Sensitivity and acclimation of *Glycine max* (L.) Merr. leaf gas exchange to CO₂ partial pressure

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Abstract

Theoretical studies suggest that partitioning leaf photosynthetic responses to CO₂ 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*) at two CO₂ partial pressures, 35 and 70 Pa, and making a suite of ecophysiological measurements on expanding and fully expanded leaves. These CO₂ treatments resulted in a variety of acclimation responses, including changes in net photosynthetic rate and capacity, stomatal conductance, transpiration, and respiration. These responses were strongly dependent on leaf age. Despite the wide variety of acclimation responses, the experimentally derived photosynthetic sensitivity did not vary with CO₂ treatments or leaf age. In addition, the photosynthetic sensitivity to ambient CO₂ partial pressure was consistent with the sensitivity to intercellular CO₂ partial pressure, indicating little effect of stomatal conductance on photosynthetic sensitivity. This study supports the theoretical conclusion that photosynthetic sensitivity is independent of growth environment and leaf age, as well as photosynthetic acclimation, even though the latter varies with both environmental and developmental factors. Accordingly, photosynthetic sensitivity may be directly extrapolated from leaf to globe to predict the increment in terrestrial carbon influx stimulated by the slowly increase in atmospheric CO₂, whereas the acclimation component must be used to adjust the overall global rate. © 1999 Elsevier Science B.V. All rights reserved.

Introduction

Leaf-level studies have shown that photosynthetic responses to CO₂ partial pressure vary strongly 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 variation 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 CO₂ partial pressure. Luo et al. (1996) have proposed that...
separating leaf photosynthetic responses to long-term growth in elevated CO\textsubscript{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\textsubscript{2} partial pressure. By contrast, the acclimation component is the long-term, CO\textsubscript{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\textsubscript{2} describes changes in the ratio of carboxylation to oxygenation of ribulose 1,5-bisphosphate (RuBP). In C\textsubscript{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\textsubscript{2} partial pressure. First, under current atmospheric CO\textsubscript{2} partial pressures, Rubisco operates well below its \( K_m \) at about 25\% of its maximum capacity, and therefore elevated CO\textsubscript{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\textsubscript{2} partial pressure increases, the ratio of carboxylation to oxygenation increases, further contributing to the photosynthetic CO\textsubscript{2} sensitivity.

By contrast the acclimation component of photosynthetic responses to elevated CO\textsubscript{2} can be defined as physiological adjustments in photosynthetic capacity resulting from long-term growth in different CO\textsubscript{2} partial pressures. While short-term (minutes to hours) increases in ambient CO\textsubscript{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\textsubscript{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\textsubscript{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, roating 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, \( J_{\text{max}} \) (maximum electron transport rate) and \( V_{\text{cmax}} \) (the maximum carboxylation rate), are used to describe the regulation of leaf photosynthetic rates by light, species, CO\textsubscript{2} and nutrient availability. Luo and Mooney (1996) have demonstrated that when only sensitivity is concerned, both parameters \( (J_{\text{max}} \) and \( V_{\text{cmax}} \)) can be eliminated mathematically from this model, resulting in a leaf-level function \( (L) \), that is independent of environmental and species variation. Furthermore, acclimation induced by growth in elevated CO\textsubscript{2} can be described by varying the values of these variables \( (J_{\text{max}} \) and \( V_{\text{cmax}} \)) 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 Poisl and Wang (1992) (termed the CO\textsubscript{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 CO₂ 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 C₅(C₅/C₅), predictable? Although Luo et al. (1996) have tested the L function with existing CO₂ 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, CO₂ 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 Mcellon 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 5(10 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 CO₂ within the low CO₂ greenhouse tracked outside ambient (36-38 Pa) while the CO₂ partial pressure in the elevated CO₂ greenhouse was maintained at 70 Pa, 24 h a day for the duration of the experiment (36 days). CO₂ 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 CO₂ 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 CO₂ concentrations well within 2% of the desired set-point. CO₂ and H₂O 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 CO₂ and H₂O 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 leaflet 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 trifoliate was placed in the gas exchange cuvette and allowed to equilibrate for a minimum of 15 min. Gases of the desired CO₂, O₂ and N₂ 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 CO₂ partial pressure (A/Ci) was measured by varying the CO₂ partial pressure of the air entering the leaf cuvette from 10 to 150 Pa in 15 roughly equal steps.

Photosynthetic parameters, Vmax and Jmax, were calculated from the measured responses of assimilation to intercellular CO₂ partial pressure (A/Ci curves) by fitting data to the Farquhar et al. (1980) model using linearized equations:

\[
(A_1 + R)(C_i + K) = V_{\text{max}}(C_i - \Gamma) \tag{1}
\]

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

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, \(\Gamma\) is the photorespiratory CO₂ compensation point, \(K\) is a kinetic coefficient for the photosynthetic enzymes, and \(J_{\text{max}}\) is calculated from \(J\) as:

\[
J_{\text{max}} = \sqrt{\frac{a^2\Gamma}{J^2}} \tag{3}
\]

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

Following the gas-exchange measurements (7 days after planting), all plants were harvested and separated into leaves, stems and roots. The leaf area of each plant was measured in a belt-driven leaf area meter (Li-3000A, Li-Cor, Lincoln, NE). The leaves were then dried to a constant mass in a convection oven and subsequently weighed. Dried leaves were ground to fine powder in a ball mill (Model 2601, Cianflone Scientific Instrument Company, Pittsburgh, PA) and analyzed in a CHN analyzer for total carbon and nitrogen (Model 2400, Perkin-Elmer, Norwalk, CT) (Horneck and Miller, 1998).

2.3. Model parameterization and calculation of photosynthetic parameters

A full description of the model development and parameterization can be found elsewhere (Luo and Mooney, 1996) but briefly, the L function describing the relative change in photosynthesis for a small change in CO₂ partial pressure is defined as:

\[
L = \frac{1}{P} \frac{dP}{d[C_{\text{CO}_2}]} \tag{4}
\]

and is derived mathematically from the photosynthesis model of Farquhar et al. (1980) based on gross photosynthetic response to intercellular CO₂ partial pressure (\(A/C_i\) curves) as:

\[
L_1(A/C_i) = \frac{15\Gamma}{(C_i - \Gamma)(4.5C_i + 10.5\Gamma)} \tag{1}
\]

and

\[
L_2(A/C_i) = \frac{K + \Gamma}{(C_i - \Gamma)(C_i + K)} \tag{2}
\]

where \(C_i\) is the intercellular CO₂ 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 CO₂ response curve (Eqs. (1) and (2)).

In order to examine the effects of stomatal conductance on the \(L\) function, we define \(L(A/C_i)\).
from the photosynthetic response to ambient CO\textsubscript{2} as:

\[ L(A/C_\text{a}) = \frac{15z\Gamma}{(zC_\text{a} - \Gamma)(4.5zC_\text{a} + 10.5\Gamma)} \]

(7)

and

\[ L(A/C_j) = \frac{z(K + \Gamma)}{(zC_j - \Gamma)(zC_j + K)} \]

(8)

where \( z = C_j/C_a \). Theoretically:

\[ L(A/C_j) = zL(A/C_\text{a}) \]

(9)

2.4. Statistical analysis

Leaf respiration was added to the net photosynthetic rate \((A)\) and the responses to intercellular CO\textsubscript{2} partial pressure \((C_j)\) or ambient CO\textsubscript{2} partial pressure \((C_\text{a})\) were used to derive experimental \(L\) values \((L_e)\) with a difference equation:

\[ L_e = \left( \frac{1}{(P_j + P_{j-1})} \right) \left( \frac{p_j - p_{j-1}}{C_{x,j} - C_{x,j-1}} \right), \quad j = 2, 3, \ldots, n \]

(10)

where \( C_j \) 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 theoretical curves of the \(L\) function (equivalent to conventional determinant coefficient, \( r^2 \)). When computing this \( r^2 \), \(L_e\) values of \( C_j < 19 \text{ Pa} \) (= 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\textsubscript{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\textsubscript{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\textsubscript{2} partial pressures were affected significantly by both age and CO\textsubscript{2}, increasing significantly under elevated CO\textsubscript{2} in the fully expanded leaves \((P = 0.042)\), and increasing with leaf age in the 70 Pa CO\textsubscript{2} treatment \((P = 0.043, \text{ Table 1})\). \(V_{\text{cmax}}\), 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\textsubscript{2}. Similar trends were seen in \(J_{\text{max}}\) which was more variable than \(V_{\text{cmax}}\); yet no statistically significant differences were found. \(J_{\text{max}}\) and \(V_{\text{cmax}}\) were correlated linearly with each other (Fig. 1).

Respiration rates were also affected significantly by leaf age but not CO\textsubscript{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\textsubscript{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\textsubscript{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>
<thead>
<tr>
<th></th>
<th>n</th>
<th>A (µmol m⁻² per s)</th>
<th>V_{max} (µmol m⁻² per s)</th>
<th>J_{max} (µmol m⁻² per s)</th>
<th>R (µmol m⁻² per s)</th>
<th>A/R</th>
<th>g_0 (mol m⁻² per s)</th>
<th>E (µmol m⁻² per s)</th>
<th>C_i/C_a</th>
<th>WUE (A/E) (µmol mmol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 Pa exp.</td>
<td>3</td>
<td>11.1 ± 1.32</td>
<td>39.9 ± 3.5</td>
<td>109.9 ± 13.2</td>
<td>-2.45 ± 0.79</td>
<td>5.4</td>
<td>0.216 ± 0.060</td>
<td>3.18 ± 0.50</td>
<td>0.70 ± 0.02</td>
<td>3.59 ± 0.57</td>
</tr>
<tr>
<td>35 Pa full</td>
<td>5</td>
<td>13.4 ± 0.58</td>
<td>48.7 ± 6.0</td>
<td>131.3 ± 21.8</td>
<td>-1.33 ± 0.12</td>
<td>10.4</td>
<td>0.204 ± 0.034</td>
<td>5.09 ± 0.59</td>
<td>0.61 ± 0.01</td>
<td>2.72 ± 0.22</td>
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<tr>
<td>70 Pa exp.</td>
<td>3</td>
<td>12.8 ± 1.16</td>
<td>32.7 ± 3.1</td>
<td>105.3 ± 11.7</td>
<td>-2.53 ± 0.12</td>
<td>5.1</td>
<td>0.099 ± 0.013</td>
<td>2.38 ± 0.26</td>
<td>0.71 ± 0.02</td>
<td>5.42 ± 0.29</td>
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<td>70 Pa full</td>
<td>5</td>
<td>17.1 ± 1.74</td>
<td>49.2 ± 6.1</td>
<td>139.6 ± 37.2</td>
<td>-1.76 ± 0.41</td>
<td>10.7</td>
<td>0.102 ± 0.013</td>
<td>2.91 ± 0.33</td>
<td>0.64 ± 0.04</td>
<td>5.90 ± 0.08</td>
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**ANOVA (probability)**

<table>
<thead>
<tr>
<th></th>
<th>CO₂</th>
<th>Age</th>
<th>CO₂ x age</th>
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<tr>
<td>Probability</td>
<td>0.064</td>
<td>0.029</td>
<td>0.475</td>
</tr>
<tr>
<td></td>
<td>0.283</td>
<td>0.001</td>
<td>0.038</td>
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<tr>
<td></td>
<td>0.910</td>
<td>0.080</td>
<td>0.615</td>
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<tr>
<td></td>
<td>0.547</td>
<td>0.041</td>
<td>0.678</td>
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<td></td>
<td>0.975</td>
<td>0.002</td>
<td>0.815</td>
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<td></td>
<td>0.005</td>
<td>0.897</td>
<td>0.804</td>
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<td></td>
<td>0.018</td>
<td>0.044</td>
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<td></td>
<td>0.051</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>0.500</td>
<td>0.034</td>
</tr>
</tbody>
</table>

*Gas exchange characteristics were measured on either fully expanded (full) or rapidly expanding (exp.) leaflets; A, net assimilation rate measured at the growth CO₂ partial pressure; V_{max}, 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 CO₂ partial pressure; A/R, ratio of photosynthesis to respiration; g_0, stomatal conductance; E, transpiration; C_i/C_a, calculated ratio of intercellular to ambient CO₂ 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_{max}) and regeneration of RuBP (J_{max}). Primary limitations calculated from CO₂ response curves of expanding and fully expanded leaves of soybean grown under two CO₂ partial pressures (35 or 70 Pa); n = 3–5 leaves per treatment; r² of the regression, 0.71.

Conductance resulted in a lower C/C₂ ratio for plants grown at 35 Pa CO₂ as compared to 70 Pa CO₂ and in fully expanded compared to expanding leaves. The amount of carbon gained per unit water lost (photosynthetic water use efficiency, WUE; μmol CO₂ mmol⁻¹ H₂O⁻¹) was increased significantly in elevated CO₂ and in fully expanded compared to expanding leaves of plants grown in 35 Pa CO₂.

Leaf nitrogen concentrations were all quite high and were not affected significantly by either age class or CO₂ (Table 2). Leaf carbon concentrations were significantly lower in fully expanded leaves compared to expanding leaves, but not influenced by CO₂, C/N ratio and specific leaf mass (g m⁻²) were both unaffected by either age class or CO₂. Photosynthetic nitrogen use efficiency (PNUE, μmol C mmol⁻¹ 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 CO₂ treatment. There were no CO₂ by age interactions in PNUE.

The CO₂ 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) or ambient (A/Cₐ) CO₂ partial pressure for expanding and expanded leaves (Figs. 2 and 3). Photosynthetic response to intercellular CO₂ partial pressure varies considerably with CO₂ treatments for expanded leaves (Fig. 2A). Derived Lvalues 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>
<thead>
<tr>
<th>n</th>
<th>SLM (g dry mass m⁻²)</th>
<th>C (µmol of dry mass)</th>
<th>N (µmol of dry mass)</th>
<th>C/N</th>
<th>PNUE (µmol C mmol⁻¹ N⁻¹)</th>
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<tbody>
<tr>
<td>1 Pa exp.</td>
<td>3</td>
<td>28.9 ± 2.3</td>
<td>42.8 ± 0.1</td>
<td>4.7 ± 0.5</td>
<td>9.1 ± 0.9</td>
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<td>1 Pa full</td>
<td>5</td>
<td>24.0 ± 1.2</td>
<td>40.9 ± 0.6</td>
<td>5.1 ± 0.2</td>
<td>8.1 ± 0.3</td>
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<tr>
<td>1 Pa exp.</td>
<td>3</td>
<td>30.6 ± 3.9</td>
<td>42.3 ± 1.0</td>
<td>5.3 ± 0.4</td>
<td>8.1 ± 0.5</td>
</tr>
<tr>
<td>1 Pa full</td>
<td>5</td>
<td>27.1 ± 2.6</td>
<td>41.2 ± 0.4</td>
<td>5.2 ± 0.2</td>
<td>8.0 ± 0.3</td>
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</table>

NOV (probability): O₄, 0.484, ge, 0.885, 0.275, 0.186, 0.632, 0.235, 0.64, 0.685, 0.192, 0.031, 0.84, 0.69, 0.488, 0.304, 0.611

Measurements were made on either fully expanded (full) or rapidly expanding (exp.) leaflets.
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 $A/C_s$ 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 $aL_e$ 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 CO2 in comparison to ambient CO2. Leaf respiration rate differs by nearly twofold between the two age groups of leaves; and photosynthetic rate differs by 15–28% between the two CO2 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 $A/C_o$ 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 CO2 partial pressure (Poglass and Wang, 1992; Luo and Mooney, 1996). The independence of the $L$ 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 CO2/O2 ratio and measurement temperature). In light of these bio-

3. Photosynthetic responses and calculated CO2 sensitivity for expanding leaves of soybean grown under two CO2 partial pressures (35 or 70 Pa). Panel (A) net photosynthetic response to intercellular CO2 partial pressure; panel (B) net photosynthetic response to ambient CO2 partial pressure; panel (C) $L$ function (calculated CO2 sensitivity) vs. intercellular CO2 partial pressure, and panel (D) $L$ function vs. ambient CO2 partial pressure. Squares, plants grown in 35 Pa CO2; inverse triangles, plants grown in 70 CO2; $n = 3–5$ leaves per treatment.
Table 3
Quantitative measures of the variation in the experimental L values that can be explained by theoretical prediction of the L function (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 values are not significantly different from the predicted range of the 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).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age</th>
<th>A/C(_i) curves</th>
<th>A/C(_a) curves</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO(_2) (Pa)</td>
<td></td>
<td>P-value</td>
<td>P-value</td>
</tr>
<tr>
<td>35</td>
<td>Expanding</td>
<td>63</td>
<td>0.89</td>
</tr>
<tr>
<td>35</td>
<td>Expanding</td>
<td>54</td>
<td>0.92</td>
</tr>
<tr>
<td>70</td>
<td>Expanding</td>
<td>43</td>
<td>0.86</td>
</tr>
<tr>
<td>70</td>
<td>Expanding</td>
<td>28</td>
<td>0.91</td>
</tr>
</tbody>
</table>

The C\(_i\) and A/C\(_a\) response curves were measured on either fully expanded or rapidly expanding leaflets. n = 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, \( \alpha = C_i/C_a \) (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 (\( \alpha \)) 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 resulting from a small change in either ambient or 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 important component of
predicting photosynthetic carbon influx. In this study, adjustments in the photosynthetic response curves (acclimation) of soybean grown under elevated CO₂ 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 CO₂ 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 CO₂ studies with soybean (e.g. Thomas and Griffin, 1994), we did not find CO₂ 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, 1992), we did not investigate experimentally these effects here. The kinetic effects of temperature on carboxylation versus oxygenation (the biochemical basis for the L function) have been well studied (Kirschbaum and Farquhar, 1984; Brooks and Farquhar, 1985) and can be used to calculate the temperature effects on Jmax and V_cmax (Harley et al., 1992), and therefore L. For example, when the temperature varies by ±5°C, Jmax varies by 19% and L varies by 4% (Luo and O’oney, 1996). This temperature dependence has strong implications for global scaling, suggesting that vegetation responses to rising CO₂ will be regionally specific (Polglass and Wang, 1992; Kirschbaum, 1994). Simulation of global carbon influx using a spatially explicit model indicates that this temperature sensitivity results in a higher global average carbon influx, approximating a mean temperature of 20°C, approximately 4°C above the current average earth surface temperature (Y. Luo, unpublished data).

The lack of a strong CO₂ 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 CO₂ as would be evidenced by a reduced initial slope of the A/C 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 Jmax and V_cmax (Table 1, Fig. 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 CO₂ partial pressure with detailed ecophysiological measurements. Growth in elevated CO₂ 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 CO₂) ratio and could be linked directly to ambient CO₂ 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 CO₂ partial pressure is further warranted.
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