Quantifying the response of photosynthesis to changes in leaf nitrogen content and leaf mass per area in plants grown under atmospheric CO_2 enrichment

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ABSTRACT

Previous modelling exercises and conceptual arguments have predicted that a reduction in biochemical capacity for photosynthesis (A_{area}) at elevated CO_2 may be compensated by an increase in mesophyll tissue growth if the total amount of photosynthetic machinery per unit leaf area is maintained (i.e. morphological upregulation). The model prediction was based on modelling photosynthesis as a function of leaf N per unit leaf area (N_{area}), where $N_{area} = N_{mass} \times LMA$. Here, N_{mass} is percentage leaf N and is used to estimate biochemical capacity and LMA is leaf mass per unit leaf area and is an index of leaf morphology. To assess the relative importance of changes in biochemical capacity versus leaf morphology we need to control for multiple correlations that are known, or that are likely to exist between CO_2 concentration, N_{area}, N_{mass}, LMA and A_{area}. Although this is impractical experimentally, we can control for these correlations statistically using systems of linear multiple-regression equations. We developed a linear model to partition the response of A_{area} to elevated CO_2 into components representing the independent and interactive effects of changes in indexes of biochemical capacity, leaf morphology and CO_2 limitation of photosynthesis. The model was fitted to data from three pine and seven deciduous tree species grown in separate chamber-based field experiments. Photosynthetic enhancement at elevated CO_2 due to morphological upregulation was negligible for most species. The response of A_{area} in these species was dominated by the reduction in CO_2 limitation occurring at higher CO_2 concentration. However, some species displayed a significant reduction in potential photosynthesis at elevated CO_2 due to an increase in LMA that was independent of any changes in N_{area}. This morphologically based inhibition of A_{area} combined additively with a reduction in biochemical capacity to significantly offset the direct enhancement of A_{area} caused by reduced CO_2 limitation in two species. This offset was 100% for *Acer rubrum*, resulting in no net effect of elevated CO_2 on A_{area} for this species, and 44% for *Betula pendula*. This analysis shows that interactions between biochemical and morphological responses to elevated CO_2 can have important effects on photosynthesis.

Key-words: carbon dioxide; leaf nitrogen; photosynthesis; structural equation model.

INTRODUCTION

Leaf-level photosynthesis (A_{area}) is often enhanced in plants grown under long-term exposure to elevated levels of atmospheric CO_2 (Gunderson & Wullschleger 1994; Curtis 1996; Drake, Gonzàlez-Meler & Long 1997). This enhancement is caused by an increase in the rate of carboxylation of ribulose-1,5-bisphosphate (RuBP) catalyzed by RuBP carboxylase/oxygenase (Rubisco) (Woodrow & Berry 1988). There are at least two fundamentally different ways in which the rate of carboxylation per unit leaf area can be modified by elevated CO_2. The first way, which we refer to as a 'direct' effect of CO_2, involves the biochemistry of photosynthesis. This direct effect consists of (i) a reduction in substrate limitation of Rubisco catalysis (Farquhar, von Caemmerer & Berry 1980) (ii) competitive reduction of RuBP oxygenation (Farquhar *et al*. 1980), and (iii) any adjustments in the photosynthetic apparatus (from

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light capture through starch and sucrose synthesis) that alter the RuBP limitation of Rubisco (Sage, Sharkey & Seemann 1989; Sage 1990). The second way in which elevated CO_2 may affect the rate of carboxylation per unit leaf area involves changes in leaf morphology and anatomy (Gunderson & Wullschleger 1994; Luo, Field & Mooney 1994). These may include changes in mesophyll cell number per unit leaf area (Vu, Allen & Bowes 1989), mesophyll thickness (Sims, Seemann & Luo 1998), carbohydrate concentration (Stitt 1991) and leaf mass per unit area (*LMA*) (Curtis 1996).

During short-term exposure to elevated CO_2, photosynthesis is frequently limited by the capacity to regenerate RuBP. In the longer-term, the amount or activity of Rubisco may decrease, thus balancing the reduction in RuBP regeneration (Sage 1990; Stitt 1991; Gunderson & Wullschleger 1994; Drake *et al.* 1997). The effect of this down-regulation of the amount or activity of Rubisco on A_{area} may be offset if it is associated with an increase in mesophyll tissue such that the amount of photosynthetic apparatus per unit leaf area is maintained (Radoglou & Jarvis 1990; Luo *et al.* 1994). Alternatively, other changes in leaf morphology or anatomy associated with an increase in *LMA* may potentially reduce A_{area} since photosynthesis and *LMA* are negatively correlated at ambient CO_2 (Reich, Walters & Ellsworth 1997; Peterson *et al.* 1999). This negative correlation is independent of any effect *LMA* may have on N_{area} or N_{mass} (Peterson *et al.* 1999) and may be due to a reduction in N allocation to photosynthetic versus non-photosynthetic compounds (Evans 1989), to greater allocation of biomass to structural versus photosynthetic components (Vitousek, Field & Mantson 1990; Lloyd *et al.* 1992), to reduced light penetration through the various layers of leaf tissue (Terashima & Hirosaka 1995), or to greater limitations to internal diffusion (Parkhurst 1994; Pachepsky *et al.* 1997).

While biochemical aspects of photosynthetic enhancement at elevated CO_2 have been studied extensively, less emphasis has been placed on understanding how those enhancements interact with changes in leaf morphology and anatomy. In an important advancement, Pachepsky & Acock (1996) developed a two-dimensional model of leaf-level photosynthesis that demonstrates how leaf anatomy can have an important effect on photosynthesis. However, the general utility of this model is limited because it requires detailed maps of mesophyll cell location and information on stomatal area and density to parameterize it. Luo *et al.* (1994) developed the Photosynthetic Acclimation to CO_2 (PAC) model which is much less data-intensive and which suggests that interactions between biochemical and morphological responses to elevated CO_2 can have important effects on leaf-level photosynthesis. The PAC model uses the biochemical model of Farquhar *et al.* (1980) to model photosynthesis as a function of nitrogen per unit leaf area (N_{area}) (e.g. Harley *et al.* 1992). The model assumes that (i) nitrogen per unit leaf mass (N_{mass}) reflects the biochemical capacity for photosynthesis (e.g. Körner & Miglietta 1994) since the majority of leaf N is found in the proteins of the Calvin cycle (including Rubisco) and the thylakoid membranes (Evans 1989) and (ii) that *LMA* summarizes aspects of leaf morphology and anatomy such as photosynthetic biomass and non-structural carbohydrate storage. The PAC model links changes in biochemical capacity and leaf morphology through the relationship $N_{area} = N_{mass} \times LMA$. The model predicts that a reduction in biochemical capacity at elevated CO_2 (estimated from a reduction in N_{mass}) may be counterbalanced by morphological upregulation if an increase in *LMA* is relatively greater than the reduction in N_{mass}. Leaf mass per unit area has been shown to be tightly correlated with leaf thickness in soybeans grown at ambient and elevated CO_2 (Sims *et al.* 1998), but there is also substantial evidence to show that increases in *LMA* at elevated CO_2 can be caused by increased storage of non-structural carbohydrates (e.g. Roumet *et al.* 1996). However, if both N_{area} and *LMA* increase while N_{mass} decreases, then the increase in *LMA* must involve additional N-bearing tissue even though N concentration decreased.

The PAC model provides valuable insights into relationships between biochemical and morphological responses to elevated CO_2 by showing how net changes in N_{mass} and *LMA* can interact to affect photosynthesis. However, because the PAC model focuses on net changes, it does not allow us to accurately assess the relative importance of biochemical versus morphological responses. This could be achieved by experimentally controlling N_{mass} or N_{area} in addition to *LMA*, but this is clearly impractical. An alternative approach would be to use a simple multiple regression of A_{area} on CO_2 concentration, N_{area} and *LMA*. Since the coefficients in a multiple regression are partial derivatives, they quantify the relationship between the dependent variable (A_{area}) and each independent variable when the other independent variables in the equation are held constant. This allows us to statistically control both N_{area} and *LMA*, and to partition the response of A_{area} among each independent variable. Using this approach, the partial regression coefficient for CO_2 represents an index of CO_2 limitation of photosynthesis because it quantifies the change in A_{area} per unit change in CO_2 when both N_{area} and *LMA* are held constant. Similarly, the partial regression coefficient for N_{area} represents an index of biochemical capacity per unit leaf area, and the coefficient for *LMA* represents an index of morphological regulation.

The multiple regression discussed above would provide useful information on the relative importance of the effects of CO_2 concentration, N_{area} and *LMA* on photosynthesis. This model is, however, too simplistic to capture the range of interactions that are known, or that are likely to occur between these variables. To start with, both N_{area} and N_{mass} are correlated with *LMA* at ambient CO_2 (Reich *et al.* 1994; Reich & Walters 1994; Peterson *et al.* 1999). This lack of independence may confound parameter estimation and interpretation in the simple regression model. In addition, the response of N_{area}, N_{mass} and *LMA* to elevated CO_2 are also likely to be correlated with each other. This complex set of correlations make it difficult to tease apart the indi-

vidual responses. These correlations can, however, be dealt with using statistical control by framing the multiple regression model in the context of a structural equation model (Hoyle 1995). A structural equation model (often referred to as path analysis or covariance structure analysis) can be specified as a system of multiple regression equations that partitions interactions among multiple dependent and independent variables (see the Methods section for details). In this paper we present a model that partitions the response of A_{area} to elevated CO_2 into independent and interactive components representing changes in an index of CO_2 limitation, an index of biochemical capacity and an index of leaf morphology. Although the model is not mechanistic, it partitions known and hypothesized relationships in a conceptually and mechanistically plausible framework based on the general concepts of the PAC model. We used this linear model to re-analyse existing data from 10 C_3 species (three pine and seven deciduous tree species) grown at ambient and elevated CO_2 concentrations. One aim of this study was to develop a computationally and data efficient tool for assessing the relative importance of changes in biochemical capacity and leaf morphology that could be applied to biogeochemical or biogeographic models of global change. We also compared responses across species to identify whether generalizations could be made from these data based on functional or taxonomic relatedness.

MATERIALS AND METHODS

Data

Data used in this analysis were obtained from 10 separate chamber-based elevated CO_2 experiments, conducted in the field, and included three pine and seven deciduous tree species (see caption to Fig. 2 for details). Data consisted of rates of net photosynthetic carbon assimilation [A_{area}, μmol (CO_2) m^{-2} (leaf) s^{-1}] measured at light saturation under growth conditions; leaf N per unit leaf area [N_{area}, g (N) m^{-2} (leaf)]; and leaf mass per unit area [LMA, g (leaf) m^{-2} (leaf)]. In most data sets leaf N was determined using the same leaves that photosynthesis was measured on, although in some cases adjacent leaves were collected for N analysis. Variation in leaf N resulted from either fertilization treatments, sun versus shade leaves, leaf developmental stage, natural variation within leaf classes, or variation due to CO_2 treatment (see citations for details). Photosynthesis measurements were made at ecologically relevant temperatures for each species (20 to 30 °C depending on species) and measurements for single species were usually controlled to within ± 2 °C. Ambient CO_2 concentration was either 350 or 360 μmol mol^{-1} and the elevated concentration was either 650 or 700 μmol mol^{-1} (see citations for details).

Structural equation model

Structural equation models are a class of general linear models that include analysis of variance (ANOVA), multiple regression, path analysis and factor analysis (Hoyle 1995). In this analysis we used a system of multiple regression equations (presented below) to approximate known and hypothesized relationships among CO_2, A_{area}, N_{area} and LMA. Path analysis (also known as covariance structure analysis) could be used in this situation but we chose the multiple regression approach for the following reasons. First, path analysis is designed to analyse hypothesized relationships among measured and latent (unmeasured) variables that are inferred from a covariance matrix (Hoyle 1995). Because there are no latent variables in our model (other than the error terms), path analysis provides no benefit over multiple regression analysis in this case. Second, even though path analysis and regression analysis give identical slope coefficients for the relationships modelled in this study, path analysis does not provide information on the intercepts, which are important for comparing responses across species.

The system of regression equations used in this analysis was

$$LMA = \beta_0 + \beta_1 CO_2 + \varepsilon_i$$
$$N_{area} = \beta_2 + \beta_3 CO_2 + \beta_4 LMA + \varepsilon_j \qquad \text{Model 1}$$
$$A_{area} = \beta_5 + \beta_6 CO_2 + \beta_7 N_{area} + \beta_8 LMA + \varepsilon_k$$

The β coefficients for each equation are partial regression coefficients and give the slope of the relationship between the appropriate dependent and independent variable when the other independent variables in that equation are held constant. Two important coefficients in these equations are β_3 (the direct effect of CO_2 on N_{area}) and β_6 (the direct effect of CO_2 on A_{area}). Even though β_3 has units of g (N) m^{-2} (leaf), it is an index of change in N concentration (N_{mass}) because it quantifies change in the mass of N per unit leaf area when leaf mass per unit area is held constant. β_6 is an index of CO_2 limitation of photosynthesis because it quantifies the effect of CO_2 on A_{area} when N_{area} and LMA are both held constant. A graphical representation of this model is shown in Fig. 1.

The model was fitted to data from each species using least squares, and 95% confidence intervals for coefficients were estimated from 2000 bootstrap replicates using the bias corrected and accelerated method of Efron & Tibshirani (1993). Bootstrapping involves resampling with replacement from the original data to simulate multiple samples from a population. It is useful when the statistical properties of the comparison being made are not well understood, as is the case for calculating confidence intervals for the indirect effects of CO_2 which are discussed below. Confidence intervals were used to assess the statistical significance of all coefficients.

Model 1 allows us to partition the effect of elevated CO_2 on A_{area} into components representing a direct effect of CO_2 (β_6) and indirect effects consisting of interactions among CO_2, N_{area} and LMA. For example, the indirect effect of CO_2 on A_{area} due to CO_2 induced changes in LMA is represented by the compound pathway $CO_2 \rightarrow LMA \rightarrow A_{area}$ (Fig. 1). In this pathway the arrows represent specific coefficients from model 1 with the first arrow
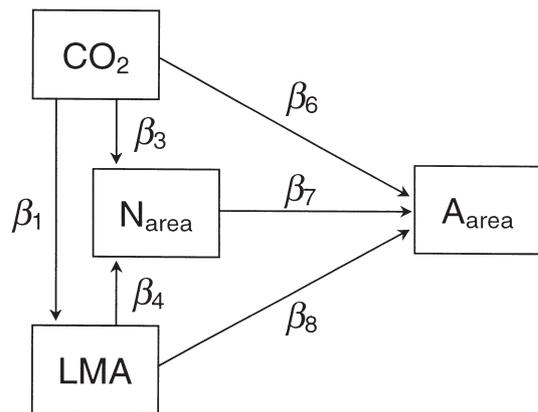
Figure 1. Graphical representation of model 1 showing the interactions among CO_2 concentration, leaf nitrogen content (N_{area}), leaf mass per area (LMA) and leaf-level photosynthesis (A_{area}). The β_i are coefficients in model 1. Intercepts and error terms have been excluded for simplicity.

being β_1 and the second arrow β_8. The magnitude of this indirect effect is the product of these coefficients. Model 1 has three different pathways representing indirect effects of CO_2 on A_{area}: (1) $CO_2 \rightarrow LMA \rightarrow A_{area}$; (2) $CO_2 \rightarrow LMA \rightarrow N_{area} \rightarrow A_{area}$; and (3) $CO_2 \rightarrow N_{area} \rightarrow A_{area}$ (Fig. 1). Each indirect effect has units of μmol (CO_2) m^{-2} (leaf) s^{-1} and the total indirect effect of CO_2 on A_{area} is the sum of these three independent effects (Sokal & Rohlf 1981). Because these indirect effects have the same units and are additive they provide comparative information on the relative importance of each set of interactions on A_{area}.

In this analysis all independent variables except CO_2 were random. Least squares regression is appropriate when independent variables are random as long as their frequency distributions are not functions of the regression coefficients (Neter, Wasserman & Kutner 1990; p 86). We assumed that this was the case for all data sets. Normality and homogeneity of residuals were checked using the original non-bootstrapped data. Because we expected correlations among the independent variables we paid particular attention to possible effects of multicolinearity on parameter estimates. Multicolinearity was checked using the original non-bootstrapped data but there was no evidence to suggest that it was a problem. We concluded that all original data sets showed adequate compliance with the assumptions of least squares regression.

Comparing responses across species

If the coefficients from model 1 were more similar within than between vegetation types (pines versus deciduous trees), then the accuracy of ecosystem and global models may be improved by incorporating the specific details of each group. We assessed the similarity of coefficients across species in two ways. First, we compared the estimated population-level frequency distributions obtained from the

bootstrap replicates. If the means and 95% confidence limits for different species were similar then those species could be grouped on the premise that they had similar responses to elevated CO_2. Otherwise those species were assumed to have different frequency distributions and therefore different responses.

Second, we used non-parametric and parametric cluster-analysis (Everitt 1980; Sokal & Rohlf 1981; Digby & Kempton 1987) on the coefficients to determine if species formed discrete groups. This would imply that the complete set of coefficients were more similar within than between groups. We used hierarchical cluster-analysis to identify potential groupings and then *k*-means cluster analysis to test whether those groups were robust. The hierarchical procedure compares the Euclidean distance between points (species) in *n*-dimensional space (where *n* is the number of coefficients in model 1) and links those points that are closest together into clusters. The *k*-means procedure is an optimization procedure that assigns observations to a pre-defined set of *k* groups so that the within group variance is minimized and the between group variance is maximized. We based *k* on the number of major groups identified by the hierarchical procedure. All coefficients were standardized to have a mean of zero and standard deviation of one, and the hierarchical clustering criterion was complete linkage using Euclidean distances.

RESULTS AND DISCUSSION

Growth at elevated CO_2 increased net photosynthesis for nine of the 10 species in this study, although this increase was marginal for both *Acer saccharum* and *Pinus radiata* (Fig. 2a). Elevated CO_2 did not have a significant effect on N_{area} for the majority of species even though there was a generally consistent trend for N_{area} to be slightly lower under elevated versus ambient CO_2 (Fig. 2b). *Alnus glutinosa* was an exception because it displayed a significant increase in N_{area} under elevated CO_2, whereas *P. radiata* displayed a significant reduction in N_{area} (Fig. 2b). In order to simplify the discussion of how these patterns in A_{area} and N_{area} are explained by interactions among CO_2 concentration, N_{mass} and LMA, we focus on general responses across species. Individual species that differed from these general patterns are discussed separately. First we present results for the effects of elevated CO_2 on LMA and N_{area}, then we link those results to the direct and indirect effects of CO_2 on A_{area}.

Effects of elevated CO_2 on *LMA* and N_{area}

All species displayed a significantly positive intercept for the regression of LMA on CO_2 concentration (Fig. 3a) and increased CO_2 resulted in an increase in LMA for the majority of species (Fig. 3b). This is a common response for C_3 plants (Curtis 1996). The N_{area} intercept was also positive for the majority of species (Fig. 4a), but elevated CO_2 generally had a negative direct effect on N_{area} when LMA was held constant (Fig. 4b). The fact that LMA was held
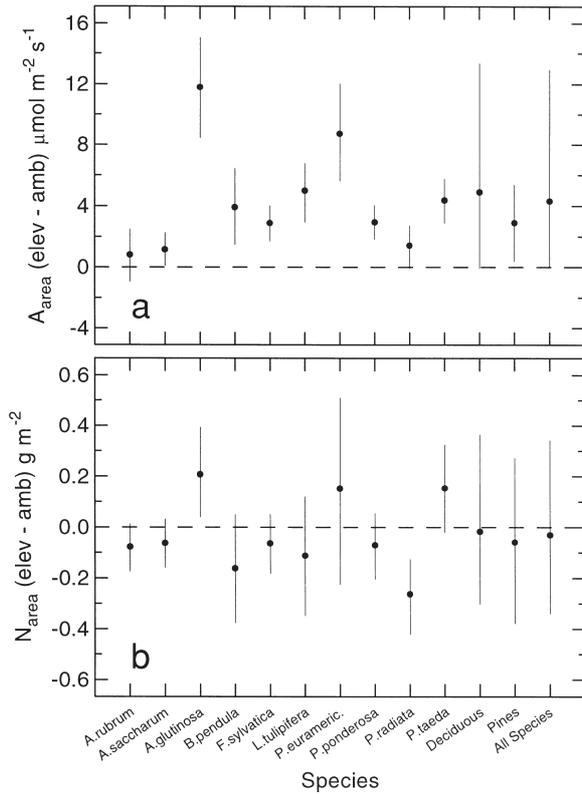
Figure 2. Mean and 95% confidence intervals for the net effect of elevated CO_2 on A_{area} (Panel a) and N_{area} (Panel b) calculated as the difference between elevated and ambient CO_2 treatments (elevated – ambient). Data citations are: *Acer rubrum* (Norby *et al.* 1997; *n* = 69); *Acer saccharum* (Norby *et al.* 1997; *n* = 72); *Alnus glutinosa* (Vogel & Curtis 1995; *n* = 23); *Betula pendula* (Rey & Jarvis 1998; *n* = 45); *Fagus sylvatica* (Forstreuter 1995; *n* = 87); *Liriodendron tulipifera* (Gunderson, Norby & Wullschleger 1993; *n* = 23); *Populus euramericana* (Curtis *et al.* 1995; *n* = 23); *Pinus ponderosa* (Tissue, Griffin & Ball 1999; *n* = 27); *Pinus radiata* (Whitehead *et al.* 1995; *n* = 33); *Pinus taeda* (Tissue, Thomas & Strain 1997; *n* = 42); Deciduous = the average for all deciduous trees, Pines = the average for all pines and All Species = the average for all species.

constant means that N concentration (N_{mass}) decreased and suggests that structural or non-structural carbohydrates increased with CO_2 (e.g. Radoglou & Jarvis 1992; Ryle, Powell & Davidson 1992; Körner & Miglietta 1994; Schechter, Proctor & Elfving 1994; Thomas & Griffin 1994). *LMA* had a positive direct effect on N_{area} when CO_2 was held constant, with N accounting for approximately 1 to 2% of the increase in leaf mass (Fig. 4c). This positive effect of *LMA* on N_{area} suggests that leaves with higher *LMA* had more mesophyll tissue at a fixed CO_2 concentration.

The indirect pathway $CO_2 \rightarrow LMA \rightarrow N_{area}$ [units, g (N) m^{-2}] quantifies how CO_2 induced changes in *LMA* affects N_{area}. This indirect effect was not significant for *Betula pendula*, *Fagus sylvatica*, *Liriodendron tulipifera*, *Populus euramericana* and *Pinus ponderosa* (Fig. 4d). Of these five species, *F. sylvatica*, *L. tulipifera* and *P. ponderosa*

did not display a significant effect of CO_2 on *LMA* (Fig. 3b), which explains why the indirect effect of CO_2 on N_{area} was not significant for these species. In contrast, *B. pendula* and *P. euramericana* both displayed higher *LMA* under elevated versus ambient CO_2 (Fig. 3b), but the non-significant indirect effect of CO_2 on N_{area} suggests that this increase in *LMA* was due largely to the accumulation of carbohydrates. *Acer rubrum*, *A. saccharum*, *Alnus glutinosa* and *Pinus taeda* all displayed positive effects of $CO_2 \rightarrow LMA \rightarrow N_{area}$ (Fig. 4d). For these four species, the CO_2-induced increase in *LMA* appeared to be due at least in part to increased mesophyll tissue growth. However, for both *A. rubrum* and *A. saccharum*, the net effect of joint variation in N_{mass} and *LMA* due to elevated CO_2 resulted in no overall change in N_{area} (Fig. 2b). *Alnus glutinosa* and *P. taeda* both tended to have higher N_{area} under elevated CO_2 (Fig. 2b). This was because both species displayed non-significant direct effects of CO_2 on N_{area} (i.e. no reduction in N_{mass}) (Fig. 4b) and large positive effects of CO_2 on *LMA* (Fig. 3b) and of *LMA* on N_{area} (Fig. 4c). These results suggest that both of these species displayed an increase in mesophyll tissue growth and little or no accumulation of carbohydrates with increasing CO_2 concentration.

Pinus radiata also differed from the majority of species because it displayed a significantly negative effect of CO_2
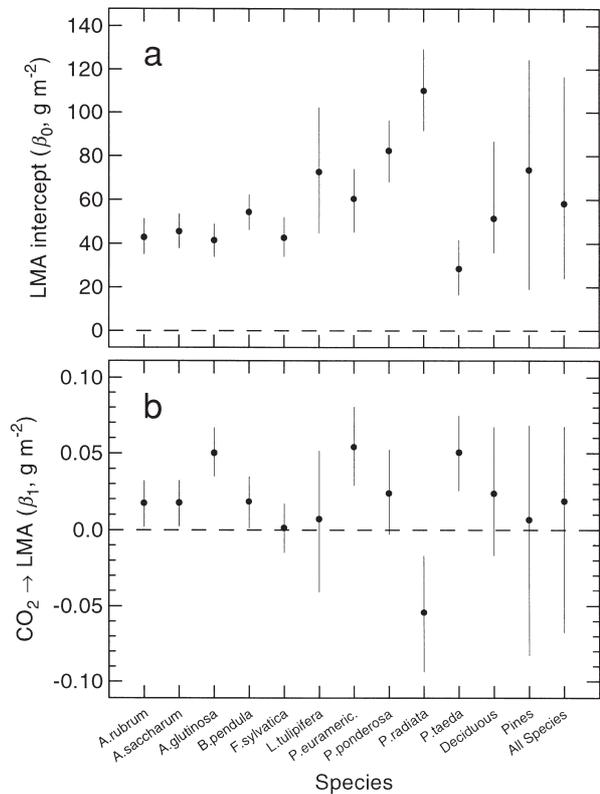
Figure 3. Regression coefficients and 95% confidence intervals for the response of *LMA* to CO_2 concentration. Panel (a) is the *LMA* intercept (β_0) and panel (b) is the effect of CO_2 on *LMA* (β_1).
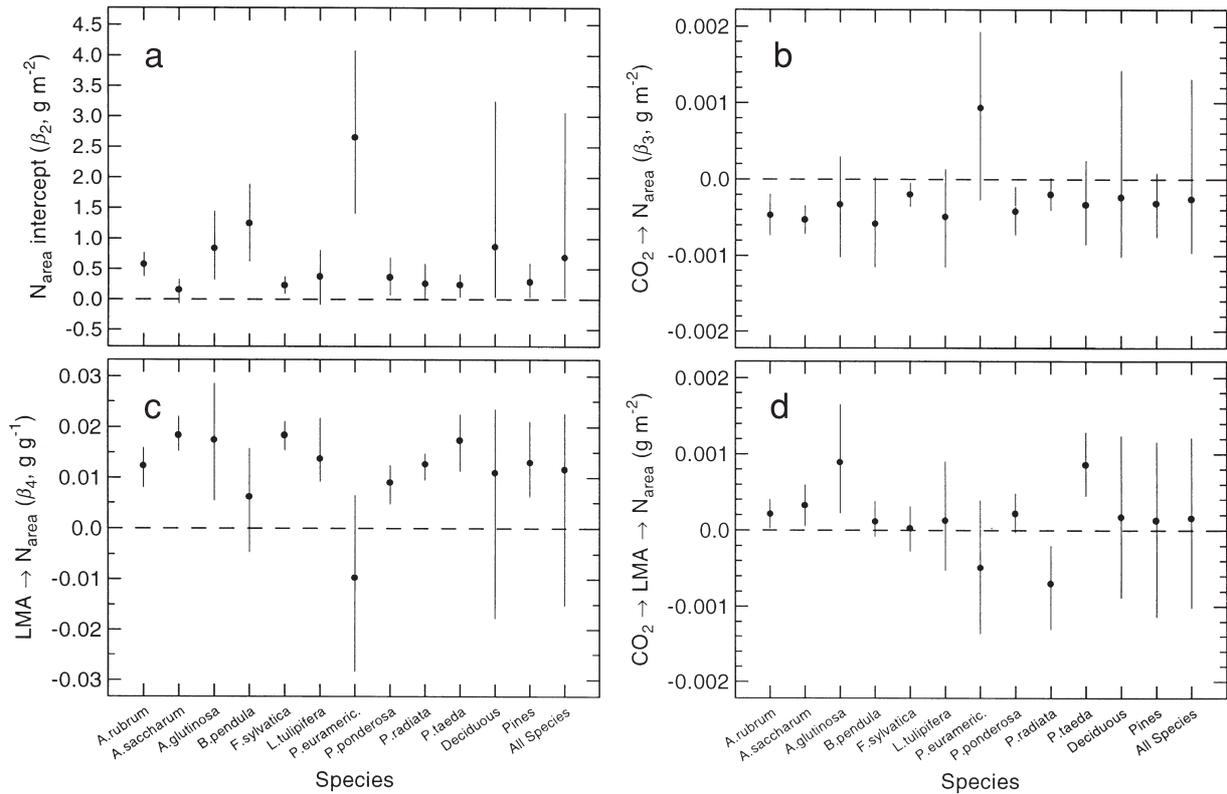
Figure 4. Partial regression coefficients and 95% confidence intervals for the response of N_{area} to CO_2 concentration and *LMA*. Panel (a) is the N_{area} intercept (β_2), panel (b) is the effect of CO_2 on N_{area} (β_3), panel (c) is the effect of *LMA* on N_{area} (β_4) and panel (d) is the indirect effect of $CO_2 \rightarrow LMA \rightarrow N_{area}$.

on *LMA* (Fig. 3b) (see also Hocking & Meyer (1991), Coleman & Bazzaz (1992), Ryle *et al.* (1992) and Knapp *et al.* (1994) for other examples of negative effects of CO_2 on *LMA*. This negative effect of CO_2 on *LMA* resulted in a significantly negative indirect effect of CO_2 on N_{area} (Fig. 4d). This negative indirect effect combined with a marginal reduction in N_{mass} to significantly reduce N_{area} at elevated CO_2 (Fig. 2b). These results for *P. radiata* suggest that this species experienced both a reduction in N_{mass} and a reduction in mesophyll tissue under elevated CO_2.

Effects of elevated CO_2 on A_{area}

There was substantial variation across species for the A_{area} intercept, with some species displaying significantly positive values while others displayed significantly negative values (Fig. 5a). All species did, however, display a positive and significant direct effect of CO_2 on A_{area} (Fig. 5b). This direct effect suggests a significant reduction in the CO_2 limitation of photosynthesis because both N_{area} and *LMA* were held constant statistically. Thus leaves grown at elevated CO_2 had higher photosynthesis for a given N_{area} and a given *LMA* compared to leaves grown at ambient CO_2. The direct effect of N_{area} on A_{area} was also positive for most species when CO_2 and *LMA* were held constant (Fig. 5c). This positive association between N_{area} and A_{area} is consistent with

many previous studies (e.g. Field & Mooney 1986; Walters & Field 1987; Evans 1989; Reich *et al.* 1994). In contrast, *LMA* tended to have either no effect or a significant negative effect on A_{area} when CO_2 and N_{area} were held constant (Fig. 5d).

The indirect effect of $CO_2 \rightarrow LMA \rightarrow A_{area}$ (Fig. 6a), which compares leaves with the same N_{area} but with *LMA* free to vary with CO_2 concentration, was either zero or significantly negative and corresponded with the direct effect of *LMA* on A_{area} discussed above. That is, species which displayed a negative and significant effect of *LMA* on A_{area} also displayed a negative and significant effect of $CO_2 \rightarrow LMA \rightarrow A_{area}$. This negative effect of CO_2 on A_{area} via *LMA* suggests the presence of a morphological mechanism of photosynthetic inhibition that is independent of any effect of CO_2 on N_{area}.

The indirect effect of $CO_2 \rightarrow LMA \rightarrow N_{area} \rightarrow A_{area}$ was not significant for most species (Fig. 6b), indicating that increased N_{area} due to greater mesophyll tissue growth under elevated CO_2 was either non-existent or too small to affect A_{area}. Species that did display a positive effect of $CO_2 \rightarrow LMA \rightarrow N_{area} \rightarrow A_{area}$ (i.e. morphological enhancement of photosynthesis) were those that also displayed a strong direct effect of N_{area} on A_{area}. In contrast, the indirect effect of $CO_2 \rightarrow N_{area} \rightarrow A_{area}$, which compares leaves with the same *LMA* but with N_{area} free to vary with CO_2
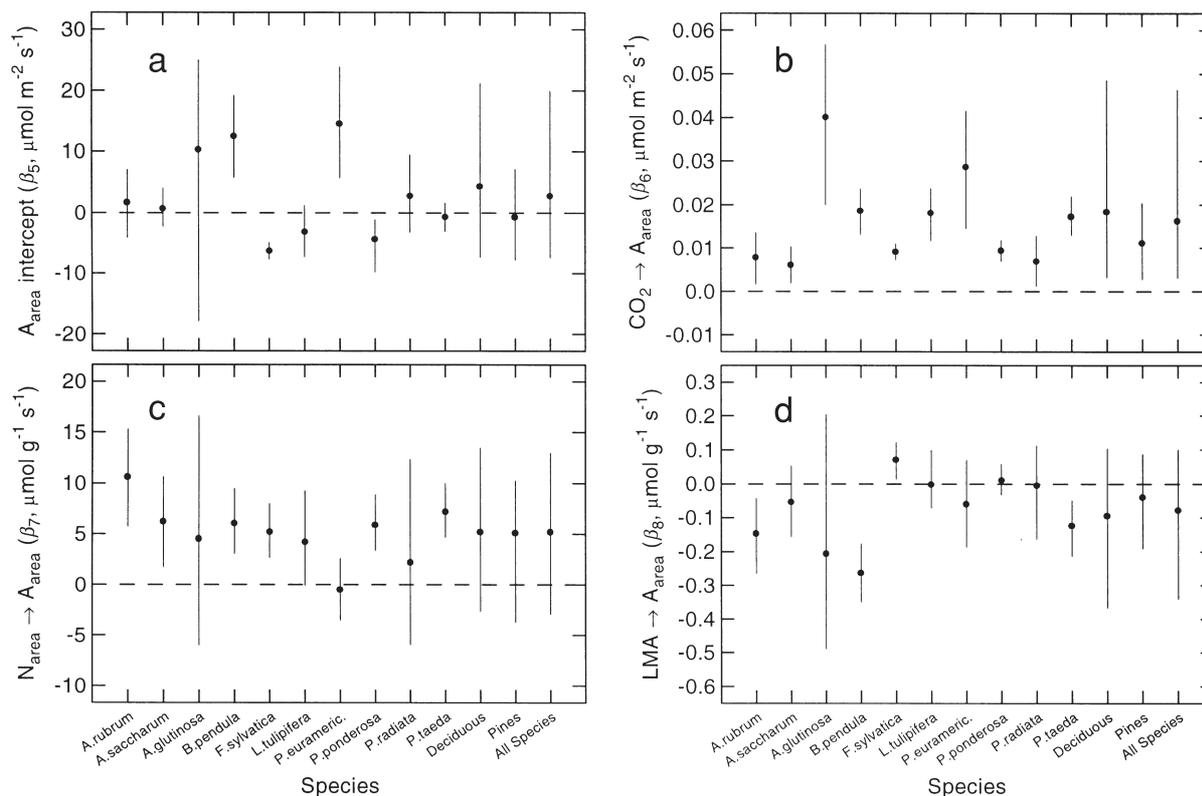
Figure 5. Partial regression coefficients and 95% confidence intervals for the response of A_{area} to CO_2 concentration, N_{area} and *LMA*. Panel (a) is the A_{area} intercept (β_5), panel (b) is the effect of CO_2 on A_{area} (β_6), panel (c) is the effect of N_{area} on A_{area} (β_7) and panel (d) is the effect of *LMA* on A_{area} (β_8).

concentration (i.e. variation in N_{mass}), was significantly or marginally negative for most species (Fig. 6c). This negative indirect effect suggests a reduction in the biochemical capacity for photosynthesis at a given *LMA*, possibly due to the dilution of N caused by increased carbohydrate storage. The total indirect effect of CO_2 on A_{area} (Fig. 6d), which is the sum of the indirect effects discussed above, was not significant for all but two species (see below). This analysis suggests that in general, the enhancement of A_{area} under elevated CO_2 was dominated by the reduction in CO_2 limitation of photosynthesis. Morphological upregulation at elevated CO_2 did not have an appreciable effect on A_{area} for the majority of species in this study.

The total indirect effect of CO_2 on A_{area} discussed above was significantly negative for both *A. rubrum* and *B. pendula* (Fig. 6d). The negative response for these two species was due to a combination of morphological reduction of photosynthesis ($CO_2 \rightarrow LMA \rightarrow A_{area}$, Fig. 6a) and a reduction in biochemical capacity ($CO_2 \rightarrow N_{area} \rightarrow A_{area}$, Fig. 6c). For *A. rubrum*, this combination cancelled the positive effect of reduced CO_2 limitation (Fig. 5b) and explains why there was no net effect of CO_2 on photosynthesis for this species (Fig. 2a). For *B. pendula*, the negative effects of morphology and lower biochemical capacity nearly halved (44% reduction) the enhancement caused by reduced CO_2 limitation, resulting in an enhancement of A_{area} under ele-

vated CO_2 that was still significant (Fig. 2a), although substantially constrained.

Comparisons across species

An important result of this analysis was the variation across species for most of the coefficients in model 1. Some of this variation may represent species differences in response to elevated CO_2, although some of it may reflect differences in experimental design such as nutritional status, tree age, tree density, the seasonal timing of data collection, the temperature at which measurements were made, or to differences among experiments in the CO_2 concentrations used for the ambient and elevated treatments (see relevant citations for details). All experiments were, however, conducted under conditions that were considered ecologically relevant but necessarily artificial. Even though the variation between species may be reduced by using standardized experimental conditions, it may still reflect important natural variation that needs to be considered in large-scale models of plant responses to global change. Nevertheless, there seems to be little evidence to suggest that the taxonomically or functionally similar species presented in this study have comparable frequency distributions for many of these coefficients. This high degree of variation across species resulted in the averages for most coefficients being
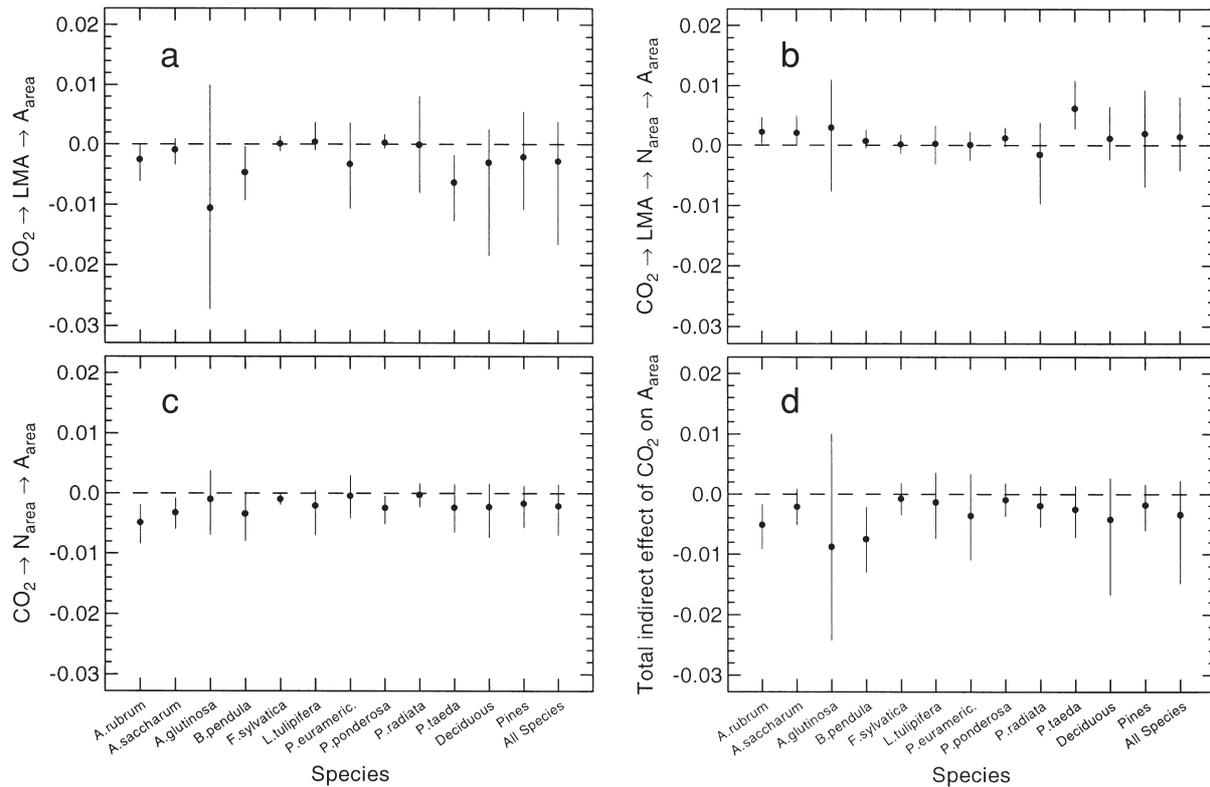
Figure 6. Coefficients and 95% confidence intervals for the indirect effects of CO_2 on A_{area}. Panel (a) is $CO_2 \rightarrow LMA \rightarrow A_{area}$, panel (b) is $CO_2 \rightarrow LMA \rightarrow N_{area} \rightarrow A_{area}$, panel (c) is $CO_2 \rightarrow N_{area} \rightarrow A_{area}$, and panel (d) is the total indirect effect of CO_2 on A_{area}, i.e. the sum of the effects presented in panels (a), (b), and (c). Units for all panels are μmol m^{-2} s^{-1}.

non-significant for each vegetation type (averages are presented in each figure). The only coefficients for which these averages were significant were the *LMA* intercept (Fig. 4a), the N_{area} intercept (Fig. 3a) and the direct effect of CO_2 on A_{area} (Fig. 5b).

Despite the variation discussed above, the hierarchical cluster analysis identified four groups that were also confirmed by the *k*-means cluster analysis. Two of these contained only one species each, namely *P. euramericana* and *P. radiata*. The response of *P. euramericana* tended to be opposite that of the other species for several coefficients. This was most notable for the N_{area} intercept, the direct effect of CO_2 on N_{area} and the direct effect of *LMA* on N_{area} (Fig. 7). *Pinus radiata* appeared to differ from the majority of species with respect to the *LMA* intercept, the effect of CO_2 on *LMA* and the direct effect of N_{area} on A_{area} (Fig. 7). The third group consisted of *A. glutinosa* and *B. pendula*. These two species differed from the remaining six species by having relatively higher intercepts for N_{area} and A_{area}, a relatively larger direct effect of CO_2 on A_{area} and a more strongly negative effect of *LMA* on A_{area} (Fig. 7). The last group included *A. rubrum*, *A. saccharum*, *F. sylvatica*, *L. tulipifera*, *P. ponderosa* and *P. taeda* (Fig. 7).

Although this analysis did identify apparently robust groupings of species, there were no clear associations based

on taxonomic or functional relatedness. In fact, the analysis showed that some taxonomically divergent species were comparable for the full set of coefficients presented in model 1. This may simply represent an artifact of the conditions used in each experiment, but we may be able to confirm or refute these patterns as additional data from newer experimental technologies such as Free Air CO_2 Enrichment become available. Nevertheless, it may be worthwhile conducting sensitivity analyses on models of global change by incorporating the range of variability observed here to determine if it has important effects on model predictions. Other sources of natural variation, such as temporal variation during the growing season and lag-effects from previous growing seasons, may also affect the relationships modelled in this study. Understanding the temporal dynamics of these relationships may be crucial for developing accurate and robust models of plant growth in changing environments.

In summary, this analysis extended the PAC model of Luo *et al.* (1994) and suggested that the general enhancement of A_{area} under elevated CO_2 was dominated by a reduction in CO_2 limitation of photosynthesis as indicated by the direct effect of CO_2 on A_{area}. Contrary to the prediction of the PAC model we failed to support the hypothesis that reduction in biochemical capacity is frequently offset by increased *LMA*. Instead, we found that increased
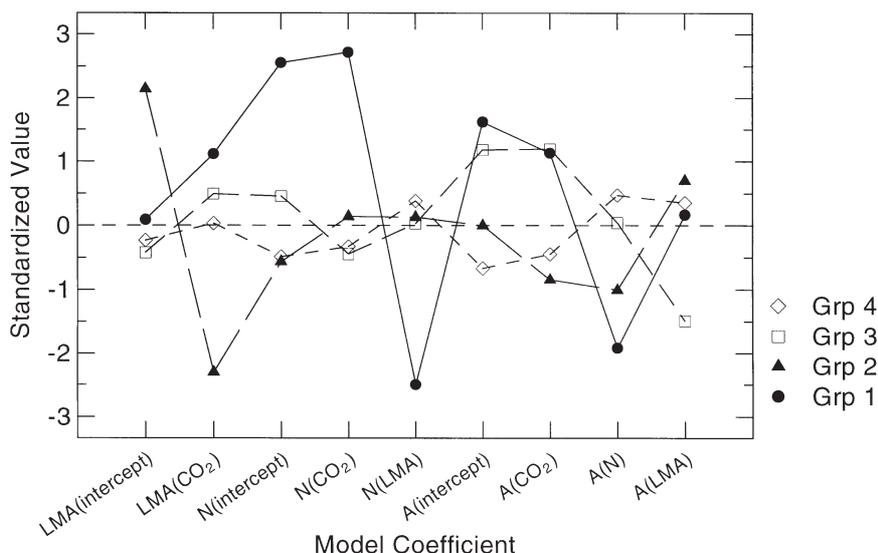
Figure 7. Standardized coefficients for each group of species identified in the cluster analyses. Symbols on the *X*-axis represent each coefficient in model 1, with the dependent variable outside the brackets and the independent variable inside the brackets, e.g. *LMA*(*CO*2) is the direct effect of CO_2 on *LMA*. Group 1 = *P. euramericana*; Group 2 = *P. radiata*; Group 3 = *A. glutinosa* and *B. pendula*; Group 4 = *A. rubrum*, *A. saccharum*, *F. sylvatica*, *L. tulipifera*, *P. ponderosa* and *P. taeda*.

LMA due to elevated CO_2 can significantly reduce photosynthesis in some species through a mechanism that appeared to be morphologically based but independent of N_{area}. The nature of this mechanism is not clear but may include a combination of factors mentioned previously, such as a reduction in N allocation to photosynthetic versus non-photosynthetic compounds (Evans 1989), to greater allocation of biomass to structural versus photosynthetic components (Vitousek *et al*. 1990; Lloyd *et al*. 1992), to greater internal shading (Terashima & Hirosaka 1995), or to greater limitations to internal diffusion (Parkhurst 1994; Pachepsky *et al*. 1997). Nevertheless, this morphological reduction in photosynthesis combined additively with a reduction in biochemical capacity to significantly reduce potential photosynthetic enhancement at elevated CO_2 in two species.

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APPENDIX

CO₂ Models/Experiments Activity for improved Links (CMEAL)

CMEAL is a collaborative project aimed at improving the representation of the CO_2 responses in ecosystem and global models. Participants are: C. B. Field (Carnegie Institution of Washington, Co-PI); J. T. Ball (Desert Research Institute, Co-PI); J. S. Amthor (Lawrence Livermore National Laboratory); B. Drake (Smithsonian Environmental Research Center); W. R. Emanuel (University of Virginia); D. W. Johnson (Desert Research Institute); P. J. Hanson (Oak Ridge National Laboratory); Y. Luo (Desert Research Institute); R. E. McMurtrie (University of New South Wales, Australia); R. J. Norby (Oak Ridge National Laboratory); W. C. Oechel (California State University, San Diego); C. E. Owensby (Kansas State University); W. J. Parton (Colorado State University); A. G. Peterson (Desert Research Institute); L. L. Pierce (California State University, Monterey Bay); E. B. Rastetter (Marine Biological Laboratory); A. Ruimy (Universite Paris-Sud); S. W. Running (University of Montana); and D. R. Zak (University of Michigan).