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Does Long-Term Elevation of CO_2 Concentration Increase Photosynthesis in Forest Floor Vegetation?[1]

Indiana Strawberry in a Maryland Forest

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As the partial pressure of CO_2 (pCO_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 pCO_2 of a forest floor habitat to 67 Pa and were paired with control chambers providing an ambient pCO_2 of 38 Pa. After 3.5 years, *D. indica* leaves grown and measured in the elevated pCO_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 pCO_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 pCO_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, but not light-limited, photosynthesis. Even so, leaves of *D. indica* grown and measured at elevated pCO_2 showed greater light-saturated photosynthetic rates than leaves grown and measured at the current atmospheric pCO_2. In situ measurements under natural forest floor lighting showed large increases in leaf photosynthesis at elevated pCO_2, relative to controls, in both summer and fall. The increase in efficiency of light-limited photosynthesis with elevated pCO_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 pCO_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 pCO_2 on light-limited photosynthesis has received little attention relative to the many studies of acclimation of light-saturated photosynthesis to elevated pCO_2 (for review, see Drake et al., 1997). The response of light-limited photosynthesis to the rising atmospheric pCO_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 pCO_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 (ϕ). ϕ is determined by the product of the

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Abbreviations: A, net rate of leaf CO_2 uptake per unit leaf area (μmol m^{-2} s^{-1}); α, leaf absorptance (dimensionless); A_{sat}, net rate of leaf CO_2 uptake per unit leaf area at light saturation; c_i, substomatal partial pressure of CO_2 (Pa); D, leaf-atmosphere water vapor deficit (kPa); J_{max}, maximum rate of whole-chain electron transport (μmol m^{-2} s^{-1}); l, stomatal limitation of net rate of leaf CO_2 uptake per unit leaf area at light saturation (%); LHC, light-harvesting complex protein; pCO_2, partial pressure of CO_2 (Pa); ϕ, maximum quantum efficiency of CO_2 per incident photon (mol mol^{-1}); ϕ_{abs}, maximum quantum efficiency of CO_2 per incident photon on the basis of absorbed photons; pO_2, partial pressure of O_2; Q, photosynthetic quantum flux density (μmol m^{-2} s^{-1}); Q_{abs}, photosynthetic quantum flux density absorbed by the leaf; Q_{lcp}, light compensation point for net photosynthesis; Ru-P_2, ribulose 1,5-bisphosphate; τ, probability of a sunfleck; T_{leaf}, leaf temperature (°C); $V_{c,max}$, maximum in vivo carboxylation activity of Rubisco (μmol m^{-2} s^{-1}).

α and the ϕ_{abs}. ϕ_{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 ϕ_{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 ϕ. 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. α 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 ϕ_{abs}, which has been reported across a wide range of C_3 species grown under different conditions (Björkman and Demmig, 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 (McMurtrie 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 ϕ_{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 ϕ 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 ϕ_{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 (ϕ_{abs} and Q_{lcp})

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 ϕ_{abs}, Q_{lcp}, and α in three different gas mixtures, following the method, equations, and calibration procedures of Long and Drake (1991). T_{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_{abs} was linear in *D. indica* for $Q_{abs} < 10$ μmol m^{-2} s^{-1}, and there was no evidence of any Kok effect (Sharp et al., 1984).

Light-Saturated Photosynthesis (A_{sat}, $V_{c,max}$, and J_{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 $V_{c,max}$ and J_{max}. The A/c_i response was determined in saturating light, with T_{leaf} at 28.8 ± 0.1°C and D at 1.6 ± 0.1 kPa. $V_{c,max}$ and J_{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 Leegood (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 ϕ_{abs} and Q_{lcp} 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 (τ) 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 $Q > 50$ μmol m^{-2} s^{-1} was used to define τ in practice. The threshold value (50 μmol m^{-2} s^{-1}) was previously found to approximate the minimum Q at which sunflecks were detected.

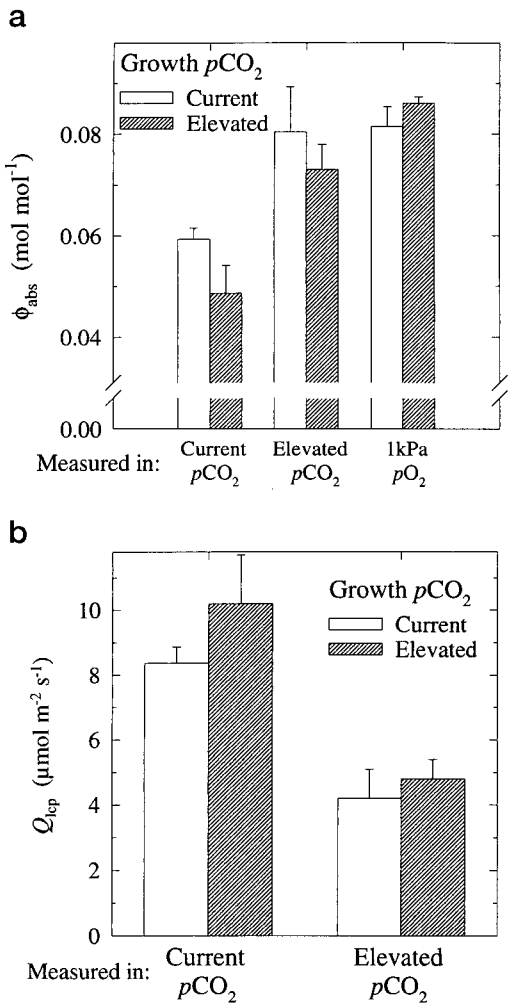

Figure 1. ϕ_{abs} (a) and Q_{lcp} (b) for *D. indica* grown in control open-top chambers with a mean pCO_2 of 38 Pa and chambers with a pCO_2 elevated to 67 Pa. Measurements were made at the control pCO_2 (Current), the elevated pCO_2 (Elevated), and the control pCO_2 with an O_2 partial pressure decreased to 1 kPa to eliminate photorespiration (1 kPa). Means (±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 ϕ_{abs} and Q_{lcp} 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 ϕ_{abs} under the nonphotorespiratory conditions of 1 kPa pO_2 (t_4 = −1.2, P = 0.30). α was 0.86 ± 0.02 in control leaves and 0.88 ± 0.03 in leaves grown at elevated pCO_2; this difference was not significant (t_4 = 0.6, P = 0.58). Subscripts 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 ϕ_{abs}, Q_{lcp}, and α were tested by two-way analysis of variance. Because α is a proportion, it was arcsine-transformed before statistical analyses were done (Sokal and Rohlf, 1981). The effect of elevated growth 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 J_{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 (ϕ_{abs} and Q_{lcp})

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

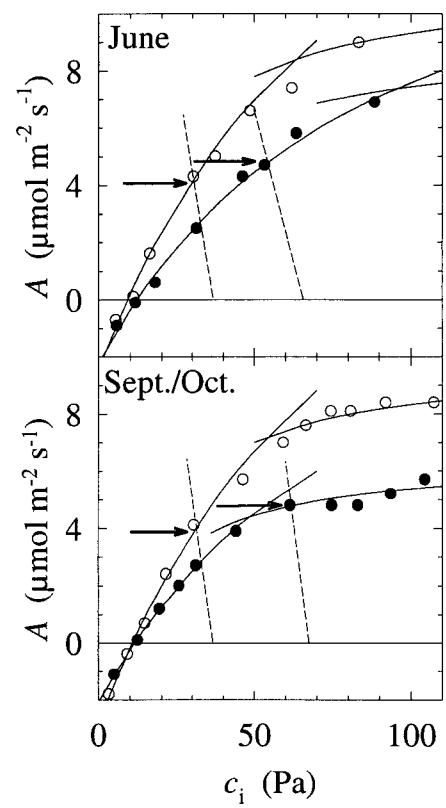

Figure 2. Representative A/c_i responses or "demand functions" for *D. indica* during June 1995 and late September through early October 1994 for plants grown in control (○) and elevated (●) pCO_2. Solid lines indicate the A/c_i response fitted to $V_{c,max}$ (below inflexion) and J_{max} (above inflexion). Also shown are supply functions for each curve (broken line), i.e. the linear rate of decrease in c_i with increasing A, determined by stomatal conductance (Farquhar and Sharkey, 1982). The intersections of the supply function with the A/c_i curves (arrows) indicate A_{sat} at the growth pCO_2.

Table I. *Photosynthetic characteristics of leaves grown at elevated and current pCO_2*

The mean A_{sat} at the growth pCO_2; l estimated from the leaf A/c_i response and A_{sat}; apparent $V_{c,max}$ and J_{max} estimated from the leaf A/c_i responses in June. *D. indica* leaves had grown either at a control or elevated pCO_2 of 38 or 67 Pa. Values of A_{sat}, l, $V_{c,max}$, and J_{max} did not differ significantly from those measured in September and October (not illustrated). Total leaf protein, Rubisco, LHC, nitrogen, total leaf chlorophyll, chlorophyll *a/b* ratio, starch, and water-soluble carbohydrate contents were determined for subsamples of the same population of leaves. All values are means ± 1 SE of the three replicate chambers of controls and the elevated pCO_2 treatment.

Characteristic	Growth pCO_2		% Control[a]
	38 Pa	67 Pa	
A_{sat} (μmol m^{-2} s^{-1})	3.3 ± 0.6	4.7 ± 0.1	142[b]
l (%)	25 ± 6	17 ± 4	68 (NS)[c]
$V_{c,max}$ (μmol m^{-2} s^{-1})	24.8 ± 1.7	18.9 ± 1.0	76[b]
J_{max} (μmol m^{-2} s^{-1})	53.1 ± 2.5	46.9 ± 0.5	88 (NS)
Total protein (mg m^{-2})	1071 ± 53	810 ± 16	75[b]
Rubisco (arbitrary units m^{-2})	439 ± 17	277 ± 8	63[d]
LHC (arbitrary units m^{-2})	475 ± 25	325 ± 7	69[e]
Nitrogen (mg m^{-2})	402 ± 20	362 ± 9	90[b]
Total chlorophyll (μmol m^{-2})	321 ± 10	276 ± 18	86[b]
Chlorophyll *a/b* ratio	2.0 ± 0.1	2.0 ± 0.1	100 (NS)
Starch (g m^{-2})	1.0 ± 0.2	1.5 ± 0.1	150[b]
Soluble carbohydrates (g m^{-2})	2.1 ± 0.3	2.3 ± 0.2	110 (NS)

[a] Each mean value at elevated pCO_2 as a percentage of the mean for the control leaves. [b] The difference between the pair of means is statistically significant at $P < 0.05$ of Student's t distribution. [c] $P > 0.05$. [d] $P \leq 0.001$. [e] $P \leq 0.01$.

There was no difference in ϕ_{abs} when pO_2 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 CO_2 assimilation was not affected by acclimation to elevated pCO_2 (Fig. 1a). α was also unaltered by treatment, despite a significant decrease in leaf chlorophyll content (Table I).

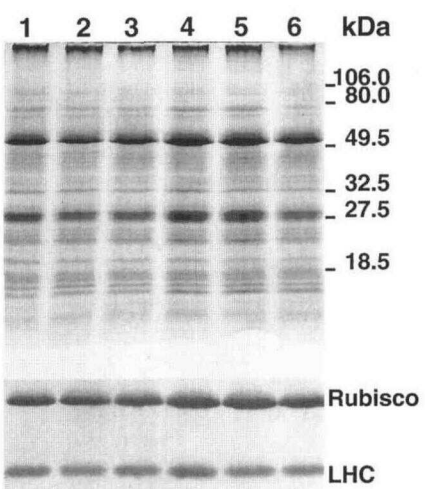

Figure 3. Rubisco and LHCs. Top, Coomassie blue-stained gel. The loaded extracts were made from the same amount of leaf area. The most intensively stained bands are the large subunit polypeptide of Rubisco at 56 kD and LHC at 27.5 kD. Lanes 1 to 3, Samples from each of the three replicate chambers at elevated pCO_2 chambers; lanes 4 to 6, samples from each of the control chambers. Bottom, Western blot showing the reaction with the large subunit polypeptide of Rubisco (monoclonal antisera raised against wheat Rubisco) and LHC (polyclonal antisera raised against pea). Lanes are as in the top panel.

Light-Saturated Photosynthesis (A_{sat}, $V_{c,max}$, and J_{max})

Figure 2 shows that leaves grown at the current pCO_2 increase in pCO_2 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 pCO_2 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 pCO_2 (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 pCO_2 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 pCO_2 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).

Table II. *Midday net leaf photosynthesis in situ at current and ambient pCO_2*

D. indica plants were grown at a mean pCO_2 of 38 or 67 Pa and measurements were made on 2 d in June 1995 and 2 d in October 1994. (*A* in situ on the forest floor at midday.) Mean values ±1 SE are for three replicate open-top chambers for each pCO_2. Repeated measures analysis of variance showed that *A* was significantly greater at elevated pCO_2 ($F_{1,4} = 11.2$; $P < 0.05$) but that *Q*, τ, T_{leaf}, and *D* were not affected by treatment ($P > 0.05$). There were significant differences in *Q*, τ, T_{leaf}, and *D* between measurement dates but no significant interaction between measurement dates and treatment ($P > 0.05$).

Variable	June 15		June 27		October 4		October 18	
	38 Pa	67 Pa	38 Pa	67 Pa	38 Pa	67 Pa	38 Pa	67 Pa
A (μmol m^{-2} s^{-1})	0.24 ± 0.21	0.49 ± 0.12	0.01 ± 0.11	0.42 ± 0.10	0.17 ± 0.17	1.16 ± 0.32	0.10 ± 0.27	0.24 ± 0.21
Q (μmol m^{-2} s^{-1})	25.7 ± 11.4	11.6 ± 0.5	7.7 ± 0.1	7.6 ± 0.0	35.8 ± 11.3	24.0 ± 6.8	17.2 ± 8.1	16.0 ± 10.5
τ	0.049 ± 0.035	0.020 ± 0.010	0 ± 0	0 ± 0	0.053 ± 0.053	0.038 ± 0.038	0.012 ± 0.020	0.066 ± 0.088
T_{leaf} (°C)	23.0 ± 0.1	22.8 ± 0.1	22.4 ± 0.2	22.6 ± 0.2	16.3 ± 0.6	15.4 ± 0.3	18.2 ± 0.4	17.6 ± 0.3
D (kPa)	1.28 ± 0.03	1.25 ± 0.09	0.62 ± 0.06	0.68 ± 0.06	0.78 ± 0.01	0.78 ± 0.01	0.97 ± 0.04	0.92 ± 0.02

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 pCO_2, 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 pCO_2 (Table I). Starch content was 50% greater in leaves grown at elevated pCO_2 than in controls, but there was no difference in soluble carbohydrate content (Table I).

Leaf CO_2 Uptake under in Situ Conditions

Net CO_2 uptake was stimulated at midday under elevated pCO_2, as shown by measurements made on randomly selected leaves in situ. The increase was statistically significant and could not be attributed to *Q*, τ, T_{leaf}, or *D*, which showed no significant differences between control and elevated pCO_2 chambers (Table II). Relative stimulation in *A* by elevated pCO_2 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 Q_{lcp} in the control leaves but was sufficient to support a positive and significant rate of net photosynthetic CO_2 uptake in the leaves growing at elevated pCO_2 (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 pCO_2 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 CO_2 uptake occurred even in the absence of sunflecks in the elevated pCO_2 chambers (Table III). The increase in CO_2 uptake at elevated CO_2 could not be attributed to an increase in *Q* or differences in T_{leaf}; indeed, *Q* was significantly lower for the leaves in the elevated pCO_2 chambers (Table III).

DISCUSSION

An increase in pCO_2 from a current forest floor mean of 38 Pa to an elevated 67 Pa increased the maximum quantum efficiency of photosynthesis (ϕ_{abs}) by 22% and decreased the Q_{lcp} by 42% in *D. indica*. Although acclimation to elevated pCO_2 significantly decreased leaf Rubisco and LHC contents, it did not decrease the stimulation of ϕ_{abs} by elevated pCO_2 (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 pCO_2, α measured in an integrating sphere showed only a 2%, statistically insignificant decrease (Fig.

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

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 pCO_2 ($F_{1,8} = 11.3$; $p = 0.03$ and $F_{1,8} = 8.0$; $P = 0.05$, respectively). There was no difference in the T_{leaf} in the two treatments ($F_{1,8} = 0.0$; $P = 0.96$). All values shown are the means ± 1 SE for the three open-top chambers.

Variable	Early October		Late October	
	38 Pa	67 Pa	38 Pa	67 Pa
A (μmol m^{-2} s^{-1})	−0.3 ± 0.1	0.1 ± 0.1	−0.0 ± 0.1	0.1 ± 0.0
Q (μmol m^{-2} s^{-1})	8 ± 1	5 ± 1	9 ± 0	7 ± 0
T_{leaf} (°C)	17.5 ± 0.6	17.5 ± 0.6	16.7 ± 0.3	16.7 ± 0.3

1). This may be explained by the hyperbolic relationship between absorptance and chlorophyll concentration for a given leaf, which predicts that, when absorptance approaches a maximum, variation in the chlorophyll concentration of the order reported here would have little effect. Assuming a leaf surface reflectance of 0.1, the measured absorptances of 0.86 to 0.88 suggest that absorption of light entering the mesophyll remained almost maximal in these leaves.

Following the biochemical model of leaf photosynthesis of Farquhar et al. (1980) and the kinetic constants for Rubisco of McMurtrie and Wang (1993), at 28°C an increase in pCO_2 from 38 to 67 Pa would increase ϕ_{abs} by 19.2%, as a result of decreased photorespiration. This is very similar to the 22% increase observed here. If mitochondrial respiration remains unchanged, a 22% increase in ϕ_{abs} must produce a reciprocal 18% decrease in Q_{lcp}. The actual decrease in Q_{lcp} of 42% is greatly in excess of the predicted value but could be explained by a decrease in the rate of mitochondrial respiration. Such decreases in mitochondrial respiration have been observed frequently in response to growth at elevated pCO_2 (Drake et al., 1997).

The stimulation in light-limited photosynthesis close to the light compensation point meant that photosynthesis in situ was significantly increased, despite 3 to 4 years of growth at elevated pCO_2. Previously, we showed that for the sun species *S. olneyi* a 22% increase in ϕ_{abs} in response to elevated pCO_2 would increase total net carbon gain by 18% over a diurnal course under clear sky conditions in the summer. This increase was independent of any increase in canopy carbon gain attributable to increased A_{sat} (Long and Drake, 1991). The influence of increased ϕ_{abs} on net canopy carbon gain increases with shade and should, therefore, have an even greater influence on total carbon gain in a shade species such as *D. indica* than in the sun species *S. olneyi*. This increase could have important implications for the ecology of *D. indica* in forest floor habitats at elevated pCO_2, since growth is commonly limited by light in forest floor herbs (Chazdon, 1988). An increase in the efficiency with which *D. indica* is able to fix carbon in the limiting diffuse light that prevails on the forest floor could lead potentially to large increases in the biomass at elevated pCO_2. This could allow the species to extend its range into more deeply shaded areas of the forest floor. This might be counteracted if the leaf area index of the forest canopy increased.

Stimulation of A in situ at elevated pCO_2 was observed both on sunny days, in which sunflecks reached *D. indica*, and on overcast days. The increase in A on sunny days could result not only from increased ϕ_{abs} and decreased Q_{lcp} but also from increased A_{sat}. Leaves from both treatments were light-saturated at $Q = 100$ μmol m^{-2} s^{-1}, and thus many sunflecks would be saturating. However, the photosynthetic rate in sunflecks is not simply determined by a capacity for light-saturated photosynthesis but also by the speed of change in photosynthetic rate in response to a step change in photon flux and by a vulnerability to photoinhibition in transient high light (Pearcy, 1988). Both could be affected by elevated pCO_2.

The highly significant decrease in Rubisco content was paralleled by a significant decrease in the apparent in vivo Rubisco activity ($V_{c,max}$) for leaves grown at elevated pCO_2 (Table I; Figs. 1–3). Acclimation removed part of the stimulation of A_{sat}, resulting from both decreased photorespiration and increased CO_2 saturation of Rubisco. However, A_{sat} for leaves grown and measured in elevated pCO_2 still exceeded that of leaves grown and measured at the current ambient pCO_2. 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 Q_{lcp} than was predicted from decreased photorespiration alone.

A decrease in leaf Rubisco content with an acclimation to elevated pCO_2 is commonly correlated with a decline in leaf nitrogen content (Long and Drake, 1992); such was the case in *D. indica* (Table II). Since Rubisco and LHC can each account for 10 to 25% of leaf nitrogen (Field and Mooney, 1986; Evans, 1989), the decrease in these proteins may explain the decline in leaf nitrogen that we observed. A decrease in photosynthetic proteins in elevated pCO_2 has been associated with repression of specific genes by soluble carbohydrates (Van Oosten et al., 1994). No increase in leaf soluble carbohydrate content was detected. This does not eliminate the possibility of carbohydrate repression, since there could be underlying changes in partitioning between different carbohydrate pools and subcellular locations in elevated pCO_2 that might affect gene expression.

In summary, acclimation to elevated pCO_2 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 pCO_2.

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LITERATURE CITED

Anderson MC (1964) Studies of the woodland climate. II. Seasonal variation in the seasonal climate. J Ecol **52:** 643–663

Bainbridge G, Madgwick P, Parmar S, Mitchell R, Paul M, Pitts J, Keys A, Parry MAJ (1995) Engineering Rubisco to change its catalytic properties. J Exp Bot **46:** 1269–1276

Björkman O, Demmig B (1987) Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta **170:** 489–504

Bowes G (1993) Facing the inevitable—plants and increasing atmospheric CO_2. Annu Rev Plant Physiol Plant Mol Biol **44:** 309–332

Britton NL, Brown HA (1970) An Illustrated Flora of the Northern United States and Canada, Vol 2. Dover Publications, New York, p 259

Chazdon RL (1988) Sunflecks and their importance to understorey plants. Adv Ecol Res **18:** 1–63

Cipollini ML, Drake BG, Whigham D (1993) Effects of elevated CO_2 on growth and carbon/nutrient balance in the deciduous woody shrub *Lindera benzoin* (L.) Blume (Lauraceae). Oecologia **96:** 339–346

Drake BG, Gonzalez-Meler M, Long SP (1997) More efficient plants? A consequence of rising atmospheric CO_2. Annu Rev Plant Physiol Plant Mol Biol **48:** 607–637

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem **28:** 350–356

Evans JR (1988) Acclimation by the thylakoid membranes to growth irradiance and the partitioning of nitrogen between soluble and thylakoid proteins. Aust J Plant Physiol **15:** 93–106

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia **78:** 9–19

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Annu Rev Plant Physiol **33:** 317–345

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta **149:** 78–90

Farrar JF (1993) Carbon partitioning. *In* DO Hall, JMO Scurlock, HR Bolhàr-Nordenkampf, RC Leegood, SP Long, eds, Photosynthesis and Production in a Changing Environment: A Field and Laboratory Manual. Chapman and Hall, London, pp 232–246

Field C, Mooney HA (1986) The photosynthesis-nitrogen relationship in wild plants. *In* TV Givnish, ed, On the Economy of Form and Function. Cambridge University Press, Cambridge, UK, pp 25–55

Fritz CC, Wolter FP, Schenkemeyer V, Herget T, Schreier PH (1993) The gene family encoding the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) small-subunit of potato. Gene **137:** 271–274

Graan T, Ort DR (1984) Quantitation of the rapid electron donors to P700, the functional plastoquinone pool, and the ratio of the photosystems in spinach chloroplasts. J Biol Chem **259:** 14003–14010

Gunderson CA, Wullschleger SD (1994) Photosynthetic acclimation in trees to rising atmospheric CO_2: a broader perspective. Photosynth Res **39:** 369–388

Leegood RC (1993) Carbon metabolism. *In* DO Hall, JMO Scurlock, HR Bolhàr-Nordenkampf, RC Leegood, SP Long, eds, Photosynthesis and Production in a Changing Environment: A Field and Laboratory Manual. Chapman and Hall, London, UK, pp 247–267

Long SP (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: has its importance been underestimated? Plant Cell Environ **14:** 729–740

Long SP, Drake BD (1991) Effect of the long-term elevation of CO_2 concentration in the field on the quantum yield of the C_3 sedge, *Scirpus olneyi*. Plant Physiol **96:** 221–226

Long SP, Drake BD (1992) Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentrations. *In* NR Baker, H Thomas, eds, Crop Photosynthesis: Spatial and Temporal Determinants. Elsevier Science, Amsterdam, The Netherlands, pp 69–103

Long SP, Postl WF, Bolhár-Nordenkampf HR (1993) Quantum yields for uptake of carbon dioxide in C_3 vascular plants of contrasting habitats and taxonomic groupings. Planta **189:** 226–234

McMurtrie RE, Wang Y-P (1993) Mathematical models of the photosynthetic response of tree stands to rising CO_2 concentrations and temperatures. Plant Cell Environ **16:** 1–13

Nie G-Y, Long SP, Garcia RL, Kimball BA, LaMorte RL, Pinter PJ Jr, Wall GW, Webber AN (1995) Effects of free-air CO_2 enrichment on the development of the photosynthetic apparatus in wheat, as indicated by changes in leaf proteins. Plant Cell Environ **18:** 855–864

Pearcy RW (1988) Photosynthetic utilization of lightflecks by understorey plants. Aust J Plant Physiol **15:** 223–238

Sharp RE, Matthews MA, Boyer JS (1984) Kok effect and the quantum yield of photosynthesis: light partially inhibits dark respiration. Plant Physiol **75:** 95–101

Sheen J (1994) Feedback control of gene expression. Photosynth Res **39:** 427–438

Sokal RR, Rohlf FJ (1981) Biometry, Ed 2. WH Freeman, San Francisco, CA

Stitt M (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant Cell Environ **14:** 741–762

Van Oosten J-J, Wilkins D, Besford RT (1994) Regulation of the expression of photosynthetic nuclear genes by CO_2 is mimicked by regulation by carbohydrates: a mechanism for the acclimation of photosynthesis to high CO_2? Plant Cell Environ **17:** 913–923

Wullschleger SD (1993) Biochemical limitations to carbon assimilation in C_3 plants—a retrospective analysis of the A/C_i curves from 109 species. J Exp Bot **44:** 902–920

Ziska LH, Hogan KP, Smith AP, Drake BG (1991) Growth and photosynthetic response of nine tropical species with long-term exposure to elevated carbon dioxide. Oecologia **86:** 383–389