemically adjust or commensurate changes in Rubisco content and activation state in these plants. The high leaf nitrogen concentrations, unchanging specific leaf mass (Table 2) and rapid growth of the plants support the former of these cases. The relationship between J_{max} and V_{cmax} (Table 1Fig. 1) suggests that nonetheless, balance was maintained between the regulatory processes, and that the overall rate of photosynthesis was roughly co-limited by both the capacity to regenerate RuBP and by Rubisco activity (carboxylation) (Wullschleger, 1993).

Overall, this study provides an experimental examination of photosynthetic sensitivity and acclimation to CO_2 partial pressure with detailed ecophysiological measurements. Growth in elevated CO_2 induced substantial changes in photosynthetic rate, stomatal conductance, transpiration rate, and respiration as regulated by leaf ages. Photosynthetic sensitivity, L, was independent of these changes. More importantly, our experimental data validate the assertion that the L function was affected little by C_i/C_a (intercellular/ambient CO_2) ratio and could be linked directly to ambient CO_2 partial pressure. Thus, the global application of the L function to predict changes in carbon influx into terrestrial ecosystems caused by an annual increment in atmospheric CO_2 partial pressure is further warranted.

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