Global Change Biology (1998) **4,** 173–183

Nonlinearity of photosynthetic responses to growth in rising atmospheric CO_2: an experimental and modelling study

YIQI LUO, DANIEL A. SIMS and KEVIN L. GRIFFIN
Biological Sciences Centre, Desert Research Institute, PO Box 60220, Reno, NV 89506, USA

Abstract

Nonlinear responses of photosynthesis to the CO_2 concentration at which plants were grown (C_g) have been often reported in the literature. This study was designed to develop mechanistic understanding of the nonlinear responses with both experimental and modelling approaches. Soybean (Glycine max) was grown in five levels of C_g (280, 350, 525, 700, 1000 ppm) with either a high or low rate of nitrogen fertilization. When the rate of nitrogen fertilization was high, the photosynthetic rate measured at C_g was highest in plants from the 700 ppm CO_2 treatment. When the rate of nitrogen fertilization was low, little variation was observed in the photosynthetic rates of plants from the different treatments measured at their respective C_g. Measurements of CO_2-induced changes in mass-based leaf nitrogen concentration (n_m, an index of changes in biochemical processes) and leaf mass per unit area (h, an index of morphological properties) were used in a model and indicate that the nonlinearity of photosynthetic responses to C_g is largely determined by relative changes in photosynthetic sensitivity, biochemical downregulation, and morphological upregulation. In order to further understand the nonlinear responses, we compiled data from the literature on CO_2-induced changes in n_m and h. These compiled data indicate that h generally increases and n_m usually decreases with increasing C_g, but that the trajectories and magnitudes of the changes in h and n_m vary with species and growth environments. Integration of these variables (n_m and h) into a biochemically based model of photosynthesis enabled us to predict diverse responses of photosynthesis to C_g. Thus a general mechanism is suggested for the highly variable, nonlinear responses of photosynthesis to C_g reported in the literature.

Keywords: biochemistry, carbon dioxide, carboxylation efficiency, global change, leaf nitrogen, morphology, modelling, photosynthesis

Received 30 September 1996; revision received 31 January 1997; accepted 9 April 1997

Introduction

When plants are grown at three or more CO_2 concentrations (growth CO_2, C_g), nonlinear responses of physiological processes have often been observed (Ackerley & Bazzaz 1995; Körner 1995). For example, when *Salix dasyclados* was grown in four levels of C_g, area-based photosynthetic rates measured at C_g increased from the 300–500 ppm treatments, reached a peak in the 700 ppm treatment, and then declined ⊤ 20% in the 1000 ppm treatment (Silvola & Ahlholm 1992). Similarly, Ball *et al.* (1994) found that the photosynthetic rates of field grown *Pinus ponderosa* were higher in a 525 ppm treatment than that in either the 350 or the 700 ppm treatments by 75% and 26%, respectively. Furthermore, Ackerley & Bazzaz (1995) summarized 25 studies reporting plant growth and reproductive responses to 223 separate three-level CO_2 gradients and demonstrated that in 32 of the 223 cases biomass responded nonlinearly to C_g.

The nonlinearities of these CO_2 responses have important implications for the extrapolation of experimental results to predict plant and ecosystem responses to global change in the natural world. Atmospheric CO_2 has gradually increased from 280 ppm in the pre-industrial time (Neftel *et al.* 1982; Friedli *et al.* 1986) to 360 ppm in 1995 (Keeling *et al.* 1995) and is expected to reach 700 ppm in the next century (IPCC 1995).

Correspondence: Dr Yiqi Luo, tel +1/ 702 674 7562, fax +1/ 702 6737485, e-mail yluo@maxey.unr.edu

While plants in natural ecosystems are experiencing a gradual increase in CO_2, most experimental research on plant and ecosystem responses are conducted at two distinct levels of CO_2 (e.g. 350 vs. 700 ppm) due to technical and economic limitations. Since plant and ecosystem responses to an incremental increase in CO_2 are likely to gradually saturate, the choice of which two CO_2 concentrations to use may change the experimental conclusions, not only on relative magnitude of the response but also on directions (i.e. positive vs. negative) of the CO_2 effects (Körner 1995). In addition, nonlinear responses at the individual plant level could translate into dramatic consequences at the community and ecosystem levels (Ackerley & Bazzaz 1995).

Despite the importance of nonlinear responses to C_g, they have not been investigated carefully. Hunt *et al.* (1991, 1993) did examine biomass responses in numerous species to four levels of C_g and analysed each species' response by fitting a hyperbolic function to the responses. However, the physiological mechanisms causing the different responses are not clear. Similarly, Ackerley & Bazzaz (1995) lumped plant responses to C_g into four categories: positive responses (biomass increasing across some portion of the three-level gradient), no response (no significant difference in any segment of the gradient); negative responses (biomass decreasing across some portion of the gradient); and nonmonotonic responses (biomass increasing in one segment and decreasing in the other segment). Again, this category method does not identify physiological mechanisms and additionally has application limits to four or more levels of C_g.

When plants are exposed to elevated CO_2, photosynthesis, which is the focus of this study, initially increases with CO_2 because carboxylation efficiency, i.e. carboxylation rate per unit of photosynthetic machinery, increases (Stitt 1991). Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) has a relatively low affinity for CO_2 and is substrate-limited at current atmospheric CO_2 and O_2 concentrations. Carboxylation is stimulated because increased CO_2 competes with O_2 and decreases oxygenation. After plants have been grown in elevated CO_2 for a few days, a suite of leaf biochemical and morphological properties may be adjusted. With increased carboxylation efficiency in elevated CO_2, photosynthesis tends to be limited by the capacity to regenerate ribulose bisphosphate (RuBP) and to recycle phosphate (P_i) in the Calvin cycle (Sage 1990), possibly resulting in a reallocation of resources within the photosynthetic apparatus (Sage 1990; Stitt 1991; Harley *et al.* 1992). The increased photosynthetic rate in elevated CO_2 leads to more carbohydrate production which may stimulate leaf mesophyll tissue growth (Vu *et al.* 1989).

Luo *et al.* (1994) have developed a **P**hotosynthetic **A**cclimation to **C**O_2 (PAC) model and proposed that CO_2-induced changes in photosynthetic capacity result from interactions between biochemical and morphological adjustments. When plants are grown at elevated CO_2, biochemical regulation at several levels (e.g. gene expression, end-product inhibition, P_i and RuBP regeneration) tends to promote a decrease in photosynthetic capacity (Sage 1990; Long 1991; Stitt 1991; Nie *et al.* 1995a, b). In contrast, changes in leaf morphology (primarily increased leaf mesophyll tissue growth) tends to increase photosynthetic capacity (Vu *et al.* 1989). The PAC model suggests that when the increase in photosynthesis resulting from additional mesophyll growth exceeds biochemically based reductions in CO_2 assimilation in elevated CO_2, photosynthetic capacity increases. Otherwise, photosynthetic capacity decreases.

The objective of this study is to apply the PAC model to understand nonlinear photosynthetic responses to C_g. We conducted experiments with five levels of CO_2 concentrations and two levels of nitrogen fertilization. Photosynthetic responses to C_g, changes of mass-based leaf nitrogen concentration (n_m) and mass per unit area (h) with C_g were measured. We also compiled data from the literature on CO_2-induced changes in n_m and h of plants grown in three or more levels of C_g. Based on the compiled data, we generalized three trajectory patterns of changes in n_m and h with a continuous CO_2 increase. The generalized changes in n_m and h were integrated into the PAC model to predict photosynthetic responses to growth in rising atmospheric CO_2.

Material and methods

Plant growth and measurements

Seeds of a non-nodulating variety of soybean, *Glycine max* (line D68–0099, Soybean Production Research, Stoneville, MS, USA), were planted in 8 L pots in a 50/50 v/v mixture of fine sand and sandy loam topsoil. The pots were placed in 5 naturally lit growth chambers inside a greenhouse at the Desert Research Institute in Reno, NV, USA. Temperature was controlled to 28 ± 2 °C in the daytime and 22 ± 1 °C at night. Relative humidity at midday was 66 ± 7%. The CO_2 concentration in each chamber was measured once every 6 min by an infrared gas analyser (model 6262, LICOR Inc., Lincoln, NE, USA). A datalogger (model CR10, Campbell Scientific, Logan, UT, USA) collected the data and controlled the CO_2 injection into the chambers on a 30 s cycle. The CO_2 setpoints of 280, 350, 525, 700, and 1000 ppm were maintained by switches between CO_2 injection and scrubbing. CO_2 scrubber boxes (14 × 45 × 56 cm) were constructed from plexiglass with two fans in the top and

an open grill covered by screening in the bottom, CO_2 in the air flowing through the boxes was absorbed by cooler pads and soda lime ('Coolpad' brand, Research Products Corp., Avondale, AZ, USA). Photon flux density averaged 22.9 ± 2.2 mol m^{-2} d^{-1} during the experiment. The nutrient treatments were randomly arranged within the CO_2 treatments. After seedling emergence, half of the plants were watered once a day with 1/2 strength Hoagland solution (7.5 mmol NO_3, 0.5 mmol PO_4, 3 mmol K, 2.5 mmol Ca, 1 mmol Mg, 1 mmol SO_4, 0.067 mmol Fe-EDTA, plus micronutrients), and the other half received a modified 1/2 strength Hoagland solution with 1/10 of the normal nitrogen concentration (0.75 mmol NO_3) with other nutrients kept at the same level.

Photosynthesis was measured in open flow gas exchange systems (model 6400, LICOR Inc., Lincoln, NE, USA) and a modified system similar to the MPH-1000 (Campbell Scientific Inc., Logan, UT, USA). The MPH-1000 system used a nickel-plated chamber with a glass window. CO_2 and O_2 concentrations in the air entering the chamber were controlled by mixing pure O_2 and N_2 with CO_2 in N_2 using mass flow controllers (model 825, Edwards High Vacuum International, Wilmington, MA, USA). Dew point of the air was controlled by a dew point humidifier (model DPH02, Armstrong Enterprises, Palo Alto, CA, USA). When the LICOR 6400 was used, the air supply was first mixed by the MPH-1000 system. The light source for all measurements was a tungsten halogen projector lamp (model ENH, 120 V-250 W, Radiac Inc., Japan) reflected off a 45 degree cold mirror. After 6 weeks growth, photosynthesis was measured at light saturation, 1400–1600 μmol m^{-2} s^{-1}, 28 °C leaf temperature, and 30 ± 2 mmol/mol water vapor concentration. Leaf disks were collected from the same leaves used in the gas exchange measurements. Prior to collection of the leaf disks, the plants were returned to the growth chambers for 1 day and then disks were collected in mid-afternoon. Collection at a consistent time of day reduced variation due to diurnal changes in carbohydrate content. Dry mass (after drying for 48 h at 60 °C), and total nitrogen content (model 2400 CHN analyser, Perkin Elmer, Norwalk, CT, USA) were measured for all samples.

The photosynthesis model

We used the PAC model (Luo *et al.* 1994) to examine the physiological mechanisms underlying the nonlinear responses of photosynthesis to a continuous increase in growth CO_2 (C_g). The PAC model suggests that the photosynthetic rate at a given C_g is determined largely by three components: Rubisco sensitivity, biochemical downregulation, and morphological upregulation (Fig. 1).

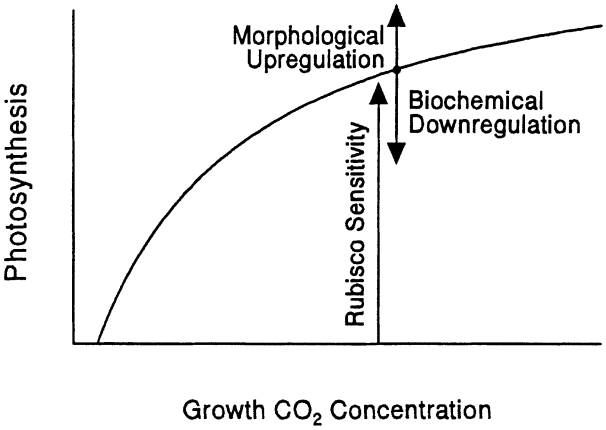

Fig. 1 Schematic representation of three processes (sensitivity, biochemical downregulation, and morphological upregulation) regulating photosynthetic responses to CO_2 concentrations at which plants are grown (growth CO_2). As growth CO_2 increases, enzymatic sensitivity of Rubisco to CO_2 consistently increases, leading to higher photosynthetic rates. In response to an increase in growth CO_2, biochemical adjustments, including gene expression, phosphorus regeneration, and end-product inhibition, tend to reduce photosynthetic capacity whereas morphological adjustments, primarily mesophyll growth, tend to increase photosynthetic capacity. Relative changes in these three components determine the net photosynthetic rate at a given growth CO_2 concentration (see text for detailed explanation).

The sensitivity of photosynthetic responses to CO_2 is caused by competition between carboxylation and oxygenation of RuBP catalysed by Rubisco. When CO_2 increases from 350 to 700 ppm, carboxylation efficiency increases by 30–70% (Stitt 1991). If inorganic phosphorus limits photosynthesis, however, photosynthetic sensitivity becomes zero or even negative (Sharkey 1985). Photosynthetic sensitivity is described by the Farquhar *et al.* (1980) model and has recently been re-examined by Luo & Mooney (1996) and Luo *et al.* (1996) in the context of global extrapolation. Biochemical regulation is the result of many processes including CO_2-induced inorganic phosphorus limitation, end-product inhibition through nonstructural carbohydrate (e.g. sugar), depressed gene expression, and reduced rubisco amount and/or activity. Regulation of these biochemical processes for plants grown in elevated CO_2 generally results in lower photosynthetic rates and capacities (i.e. downregulation) and is often indicated by changes in mass-based leaf nitrogen concentrations (n_m). Morphological upregulation is CO_2-stimulated mesophyll tissue growth, possibly including expanded cell volumes and cell layers (Vu *et al.* 1989) and can be represented by leaf mass per unit area (h).

At any given C_g, photosynthetic capacity, or the maximum carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) in the Farquhar *et al.* (1980)

model, is linearly correlated with leaf nitrogen per unit leaf area (n_a, g m^{-2}) (Field 1983; Harley *et al.* 1992). The latter is the product of n_m (g g^{-1}) and h (g m^{-2}). Similarly, phosphate release rate from triose phosphate utilization (TPU) and dark respiration (ρ) are also linear functions of n_a. Using these photosynthesis–nitrogen relationships, it is possible to incorporate both the biochemical and morphological components into a model to predict photosynthetic responses to growth in rising atmospheric CO_2 (Appendix). Breaking n_a into two terms n_m and h is arithmetically trivial but physiologically meaningful since these parameters represent two distinct types of processes (biochemical and morphological) and respond to increasing CO_2 in opposite ways (Luo *et al.* 1994).

Results

Experimental results and model validation

When plants were grown in high nitrogen, area-based photosynthetic rate measured at growth CO_2 (C_g) increased from 19.1 μmol m^{-2} s^{-1} in the 280 ppm treatment to 32.3 μmol m^{-2} s^{-1} in the 700 ppm treatment, and declined to 25.5 μmol m^{-2} s^{-1} in the 1000 ppm treatment (Fig. 2a). When plants were grown in low nitrogen, the photosynthetic rate at C_g increased from 14.5 μmol m^{-2} s^{-1} in the 280 ppm treatment to 16.5 μmol m^{-2} s^{-1} in the 350 ppm treatment and declined to 13.5 μmol m^{-2} s^{-1} in the 1000 ppm treatment. None of these changes were statistically significant (P = 0.163) with the low nitrogen fertilization. Mass-based photosynthetic rate, converted from the area-based photosynthetic rate by dividing it by leaf mass per unit area, was the highest in the 280 ppm treatment with both the low and high rates of nitrogen fertilization and gradually decreased with C_g (data not shown).

Based on a modelling study that suggested photosynthetic responses to C_g are largely determined by relative changes in mass-based leaf nitrogen concentration (n_m) and leaf mass per unit area (h) (Luo *et al.* 1994), we measured these two parameters. n_m was 4.9% of dry mass for plants grown in the 280 ppm treatment with high nitrogen (Fig. 2b) and decreased with C_g to 3.1% for plants grown in the 1000 ppm treatment. When plants were grown in low nitrogen, n_m was consistently lower than that in plants with high nitrogen and decreased with C_g from 2.1% in the 280 ppm treatment to 1.2% in the 1000 ppm treatment. Leaf mass per unit area (h) increased with C_g, from 40 to 65 g m^{-2} (Fig. 2c) regardless of nitrogen supply (P = 0.342).

We parameterized the PAC model with data from another independent study with soybean grown in two CO_2 concentrations (350 and 700 ppm), two rates of nitrogen fertilization (1/2 and 1/20 Hoagland solution in nitrogen), and two light levels (100 and 15% of natural light). The model was then used to predict photosynthetic responses in this study. The predicted rate of photosynthesis was generally consistent with the observed data (r^2 = 0.823 where r^2 is the determinant coefficient, Fig. 3). However, the model underpredicted leaf photosynthesis for plants grown in low nitrogen.

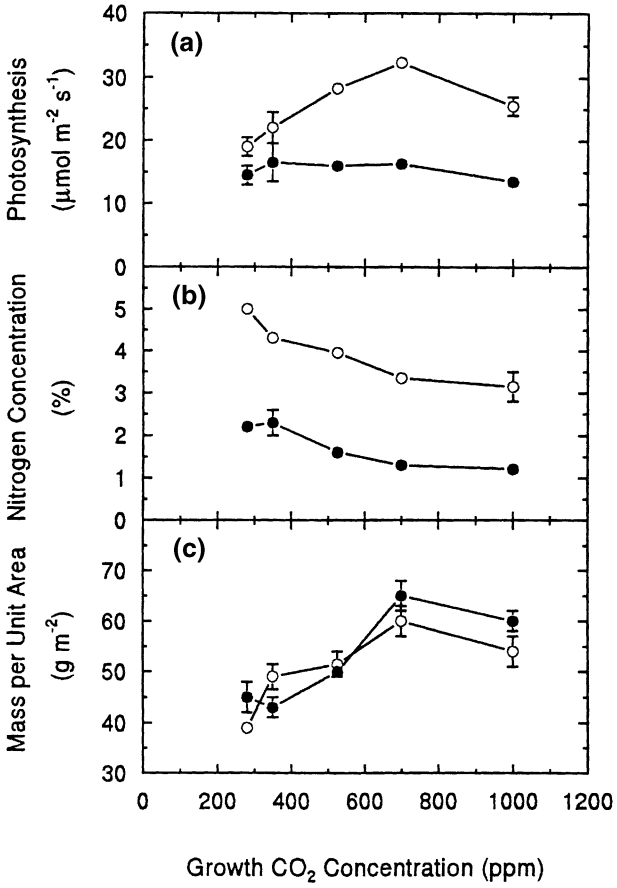

Fig. 2 Experimental results of: (a) photosynthetic rate measured at the CO_2 concentration at which plants were grown; (b) mass-based leaf nitrogen concentration; and (c) leaf mass per unit area for plants grown in high nitrogen (open circles) and low nitrogen (solid circles) (mean ± 1 SE, n = 4–6). Soybean plants were grown in five CO_2 concentrations 280, 350, 525, 700, and 1000 ppm) for 6 weeks.

Variation of n_m and h with growth CO_2

In order to develop more general understanding of photosynthetic responses to plant growth in a variety of CO_2 concentrations beyond the conditions in this experimental study, we compiled data on CO_2-induced changes in mass-based leaf nitrogen concentration (n_m) and mass per unit area (h) from the literature. Consistent with our own data in Fig. 2(b,c), n_m generally decreased and h usually increased with C_g (Fig. 4a,b). The magnitude

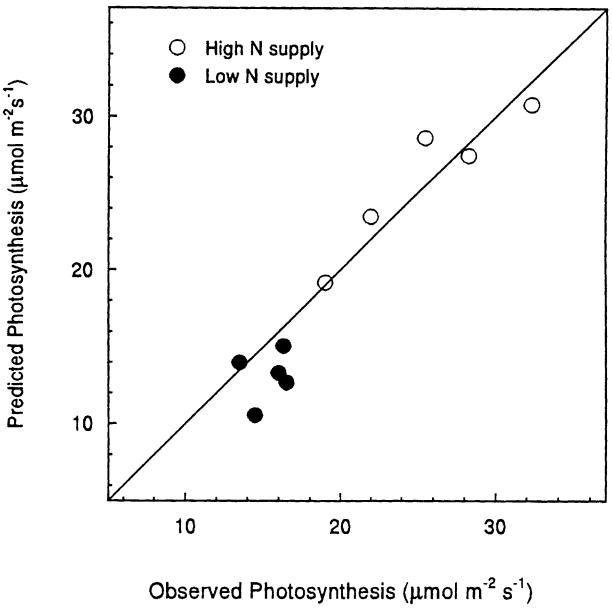

Fig. 3 Correlation of the predicted with the observed rates of photosynthesis presented in Fig. 2(a). The model and its parameterization are described in the Appendix. Model predictions are lower than the measured photosynthetic rates for plants grown in low nitrogen, possibly resulting from nitrogen reallocation towards photosynthetic apparatus and away from nonphotosynthetic processes. By adjusting values of parameters k_1 – k_8 in equation (A6), the model predictions for the plants grown in low nitrogen are improved.

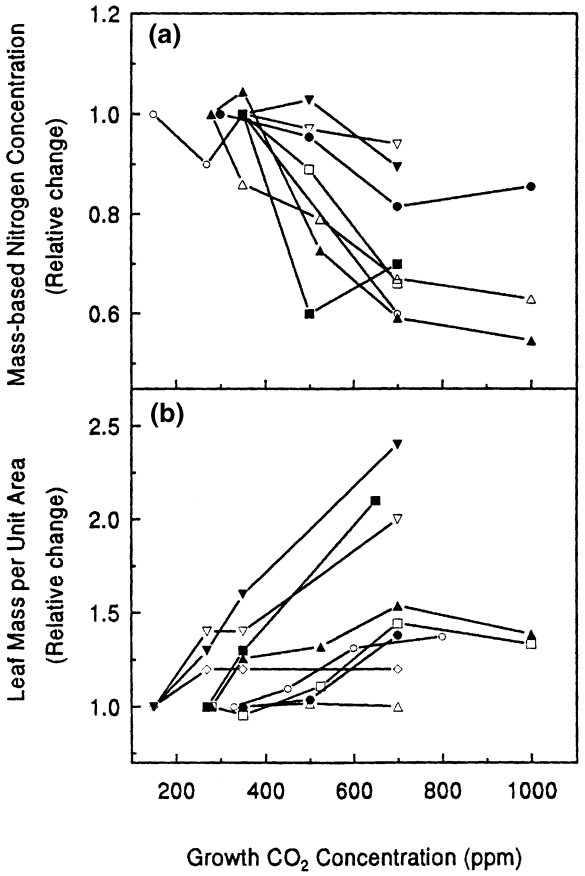

Fig. 4 (a) Trajectories of relative change in mass-based leaf nitrogen concentration with changes in growth CO_2 concentrations. Data were compiled from the literature: open circles for *Abutilon theophrasti* (Tissue *et al.* 1994), solid circles for *Glycine max* (Vu *et al.* 1989), open squares for *Abutilon theophrasti* (Garbutt *et al.* 1990), solid squares for *Pinus taeda* (Teskey 1995); open and solid triangles for *Glycine max* derived from Fig. 2b with high and low nitrogen fertilization, respectively; open inverse triangles for *Chenopodium alba* (Garbutt *et al.* 1990); solid inverse triangles for *Ambrosia artemisiifolia* (Garbutt *et al.* 1990). (b) Trajectories of the relative change in leaf mass per unit area with changes in growth CO_2 concentrations. Data were compiled from the literature: open circles for *Salix* × *dasyclados* (Silvola & Ahlholm 1992); solid circles for *Abutilon theophrasti* (Garbutt *et al.* 1990), open squares for *Glycine max* derived from Fig. 2c with low nitrogen supply, solid squares for *Gossypium hirsutum* (Thomas & Strain 1991); open triangles for *Setaria lutescens* (Garbutt *et al.* 1990); solid triangles for *Glycine max* derived from Fig. 2b with high nitrogen fertilization; solid inverse triangles, open inverse triangles, and open diamonds for *Abutilon theophrasti* measured at Day 14, 28, and 35, respectively, after CO_2 treatments (Tissue *et al.* 1994).

and trajectories of CO_2-induced changes in n_m and h, however, varied with species and growth environments. For example, n_m substantially decreased in the low range of C_g but changed little in the high range of C_g in *Glycine max* (Fig. 4a). In the contrast, n_m of *Ambrosia aremisiifolia* did not change much in the low range of C_g but was considerably reduced in the high range of C_g. For most species, n_m declined gradually as C_g increased (Fig. 4a). Leaf mass per unit area (h) generally increased with C_g but the increase also occurred at different levels of C_g and with varying magnitudes (Fig. 4b). h changed little for *Setaria lutescens* when C_g increased from 350 to 700 ppm but increased by 140% for *Abutilon theophrasti* when C_g increased from 150 to 700 ppm (Fig. 4b).

To facilitate modelling studies, we generalized CO_2-induced variation in n_m and h each into three trajectory patterns (Fig. 5a,b). Pattern 1 (denoted n1 for leaf nitrogen concentration and h1 for leaf mass per unit area) represented little change in n_m and h in the low range of C_g and moderate changes in the high range of C_g. Pattern 2 (n2 and h2) represented a gradual change with an increase in C_g. Pattern 3 (n3 and h3) represented a substantial change in the low range of C_g and little change in the high range of C_g. The three patterns were chosen to reflect three features of CO_2-induced changes in n_m and h: 1) n_m decreases and h increases with C_g; 2) the decrease in n_m and increase in h may occur at various magnitudes; and 3) the decrease in n_m and increase in h may occur at different levels of C_g (Fig. 5). In addition, integration of the three sets of curves in n_m and h

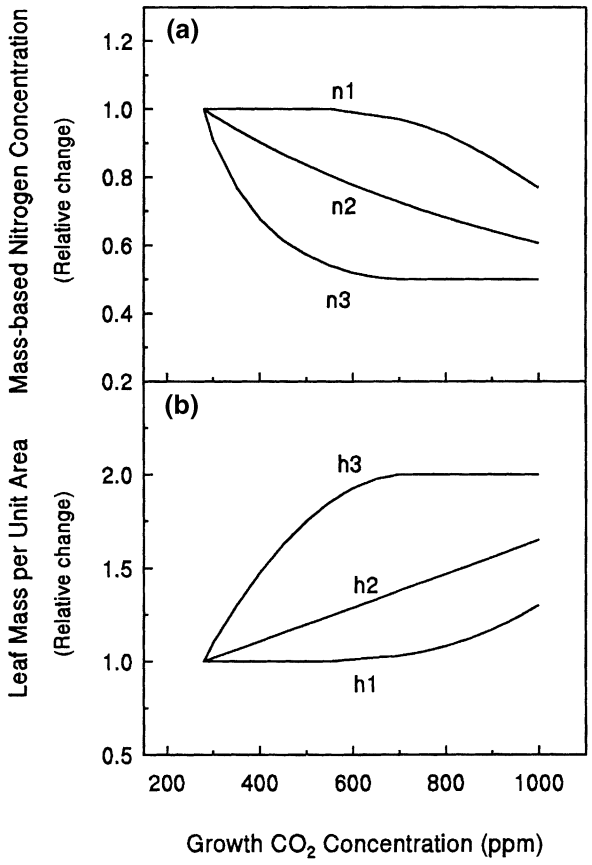

Fig. 5 Generalized trajectory patterns of (a) mass-based leaf nitrogen concentration (n_m) and (b) leaf mass per unit area (h) with increasing growth CO_2 concentrations. Notation n1–n3 and h1–h3 indicates three patterns of changes in n_m and h, respectively. The patterns are generalized in reference to the literature data in Fig. 4.

into the PAC model would generate diverse nonlinear responses of photosynthesis to C_g (Fig. 6).

Photosynthetic response to growth CO_2: Modelling studies

The PAC model was aimed to extend the experimental results and to explore the possibility that interactions among the three components of photosynthetic responses to C_g illustrated in Fig. 1 may generate other nonlinear responses than these shown in Fig. 2. In a hypothetical case, for example, biochemical downregulation is perfectly counterbalanced by morphological upregulation (the trajectory pattern n1 with h1, n2 with h2, or n3 with h3 in Fig. 5a,b), and no changes in photosynthetic capacity occur. Net photosynthesis increases with C_g simply due to sensitivity (increased carboxylation efficiency) (Fig. 6a,e,i). When morphological upregulation is larger than biochemical downregulation, photosynthetic capacity increases. The increase in photosynthetic rates with increasing C_g results from a combination of sensitivity and increased photosynthetic capacity (Fig. 6b,c,f). If morphological upregulation is smaller than biochemical downregulation, photosynthetic capacity decreases and the photosynthetic rate increases slightly or even decreases with C_g (Fig. 6d,g,h). The decreasing segment in Fig. 6g is caused by a strong reduction in n_m which is larger than the increase in carboxylation efficiency.

Discussion

When photosynthesis is compared among plants grown in a variety of CO_2 concentrations, many nonlinear responses are reported (Silvola & Ahlholm 1992; Ball *et al.* 1994; Körner 1995). However, the underlying mechanisms of these nonlinearities have not been identified. This combined modelling and experimental study attempted to develop a predictive understanding of the nonlinear responses of photosynthesis to the CO_2 concentrations at which plants were grown (C_g). Both the experimental results and model simulations in this study suggested that interactions among the three well-studied processes (photosynthetic sensitivity, biochemical downregulation, and morphological upregulation) may result in various nonlinear responses.

When soybean plants were grown in five levels of C_g from 280 to 1000 ppm with high nitrogen, mass-based leaf nitrogen concentration (n_m) declined continuously with C_g whereas leaf mass per unit area (h) increased with C_g until 700 ppm and then slightly (but not significantly) decreased (Fig. 2). Therefore, biochemical downregulation was roughly counterbalanced by morphological upregulation when C_g increased from 280 to 700 ppm. Photosynthetic capacity changed little and the photosynthetic rate increased with C_g due to increased carboxylation efficiency (i.e. the sensitivity component). An increase in C_g from 700 to 1000 ppm reduced biochemical activity but did not stimulate morphological growth. The resulting reduction in photosynthetic capacity was larger than the increase in photosynthetic rate due to the sensitivity component. As a consequence, the net photosynthetic rate decreased when C_g increased from 700 to 1000 ppm. With low nitrogen, the relative decrease in n_m was larger than the relative increase in h, and photosynthetic capacity decreased continuously with C_g. This decrease in the capacity was equivalent to the increase in carboxylation efficiency, leading to a relatively flat photosynthetic response over the entire range of C_g (Fig. 2).

This modelling exercise extended the experimental results and demonstrated that the PAC model, which incorporates the three photosynthetic components (sensitivity, biochemical downregulation, and morphological upregulation), is capable of generating diverse

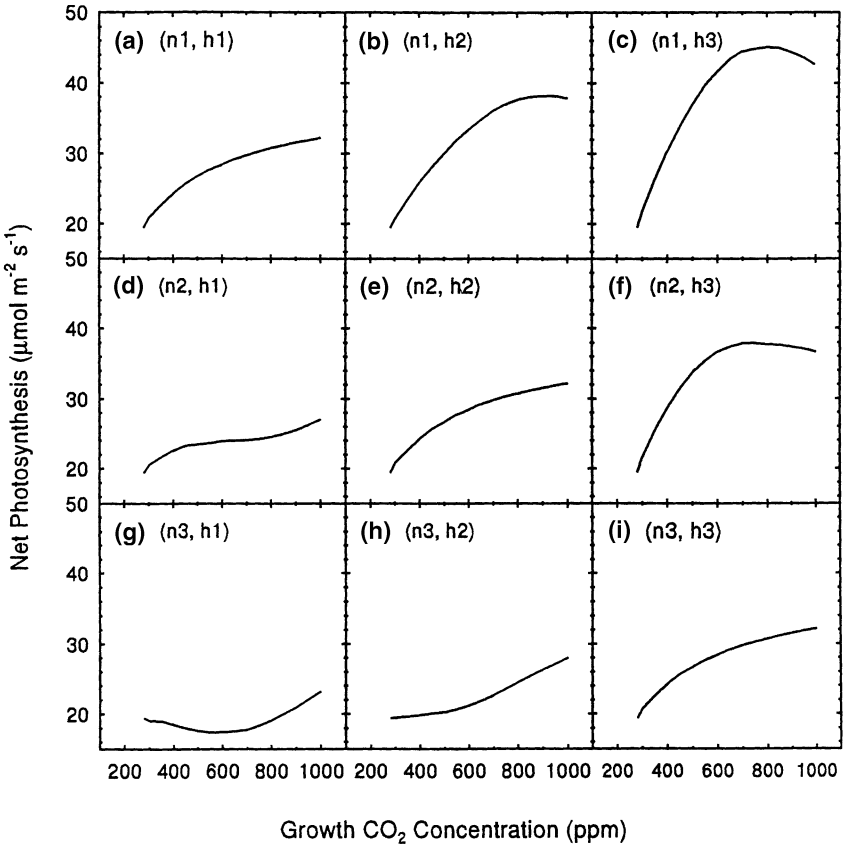

Fig. 6 Responses of net photosynthesis to growth CO_2 concentration with varying degrees of relative changes in n_m and h. The matrix of 9 response curves results from combining the three trajectory patterns of n_m with the three trajectory patterns of h (See Fig. 5). In the three cases along the diagonal (panel a, e, and i), biochemical downregulation is counterbalanced by morphological upregulation, photosynthetic capacity remains unchanged, and the photosynthetic rate increases with growth CO_2 due to photosynthetic sensitivity. In the three cases in the upper-right corner (panel b, c, and f), biochemical downregulation is smaller than morphological upregulation, photosynthetic capacity is regulated upward and the rate of photosynthesis increases with growth CO_2 much more than that in the diagonal panels. In the three cases in the lower-left corner (panel d, g, and h), biochemical downregulation is larger than morphological upregulation, photosynthetic capacity is regulated downward and the rate of photosynthesis slightly increases or even decreases with growth CO_2 concentrations, dependent on relative changes in n_m and h.

nonlinear responses of photosynthesis to C_g (Fig. 6). For example, the predicted photosynthetic rate increased dramatically in the low range of C_g (Fig. 6c) because of a combined increase in photosynthetic capacity and carboxylation efficiency. As C_g increased, the photosynthetic rate decreased (Fig. 6c) due to a decrease in the photosynthetic capacity that was larger than the increase in carboxylation efficiency. In contrast to the photosynthetic response to C_g in Fig. 6c, the predicted photosynthetic rate decreased in the low range of C_g and increased in the high range of C_g (Fig. 6g) because an increase in carboxylation efficiency was less than a decrease in the photosynthetic capacity in the low range of C_g.

Although the physiological mechanisms regulating photosynthesis, biomass, and reproduction are greatly different, the predicted diversity of trajectories in Fig. 6 are similar to the compiled patterns of biomass and reproduction responses by Ackerley & Bazzaz (1995). The positive response pattern classified by Ackerley & Bazzaz (1995) accounted for 73 of the 223 cases studied and corresponds to the predicted patterns in Fig. 6a,b,e,h,i. The negative response pattern is equivalent to the prediction in Fig. 6g if the three levels of C_g are chosen to be in the range from 280 to 700 ppm. The nonmonotonic pattern in Ackerley & Bazzaz (1995) is comparable to the predictions in Fig. 6c,f,g. If plants were grown at three levels of C_g from 400 to 800 ppm, the predicted pattern in Fig. 6(d) is relatively flat. If h is assumed not to change over the entire range of C_g and n_m changes in a trajectory between n2 and n3 in Fig. 5a, predicted photosynthetic responses to C_g could be completely flat over the range from 280 to 1000 ppm.

Among the three components regulating photosynthetic response to C_g illustrated in Fig. 1, sensitivity has been the most well studied. Sensitivity is determined by competition between carboxylation and oxygenation of ribulose-bisphosphate (RuBP). Increased CO_2 competes with oxygen, decreasing the oxygenase activity of Rubisco (Lawlor 1993) and increasing the ratio of carboxylation to oxygenation. Photosynthetic sensitivity to CO_2 varies with temperature (Kirschbaum & Farquhar 1984; Brooks & Farquhar 1985), but is similar among species (Allen *et al.* 1987), and has been shown to be not affected by growth environments (Luo *et al.* 1996).

Many facets of biochemical downregulation have been extensively studied. Plant growth in elevated CO_2 may cause redistribution of nitrogen away from Rubisco toward RuBP or P_i regeneration capacity (Sage *et al.* 1989; Sage 1990). Growth in elevated CO_2 also increases leaf soluble carbohydrate and starch contents (Long & Drake 1992; Baxter *et al.* 1995; Jacob *et al.* 1995). In plants grown

in elevated CO_2, increased carbohydrate production may exceed the demand from carbon sinks (Stitt 1991), leading to depressed gene expression (Nie *et al.* 1995a,b), reduction in mRNA (Krapp *et al.* 1993; Jang & Sheen 1994), and photosynthetic enzymes (Besford *et al.* 1990; Krapp *et al.* 1991; Socias *et al.* 1993). These biochemical adjustments generally result in a decrease in photosynthetic capacity.

Morphological adjustments have been relatively less well studied. Growth at elevated CO_2 concentration has been shown to stimulate an extra layer of mesophyll tissue (Vu *et al.* 1989), increase cell and chloroplast volumes (Robertson & Leech 1995), and thicken leaves (Thomas & Harvey 1983; Leadley *et al.* 1987; Radoglou & Jarvis 1990). These morphological regulations generally result in an increase in photosynthetic capacity. Although photosynthetic sensitivity has been well studied, this work highlights the additional importance of understanding mechanisms regulating biochemical downregulation and morphological upregulation, especially how, when, and how much these processes are adjusted by growth in elevated CO_2.

In the PAC model, n_m is used to indicate biochemical activities and h is used to quantify morphological structure. This model is effective despite its simplicity and absence of interactions and/or feedbacks among the biochemical and morphological processes. For example, starch accumulation in elevated CO_2 itself may not represent biochemical downregulation or morphological upregulation but may influence the parameters n_m and h. This can be seen in the data of Wong (1990) where nitrogen concentrations were presented with and without the subtraction of nonstructural carbohydrates (NSC). After NSC was subtracted, CO_2-induced changes in n_m were reduced and, similarly, CO_2-induced changes in h should be smaller. Subtraction of NSC, however, does not affect area-based nitrogen concentration (n_a) and thus predictions of photosynthetic responses to C_g. In addition, the PAC model is not able to mechanistically predict nitrogen reallocation within a leaf but can simulate it by adjusting parameter values for k_1–k_8 in equation A6 (Appendix).

This study also illustrates important differences between photosynthetic responses to the CO_2 concentrations in a leaf cuvette where the net photosynthetic rate is measured (i.e. measurement CO_2) vs. the CO_2 concentrations at which plants are grown (i.e. growth CO_2). During the short-term measurements of net photosynthesis in response to increasing CO_2 concentrations, the photosynthetic rate initially increases rapidly and then the rate of the increase slows. As the measurement CO_2 further increases, the photosynthetic rate may plateau. The plateau represents the maximum photosynthetic rate or photosynthetic capacity and is correlated with area-based leaf nitrogen concentration (n_a) (Field & Mooney 1986). The response of photosynthesis to growth in a variety of CO_2 concentrations does not necessarily follow this trajectory (Fig. 6). Most notably, photosynthesis does not necessarily plateau at high C_g. For example, when biochemical processes are strongly downregulated by growth in elevated CO_2 and n_a decreases, the photosynthetic rate may be highest in plants grown in a medium C_g and then decreases in plants grown in a high C_g (e.g. Fig. 6c). Alternatively if n_a is not affected by C_g, the photosynthetic rates will increase in plants grown in elevated CO_2 in a trajectory similar to that of photosynthetic responses to the short-term response described above (e.g. Fig. 6a,e,i). If additional leaf mesophyll growth is greatly stimulated by C_g and n_a substantially increases, the photosynthetic rate will continuously increase in plants grown in elevated CO_2 concentrations.

In summary, this study has experimentally demonstrated that when plants were grown in five CO_2 concentrations, nonlinear photosynthetic responses were observed. The nonlinearity of the responses can be predicted by a photosynthetic acclimation to CO_2 model which has integrated CO_2-induced relative changes in three components: photosynthetic sensitivity, biochemical downregulation, and morphological upregulation. While the photosynthetic sensitivity to CO_2 is relatively well understood, biological mechanisms controlling biochemical and morphological adjustments are not fully investigated but important to understanding the often reported nonlinear responses to growth in elevated CO_2 concentrations.

Acknowledgements

We thank three anonymous reviewers for their very helpful comments. This study was financially supported by the USDA NRICGP grant 94–37100–0345, and NSF/NASA/DOE/EPA TECO grant NSF-IBN-9524036 and DOE grant DE-FG05–95ER62083 to Duke Forest FACE project.

References

Ackerley DD, Bazzaz FA (1995) Plant growth and reproduction along CO_2 gradients: non-linear responses and implications for community change. *Global Change Biology*, **1,** 199–207.

Allen LH Jr, Boote KJ, Jones JW, Jones PH, Valle RR, Acock B, Rogers HH, Dalhman RC (1987) Response of vegetation to rising carbon dioxide: Photosynthesis, biomass, and seed yield of soybean. *Global Biogeochemical Cycles*, **1,** 1–14.

Ball JT, Johnson DW, Henderson PS *et al.* (1994) *Forest Response to CO_2, Annual report for* 1993. Desert Research Institute, Reno, NV.

Ball JT, Woodrow IE, Berry JA (1987) A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environment conditions. In:

Progress in Photosynthesis Research, Vol IV (ed. Giggins I), pp. 221–224. Martinus Nijhoff Publishers, Netherlands.

Baxter R, Bell SA, Sparks TH, Ashenden TW, Farrar JF (1995) Effects of elevated CO_2 concentrations on three montane grass species. III. Source leaf metabolism and whole plant carbon partitioning. *Journal of Experimental Botany,* **46,** 917–929.

Besford RT, Ludwig LJ, Withers AC (1990) The greenhouse effect: Acclimation of tomato plants growing in high CO_2, photosynthesis and Ribulose-1,5-bisphosphate carboxylase protein. *Journal of Experimental Botany,* **41,** 925–931.

Brooks A, Farquhar GD (1985) Effect of temperature on the CO/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Estimates from gas-exchange measurements on spinach. *Planta*, **165,** 397–406.

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta*, **149,** 79–90.

Field CB (1983) Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. *Oecologia.* **56,** 341–347.

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

Friedli H, Loestcher H, Oeschger H, Siegenthaler U, Stauffer B (1986) Ice core record of the $^{13}C/^{12}C$ record of atmospheric CO_2 in the past two centuries. *Nature*, **324,** 237–238.

Garbutt K, Williams WE, Bazzaz FA (1990) Analysis of the differential response of five annuals to elevated CO_2 during growth. *Ecology*, **71,** 1185–1194.

Harley PC, Thomas RB, Reynolds JF, Strain BR (1992) Modelling photosynthesis of cotton grown in elevated CO_2. *Plant, Cell and Environment*, **15,** 271–282.

Hunt R, Hand DW, Hannah MA, Neal AM (1991) Response to CO_2 enrichment in 27 herbaceous species. *Functional Ecology*, **5,** 410–421.

Hunt R, Hand DW, Hannah MA, Neal AM (1993) Further response to CO_2 enrichment in British herbaceous species. *Functional Ecology*, **7,** 661–668.

IPCC (Intergovernmental Panel on Climate Change) (1996) *Climate change* 1995*: The science of climate change*. Cambridge University Press, Cambridge, UK, 572pp.

Jacob J, Greitner C, Drake BG (1995) Acclimation of photosynthesis in relation to Rubisco and non-structural carbohydrate contents and *in situ* carboxylase activity in *Scirpus olneyi* grown at elevated CO_2 in the field. *Plant, Cell and Environment*, **18,** 875–884.

Jang JC, Sheen J (1994) Sugar sensing in higher plants. *The Plant Cell*, **6,** 1665–1679.

Keeling CD, Whorf TP, van Wahlen M, der Plicht J (1995) Interannual extremes in the rate of rise of atmospheric carbon dioxide since 1980. *Nature*, **375,** 666–670.

Kirschbaum MUF Farquhar GD (1984) Temperature dependence of whole-leaf photosynthesis in *Eucalyptus pauciflora* Sieb. ex Spreng. *Australian Journal of Plant Physiology,* **11,** 519–538.

Körner Ch. (1995) Towards a better experimental basis for upscaling plant responses to elevated CO_2 and climate warming. *Plant, Cell and Environment*, **18,** 1101–1110.

Krapp A, Hofmann B, Schafer C, Stitt M (1993) Regulation of the expression of rbcS and other photosynthetic genes by carbohydrate: a mechanism for the 'sink regulation' of photosynthesis? *Plant Journal*, **3,** 817–828.

Krapp A, Quick WP, Stitt M (1991) Ribulose-1,5-bisphosphate carboxylase-oxygenase, other photosynthetic enzymes and chlorophyll decrease when glucose is supplied to mature spinach leaves via the transpiration stream. *Planta*, **186,** 58–69.

Lawlor DW (1993) *Photosynthesis: Molecular, Physiological and Environmental Process*, 2th edn. Longman, White Plains, NY, 318pp.

Leadley PW, Reynolds JA, Thomas JF, Reynolds JF (1987) Effects of CO_2 enrichment on internal leaf surface area in soybeans. *Botanical Gazette*, **148,** 137–140.

Leuning R (1995) A critical appraisal of a combined stomatal-photosynthesis model for C_3 plants. *Plant, Cell and Environment*, **18,** 339–355.

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 and Environment*, **14,** 729–739.

Long SP, Drake BG (1992) Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentration. In: *Crop Photosynthesis: Spatial and Temporal Determinants* (eds Baker NR, Thomas H), pp. 69–95. Elsevier Science Publishers, Amsterdam.

Luo Y, Field CB, Mooney HA (1994) Predicting responses of photosynthesis and root fraction to elevated CO_2: Interactions among carbon, nitrogen, and growth. *Plant, Cell and Environment*, **17,** 1195–1204.

Luo Y, Mooney HA (1996) Stimulation of global photosynthetic carbon influx by an increase in atmospheric carbon dioxide concentration. In: *Carbon Dioxide and Terrestrial Ecosystems* (eds Koch GW, Mooney HA), pp. 381–397. Academic Press, San Diego, CA.

Luo Y, Sims DA, Thomas RB, Tissue DT, Ball JT (1996) Sensitivity of leaf photosynthesis to CO_2 concentration is an invariant function for C_3 plants: A test with experimental data and global applications. *Global Biogeochemical Cycles*, **10,** 209–222.

Neftel A, Oeschger H, Schwander J, Stauffer B, Zumbrunn R (1982) Ice core measurements give atmospheric CO_2 content during past 40,000 years. *Nature*, **295,** 222–223.

Nie GY, Hendrix DL, Webber AN, Kimball BA, Long SP (1995a) Increased accumulation of carbohydrates and decreased photosynthetic gene transcript levels in wheat grown at an elevated CO_2 concentration in the field. *Plant Physiology,* **108,** 975–983.

Nie GY, Long SP, Garcia RL, Kimball BA, Lamorte RL, Pinter PJ, Wall GW, Webber AN (1995b) 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 and Environment*, **18,** 855–864.

Radoglou KM, Jarvis PG (1990) Effects of CO_2 enrichment on four poplar clones. I. Growth and leaf anatomy. *Annals of Botany,* **65,** 617–626.

Robertson EJ, Leech RM (1995) Significant changes in cell and

chloroplast development in young wheat leaves (*Triticum aestivum* cv Hereward) grown in elevated CO_2. *Plant Physiology*, **107,** 63–71.

Sage RF (1990) A model describing the regulation of ribulose-1,5-bisphosphate carboxylase, electron transport and triose-phosphate use in response to light intensity and CO_2 in C_3. *Plant Physiology*, **94,** 1728–1734.

Sage RF, Sharkey TD, Seemann JR (1989) Acclimation of photosynthesis to elevated CO_2 in five C_3 species. Plant Physiology **89,** 590–596.

Sharkey TD (1985) Photosynthesis in intact leaves of C_3 plants: Physics, physiology, and rate limitations. *Botanical Review*, **51,** 53–105.

Silvola J, Ahlholm U (1992) Photosynthesis in willows (*Salix x dasyclados*) grown at different CO_2 concentrations and fertilization levels. *Oecologia*, **91,** 208–213.

Socias FX, Medrano H, Sharkey TD (1993) Feedback limitation of photosynthesis of *Phaseolus vulgaris* L. grown in elevated CO_2. *Plant, Cell and Environment*, **16,** 81–86.

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

Teskey RO (1995) A field study of the effects of elevated CO_2 on carbon assimilation, stomatal conductance and leaf and branch growth of *Pinus taeda* trees. *Plant, Cell and Environment*, **18,** 565–573.

Thomas JF, Harvey CN (1983) Leaf anatomy of four species grown under continuous CO_2 enrichment. *Botanical Gazette*, **144,** 303–309.

Thomas RB, Strain BR (1991) Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon dioxide. *Plant Physiology*, **96,** 627–634.

Tissue DT, Griffin KL, Thomas RB, Strain BR (1994) Effects of low and elevated CO_2 on C_3 and C_4 annuals. II. Photosynthesis and biochemistry. *Oecologia*, **101,** 21–28.

Vu JCV Allen LH Jr, Bowes G (1989) Leaf ultrastructure, carbohydrates and protein of soybeans grown under CO_2 enrichment. *Environmental and Experimental Botany*, **29,** 141–147.

Wong SC (1990) Elevated atmospheric partial pressure of CO_2 and plant growth. II. Non-structural carbohydrate content in cotton plants and its effect on growth parameters. *Photosynthetic Research*, **23,** 171–180.

Appendix

*The **P**hotosynthetic **A**cclimation to CO_2 (PAC) model and parameter values*

The PAC model conceptually has three components: the photosynthetic sensitivity, biochemical downregulation and morphological upregulation. The CO_2 sensitivity is described by the Farquhar *et al.* (1980) model that assumes that photosynthesis is limited either by Rubisco or by the light-driven regeneration of RuBP:

$$P_1 = V_{cmax}\frac{C_i - \Gamma}{C_i + k_c(1 + O/k_o)} - \rho \quad \text{(A1)}$$

$$P_2 = J\frac{C_i - \Gamma}{4.5\,C_i + 10.5\,\Gamma} - \rho \quad \text{(A2)}$$

where V_{cmax} is the maximum RuBP carboxylase activity (μmol m^{-2} s^{-1}), C_i is the intercellular CO_2 concentration (μmol mol^{-1}), Γ is the CO_2 compensation point (= 45 μmol mol^{-1} at 27 °C in this study) without dark respiration, k_c and k_o are the Michaelis–Menten constants for CO_2 and oxygen and equal to 34.2 μmol mol^{-1} and 43.5 mmol mol^{-1}, respectively, at 27 °C, O is the partial pressure of oxygen at the site of carboxylation (= 0.21 mol mol^{-1}), ρ is dark respiration (μmol m^{-2} s^{-1}), and J is:

$$J = \frac{J_{max} I}{I + 2.1\,J_{max}} \quad \text{(A3)}$$

where J_{max} is the light saturated rate of electron transport (μmol m^{-2} s^{-1}) and I is the instantaneous photon flux density, equaling 1500 μmol m^{-2} s^{-1} in this study.

Photosynthetic sensitivity to CO_2 may be depressed by a potential phosphate limitation (Sharkey 1985). Harley *et al.* (1992) integrates the phosphate limitation into the Farquhar et al. model by

$$P_3 = 3\,TPU - \rho, \quad \text{(A4)}$$

where TPU is the rate of phosphate release in triose phosphate utilization (μmol m^{-2} s^{-1}). Thus, net photosynthesis (P) is the minimum of the three processes limited by Rubisco, electron transport, and triose phosphate utilization as

$$P = \min\{P_1, P_2, P_3\}. \quad \text{(A5)}$$

Growth CO_2 may induce morphological modification and biochemical adjustments. Morphological changes can be represented by leaf mass per unit area (h, g m^{-2}) and biochemical adjustments can be quantified by mass-based leaf nitrogen concentration (n_m, g g^{-1}). The product of h and n_m is area-based leaf nitrogen concentration (n_a, g m^{-2}) which is well correlated with parameters V_{cmax}, J_{max}, TPU, and ρ (Field 1983; Harley *et al.* 1992) as

$$\begin{aligned} V_{cmax} &= k_1 n_a + k_2 \\ J_{max} &= k_3 n_a + k_4 \\ TPU &= k_5 n_a + k_6 \\ \rho &= k_7 n_a + k_8, \end{aligned} \quad \text{(A6)}$$

where k_1 through $_8$ are constants. k_1 – k_4 equal to 48.4, – 5.8, 123.1, and – 5.2, respectively, as determined in our

other independent experiment with *Glycine max*. k_5 – k_8 equal 6.8, 0.3, 0.775, and –0.238 from Field (1983) and Harley *et al.* (1992) with a small modification to fit data.

The intercellular CO_2 concentration is iteratively calculated from the CO_2 concentration at which photosynthesis is measured (C_m, μmol mol^{-1}) and the stomatal conductance (g_s, μmol m^{-2} s^{-1}; Ball *et al.* 1987; Leuning 1995) by

$$C_i = C_m - P/g_s \quad \text{(A7)}$$

$$g_s = \frac{a_1 P h_s}{C_m} + b, \quad \text{(A8)}$$

where a and b are coefficients, equaling 9.31 and 0.001, respectively (Ball *et al.* 1987), h_s is the relative humidity in the cuvette, equalling to 66% in this study.