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As the partial pressure of CO$_2$ ($p$CO$_2$) in the atmosphere rises, photorespiratory loss of carbon in C$_3$ photosynthesis will diminish and the net efficiency of light-limited photosynthetic carbon uptake should rise. We tested this expectation for Indiana strawberry (Duchesnea indica) growing on a Maryland forest floor. Open-top chambers were used to elevate the $p$CO$_2$ of a forest floor habitat to 67 Pa and were paired with control chambers providing an ambient $p$CO$_2$ of 38 Pa. After 3.5 years, D. indica leaves grown and measured in the elevated $p$CO$_2$ showed a significantly greater maximum quantum efficiency of net photosynthesis (by 22%) and a lower light compensation point (by 42%) than leaves grown and measured in the control chambers. The quantum efficiency to minimize photorespiration, measured in 1% O$_2$, was the same for controls and plants grown at elevated $p$CO$_2$. This showed that the maximum efficiency of light-energy transduction into assimilated carbon was not altered by acclimation and that the increase in light-limited photosynthesis at elevated $p$CO$_2$ was simply a function of the decrease in photorespiration. Acclimation did decrease the ribulose-1,5-bisphosphate carboxylase/oxygenase and light-harvesting chlorophyll protein content of the leaf by more than 30%. These changes were associated with a decreased capacity for light-saturated photosynthesis. Even so, leaves of D. indica grown and measured at elevated $p$CO$_2$ showed greater light-saturated photosynthetic rates than leaves grown and measured at the current atmospheric $p$CO$_2$. In situ measurements under natural forest floor lighting showed large increases in leaf photosynthesis at elevated $p$CO$_2$, relative to controls, in both summer and fall. The increase in efficiency of light-limited photosynthesis with elevated $p$CO$_2$ allowed positive net photosynthetic carbon uptake on days and at locations on the forest floor that light fluxes were insufficient for positive net photosynthesis in the current atmospheric $p$CO$_2$.

Despite the fact that all plants assimilate some of their carbon under light-limiting conditions and some plants assimilate all of their carbon under light-limiting conditions, the effects of increasing atmospheric $p$CO$_2$ on light-limited photosynthesis has received little attention relative to the many studies of acclimation of light-saturated photosynthesis to elevated $p$CO$_2$ (for review, see Drake et al., 1997). The response of light-limited photosynthesis to the rising atmospheric $p$CO$_2$ has special significance to plants of the forest floor. Photosynthetic carbon gain by the leaves of forest floor herbs depends on their capacity for both light-limited photosynthesis, when they are shaded from direct sunlight, and light-saturated photosynthesis, when sunflecks penetrate gaps in the overlying tree canopy. Although different endogenous factors determine photosynthetic capacity under light-limiting and light-saturating conditions, rising $p$CO$_2$ is expected to increase photosynthesis under both conditions (Long and Drake, 1991; Bowes, 1993).

The key measure of photosynthetic capacity when photosynthesis is strictly light-limited, as in the deep shade of a forest floor, is the initial slope of the response of photosynthetic CO$_2$ uptake ($A$) to the incident photon flux ($Q$), i.e. the maximum efficiency with which photons are used in CO$_2$ fixation ($\phi$). $\phi$ is determined by the product of the

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**Abbreviations:** $A$, net rate of leaf CO$_2$ uptake per unit leaf area ($\mu$mol m$^{-2}$ s$^{-1}$); $\alpha$, leaf absorbance (dimensionless); $A_{max}$, net rate of leaf CO$_2$ uptake per unit leaf area at light saturation; $c_v$, substomatal partial pressure of CO$_2$ (Pa); $D$, leaf-atmosphere water vapor deficit (kPa); $I_{max}$, maximum rate of whole-chain electron transport ($\mu$mol m$^{-2}$ s$^{-1}$); $I$, stomatal limitation of net rate of leaf CO$_2$ uptake per unit leaf area at light saturation (%); LHC, light-harvesting complex protein; $p$CO$_2$, partial pressure of CO$_2$ (Pa); $\phi$, maximum quantum efficiency of CO$_2$ per incident photon (mol mol$^{-1}$); $\phi_{max}$, maximum quantum efficiency of CO$_2$ per incident photon on the basis of absorbed photons; $p$O$_2$, partial pressure of O$_2$; $Q$, photosynthetic quantum flux density ($\mu$mol m$^{-2}$ s$^{-1}$); $Q_{abs}$, photosynthetic quantum flux density absorbed by the leaf; $Q_{cfr}$, light compensation point for net photosynthesis; Ru-P$_2$, ribulose 1,5-bisphosphate; $T$, probability of a sunfleck; $T_{leaf}$, leaf temperature (°C); $T_{max}$, maximum in vivo carboxylation activity of Rubisco ($\mu$mol m$^{-2}$ s$^{-1}$).
$\alpha$ and the $\phi_{abs}$, $\phi_{abs}$ is determined by the product of the efficiencies with which (a) absorbed light energy is transduced into NADPH and ATP and (b) NADPH and ATP are used to assimilate CO$_2$ into carbohydrate. The major cause of inefficiency of the use of NADPH and ATP in net photosynthesis is diversion of this reductive and phosphorylating power into photorespiration. The rate of photorespiration relative to photosynthesis is determined by the ratio of the Rubisco-catalyzed velocities of oxygenation to carboxylation, which in limiting light is directly proportional to the ratio of pO$_2$/pCO$_2$ at Rubisco and inversely proportional to the specificity of the enzyme for CO$_2$ relative to O$_2$ (Long, 1991). As the pCO$_2$ of the atmosphere rises, the efficiency of light-limited net photosynthesis will rise. If the rate of mitochondrial respiration does not increase, then an increase in $\phi_{abs}$ will result in a decrease in $Q_{lcp}$ (Long and Drake, 1991). For forest floor vegetation growing at photon fluxes close to $Q_{lcp}$, an increase in pCO$_2$ would extend the period of the day and the number of days in which leaves could maintain positive net assimilation of CO$_2$. This prediction assumes that acclimation to elevated pCO$_2$ does not offset the increase in efficiency resulting from decreased photorespiration.

Photosynthetic acclimation can be defined as biochemical and physiological changes in the photosynthetic apparatus with development in an altered environment, in the current context, elevated pCO$_2$ (Gunderson and Wullschleger, 1994). A decrease in one of four factors with acclimation to elevated pCO$_2$ could offset the predicted increase in $\phi$. These are (a) the efficiency of light absorption by the leaf, (b) the efficiency of energy transduction into ATP and NADPH, (c) the diffusive conductance to CO$_2$, and (d) the specificity of Rubisco for CO$_2$. $\alpha$ could decrease if large decreases in the chlorophyll content occur or if leaf spectral properties are altered by growth in elevated pCO$_2$. In the absence of photorespiration, the constancy of $\phi_{abs}$, which has been reported across a wide range of C$_3$ species grown under different conditions (Björkman and Demmg, 1987; Long et al., 1993), suggests that decreased efficiency of energy transduction into ATP and NADPH is unlikely. The specificity of Rubisco for CO$_2$ is normally regarded as a constant within a species at a given temperature (McMurryie and Wang, 1993; Bainbridge et al., 1995). However, the discovery of differentially expressed gene families for the small subunit of Rubisco provides one possible mechanism by which a change in the environment might induce a change in the kinetic properties of the holoenzyme (Fritz et al., 1993). Anatomical and stomatal conductance changes are commonly observed during acclimation to elevated pCO$_2$ (Long and Drake, 1992); if these significantly decrease the diffusive conductance to CO$_2$, they will increase pO$_2$/pCO$_2$ at Rubisco within the photosynthesizing leaf. However, since pCO$_2$ within the leaf will equal that outside at the $Q_{lcp}$, change in conductance with acclimation to rising pCO$_2$ could not offset the decline in $Q_{lcp}$ that will result from decreased photorespiration.

After 3 years of growth in elevated pCO$_2$, the increase in $\phi_{abs}$ in the sedge Scirpus olneyi was identical to that observed when control plants were transferred to the same elevated pCO$_2$ (Long and Drake, 1991). This suggested that no significant acclimation had occurred in any of the factors controlling capacity for light-limited photosynthesis. However, S. olneyi is a species of open habitat and may have little capacity for acclimation of light-limited photosynthesis. Furthermore, after 3 years of growth in elevated pCO$_2$ these plants showed no acclimation of light-saturated photosynthetic capacity (Ziska et al., 1991). Thus, these plants may have been a poor subject in which to test for acclimation in light-limited photosynthesis. An increase in $\phi$ and a decrease in $Q_{lcp}$ at elevated pCO$_2$ would be of much greater significance to the carbon balance of plant communities that are naturally light-limited throughout much or all of their life cycle, such as herbaceous species of the forest floor.

Photosynthesis that takes place in sunflecks may provide 30 to 60% of the daily carbon gain in leaves of forest floor species (Pearcy, 1988). As a result, $A_{sat}$ can be an important determinant of CO$_2$ uptake in leaves growing on the forest floor. When measured at the current ambient pCO$_2$, $A_{sat}$ will often be lower for plants grown in elevated pCO$_2$ than for plants grown at the current ambient pCO$_2$. This acclimation commonly involves a decrease in the activity of Rubisco and may involve decreased capacity for Ru-P$_2$ regeneration (Long and Drake, 1992). Neither the loss of Rubisco activity nor the decrease in capacity for regeneration of Ru-P$_2$ can offset increases in $\phi_{abs}$ at elevated pCO$_2$ and low light, but both could affect $A$ of shade species during sunflecks, as could any change in stomatal limitation. Acclimatory decrease in both Rubisco and the capacity for Ru-P$_2$ regeneration is associated commonly with an increase in leaf carbohydrate concentration (Stitt, 1991; Sheen, 1994; Van Oosten et al., 1994). Since plants growing in deep shade are light-limited (Chazdon 1988), an accumulation of carbohydrates in leaves might seem unlikely even under elevated pCO$_2$, and thus acclimation in light-saturated photosynthesis is not expected. Therefore, both light-limited and light-saturated photosynthesis may be expected to increase in plants of the forest floor in response to the increasing atmospheric pCO$_2$.

In this study we tested this expectation using Duchesnea indica, a herbaceous perennial of the Rosaceae with trifoliate leaves and an indeterminate, clonal growth pattern. The plant spreads by means of surface runners, retains its leaves throughout the year, and continues to grow throughout the summer and autumn, when the overlying forest canopy imposes deep shade (Britton and Brown, 1970). D. indica was a major component of the ground flora in the open-top chambers used to elevate the pCO$_2$ of the understory vegetation in a deciduous forest for 4 years. Measurements were made in (a) late June, when D. indica was fruiting, and (b) late September to early October, when the plant was still growing vigorously and quantum flux at the forest floor reaches the yearly minimum (Anderson, 1964).

**MATERIALS AND METHODS**

**Plant Material and the Experimental Site**

All measurements were made within a long-term investigation of the effects of elevated pCO$_2$ on an understory
community, in a mixed, deciduous woodland on sandy loam soil at the Smithsonian Environmental Research Center (Edgewater, MD). A mean elevated pCO$_2$ of 67 Pa was provided beginning in 1991 in three cylindrical, open-top chambers that were 3.4 m in height and 3.8 m in diameter (Cipollini et al., 1993). Each treatment chamber was paired with an equivalent control chamber with a mean pCO$_2$ of 38 Pa in the forest understory. The overstory consisted of predominantly mature Liriodendron tulipifera (L.) and Liquidambar styraciflua (L.), with a canopy height of about 30 m. An understory was formed largely by the shrub Lindera benzoin (L.), below which grew a significant community of forest floor perennial herbs (Cipollini et al., 1993). Duchesnea indica (Andrzejowski) Focke. was abundant within this forest floor community and covered up to 30% of the ground surface. Full details of the experimental site, vegetation, and open-top chambers that were used were provided by Cipollini et al. (1993).

For this study, leaves were sampled June through October, 1994 and 1995, i.e. in the 3rd and 4th years of treatment. The central leaflet of the youngest fully expanded leaf on randomly selected ramets of _D. indica_ was used for all of the measurements. Leaves showing physical signs of senescence, damage, or disease or those growing within 30 cm of the chamber wall were not used.

### Light-Limited Photosynthesis (\(\phi_{\text{abs}}\) and \(Q_{\text{top}}\))

An Ulbricht integrating sphere leaf chamber (PP Systems, Hitchin, UK; Long et al., 1993) was incorporated into an open gas-exchange system and used to estimate \(\phi_{\text{abs}}\), \(Q_{\text{top}}\), and \(a\) in three different gas mixtures, following the method, equations, and calibration procedures of Long and Drake (1991). \(T_{\text{leaf}}\) was maintained at 28.0 ± 0.1°C (mean ± 1 se) and \(D\) was maintained at about 1.2 kPa. The response of \(A\) to \(Q_{\text{abs}}\) was linear in _D. indica_ for \(Q_{\text{abs}} < 10 \mu\text{mol m}^{-2}\text{s}^{-1}\), and there was no evidence of any Kok effect (Sharp et al., 1984).

### Light-Saturated Photosynthesis (\(A_{\text{sat}}\), \(V_{\text{c,max}}\), and \(I_{\text{max}}\))

The response of \(A\) to \(c_i\) was determined using a portable, open gas-exchange system (CIRAS-1, PP Systems) and was used to estimate \(V_{\text{c,max}}\) and \(I_{\text{max}}\). The \(A/c_i\) response was determined in saturating light, with \(T_{\text{leaf}}\) at 28.8 ± 0.1°C and \(D\) at 1.6 ± 0.1 kPa. \(V_{\text{c,max}}\) and \(I_{\text{max}}\) were calculated by the method of Wullschleger (1993), incorporating the temperature correction of McMurtrie and Wang (1993). \(I\) in the growth pCO$_2$ was calculated from the response of \(A\) to \(c_i\) by the method of Farquhar and Sharkey (1982).

### Leaf Proteins, Chlorophylls, Nonstructural Carbohydrates, and Nitrogen Contents

Samples of leaves from each of the six chambers were taken in parallel with photosynthetic measurements in June 1995, frozen in liquid nitrogen in situ, and stored at −80°C until subsequent analysis. Total leaf proteins were extracted and separated by SDS-PAGE, as described previously (Nie et al., 1995). Western analysis was used to identify LHC and the large and small subunits of Rubisco. Amounts of these proteins were quantified from the blots by the procedure of Nie et al. (1995). Total leaf nitrogen was determined in the same samples. Leaf material was ground to a fine powder and dried to a constant mass at 57°C. Nitrogen content of ground samples was determined by combustion and chromatographic separation in an elemental analyzer (PE 2400 series II CHNS/O analyzer, Perkin-Elmer Cetus). The measurement system was first calibrated against acetanilide standards. Chlorophyll was extracted from parallel samples using the method of Lee-good (1993) and quantified following the method of Graan and Ort (1984). Sampling for carbohydrate analysis took place between 5 and 6 PM, shortly after the period of maximum photosynthesis and the point in the day when the carbohydrate content should be greatest. Soluble sugars and starch were extracted according to the method of Farrar (1993) and quantified using the method of Dubois et al. (1956).

### Photosynthesis under In Situ Conditions

Leaf CO$_2$ uptake on the forest floor was measured between 10:30 AM and 5 PM with the portable, open gas-exchange system described above (CIRAS-1) under the natural lighting of the forest floor. Measurements were made at 67 ± 3 Pa for leaves in the elevated pCO$_2$ chambers and at 38 ± 1.5 Pa for leaves in the control chambers. Leaves were selected by a fully randomized design; therefore, measurements were made in the range of \(Q\) representative of the forest floor environment, including both sunflecks and diffuse shade light.

To determine whether the changes in \(\phi_{\text{abs}}\) and \(Q_{\text{top}}\) resulting from increased pCO$_2$ led to effects on light-limited photosynthesis in situ, additional measurements of \(A\) were made in areas where sunflecks were absent, and \(Q\) was close to the light-compensation point for photosynthesis. \(Q\) was measured using a quantum sensor (LI-189 and LI-1905A, Li-Cor, Lincoln, NE) immediately after the photosynthetic rate of an individual leaf was measured. To avoid errors associated with the high spatial heterogeneity in \(Q\) on the forest floor, the sensor was placed on the leaf chamber window and immediately above the leaf.

### Light and Sunflecks in Situ

Spatially averaged photon flux measurements were made to test for any differences between amounts of light experienced by the _D. indica_ leaf populations in the control and in elevated pCO$_2$ chambers. Spatially averaged photon flux (\(Q\)) and the proportion of sunflecks (\(\tau\)) at the surface of the _D. indica_ canopies were estimated with a 0.4-m line quantum sensor and sunfleck ceptometer (Decagon Devices, Pullman, WA), in parallel with gas-exchange measurements. The proportion of the ceptometer sensor array in which \(Q > 50 \mu\text{mol m}^{-2}\text{s}^{-1}\) was used to define \(\tau\) in practice. The threshold value (50 \(\mu\text{mol m}^{-2}\text{s}^{-1}\)) was previously found to approximate the minimum \(Q\) at which sunflecks were detected.
The effect of measurement $pCO_2$ on other variables was examined by repeated-measures analysis of variance or the Student's $t$ test for paired samples. One-tailed tests were used to test the hypotheses, predicted from previous studies of acclimation, that growth at elevated $pCO_2$ decreased $V_{c,max}$ and $I_{max}$; decreased leaf protein, chlorophyll, and nitrogen contents; and increased leaf carbohydrate contents. Where the variance ratio indicated heterogeneity of variances, a $z$ test was used in place of the Student's $t$ test (Sokal and Rohlf, 1981).

RESULTS

Light-Limited Photosynthesis ($\phi_{abs}$ and $Q_{lep}$)

Leaves grown and measured at elevated $pCO_2$ showed a 22% stimulation of $\phi_{abs}$ and a 42% reduction in $Q_{lep}$ by comparison with controls grown and measured at the current ambient $pCO_2$ (Fig. 1). Elevation of $pCO_2$ in the measuring atmosphere increased $\phi_{abs}$ to the same degree in control leaves as in leaves grown at elevated $pCO_2$ (Fig. 1a).

Figure 1. $\phi_{abs}$ (a) and $Q_{lep}$ (b) for $D$. indica grown in control open-top chambers with a mean $pCO_2$ of 38 Pa and chambers with a $pCO_2$ elevated to 67 Pa. Measurements were made at the control $pCO_2$ (Current), the elevated $pCO_2$ (Elevated), and the control $pCO_2$ with an O2 partial pressure decreased to 1 kPa to eliminate photorespiration (1 kPa). Means ($\pm 1$ se) are indicated for the three replicate open-top chambers during late September and early October (1994). The effect of measurement $pCO_2$ on $\phi_{abs}$ and $Q_{lep}$ was highly significant ($F_{1,8} = 14.5, P = 0.01$ and $F_{1,8} = 24.9, P = 0.001$, respectively), whereas the effect of growth $pCO_2$ ($F_{1,8} = 2.4, P = 0.16$ and $F_{1,8} = 1.8, P = 0.22$) and the interaction between growth and measurement $pCO_2$ ($F_{1,8} = 0.1, P = 0.76$ and $F_{1,8} = 0.5, P = 0.51$) were not statistically significant. Growth $pCO_2$ had no effect on $\phi_{abs}$ under the nonphotorespiratory conditions of 1 kPa $pO_2$ ($t_4 = -1.2, P = 0.30$); $\alpha$ was $0.86 \pm 0.02$ in control leaves and $0.88 \pm 0.03$ in leaves grown at elevated $pCO_2$; this difference was not significant ($t_4 = 0.6, P = 0.58$). Subscript of $F$ and $t$ are the degrees of freedom determining the critical values of each statistic.

Statistical Analyses

For all statistical analyses, the sample was considered as the chamber rather than the individual plant. Effects of both growth $pCO_2$ and measurement $pCO_2$ on $\phi_{abs}$, $Q_{lep}$, and $\alpha$ were tested by two-way analysis of variance. Because $\alpha$ is a proportion, it was arcsine-transformed before statistical analyses were done (Sokal and Rohlf, 1981).
Table 1. Photosynthetic characteristics of leaves grown at elevated and current pCO₂

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Growth pCO₂</th>
<th>% Control*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38 Pa</td>
<td>67 Pa</td>
</tr>
<tr>
<td>A sat (μmol m⁻² s⁻¹)</td>
<td>3.3 ± 0.6</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>I (%)</td>
<td>25 ± 6</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>V c,max (μmol m⁻² s⁻¹)</td>
<td>24.8 ± 1.7</td>
<td>18.9 ± 1.0</td>
</tr>
<tr>
<td>J max (μmol m⁻² s⁻¹)</td>
<td>53.1 ± 2.5</td>
<td>46.9 ± 0.5</td>
</tr>
<tr>
<td>Total protein (mg m⁻²)</td>
<td>1071 ± 53</td>
<td>810 ± 16</td>
</tr>
<tr>
<td>Rubisco (arbitrary units m⁻²)</td>
<td>439 ± 17</td>
<td>277 ± 8</td>
</tr>
<tr>
<td>LHC (arbitrary units m⁻²)</td>
<td>475 ± 25</td>
<td>325 ± 7</td>
</tr>
<tr>
<td>Nitrogen (mg m⁻²)</td>
<td>402 ± 20</td>
<td>362 ± 9</td>
</tr>
<tr>
<td>Total chlorophyll (μmol m⁻²)</td>
<td>321 ± 10</td>
<td>276 ± 18</td>
</tr>
<tr>
<td>Chlorophyll a/b ratio</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>Starch (g m⁻²)</td>
<td>1.0 ± 0.2</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Soluble carbohydrates (g m⁻²)</td>
<td>2.1 ± 0.3</td>
<td>2.3 ± 0.2</td>
</tr>
</tbody>
</table>

* Each mean value at elevated pCO₂ as a percentage of the mean for the control leaves.

There was no difference in φabs when pO₂ was lowered to 1 kPa to eliminate photorespiration. The absence of a difference when photorespiration was eliminated indicated that the maximum capacity for energy transduction in CO₂ assimilation was not affected by acclimation to elevated pCO₂ (Fig. 1a). α was also unaltered by treatment, despite a significant decrease in leaf chlorophyll content (Table I).

Light-Saturated Photosynthesis (A sat, V c,max, and J max)

Figure 2 shows that leaves grown at the current pCO₂ increase in pCO₂ from 38 to 67 Pa in the measuring atmosphere increased A sat substantially. Averaged across all of the control leaves measured in June, this increase was 56%. However, A sat in leaves grown at elevated pCO₂ was always lower for a given c i than in the control leaves (Fig. 2; Table I). This acclimation lowered the average increase in A sat to 42% for leaves grown and measured at elevated pCO₂ (Table I). Acclimation of A sat results from the apparent decreases in both V c,max and J max (Table I), which determine the initial slope of the response of A sat to c i and the A sat at saturating c i respectively (Fig. 2). Values of V c,max, J max and A sat from the two treatments in September and October 1994 (not shown) did not differ significantly (P > 0.05) from those measured in June 1995 (Table I). Decreased A sat at elevated pCO₂ would also occur if stomatal limitation increased; however, no significant changes in stomatal limitation were detected (Table I).

Leaf Proteins, Chlorophylls, Nonstructural Carbohydrates, and Nitrogen Contents

Significant decreases in both Rubisco and LHC contents per unit leaf area were observed in the leaves grown at elevated pCO₂ compared with controls (Fig. 3; Table I). Relative decreases in Rubisco and LHC were greater than for total leaf protein and nitrogen contents, suggesting a selective loss of these photosynthetic proteins (Table I).
This was confirmed when gels were loaded with an equal amount of total protein in each lane. On these gels the amount of Rubisco was decreased on average by 16% in protein extracts from the leaves grown at elevated pCO₂ relative to control leaves (data not shown). Decreases in leaf proteins were accompanied by statistically significant decreases in chlorophyll content, but the chlorophyll a/b ratio was unaffected by growth at elevated pCO₂ (Table I). Starch content was 50% greater in leaves grown at elevated pCO₂ than in controls, but there was no difference in soluble carbohydrate content (Table I).

### Leaf CO₂ Uptake under in Situ Conditions

Net CO₂ uptake was stimulated at midday under elevated pCO₂ as shown by measurements made on randomly selected leaves in situ. The increase was statistically significant and could not be attributed to Q, τ, Tleaf or D, which showed no significant differences between control and elevated pCO₂ chambers (Table II). Relative stimulation in A by elevated pCO₂ varied between 100 and 580% on the 3 cloudless days on which measurements were made, i.e. June 15 and both days in October. Under the overcast conditions of June 27, the mean photon flux at the forest floor at approximately midday was close to the Qλcp in the control leaves but was sufficient to support a positive and significant rate of net photosynthetic CO₂ uptake in the leaves growing at elevated pCO₂ (Table II).

Measurements made on leaves at positions in the chambers where no sunflecks occurred, and therefore made at photon fluxes close to the light compensation point of photosynthesis (mean Q = 5–9 μmol m⁻² s⁻¹), also showed a large relative increase in A at elevated pCO₂ compared with controls (Table III). Leaves in the control chambers were unable to maintain positive rates of photosynthesis in this limiting light, but positive rates of CO₂ uptake occurred even in the absence of sunflecks in the elevated pCO₂ chambers (Table III). The increase in CO₂ uptake at elevated CO₂ could not be attributed to an increase in Q or differences in Tleaf; indeed, Q was significantly lower for the leaves in the elevated pCO₂ chambers (Table III).

### DISCUSSION

An increase in pCO₂ from a current forest floor mean of 38 Pa to an elevated 67 Pa increased the maximum quantum efficiency of photosynthesis (φ₅₀₅) by 22% and decreased the Qλcp by 42% in D. indica. Although acclimation to elevated pCO₂ significantly decreased leaf Rubisco and LHC contents, it did not decrease the stimulation of φ₅₀₅ by elevated pCO₂ (Fig. 1a). These findings suggest that none of the potential mechanisms that could cause acclimation in light-limited photosynthetic capacity are realized. The response of light-limited photosynthesis in this shade species is essentially that found previously in the sun species S. olneyi (Long and Drake, 1991). Although leaf chlorophyll content showed a significant 14% decrease with growth at elevated pCO₂, α measured in an integrating sphere showed only a 2%, statistically insignificant decrease (Fig.

### Table II. Midday net leaf photosynthesis in situ at current and ambient pCO₂

<table>
<thead>
<tr>
<th>Variable</th>
<th>June 15</th>
<th>June 27</th>
<th>October 4</th>
<th>October 18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38 Pa</td>
<td>67 Pa</td>
<td>38 Pa</td>
<td>67 Pa</td>
</tr>
<tr>
<td>A (μmol m⁻² s⁻¹)</td>
<td>2.04 ± 0.21</td>
<td>0.49 ± 0.12</td>
<td>0.01 ± 0.11</td>
<td>0.42 ± 0.10</td>
</tr>
<tr>
<td>Q (μmol m⁻² s⁻¹)</td>
<td>25.7 ± 11.4</td>
<td>11.6 ± 0.5</td>
<td>7.2 ± 0.1</td>
<td>7.6 ± 0.0</td>
</tr>
<tr>
<td>τ</td>
<td>0.049 ± 0.035</td>
<td>0.020 ± 0.010</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Tleaf (°C)</td>
<td>23.0 ± 0.1</td>
<td>22.8 ± 0.1</td>
<td>22.4 ± 0.2</td>
<td>22.6 ± 0.2</td>
</tr>
<tr>
<td>D (kPa)</td>
<td>1.28 ± 0.03</td>
<td>1.25 ± 0.09</td>
<td>0.62 ± 0.06</td>
<td>0.68 ± 0.06</td>
</tr>
</tbody>
</table>

### Table III. Net leaf photosynthesis in the shade at midday

<table>
<thead>
<tr>
<th>Variable</th>
<th>Early October</th>
<th>Late October</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38 Pa</td>
<td>67 Pa</td>
</tr>
<tr>
<td>A (μmol m⁻² s⁻¹)</td>
<td>-0.3 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Q (μmol m⁻² s⁻¹)</td>
<td>8 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Tleaf (°C)</td>
<td>17.5 ± 0.6</td>
<td>17.5 ± 0.6</td>
</tr>
</tbody>
</table>

D. indica plants were grown in continuous shade in situ. Treatments are described in Table II. Measurements were made between 11 AM and 3:45 PM, selecting leaves by randomized design but excluding positions that would receive a sunfleck. Measurement dates were “Early October” (October 7 and 8, 1994) and “Late October” (October 17 and 25, 1994). Repeated measures analysis of variance showed that A was significantly greater and Q significantly lower for leaves grown at elevated pCO₂ (F₁,₈ = 11.3; p = 0.03 and F₁,₈ = 8.0; p = 0.05, respectively). There was no difference in the Tleaf in the two treatments (F₁,₈ = 0.0; p = 0.96). All values shown are the means ± 1 standard error for the three open-top chambers.
photosynthesis of forest floor vegetation under elevated CO₂.

The highly significant decrease in Rubisco content was paralleled by a significant decrease in the apparent in vivo Rubisco activity (Vₐₛₘₐₓ) for leaves grown at elevated pCO₂ (Table I; Figs. 1–3). Acclimation removed part of the stimulation of Aₛₜₛₑ, resulting from both decreased photorespiration and increased CO₂ saturation of Rubisco. However, Aₛₜₛₑ for leaves grown and measured in elevated pCO₂ still exceeded that of leaves grown and measured at the current ambient pCO₂. A decrease in both the Rubisco and the LHC content of the leaf through acclimation would have reduced the respiratory requirement for maintaining these major leaf proteins without decreasing photosynthetic carbon uptake in low light (Evans, 1988). This may, in part, explain the lower mitochondrial respiration rates that would be needed to explain the greater decrease in Qₑ than was predicted from decreased photorespiration alone.

In summary, acclimation to elevated pCO₂ in D. indica has removed none of the stimulation of light-limited photosynthesis resulting from decreased photorespiration and yet has significantly decreased leaf nitrogen content. Thus, the leaf is not only more efficient in its use of light but also in its use of nitrogen. Both factors suggest that if D. indica is typical of perennial herbs of the forest floor, then the potential range of habitats that such species could occupy will expand considerably with rising atmospheric pCO₂.

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LITERATURE CITED


Long SP (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO₂ concentrations: has its importance been underestimated? Plant Cell Environ 14: 729–740


Van Oosten J-J, Wilkins D, Besford RT (1994) Regulation of the expression of photosynthetic nuclear genes by CO₂ is mimicked by regulation by carbohydrates: a mechanism for the acclimation of photosynthesis to high CO₂? Plant Cell Environ 17: 913–923
