

Meta-analysis reveals profound responses of plant traits to glacial CO₂ levels

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Abstract

A general understanding of the links between atmospheric CO₂ concentration and the functioning of the terrestrial biosphere requires not only an understanding of plant trait responses to the ongoing transition to higher CO₂ but also the legacy effects of past low CO₂. An interesting question is whether the transition from current to higher CO₂ can be thought of as a continuation of the past trajectory of low to current CO₂ levels. Determining this trajectory requires quantifying the effect sizes of plant response to low CO₂. We performed a meta-analysis of low CO₂ growth experiments on 34 studies with 54 species. We quantified how plant traits vary at reduced CO₂ levels and whether C₃ versus C₄ and woody versus herbaceous plant species respond differently. At low CO₂, plant functioning changed drastically: on average across all species, a 50% reduction in current atmospheric CO₂ reduced net photosynthesis by 38%; increased stomatal conductance by 60% and decreased intrinsic water use efficiency by 48%. Total plant dry biomass decreased by 47%, while specific leaf area increased by 17%. Plant types responded similarly: the only significant differences being no increase in SLA for C₄ species and a 16% smaller decrease in biomass for woody C₃ species at glacial CO₂. Quantitative comparison of low CO₂ effect sizes to those from high CO₂ studies showed that the magnitude of response of stomatal conductance, water use efficiency and SLA to increased CO₂ can be thought of as continued shifts along the same line. However, net photosynthesis and dry weight responses to low CO₂ were greater in magnitude than to high CO₂. Understanding the causes for this discrepancy can lead to a general understanding of the links between atmospheric CO₂ and plant responses with relevance for both the past and the future.

Introduction

Atmospheric CO₂ concentration has varied tremendously over geological time, from as high as 3000 ppm in the lower Devonian (Royer 2006) to as low as 180–280 ppm during the past 2.1 Ma of the Pleistocene (Honisch et al. 2009). About 17.5 Ka ago, atmospheric CO₂ concentration started to rise from 180 ppm, levelled off at 280 ppm around 15 Ka ago and broadly remained at 280 ppm until the Industrial Revolution. Since the start of the Industrial Revolution, CO₂ levels have risen to 390 ppm today, levels not experienced by plants for over 25 Ma (Royer 2006) and are expected to increase even further; common model estimates go up to 700 ppm by 2100 (IPCC 2007). A CO₂ atmosphere of 700 ppm has not been observed since 42 million years ago (Royer

2006). The atmosphere today and as predicted for the end of the century is thus increasingly different from that experienced by plants during a large part of the recent past.

CO₂ plays a pivotal role in a number of important eco-physiological processes: it is an essential ingredient for photosynthesis and plant growth, and it is highly likely that plants' morphological and physiological traits and their plastic responses to the CO₂ concentrations are more tuned to the range of CO₂ concentrations they have experienced recently. Because adjustment to changing CO₂ involves changes in photosynthetic rates, nitrogen allocation, and other physiological properties (Curtis and Wang 1998; Korner 2000; Cowling 2001; Poorter and Navas 2003; Ainsworth and Long 2005), this trait adjustment has the potential to create a feedback that could

affect the global carbon cycle (Beerling *et al.* 2012). Connecting the performance of plants of different species at low, ambient and future high CO₂ is thus an important part of understanding the links between the atmospheric CO₂ concentrations and the terrestrial biosphere in the past, present, and future.

At low CO₂, photosynthesis is limited by the amount of available carbon by limiting carboxylase activity of the enzyme RuBisCO. Conversely, at higher CO₂ concentrations, the rate at which CO₂ can be taken up (photosynthetic capacity) becomes limiting (Sage 1994). CO₂ levels in the past could thus have selected for RuBisCO carboxylase activity or efficiency. At current and future CO₂ concentrations, other factors such as water and nutrient uptake will become more limiting than CO₂ (Ward *et al.* 1999; Campbell and Sage 2006; Reich *et al.* 2006). This implies that trait states useful in a low CO₂ atmosphere can be redundant or suboptimal in a high CO₂ atmosphere. For example, high investment in RuBisCO, useful at low CO₂, in a high CO₂ environment requires a high N investment, which could otherwise be used in other N-limited steps. High activity of enzymes facilitating transport and binding of CO₂ is a lower priority at high CO₂ compared with the need for more sink capacity of photosynthates to take advantage of increased photosynthetic rates (Sage and Coleman 2001). Favorable traits in low CO₂ thus do not necessarily mirror those in high CO₂.

For obvious reasons, considerable scientific effort has gone into examining the response of plants to high levels of CO₂ as projected for the latter half of this century. Several recent meta-analyses have found that, despite methodological differences among studies, a few main results are apparent: at high (500+ ppm) CO₂, there is an increase in carbon assimilation and growth and decrease in stomatal conductance, nitrogen content and specific leaf area (Poorter 1993; Curtis and Wang 1998; Poorter and Navas 2003). The increase in biomass is about +45% for C₃ species and +12% for C₄ species at a 50% increase in CO₂ concentration (Poorter and Navas 2003). The response of C₄ species to increased CO₂ is smaller than that of C₃ species, probably because the carbon concentrating mechanism of C₄ plants already concentrates CO₂ around RuBisCO leaving less room for increased photosynthetic rate (Bowes 1993; Ghannoum *et al.* 2000). Overall, woody species showed a greater response to elevated CO₂ than herbaceous species (Curtis and Wang 1998; Poorter and Navas 2003; Ainsworth and Long 2005; Lee *et al.* 2011).

In contrast to the large amount of studies on plant responses to elevated CO₂, less research has been carried out on the response of plants to subambient, Pleistocene levels of CO₂. Several individual experiments reveal that the influence of low CO₂ acts on multiple biotic levels,

ranging from leaf level to plant level and ecosystem level (Gerhart and Ward 2010). The emergence of agriculture has even been linked to the increase in CO₂ to 280 ppm 17.5 Ka ago, as higher levels of CO₂ lead to higher yields (Sage 1995). Understanding how plants have adapted to the low CO₂ of their recent evolutionary history can aid us in understanding plants response to future high CO₂ (Gerhart and Ward 2010; Beerling 2012; Leakey and Lau 2012). Recent research has shown CO₂ uptake and water use are highly consistent across CO₂, from low to high (Franks *et al.* 2013). Thus, there is clearly a need to integrate the knowledge available so far on low CO₂ responses to determine whether more traits follow a predictable pattern.

Some qualitative expectations can be made as to how plants are likely to respond to low CO₂. A lowering of CO₂ will likely lead to a reduction in photosynthetic rates (Farquhar *et al.* 1980) and plant biomass (Overdieck *et al.* 1988; Cunniff *et al.* 2008). Next, an increase in leaf nitrogen concentration as RuBisCO may ameliorate some of the reduction in total C assimilation rate (Sage and Coleman 2001). Differences in response among different plant types can also be expected. C₄ metabolism, which concentrates CO₂ around RuBisCO, could partly compensate the potential reduction in growth as experienced by C₃ plants. At lower atmospheric CO₂ concentration, one of the mechanisms to maintain a high enough internal CO₂ concentration is to open stomata wider, allowing water to escape at a faster rate (Farquhar and Sharkey 1982). Because of the carbon concentrating mechanism in C₄ species, the diffusion gradient of CO₂ across the stomata can be much steeper. At reduced CO₂, this should allow C₄ plants to maintain a smaller stomatal aperture than C₃ plants, giving a smaller increase in stomatal conductance and a smaller decrease in water use efficiency (Farquhar and Sharkey 1982). As woody plants invest more biomass in stems than herbaceous plants (Poorter *et al.* 2012) and as stems usually do not contribute substantially to photosynthesis, it is possible that this constrains the ability to adjust carbon allocation at low atmospheric CO₂. In response to a reduction in growth, a complex suite of trait adjustments, with differences among plant types, is expected at all physiological levels, varying from photosynthesis to biomass allocation. While the directions of all these responses to low CO₂ have empirical support (Gerhart and Ward 2010), they have not yet been quantified in general terms across studies and species.

Here, we present the results of a global meta-analysis synthesizing data from currently available low CO₂ experiments to quantify general patterns of morphological and ecophysiological trait responses to subambient CO₂. In particular, together with the body of work on high CO₂,

we aim to build toward a general, quantitative understanding of the response of plant traits to a range of CO₂.

Thus, we address the following research questions and hypotheses:

- 1 How much do plant traits vary with decreased CO₂ concentration?

We hypothesize that lower photosynthetic rates will lead to reduced growth at low CO₂. To acclimate to a low CO₂ environment and keep up photosynthetic rates, plants will have higher leaf nitrogen and larger stomatal conductance.

- 2 How much do plant functional groups differ in their response to low CO₂?

Because of the carbon concentrating mechanism of C₄ plants, we hypothesize (a) that the negative effects of low CO₂ on their photosynthesis and growth will be reduced as compared to those in C₃ plants; and (b) that woody species will invest more of their biomass in nonphotosynthetic tissue leading to a greater reduction in biomass accumulation than herbaceous species.

- 3 Is plant trait response to low CO₂ similar in magnitude to the response to elevated CO₂?

Atmospheric CO₂ is on a trajectory from low during glacial times to very high CO₂ in the future. We aim to shed light on whether plant traits adjust similarly from low to ambient as from ambient to high. Given the saturating nature of the photosynthetic response to CO₂, we expect photosynthetic traits to respond to low CO₂ through a greater magnitude shift compared with the high CO₂ response. For other traits, we are curious if they follow the saturating response of photosynthesis or if they respond more proportional to CO₂ changes.

Methods

We performed a literature review on plant science journals searching Web of Science using keywords “subambient CO₂,” “low CO₂,” “reduced CO₂” and “glacial CO₂.” This resulted in 33 papers that reported on studies with experimentally lowered atmospheric CO₂ concentration for 54 species in total. In these experiments, plants were grown in greenhouses, climate chambers, or outdoor tubes after Mayeux *et al.* (1993). For the climate chambers and greenhouses, CO₂ concentrations were reduced by passing air through some kind of filter or adsorbent (e.g., Soda lime) or through a reactive solution (e.g., NaOH).

For each study, we recorded sample size, duration, growing conditions, low CO₂ treatment type, and germination conditions along with measures of plant physiological traits at the CO₂ concentrations used

(Table S1, Data S1 and S2). A full list of the studies found with which species and trait data they reported can be found in Table S1, Data S1 and S2. When data and errors were not present in tabular form, they were extracted from graphs using Datathief 3 (Tummers 2006). All papers reported trait means in response to CO₂ concentration and most reported a measure of error (standard deviation, standard error, or confidence interval) for the trait in each CO₂ treatment. Three of the published papers dealt with response to low CO₂ at varying resource conditions (P limitation: Campbell and Sage 2006; Lewis *et al.* 2010; Drought: Ward *et al.* 1999). In order to exclude confounding factors, only those results at high nutrients and well-watered conditions were included. C₃–C₄ intermediates were grouped together with C₄ species.

From the studies found, only 6 traits emerged with 10 or more species analyzed and only 20 traits with 3 or more species. Of these 20 traits, 12 were related to growth and development. These traits included specific leaf area (as SLA or leaf mass per area, which was recalculated to SLA, m²·g⁻¹) and (components of) plant biomass (DW, g dry weight). In some cases, plant biomass was divided into above- and belowground mass. Aboveground mass was divided into leaf and stem mass. All of these masses can be expressed either in absolute terms or as allocation, that is, relative to plant mass. Number of stomata and stomatal pore size (μm) were infrequently reported. Five traits related to photosynthesis included photosynthetic rate either as maximum, at saturating light levels (A_{\max} , μmol·m⁻²·s⁻¹), and/or net, at growth conditions, photosynthesis (A_{net} , μmol·m⁻²·s⁻¹), stomatal conductance (g_s , mol·m⁻²·s⁻¹), the ratio of internal to external CO₂ concentration (C_i/C_a) and water use efficiency (WUE; A_{net}/g_s , mmol·mol⁻¹). Lastly, four traits were related to chemical composition, namely nitrogen content either area-based (g·m⁻²) or mass-based (g·g⁻¹), chlorophyll content (μmol·g⁻¹) and RuBisCO content (g·m⁻²).

To examine the effect of CO₂ among all species in the study, we performed a weighted ANCOVA for each trait with CO₂ concentration as covariate, species as a factor, and a potential interaction between the two. To determine the overall effect of CO₂ on a plant trait, a model without differing slopes between species was fitted when species by CO₂ interaction was not significant or the average slope from all species was calculated when species by CO₂ interactions were significant. We used the inverse of the square root of the standard error from the original study as the weighting factor for uncertainty, as is standard in meta-analyses (Hedges and Olkin 1985; Sokal and Rohlf 1995) For trait values reported without an error term, the average standard deviation in the trait was used to calculate the weighting factor. All traits and CO₂ concentrations were log-transformed, which improved the

normality of the residuals and allowed the output to be considered as scaling slopes (Renton and Poorter 2011). We investigated both which traits responded to low CO₂ and, for those traits that did, what the effect size of that adjustment was.

In the ANCOVA framework, the scaling slope of the trait-CO₂ relationship then indicates the proportional change in trait value in the following way:

$$\text{Trait change} = \text{CO}_2\text{change}^\beta - 1 \quad (1)$$

where CO₂ change is the proportional change in CO₂ concentration and β is the slope in the log-log plot. For example, if β were 1 then a 50% reduction in CO₂ concentration would result in a 50% reduction in trait amount. When β is less or >1, a 50% reduction in CO₂ will result in a less or more than 50% reduction in trait amount, respectively. A negative slope indicates an increase in trait value with a decrease in CO₂ concentration. Subsequent to the ANCOVA analysis, differences in slope between C₃ and C₄ herbs and woody and herbaceous C₃ plants were assessed by 2 sample t-tests weighted by 1/SE of the species. All statistics were performed using R, version 2.14.0 (R Foundation for Statistical Computing, Vienna, Austria). Due to the limited number of species for many traits, statistical power tended to be low; however, we judge it important enough to report those results as they reflect the current state of knowledge and to show the lack of data in important traits.

Rather than performing a meta-analysis on the available high CO₂ experiments ourselves, we searched the literature for highly cited large-scale meta-analyses on plant traits in experimentally elevated CO₂. From our survey, 5 large meta-analyses emerged involving tens to hundreds of plant species reporting various traits including the 6 traits that were reported for 10 or more species at reduced CO₂ (Curtis and Wang 1998; Poorter and Navas 2003; Ainsworth and Long 2005; Ainsworth and Rogers 2007; Wang *et al.* 2012). From the meta-analyses, we extracted the shift in trait value at nonlimiting resources when available. We then compared this to the projected trait shift when assuming the same proportional response as to low CO₂.

Results

Of the 21 traits that were reported for 3 or more species, 14 showed either significant variation with CO₂ or species response to CO₂. Percentage values in the text below show the proportional change in trait value \pm SE upon a 50% reduction in growth CO₂ concentration (eqn 1). For each trait, species could respond to CO₂ (adjust their trait value), show consistent variation in trait value between species over a CO₂ gradient (species intercept or elevation

of species line in trait vs. CO₂ plot) and show significant variation in how species responded to CO₂ (CO₂*species interaction; Table 1). A nonsignificant interaction of species*CO₂ for a given trait indicates that different species adjust the trait by the same proportional amount.

Photosynthesis-related traits

Across the species studied, a 50% reduction in CO₂ did on average reduce maximum photosynthesis (A_{max} , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) by $33 \pm 4\%$ ($P < 0.05$, 15 species; Fig. 1A) and net photosynthesis (A_{net} , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) comparably by $38 \pm 5\%$ ($P < 0.001$, 25 species; Fig. 1B). Next to this, stomatal conductance (g_s , $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) increased by $60 \pm 14\%$ ($P < 0.01$, 17 species; Fig. 1C). The ratio of water loss to carbon gain, intrinsic water use efficiency (A_{net} over g_s , WUE), decreased by $48 \pm 4\%$ ($P < 0.001$, 26 species; Fig. 1D). For A_{net} , WUE and g_s species showed significant variation in trait elevation ($P < 0.001$) and response to CO₂ (interaction, $P < 0.01$). A_{max} however showed only significant variation in species trait elevation ($P < 0.001$).

Growth and allocation

A halving of growth CO₂ concentration resulted in a corresponding reduction in plant dry weight (DW) by $47 \pm 6\%$ ($P < 0.001$, 25 species; Fig. 1E). Aboveground biomass was less reduced than belowground biomass as Shoot DW was reduced by $35 \pm 7\%$ ($P < 0.01$, 7 species) and Root DW by $61 \pm 8\%$ ($P < 0.001$, 5 species). This pattern was reflected in a reduced root/shoot dry matter ratio (r/s ratio) of $21 \pm 6\%$ ($P < 0.001$, 9 species). In contrast, specific leaf area (SLA, $\text{m}^2\cdot\text{g}^{-1}$) increased by $17 \pm 6\%$ ($P < 0.001$, 22 species; Fig. 1f) at reduced CO₂. For root/shoot ratio, there was both significant variation in species trait elevation ($P < 0.001$) and response to CO₂ ($P < 0.01$). For SLA and plant biomass, species showed significant variation in trait elevation but did not respond differently to reduced CO₂.

Chemical composition

Only few data on chemical composition were available, strongly limiting statistical power of our test. The concentration of chlorophyll ($\mu\text{mol}\cdot\text{g}^{-1}$, 4 species) and amount of RuBisCO ($\text{g}\cdot\text{m}^{-2}$, 3 species) were not significantly affected by CO₂. Nitrogen levels in the leaf showed a contrasting response to halving CO₂ where leaf nitrogen percent ($\text{g}\cdot\text{g}^{-1}$) increased by 18 ± 8 ($P < 0.001$, 10 species) whereas nitrogen content per area ($\text{g}\cdot\text{m}^{-2}$) decreased by $15 \pm 6\%$ ($P < 0.05$, 4 species). Neither for nitrogen per unit mass nor per unit leaf area did species have significantly different responses to reduced CO₂.

Table 1. Overview of ANCOVA results on log(trait data) versus log(CO₂) concentration with species as covariate and as weighting factor. Traits are ordered by number of species analyzed. Slope indicates the average slope of log(trait) versus log(CO₂) including SE. -50% CO₂ gives the proportional change in trait given a 50% reduction in CO₂ concentration as per $Trait\ change = CO_2\ change^{\beta-1}$ where β is the slope. Values are calculated by slope \pm SE.

Trait	#Species	#Studies	Slope	-50% CO ₂	p(CO ₂)	p(species)	p(CO ₂ *species)
WUE (mmol mol ⁻¹)	26	8	0.95 \pm (0.1)	-48.3% \pm (3.5)	***	***	***
A _{net} (μ mol·m ⁻² ·s ⁻¹)	25	15	0.7 \pm (0.11)	-38.3% \pm (4.5)	***	***	***
DW (g)	25	14	0.91 \pm (0.16)	-46.9% \pm (5.8)	***	***	ns
SLA (m ² ·g ⁻¹)	22	17	-0.2 \pm (0.08)	+17.2% \pm (6.4)	***	***	ns
g _s (mol·m ⁻² ·s ⁻¹)	17	11	-0.7 \pm (0.13)	+59.8% \pm (13.9)	**	***	**
A _{max} (μ mol·m ⁻² ·s ⁻¹)	15	9	0.58 \pm (0.09)	-33.1% \pm (4.3)	*	***	ns
% Leaf N (g·g ⁻¹)	10	9	-0.2 \pm (0.1)	+17.8% \pm (8)	***	***	ns
PNUE (μ mol·mmol ⁻¹ ·s ⁻¹)	10	2	0.22 \pm (0.36)	-14.2% \pm (21.6)	†	*	ns
r/s ratio	9	3	0.34 \pm (0.11)	-21% \pm (6.1)	***	***	**
Shoot DW (g)	7	4	0.62 \pm (0.14)	-35.1% \pm (6.5)	***	***	ns
% Leaf mass	5	4	-0.1 \pm (0.2)	+9% \pm (15)	*	***	***
Root DW (g)	5	4	1.34 \pm (0.27)	-60.6% \pm (7.5)	***	***	ns
Leaf DW (g)	5	4	0.83 \pm (0.54)	-43.9% \pm (21.5)	***	***	**
C _i /C _a	4	4	-0.1 \pm (0.12)	+6% \pm (8.8)	ns	ns	ns
Chlorophyll (μ mol·g ⁻¹)	4	4	0.19 \pm (0.09)	-12.4% \pm (5.4)	ns	***	ns
Leaf N (g·m ⁻²)	4	4	0.23 \pm (0.11)	-14.8% \pm (6.3)	*	ns	ns
% Stem mass	4	3	0.08 \pm (0.08)	-5.4% \pm (5)	ns	*	ns
Stem DW (g)	4	3	1.72 \pm (0.83)	-69.7% \pm (18.5)	***	***	***
# Stomata	4	2	0.08 \pm (0.11)	-5.7% \pm (7.3)	ns	***	ns
RuBisCO (g·m ⁻²)	3	3	0.37 \pm (0.14)	-22.7% \pm (7.3)	ns	**	ns
Pore size (μ m)	3	1	0.12 \pm (0.13)	-7.8% \pm (8.2)	ns	***	ns

P-values are ns: not significant; †<0.1, *<0.05, **<0.01, ***<0.001.

Differential responses of plant types

Due to the small number of available species, the statistical power of the comparison between plant types was limited. Thus, in a few cases, there was only a trend of differential response among C₃, C₄ and woody and herbaceous species. Nevertheless, interesting contrasts and similarities emerged. Figure 2 shows the contrasting slopes between plant types for the eight plant traits with nine or more species. Between C₃ and C₄ herbs, only the greater increase in SLA for C₃ herbs at reduced CO₂ was significant ($P < 0.01$). C₄ plants showed on average a negligible SLA response to CO₂. Net photosynthesis and dry weight seemed to be less reduced for C₄ herbs although with small sample size and large variation this was not significant. Interestingly, plant dry weight of woody species was reduced less by 16% than that of herbaceous C₃ plants ($P < 0.05$). Overall, for the traits shown in Figure 2, different plant types appear to show rather similar responses to reduced CO₂.

Comparison with elevated CO₂ experiments

Table 2 highlights the comparison between trait responses at low CO₂ to the response at elevated CO₂ of the six traits that had the most data. When comparing the trait

shifts found in five large meta-analyses (Curtis and Wang 1998; Poorter and Navas 2003; Ainsworth and Long 2005; Ainsworth and Rogers 2007; Wang et al. 2012) to trait shifts extrapolated from the response to low CO₂, a few interesting contrasts and similarities emerged. With increasing CO₂, the magnitude of shift in A_{net} deviated more from the response to low CO₂; for g_s, the shift was similar in magnitude to results found in FACE studies and one growth chamber meta-analysis but not another. While we found no differences between C₃ and C₄ plants in water use efficiency at low CO₂, at high CO₂ large differences are found. The increase in WUE at high CO₂ found for C₃ plants at FACE sites is comparable to our extrapolated response. Whole plant dry weight (DW) appears to increase much less at high CO₂ than expected based on the low CO₂ response. SLA, however, seems to be adjusted in a similar magnitude as expected from the response to low CO₂.

Discussion

This meta-analysis seeks to quantify and aggregate current knowledge on plant traits in low CO₂. Few traits were measured for many species, and data were found for a limited number, 45, of species (Table 1). Due to the limited number of species and trait measurements,

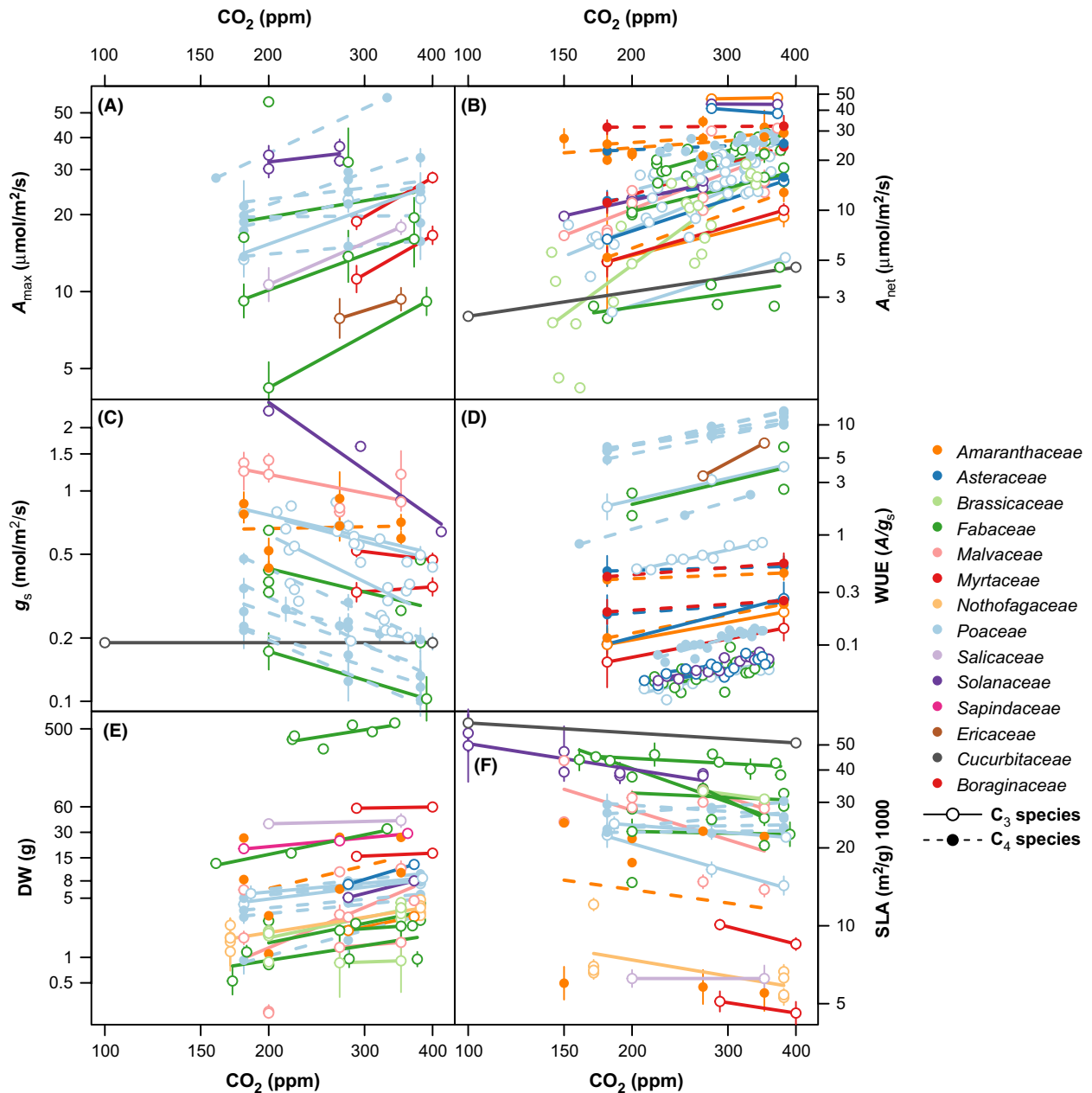


Figure 1. Plant trait versus growth CO₂ concentration (note the double-log scale) of the 6 traits with the highest number of species. (A) Maximum photosynthesis (A_{max}), (B) Net photosynthesis (A_{net}), (C) Stomatal conductance (g_s), (D) Intrinsic water use efficiency (net photosynthesis over g_s , WUE), (E) Plant dry weight, (F) Specific leaf area (SLA). Each line represents the response of a single species. Open symbols: C₃ metabolism, solid symbols: C₄ metabolism. Error bars give SE. Different colors represent the different families the species belong to.

comparison between plant types, woody–herbaceous, C₃–C₄, was difficult. This limited data set should be taken into account when reviewing the results. Interesting results do however emerge. In response to reduced CO₂, plants adjusted both physiological and morphological traits (Figure 1, Table 1). The magnitude of trait adjustment varied among species for 6 of 20 traits examined (Table 1). This suggests that in order to cope with low

CO₂ different species adjust different traits. This species-level heterogeneity in response though was not clearly based on simple functional groups (Figure 2).

Below, we discuss trait shifts at low CO₂ moving from leaf to ecosystem scales, keeping in mind that all the results discussed below are all short-time scale, plastic responses (Gerhart and Ward 2010). Plant plastic response to changing conditions occurs at different levels

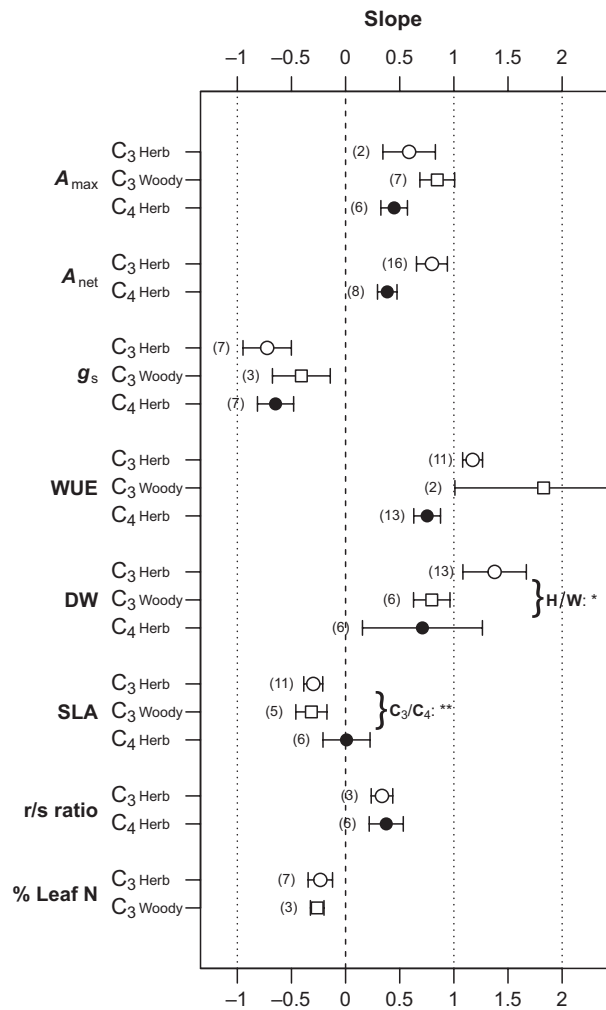


Figure 2. Slope of $\log(\text{trait})$ versus $\log(\text{CO}_2 \text{ concentration})$ for different plant types, C₃/C₄ and woody/herbaceous. Open circles, C₃ herbaceous type; solid circles, C₄ herbaceous type; open square, C₃ woody type. ± 1 indicates a 1:1 change in a trait for a change in CO₂ concentration with a negative slope indicating an increase in trait value and a positive slope indicating a decrease in trait value. A_{max} , maximum photosynthesis ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); A_{net} , net photosynthesis ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); g_s , stomatal conductance ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); WUE, water use efficiency ($\text{mmol}\cdot\text{mol}^{-1}$); DW, plant dry weight (g); SLA, specific leaf area ($\text{m}^2\cdot\text{g}^{-1}$); r/s ratio, root DW to shoot DW ($\text{g}\cdot\text{g}^{-1}$). Numbers between brackets gives the number of species for each plant type. P -values between plant types are * < 0.05 , ** < 0.01 .

of organization after different periods of time (Nicotra et al. 2010).

Leaf-level responses to low CO₂

We found support for our hypotheses at the leaf level. We hypothesized that low CO₂ would lead to lower photosynthetic rates (A), which leads to reduced growth, and that in order to partially ameliorate the photosyn-

thetic rate reduction, plants would have higher leaf nitrogen and larger stomatal conductance (g_s) (Sage and Reid 1992; Medlyn et al. 2011). Focussing first on A and g_s , A_{net} substantially decreased at a 50% decrease in CO₂ and g_s increased considerably, indicating that the increase in stomatal conductance is not enough to keep up with lower atmospheric carbon concentration.

Water use efficiency (WUE) decreased proportionally with CO₂ (following Franks et al. 2013) but did show significant variation between species. Such a strong increase in water demand suggests great consequences for plants experiencing drought stress. However, recovery from drought at low CO₂ was found to be similar between C₃ (*Abutilon theophrasti*) and C₄ (*Amaranthus retroflexus*) plants at low CO₂, due to less leaf loss and stomatal closure than expected for C₃ species (Ward et al. 1999). Suggesting that there are trait shifts that mitigate some ill effects.

When comparing the magnitude of the response of A , g_s and WUE at high CO₂ to the responses, we found at low CO₂ a contrasting picture emerges. At low CO₂, no difference in WUE was found between plant types, although at high CO₂, C₄ plants increase their WUE less than C₃ plants (Poorter and Navas 2003). For C₃ plants, the response to high CO₂ seems similar in magnitude as to low CO₂ for WUE. For the components of WUE, at high CO₂, the adjustment of A_{net} was greater in magnitude as to low CO₂, whereas the effect on g_s was comparable to low CO₂, although one meta-analysis reported a far lower decrease in g_s at high CO₂ (Table 2). The extent to which g_s can be reduced might be limited though. Leaf thermal regulation, which is impaired at very low stomatal conductance, could put a limit on the possible decrease. Paleo-evidence suggests this may have been relevant during a previous “rapid” transition to a high CO₂ atmosphere at the Triassic–Jurassic boundary (Mcelwain et al. 1999).

At the level of enzymes and leaf chemical composition, we found no evidence that RuBisCO content and chlorophyll content were affected by reduced CO₂; the power of our test was limited because few studies measured those traits (Table 1). Leaf nitrogen (N) content per mass did increase but N content per area decreased in response to low CO₂; this contrasting response might be explained by the higher specific leaf area (SLA) in low CO₂. A higher SLA indicates lower nitrogen containing mass per area. Whether or not the nitrogen increase per mass is the result of an increase in certain nitrogen-rich chemical compounds or a decrease in carbon-rich compounds, as carbon is a limiting resource, is unclear. Clearly more work is needed on the enzymatic and chemical response of plants to low CO₂.

Plants adjust their leaf morphology in order to cope with low concentrations of atmospheric CO₂. At half of ambient CO₂, leaf SLA increased by 15%. This increase

Table 2. Comparison of trait shift at high CO₂ extrapolated from low CO₂ response (bold values) to actual changes found in three meta-analyses. If the trait adjustments are proportional from past low to future high CO₂, the predictions from the low CO₂ experiments should match the measured values from the high CO₂ experiments. The measured trait shifts are from chamber studies, GC1: Curtis and Wang 1998 (700 ppm), GC2: Poorter and Navas 2003 (690 ppm), GC 3: Wang *et al.* 2012 (700 ppm) and FACE experiments, FACE: Ainsworth and Long 2005; Ainsworth and Rogers 2007 (560 ppm). Percentage values indicate magnitude of trait shift as compared to current, levels of CO₂.

Trait	GC1 (700 ppm)		GC2 (690 ppm)		GC3 (700 ppm)		FACE (560 ppm)	
	Actual	Extrapolated	Actual	Extrapolated	Actual	Extrapolated	Actual	Extrapolated
A_{\max}							+31%	+27% ± (5)
A_{net}	+28%	+63% ± (12)	+28%	+61% ± (12)	+14%	+63% ± (12)	+26%	+34% ± (6)
g_s	-11%	-38% ± (6)			-32%	-38% ± (6)	-21%	-25% ± (4)
WUE							+68%(C ₃)	+48% ± (6)
							+6%(C ₄)	
DW	+28%	+88% ± (21)	+48%(C ₃)	+86% ± (20)	+25%(C ₃)	+88% ± (21)		
			+12%(C ₄)		-3%(C ₄)			
SLA			-13%	-15% ± (5)	-10%	-15% ± (5)	-6%	-9% ± (3)

has two potential advantages to the plant: first, when CO₂ is limiting, more leaf area per unit C invested in leaves allows for lower carbon costs per unit carbon capture; second, at the leaf level, higher SLA leaves may improve mesophyll conductance (Vitousek *et al.* 1990). While increased g_s at low CO₂ can maintain internal CO₂ concentration up to a point, at low CO₂ concentrations the diffusion of CO₂ inside the leaf can become limiting to photosynthesis as well (Keenan *et al.* 2010). Higher SLA indicates either thinner or less dense leaves with more internal air space, which in many cases leads to greater mesophyll conductance to CO₂ (Loreto *et al.* 1992). However, a higher SLA could also be a result of less starch or less other nonstructural carbohydrate present in the leaf at low CO₂ concentrations (Poorter *et al.* 2009). It is interesting to find that at high CO₂, SLA follows the same trend as at low CO₂. The relative contributions of the above factors to reducing and increasing SLA are an interesting avenue to pursue further.

Plant-level responses to low CO₂

While plants adjust their gas exchange and leaf morphology in response to reduced CO₂, photosynthetic rates are nevertheless reduced, resulting in less biomass but also shifts in allocation between root and shoot. Plant biomass (g dry weight) decreases proportionally at a 50% CO₂ reduction, which is more pronounced in belowground biomass as is illustrated by a reduction in root-to-shoot ratio. This could be the result of plants balancing their nutrient gain and their carbon gain to the now more limiting carbon resource (Bloom *et al.* 1985; Chapin *et al.* 1987) or some specific source-sink relationship between root and shoot, that is, fixed in the plant's metabolism, similar to the idea of a fixed c_i/c_a ratio for species across

time (Ehleringer and Cerling 1995; Gerhart *et al.* 2012; Franks *et al.* 2013). At low CO₂, photosynthesis per area is lower, so the amount of sugars available for the roots is less per unit of shoot biomass. This shift in allocation at low CO₂ may have important implications for species interactions, particularly in tree-savanna grass interactions (Bond and Midgley 2012): at low CO₂, the regrowth capacity following disturbance of tree species is much diminished, adding weight to the importance of fire and herbivory as ecosystem shaping factors (Kgope *et al.* 2010; Bond and Midgley 2012). Next to allocation, the increased SLA at low CO₂ might lead to greater food availability and thus pressure from herbivores as high SLA leaves are eaten more readily (Poorter *et al.* 2009). Faster decomposition rates and nutrient cycling of high SLA leaves would also have large ecosystem effects by allowing faster nutrient cycling (Cornelissen *et al.* 1999).

Plant types, growth environments, and low CO₂ response

Differences between C₃, C₄, woody, and herbaceous species were not as pronounced as hypothesized. Results suggested differences between plant types although the responses of only few traits were significantly different. C₃ herbs had a significantly greater increase in SLA than C₄ herbs, which on average showed no response. Decreases in biomass diminished starting from herbaceous C₃, woody C₃ to herbaceous C₄. However, only the difference between woody and herbaceous C₃ was significant with woody plants having a smaller reduction in biomass at low CO₂. This result mirrors that at high CO₂ experiments where increased CO₂ generally leads to a greater relative growth rate (RGR) increase for fast growing, herbaceous, plants than slow growing, woody, plants

(Poorter and Navas 2003). As fast growers “win” more at high CO₂ they “lose” more at low CO₂. While the smaller decrease in biomass for herbaceous C₄ than for C₃ is not significant, it should be noted that the average reduction in A_{net} and A_{max} is also lower than in C₃ herbs. This may point to a smaller reduction in biomass accumulation for C₄ herbs via a smaller reduction in photosynthesis. With more data on woody and C₄ species, such differences between plant types and their underlying mechanism may become more apparent.

The results presented here summarize the effects of low CO₂ at high water and high nutrients. One important caveat to consider, however, is that there are many potential interactions between CO₂, water, and nutrients as is shown by some studies. Low nutrients in the form of low P limited photosynthetic rates even further at low CO₂ in *Lupinus albus* (Campbell and Sage 2006) and *Populus deltoides* (Lewis *et al.* 2010). C₃ (*Abutilon theophrasti*) and C₄ (*Amaranthus retroflexus*) plants recovered similarly from drought at low CO₂ (Ward *et al.* 1999). This shows that other environmental factors strongly influence the effect of CO₂ on plant traits. However, most data were available for well-watered, high-nutrient growth experiments. The interactions between CO₂, light, nutrients and water are clearly important when extrapolating from growth chamber experiments to glacial environments, but a full understanding of the interactions would require many more or more extensive studies.

Experimental results presented here on plastic responses need to be put into context with other sources of information on plant traits in the past including measurements on paleomaterials. In the past, CO₂ has proven to be a strong selective agent altering worldwide floristic composition (McElwain *et al.* 2005). Thus, evolution and selection have likely occurred with increasing CO₂, and it is therefore important to determine whether the traits of modern plants grown under low CO₂ compare to the traits of plants that lived in a low CO₂ atmosphere. The regeneration of *Silene stenophylla* buried in Siberian permafrost for over 30 ka (Yashina *et al.* 2012) provides an interesting opportunity for testing the response of modern plants and ancient plants to low CO₂ and how similar they are. It is also likely that in the period since the low CO₂ in the Last Glacial Maximum (LGM), species with short generation turnover have evolved more compared with long generation turnover species, although the drastic increase from 280 to 700 ppm within 250 years (1850–2100) will likely constrain the values to which traits have been adjusted. A full understanding of plant response to the transition from the LGM to current and future CO₂ levels must include both evolutionary adaptation and plastic responses.

Conclusion

In conclusion, we found that, despite the more limited set of low CO₂ studies compared with high CO₂ studies, a general response is emerging. Plant response to reduced atmospheric CO₂ involves a complex suite of trait adjustments. In order to diminish the effects of reduced CO₂ plants open their stomata wider, invest more in above-ground biomass and increase their SLA. Despite these adjustments, photosynthetic rate is nevertheless reduced, leading to a proportional reduction in biomass accumulation. Both trait adjustment and growth effect varies among species, but this variation does not appear to be a function of simple plant functional groups. Trait adjustments at low CO₂ as compared to high CO₂ were proportionally similar for g_s , WUE and SLA but responses at low CO₂ were greater than proportional for A_{net} and biomass. In other words, the data suggest that in terms of water relations and leaf morphology, the responses to low and high CO₂ are proportional and opposite. Carbon gain and whole plant growth rate are more complex—responses to low CO₂ in these cases are more extreme. At high CO₂, other factors such as nutrient and light availability could control these traits. To understand the response of plants to future high CO₂, it is important to understand how and when other factors become drivers for certain traits. Our understanding of plant response to CO₂ benefits from data from both low and high CO₂ conditions. The shape of that response will become increasingly relevant in a high CO₂ future.

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Conflict of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Overview of trait measurements reported for three or more species. **V:** Traits reported with error value, **X:** Traits reported without SE. Traits are ordered from most to least measured in low CO₂ studies. SLA/LMA, specific leaf area or recalculated leaf mass per area (g·m⁻²); g_s, stomatal conductance (mol·m⁻²·s⁻¹); DW, plant dry weight (g); A_{max}, maximum photosynthesis (μmol·m⁻²·s⁻¹); A_{net}, net photosynthesis (μmol·m⁻²·s⁻¹); WUE, water use efficiency (mmol·mol⁻¹); PNUE, photosynthetic nitrogen use efficiency (μmol·mmol·N⁻¹·s⁻¹); r/s ratio, root-to-shoot ratio DW; C_i/C_a, ratio intercellular to atmospheric CO₂; % Leaf N, percentage N per weight; % Leaf mass, percentage leaf DW of total DW; Root DW, root dry weight (g); Leaf DW, leaf dry weight (g); Chlorophyll, chlorophyll content (μmol·g⁻¹); Leaf N, N per area (g·m⁻²); %Stem mass, percentage stem mass of total weight; RuBisCO, RuBisCO content (g·m⁻²); Shoot DW, shoot dry weight (g); %Root DW, percentage root DW of total DW; Pore size, diameter of stomatal pore (μm).

Data S1. Meta-analysis data sheet compiled and used for this paper.

Data S2. Column headers used in the meta-data file with their description and meaning.