Plant Traits and Production Rates from CO2 Starvation to Saturation

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Chapter 2

Meta-Analysis Reveals Profound Responses of Plant Traits to Glacial CO$_2$ Levels

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Abstract

A general understanding of the links between atmospheric CO_2 concentration and the functioning of the terrestrial biosphere requires not only an understanding of plant trait responses to the ongoing transition to higher CO_2 but also the legacy effects of past low CO_2. An interesting question is whether the transition from current to higher CO_2 can be thought of as a continuation of the past trajectory of low to current CO_2 levels. Determining this trajectory requires quantifying the effect sizes of plant response to low CO_2. We performed a meta-analysis of low CO_2 growth experiments on 34 studies with 54 species. We quantified how plant traits vary at reduced CO_2 levels and whether C_3 versus C_4 and woody versus herbaceous plant species respond differently. At low CO_2, plant functioning changed drastically: on average across all species, a 50% reduction of current atmospheric CO_2 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 C_4 species and a 16% smaller decrease in biomass for woody C_3 species at glacial CO_2. Quantitative comparison of low CO_2 effect sizes to those from high CO_2 studies showed that the magnitude of response of stomatal conductance, water use efficiency and SLA to increased CO_2 can be thought of as continued shifts along the same line. However, for net photosynthesis and dry weight responses to low CO_2 were greater in magnitude than to high CO_2. Understanding the causes for this discrepancy can lead to a general understanding of the links between atmospheric CO_2 and plant responses with relevance for both the past and the future.

Introduction

Atmospheric CO_2 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 CO_2 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 CO_2 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 CO_2 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.

CO_2 plays a pivotal role in a number of important ecophysiological 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 CO_2 concentrations are more tuned to the range of CO_2 concentrations they have experience recently. Because adjustment to changing CO_2 involves changes in photosynthetic rates, nitrogen allocation, and other physiological properties (Ainsworth & Long 2005, Curtis & Wang 1998, Korner 2000, Cowling 2001, Poorter & Navas 2003), 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 CO_2 is thus an important part of understanding the links between the atmospheric CO_2 concentrations and the terrestrial biosphere in the past, present and future.

At low CO_2 photosynthesis is limited by the amount of available carbon by limiting carboxylase activity of the enzyme RuBisCO. Conversely, at higher CO_2 concentrations the rate at which CO_2 can be taken up (photosynthetic capacity) becomes limiting (Sage 1994). CO_2 levels in the past could thus have selected for RuBisCO carboxylase activity or efficiency. At current and future CO_2 concentrations other factors such as water and nutrient uptake will become more limiting than CO_2 (Campbell & Sage 2006, Reich *et al.* 2006, Ward *et al.* 1999). This implies that trait states useful in a low CO_2 atmosphere can be redundant or suboptimal in a high CO_2 atmosphere. For example, high investment in RuBisCO, useful at low CO_2, in a high CO_2 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 CO_2 is a lower priority at high CO_2 compared to the need for more sink capacity of photosynthates to take advantage of increased photosynthetic rates (Sage & Coleman 2001). Favourable traits in low CO_2 thus do not necessarily mirror those in high CO_2.

For obvious reasons, considerable scientific effort has gone into examining the response of plants to high levels of CO_2 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) CO_2 there is an increase in carbon assimilation and growth and decreases in stomatal conductance, nitrogen content and specific leaf area (Curtis & Wang 1998, Poorter 1993, Poorter & Navas 2003). The increase in biomass is about +45% for C_3 species and +12% for C_4 species at a 50% increase in CO_2 concentration (Poorter & Navas 2003). The response of C_4 species to increased CO_2 is smaller than that of C_3 species, probably because the carbon concentrating mechanism of C_4 plants already concentrates CO_2 around RuBisCO leaving less room for increased photosynthetic rate (Bowes 1993, Ghannoum *et al.* 2000). Overall, woody species showed a greater response to elevated CO_2 than herbaceous species (Ainsworth & Long 2005, Curtis & Wang 1998, Lee *et al.* 2011, Poorter & Navas 2003).

In contrast to the large amount of studies on plant responses to elevated CO_2, less research has been done on the response of plants to sub-ambient, Pleistocene levels of CO_2. Several individual experiments reveal that the influence of low CO_2 acts on multiple biotic levels, ranging from leaf level to plant level and ecosystem level (Gerhart & Ward 2010). The emergence of agriculture has even been linked to the increase in CO_2 to 280ppm 17.5 Ka ago, since higher levels of CO_2 lead to higher yields (Sage 1995). Understanding how plants have adapted to the low CO_2 of their recent evolutionary history can aid us in understanding plants response to future high CO_2 (Gerhart & Ward 2010, Beerling 2012, Leakey & Lau 2012). Recent research has shown CO_2 uptake and water use are highly consistent across CO_2, from low to high (Franks *et al.* 2013) Thus, there is clearly a need to integrate the knowledge available so far on low CO_2 responses to determine if more traits follow a predictable pattern.

Some qualitative expectations can be made as to how plants are likely to respond to low CO_2. A lowering of CO_2 will likely lead to a reduction in photosynthetic rates (Farquhar *et al.*

1980) and plant biomass (Cunniff *et al.* 2008, Overdieck *et al.* 1988). Next, an increase in leaf nitrogen concentration as RuBisCO may ameliorate some of the reduction in total C assimilation rate (Sage & Coleman 2001). Differences in response among different plant types can also be expected. C_4 metabolism, which concentrates CO_2 around RuBisCO, could partly compensate the potential reduction in growth as experienced by C_3 plants. At lower atmospheric CO_2 concentration one of the mechanisms to maintain a high enough internal CO_2 concentration is to open stomata wider, allowing water to escape at a faster rate (Farquhar & Sharkey 1982). Because of the carbon concentrating mechanism in C_4 species, the diffusion gradient of CO_2 across the stomata can be much steeper. At reduced CO_2 this should allow C_4 plants to maintain a smaller stomatal aperture than C_3 plants, giving a smaller increase in stomatal conductance and a smaller decrease in water use efficiency (Farquhar & 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 CO_2. 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 CO_2 have empirical support (Gerhart & 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 synthesising data from currently available low CO_2 experiments to quantify general patterns of morphological and ecophysiological trait responses to sub-ambient CO_2. In particular, together with the body of work on high CO_2, we aim to build toward a general, quantitative understanding of the response of plant traits to a range of CO_2.

Thus, we address the following research questions and hypotheses:

1. How much do plant traits vary with decreased CO_2 concentration?

We hypothesise that lower photosynthetic rates will lead to reduced growth at low CO_2. To acclimate to a low CO_2 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 CO_2?

Because of the carbon concentrating mechanism of C_4 plants we hypothesise (a) that the negative effects of low CO_2 on their photosynthesis and growth will be reduced as compared to those in C_3 plants; and (b) that woody species will invest more of their biomass in non-photosynthetic tissue leading to a greater reduction in biomass accumulation then herbaceous species.

3. Are plant trait responses to low CO_2 similar in magnitude to the response to elevated CO_2?

Atmospheric CO_2 is on a trajectory from low during glacial times to very high CO_2 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 CO_2,

we expect photosynthetic traits to respond to low CO_2 through a greater magnitude shift compared to the high CO_2 response. For other traits we are curious if they follow the saturating response of photosynthesis or if they respond more proportional to CO_2 changes.

Methods

We performed a literature review on plant science journals searching Web of Science using keywords "sub-ambient CO_2", "low CO_2", "Reduced CO_2" and "Glacial CO_2". This resulted in 33 papers that reported on studies with experimentally lowered atmospheric CO_2 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, CO_2 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 CO_2 treatment type and germination conditions along with measures of plant physiological traits at the CO_2 concentrations used (appendix 1.2). A full list of the studies found with which species and trait data they reported can be found in appendix 1.1. 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 CO_2 concentration, and most reported a measure of error (standard deviation, standard error or confidence interval) for the trait in each CO_2 treatment. Three of the published papers dealt with response to low CO_2 at varying resource conditions (P limitation: Campbell *et al.* 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. C_3-C_4 intermediates were grouped together with C_4 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, $m^2 g^{-1}$) 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, i.e. relative to plant mass. Number of stomata and stomatal pore size (μm) were infrequently reported. Five traits related to photosynthesis included photosynthesic 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 CO_2 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 CO_2 among all species in the study, we performed a weighted ANCOVA for each trait with CO_2 concentration as covariate, species as a factor, and a potential interaction between the two. To determine the overall effect of CO_2 on a plant trait, a model without differing slopes between species was fitted when species by CO_2 interaction

was not significant or the average slope from all species was calculated when species by CO_2 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 & Olkin 1985, Sokal & 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 CO_2 concentrations were log-transformed, which improved the normality of the residuals and allowed the output to be considered as scaling slopes (Renton & Poorter 2011). We investigated both which traits responded to low CO_2 and, for those traits that did, what the effect size of that adjustment was.

In the ANCOVA framework, the scaling slope of the trait-CO_2 relationship then indicates the proportional change in trait value in the following way:

Trait change = CO_2 change$^\beta$ – 1 (eq.1)

where CO_2 change is the proportional change in CO_2 concentration and β is the slope in the log-log plot. For example, if β were 1 then a 50% reduction in CO_2 concentration would result in a 50% reduction in trait amount. When β is less or greater than 1 a 50% reduction in CO_2 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 CO_2 concentration. Subsequent to the ANCOVA analysis, differences in slope between C_3 and C_4 herbs and woody and herbaceous C_3 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, 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 CO_2 experiments ourselves, we searched the literature for highly cited large scale meta-analyses on plant traits in experimentally elevated CO_2. 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 CO_2. (Ainsworth & Long 2005, Curtis & Wang 1998, Poorter & Navas 2003, Ainsworth & Rogers 2007, Wang *et al.* 2012). From the meta-analyses we extracted the shift in trait value at non-limiting resources when available. We then compared this to the projected trait shift when assuming the same proportional response as to low CO_2.

Results

Out of the 21 traits that were reported for 3 or more species 14 showed either significant variation with CO_2 or species response to CO_2. Percentage values in the text below show the proportional change in trait value±SE upon a 50% reduction in growth CO_2 concentration (eq. 1). For each trait, species could respond to CO_2 (adjust their trait value), show consistent variation in trait value between species over a CO_2 gradient (species intercept or elevation of species line in trait vs CO_2 plot) and show significant variation in how species responded to CO_2 (CO_2*species interaction) **(Table 2.1)**. A non-significant interaction of

species*CO_2 for a given trait indicates that different species adjust the trait by the same proportional amount.

Table 2.1: Overview of ANCOVA results on log(trait data) vs log(CO_2) 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) vs log(CO_2) including SE. -50% CO_2 gives the proportional change in trait given a 50% reduction in CO_2 concentration as per *Trait change = CO_2 change$^\beta$-1* where β is the slope. Values are calculated by slope±SE.

Trait	#Spec.	#Stud.	Slope	-50% CO_2	p(CO_2)	p(spec.)	p(CO_2*spec.)
WUE (mmol mol^{-1})	26	8	0.95 ± (0.1)	-48.3% ± (3.5)	***	***	***
A_{net} (µmol m^{-2} s^{-1})	25	15	0.7 ± (0.11)	-38.3% ± (4.5)	***	***	***
DW (g)	25	14	0.91 ± (0.16)	-46.9% ± (5.8)	***	***	ns
SLA (m^2 g^{-1})	22	17	-0.23 ± (0.08)	+17.2% ± (6.4)	***	***	ns
g_s (mol m^{-2} s^{-1})	17	11	-0.68 ± (0.13)	+59.8% ± (13.9)	**	***	**
A_{max} (µmol m^{-2} s^{-1})	15	9	0.58 ± (0.09)	-33.1% ± (4.3)	*	***	ns
% Leaf N (g g^{-1})	10	9	-0.24 ± (0.1)	+17.8% ± (8)	***	***	ns
PNUE (µmol mmol N^{-1} s^{-1})	10	2	0.22 ± (0.36)	-14.2% ± (21.6)	†	*	ns
r/s ratio	9	3	0.34 ± (0.11)	-21% ± (6.1)	***	***	**
Shoot DW (g)	7	4	0.62 ± (0.14)	-35.1% ± (6.5)	***	***	ns
% Leaf mass	5	4	-0.12 ± (0.2)	+9% ± (15)	*	***	***
Root DW (g)	5	4	1.34 ± (0.27)	-60.6% ± (7.5)	***	***	ns
Leaf DW (g)	5	4	0.83 ± (0.54)	-43.9% ± (21.5)	***	***	**
C_i/C_a	4	4	-0.08 ± (0.12)	+6% ± (8.8)	ns	ns	ns
Chlorophyll (µmol g^{-1})	4	4	0.19 ± (0.09)	-12.4% ± (5.4)	ns	***	ns
Leaf N (g m^{-2})	4	4	0.23 ± (0.11)	-14.8% ± (6.3)	*	ns	ns
% Stem mass	4	3	0.08 ± (0.08)	-5.4% ± (5)	ns	*	ns
Stem DW (g)	4	3	1.72 ± (0.83)	-69.7% ± (18.5)	***	***	***
# Stomata	4	2	0.08 ± (0.11)	-5.7% ± (7.3)	ns	***	ns
RuBisCO (g m^{-2})	3	3	0.37 ± (0.14)	-22.7% ± (7.3)	ns	**	ns
Pore size (µm)	3	1	0.12 ± (0.13)	-7.8% ± (8.2)	ns	***	ns

p values are ns: not significant, †: <0.1, *:<0.05,**:<0.01,***:<0.001.

Photosynthesis-related traits

Across the species studied, a 50% reduction in CO_2 did on average reduce maximum photosynthesis (A_{max}, µmol m^{-2} s^{-1}) by 33±4% (p<0.05, 15 species) (**Fig. 2.1a**) and net photosynthesis (A_{net}, µmol m^{-2} s^{-1}) comparably by 38±5% (p<0.001, 25 species) (**Fig. 2.1b**). Next to this, stomatal conductance (g_s, mol m^{-2} s^{-1}) increased by 60±14% (p<0.01, 17 species) (**Fig. 2.1c**). The ratio of water loss to carbon gain, intrinsic water use efficiency (A_{net} over g_s, WUE), decreased by 48±4% (p<0.001, 26 species) (**Fig. 2.1d**). For A_{net}, WUE and g_s species showed significant variation in trait elevation (p<0.001) and response to CO_2 (interaction, p<0.01). A_{max} however showed only significant variation in species trait elevation (p<0.001).
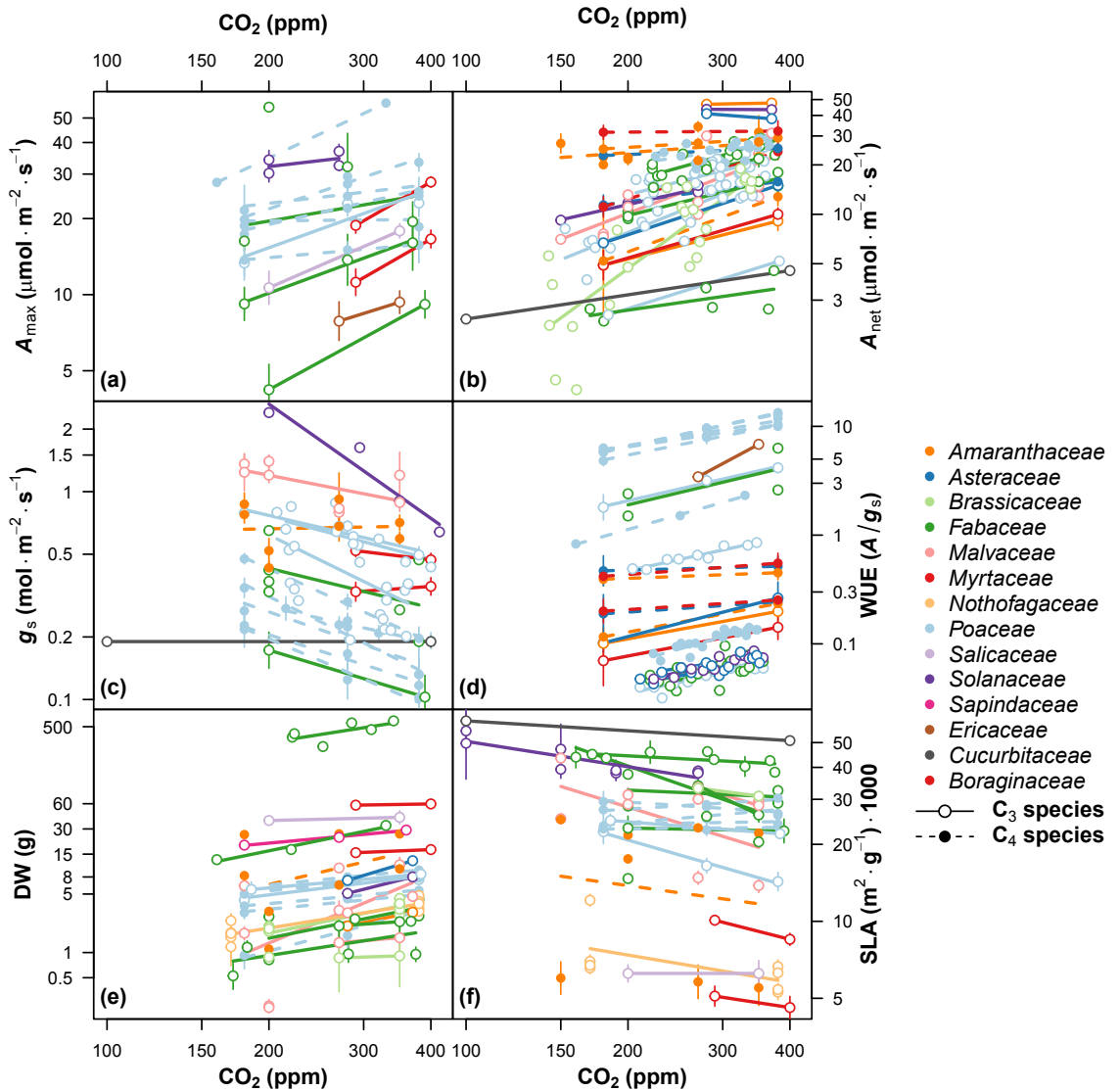
Figure 2.1 Plant trait vs growth CO_2 concentration (note the double-log scale) of the 6 traits with the highest number of species. **(a)** Maximum photosynthesis (A_{max}), **(b)** Net photosynthesis (A_{net}) **(c)** Stomatal conductance (g_s) **(d)** Intrinsic water use efficiency (net photosynthesis over g_s, WUE), **(e)** Plant dry weight. **(f)** Specific leaf area (SLA). Each line represents the response of a single species. Open symbols: C_3 metabolism, solid symbols: C_4 metabolism. Error bars give SE. Different colours represent the different families the species belong to.

Growth and allocation

A halving of growth CO_2 concentration resulted in a corresponding reduction of plant dry weight (DW) by $47\pm6\%$ ($p<0.001$, 25 species) (**Fig. 2.1e**). Aboveground biomass was less reduced than belowground biomass as Shoot DW was reduced by $35\pm7\%$ ($p<0.01$, 7 species) and Root DW by $61\pm8\%$ ($p<0.001$, 5 species). This pattern was reflected in a reduced root/shoot dry matter ratio (r/s ratio) of $21\pm6\%$ ($p<0.001$, 9 species). In contrast, specific

leaf area (SLA, $m^2 g^{-1}$) increased by $17\pm6\%$ ($p<0.001$, 22 species) (**Fig. 2.1f**) at reduced CO_2. For root/shoot ratio there was both significant variation in species trait elevation ($p<0.001$) and response to CO_2 ($p<0.01$). For SLA and plant biomass species showed significant variation in trait elevation but did not respond differently to reduced CO_2.

Chemical composition

Only few data on chemical composition were available, strongly limiting statistical power of our test. The concentration of chlorophyll ($\mu mol\ g^{-1}$, 4 species) and amount of RuBisCO ($g\ m^{-2}$, 3 species) were not significantly affected by CO_2. Nitrogen levels in the leaf showed a contrasting response to halving CO_2 where leaf nitrogen percent ($g\ g^{-1}$) increased by 18 ± 8 ($p<0.001$, 10 species) whereas nitrogen content per area ($g\ m^{-2}$) decreased by $15\pm6\%$ ($p<0.05$, 4 species). Neither for nitrogen per unit mass nor per unit leaf area did species have significantly different responses to reduced CO_2.

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 C_3, C_4 and woody and herbaceous species. Nevertheless, interesting contrasts and similarities emerged. Figure **2.2** shows the contrasting slopes between plant types for the 8 plant traits with 9 or more species. Between C_3 and C_4 herbs only the greater increase in SLA for C_3 herbs at reduced CO_2 was significant ($p<0.01$). C_4 plants showed on average a negligible SLA response to CO_2. Net photosynthesis and dry weight seemed to be less reduced for C_4 herbs though 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 C_3 plants ($p<0.05$). Overall for the traits shown in figure **2.2** different plant types appear to show rather similar responses to reduced CO_2.

Table 2.2 Comparison of trait shift at high CO_2 extrapolated from low CO_2 response (bold values) to actual changes found in three meta-analyses. If the trait adjustments are proportional from past low to future high CO_2, the predictions from the low CO_2 experiments should match the measured values from the high CO_2 experiments. The measured trait shifts are from chamber studies, GC1: Curtis & Wang 1998 (700ppm), GC2: Poorter & Navas 2003 (690ppm), GC 3: Wang *et al.* 2012 (700ppm) and FACE experiments, FACE: Ainsworth & Long 2005, Ainsworth & Rogers 2007 (560ppm). Percentage values indicate magnitude of trait shift as compared to current levels of CO_2.

	GC1 (700ppm)		GC2 (690ppm)		GC3 (700ppm)		FACE (560ppm)	
Trait	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) +6% (C_4)	**+48% ± (6)**
DW	+28%	**+88%** ± **(21)**	+48% (C_3) +12% (C_4)	**+86% ± (20)**	+25% (C_3) -3% (C_4)	**+88%** ± **(21)**		
SLA			-13%	**-15% ± (5)**	-10%	**-15% ± (5)**	-6%	**-9% ± (3)**

Comparison with elevated CO₂ experiments

Table **2.2** highlights the comparison between trait responses at low CO_2 to the response at elevated CO_2 of the six traits that had the most data. When comparing the trait shifts found in five large meta-analyses (Curtis & Wang 1998, Poorter & Navas 2003, Ainsworth & Long 2005, Ainsworth & Rogers 2007, Wang *et al.* 2012) to trait shifts extrapolated from the response to low CO_2 a few interesting contrasts and similarities emerged. With increasing CO_2 the magnitude of shift in A_{net} deviated more from the response to low CO_2, 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 C_3 and C_4 plants in water use efficiency at low CO_2, at high CO_2 large differences are found. The increase in WUE at high CO_2 found for C_3 plants at FACE sites is comparable to our extrapolated response. Whole plant dry weight (DW) appears to increase much less at high CO_2 then expected based on the low CO_2 response. SLA, however, seems to be adjusted in a similar magnitude as expected from the response to low CO_2.
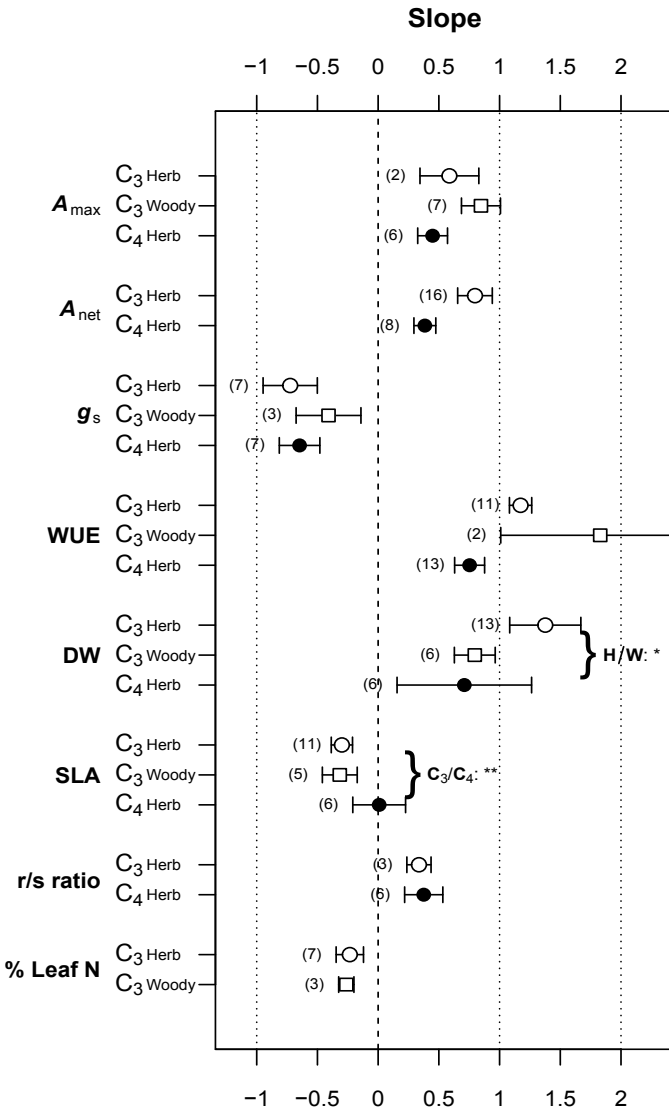
Figure 2.2 Slope of log(trait) vs log(CO_2 concentration) for different plant types, C_3/C_4 and woody/herbaceous. Open circles, C_3 herbaceous type; solid circles, C_4 herbaceous type; open square, C_3 woody type. ±1 indicates a 1:1 change in a trait for a change in CO_2 concentration with a negative slope indicating an increase in trait value and a positive slope indicating a decrease in trait value. A_{max}, maximum photosynthesis (μmol m⁻² s⁻¹); A_{net}, net photosynthesis (μmol m⁻² s⁻¹); g_s, stomatal conductance (mol m⁻² s⁻¹); WUE, water use efficiency (mmol mol⁻¹); DW, plant dry weight (g); SLA, specific leaf area (m² g⁻¹); r/s ratio, root DW to shoot DW (g g⁻¹). Numbers between brackets gives the number of species for each plant type. *:p<0.05, **p<0.01

Discussion

This meta-analysis seeks to quantify and aggregate current knowledge on plant traits in low CO_2. Few traits were measured for many species and data was found for a limited number, 45, of species (**Table 2.1**). Due to the limited number of species and trait measurements comparison between plant types, woody-herbaceous, C_3-C_4, was difficult. This limited data-set should be taken into account when reviewing the results. Interesting results do however emerge. In response to reduced CO_2 plants adjusted both physiological and morphological traits (**Fig. 2.1, Table 2.1**). The magnitude of trait adjustment varied among species for 6 out of 20 traits examined (**Table 2.1**). This suggests that on order to cope with low CO_2 different species adjust different traits. This species-level heterogeneity in response though was not clearly based on simple functional groups (**Fig. 2.2**).

Below we discuss trait shifts at low CO_2 moving from leaf to ecosystem scales, keeping in mind that all the results discussed below are all short-time scale, plastic responses (Gerhart & Ward 2010). Plant plastic response to changing conditions occurs at different levels of organization after different periods of time (Nicotra *et al.* 2010).

Leaf-level responses to low CO_2

We found support for our hypotheses at the leaf level. We hypothesized that low CO_2 would lead to lower photosynthetic rates (*A*) which leads to reduced growth and that in order to partially ameliorate the photosynthetic rate reduction, plants would have higher leaf nitrogen and larger stomatal conductance (g_s) (Medlyn *et al.* 2011, Sage & Reid 1992). Focussing first on A and g_s, A_{net} substantially decreased at a 50% decrease in CO_2 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 CO_2 (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 CO_2 was found to be similar between C_3 (*Abutilon theophrasti*) and C_4 (*Amaranthus retroflexus*) plants at low CO_2, due to less leaf loss and stomatal closure than expected for C_3 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, gs and WUE at high CO_2 to the responses we found at low CO_2 a contrasting picture emerges. At low CO_2 no difference in WUE was found between plant types, though at high CO_2 C_4 plants increase their WUE less than C_3 plants (Poorter & Navas 2003). For C_3 plants the response to high CO_2 seems similar in magnitude as to low CO_2 for WUE. For the components of WUE, at high CO_2 the adjustment of A_{net} was greater in magnitude as to low CO_2 whereas the effect on g_s was comparable to low CO_2, though one meta-analysis reported a far lower decrease in g_s at high CO_2 (**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 CO_2 atmosphere at the Triassic-Jurassic boundary (McElwain *et*

al. 1999).

At the level of enzymes and leaf chemical composition, we found no evidence that RuBis-CO content and chlorophyll content were affected by reduced CO_2, 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 CO_2; this contrasting response might be explained by the higher specific leaf area (SLA) in low CO_2. 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 CO_2.

Plants adjust their leaf morphology in order to cope with low concentrations of atmospheric CO_2. At half of ambient CO_2 leaf SLA increased by 15%. This increase has two potential advantages to the plant: first, when CO_2 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 CO_2 can maintain internal CO_2 concentration up to a point, at low CO_2 concentrations the diffusion of CO_2 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 CO_2 (Loreto *et al.* 1992). However, a higher SLA could also be a result of less starch or less other non-structural carbohydrate present in the leaf at low CO_2 concentrations (Poorter *et al.* 2009). It's interesting to find that at high CO_2 SLA follows the same trend as at low CO_2. The relative contributions of the above factors to reducing and increasing SLA are an interesting avenue to pursue further.

Plant-level responses to low CO_2

While plants adjust their gas exchange and leaf morphology in response to reduced CO_2, 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% CO_2 reduction which is more pronounced in below ground 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 & Cerling 1995, Franks *et al.* 2013, Gerhart *et al.* 2012). At low CO_2 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 CO_2 may have important implications for species interactions, particularly in tree-savanna grass interactions (Bond & Midgley 2012): at low CO_2 the regrowth capacity following disturbance of tree species is much diminished, adding weight to the importance of fire and herbivory as ecosystem shaping factors (Bond & Midgley 2012, Kgope *et al.* 2010). Next to allocation, the increased SLA at low CO_2 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 CO_2 response

Differences between C_3, C_4, woody and herbaceous species were not as pronounced as hypothesized. Results suggested differences between plant types though the responses of only few traits were significantly different. C_3 herbs had a significantly greater increase in SLA than C_4 herbs, which on average showed no response. Decreases in biomass diminished starting from herbaceous C_3, woody C_3 to herbaceous C_4. However, only the difference between woody and herbaceous C_3 was significant with woody plants having a smaller reduction in biomass at low CO_2. This result mirrors that at high CO_2 experiments where increased CO_2 generally leads to a greater Relative Growth Rate (RGR) increase for fast growing, herbaceous, plants than slow growing, woody, plants (Poorter & Navas 2003). As fast growers "win" more at high CO_2 they "lose" more at low CO_2. While the smaller decrease in biomass for herbaceous C_4 than for C_3 is not significant, it should be noted that the average reduction in A_{net} and A_{max} is also lower than in C_3 herbs. This may point to a smaller reduction in biomass accumulation for C_4 herbs via a smaller reduction in photosynthesis. With more data on woody and C_4 species such differences between plant types and their underlying mechanism may become more apparent.

The results presented here summarize the effects of low CO_2 at high water and high nutrients. One important caveat to consider, however, is that that there are many potential interactions between CO_2, water, and nutrients as is shown by some studies. Low nutrients in the form of low P limited photosynthetic rates even further at low CO_2 in *Lupinus albus* (Cambell *et al.* 2006) and *Populus deltoides* (Lewis *et al.* 2010). C_3 (*Abutilon theophrasti*) and C_4 (*Amaranthus retroflexus*) plants recovered similarly from drought at low CO_2 (Ward *et al.* 1999). This shows that other environmental factors strongly influence the effect of CO_2 on plant traits. However, most data was available for well-watered, high-nutrient growth experiments. The interactions between CO_2, 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 paleo-materials. In the past CO_2 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 CO_2 and it is therefore important to determine if the traits of modern plants grown under low CO_2 compare to the traits of plants that lived in a low CO_2 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 CO_2 and how similar they are. It is also likely that in the period since the low CO_2 in the Last Glacial Maximum (LGM), species with short generation turnover have evolved more compared to long generation turnover species, though the drastic increase from 280 to 700pmm 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 CO_2 levels must include both evolutionary adaptation and plastic responses.

Conclusion

In conclusion, we found that, despite the more limited set of low CO_2 studies compared to high CO_2 studies, a general response is emerging. Plant response to reduced atmospheric CO_2 involves a complex suite of trait adjustments. In order to diminish the effects of reduced CO_2 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 CO_2 as compared to high CO_2 were proportionally similar for g_s, WUE and SLA but responses at low CO_2 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 CO_2 are proportional and opposite. Carbon gain and whole plant growth rate are more complex—responses to low CO_2 in these cases are more extreme. At high CO_2 other factors such as nutrient and light availability could control these traits. To understand the response of plants to future high CO_2 it is important to understand how and when other factors become drivers for certain traits. Our understanding of plant response to CO_2 benefits from data from both low and high CO_2 conditions. The shape of that response will become increasingly relevant in a high CO_2 future.