

Research article

Increases in CO₂ from past low to future high levels result in “slower” strategies on the leaf economic spectrum

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ABSTRACT

Depending on resource availability plants exhibit a specific suite of traits. At the interspecific level these traits follow the leaf economic spectrum (LES), traits related to slow turnover when resources are poor and fast turnover when resources are plentiful. Limited data shows that within species, CO₂ availability, low in the recent geologic past, high in the near future, has led to plants shifting their trait levels on the LES towards faster traits. We asked whether adjustments of physiological traits could underpin faster growth from low to high CO₂ and how these responses varied among plant functional types. We analysed the trait response of seedlings of up to 28 C3 plant species grown at low (160 ppm), near-ambient (450 ppm), and high (750 ppm) CO₂. We measured growth, specific leaf area (SLA), leaf gas exchange, chemical composition and stomatal traits. On average photosynthesis was reduced by 59% at low CO₂ and increased by 14% at high CO₂ compared to ambient CO₂. Respiration decreased by 21% at low CO₂ and increased by 39% at high CO₂. Nitrogen content (N) per mass increased by 50% at low CO₂ and decreased by 9% at high CO₂. Plants drastically increased SLA at low CO₂ so that despite lower carbon gain per area, carbon gain per unit mass was not reduced as much. Contrary to the responses to other resources, plant traits along the LES are adjusted towards the “fast” end of the spectrum (higher SLA, higher N) at low CO₂ and towards the “slow” end (lower SLA, lower N) with increasing CO₂. For a limited number of species photosynthesis per unit mass showed the same, increase at low CO₂. From a resource economics perspective plants thus adjust the cost for growth towards the availability of carbon and the rate of assimilation: at lower CO₂ the carbon costs decrease due to decreased respiration and lower leaf mass per area (higher SLA thinner leaves). At higher CO₂ the carbon costs increase due to increased respiration and higher leaf mass per area (lower SLA thicker leaves). This suggests that CO₂ increases from the past to the future are allowing plant species globally to combine faster growth with more robust, resource conservative leaves.

1. Introduction

When plant growth is limited by the availability of one resource, economic theory dictates that in successful individuals the capacity to acquire other more plentiful resources should be limited; this is because it does not pay to have excess capacity in one area when resources in another cannot keep up (Bloom and Mooney, 1985; Chapin et al., 1987). Thus, the Leaf Economic Spectrum (LES) predicts that habitats of poor resource availability host plant species that exhibit a concerted predictable suite of traits that can be linked to slow matter cycling (slow leaf turnover, low nutrient content, thick tough leaves of low specific leaf area (SLA), slow growth), while habitats where resources are plentiful host species linked to fast cycling (high leaf turnover, high

nutrient content, thin “throw away” leaves of high SLA, fast growth) (Lambers and Poorter, 1992; Wright et al., 2004; Reich, 2014; Niinemets, 2015).

The availability of carbon, as an important growth-limiting resource, has greatly increased from a Pleistocene low (~180 ppm CO₂) (Hönisch et al., 2009) to current levels (~400 ppm) and will increase even more moving to high levels (possibly even >> 800 ppm) towards the end of this century (Ciais et al., 2013). From a carbon centric point of view growth rate of plants is dependent on plant morphology related to carbon uptake and plant physiology related to carbon processing (Evans, 1972; Lambers and Poorter, 1992). Up to date, different studies have assessed how plants adjust traits to carbon availability (Gerhart and Ward, 2010; Temme et al., 2013, others), as well as how the

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direction of these adjustments relates to variation in growth rate (Temme et al., 2015).

With current global change featuring rising CO₂ and increasing temperatures, most research in this field has focused on plants' response to high CO₂ (Gerhart and Ward, 2010; Temme et al., 2013). However, the effects of high CO₂ also the effects of low CO₂, as common for the past 10 Ma before the Industrial Revolution, should be considered in order to obtain a full picture of plants' responsiveness to CO₂. Plant physiology is strongly adjusted by CO₂ concentrations from transient to evolutionary time-scales (Medlyn and McMurtrie, 2005) with potential legacy effects of evolution in a low CO₂ atmosphere (Sage and Coleman, 2001; Zhu et al., 2004). Experiments have shown that plant trait responses to low CO₂ are far greater in magnitude than those to high CO₂ (Hattenschwiler and Korner, 2000; Quirk et al., 2013; Temme et al., 2013, 2015). However, unlike for other resources it has been found that, among plant species, there does not appear to be a trade-off in traits relating to carbon acquisition from Pleistocene low to future high CO₂ concentration, i.e. no species appears particularly suited to either low or high CO₂ (Temme et al., 2015). This is likely caused by carbon availability in open vegetation varying only little in space and only slowly and gradually in time until the Industrial Revolution (Hönisch et al., 2009). Carbon dioxide can be a selective agent on plant functioning, as indicated for instance by the repeated evolutionary appearance of C₄ plants with carbon-concentrating mechanisms for photosynthesis (Edwards et al., 2010; Christin and Osborne, 2014) as well as by the dominance of C₃ plants in the Holocene (Huang et al., 2006). However, we do not know whether the very rapid increase in CO₂ concentration since pre-industrial low levels and into the near future could show a similar trait CO₂ responsiveness to those in the Holocene (Sage and Coleman, 2001; Franks et al., 2013).

In a multispecies study on plant responses to a range from low to high CO₂ we confirmed that among species, at ambient CO₂ conditions, high SLA is linked with fast growth (Temme et al., 2015), as in previous studies (Lambers and Poorter, 1992; Cornelissen et al., 1996; Reich, 2014). However, contrary to what one would expect within species, low (past) carbon availability resulted in a “faster” morphology (high SLA) compared to that at ambient CO₂ but was at the same time associated with slower growth. Correspondingly, higher (future) carbon availability resulted in a “slower” morphology (lower SLA) but also faster growth than at ambient CO₂. Together, these responses go so far as to result in a decoupling of growth rate response from morphology response from low to high CO₂ (Temme et al., 2015). However, how CO₂ from past low to future high affects physiological traits (gas exchange traits, stomatal traits, respiration, and chemical composition) and how leaf morphological and those physiological traits along the LES combine to affect growth performance of plants at a range of low to high CO₂ has remained an open question so far (Medlyn and McMurtrie, 2005; Smith et al., 2012; Sack et al., 2013).

Experiments manipulating atmospheric CO₂ levels have shown a consistent adjustment in physiological traits within species. Low CO₂ strongly reduces photosynthetic rates and increases stomatal conductance and transpiration (Gerhart and Ward, 2010; Temme et al., 2013) whereas high CO₂ increases photosynthetic rate and decreases stomatal conductance and transpiration to a far lesser extent (Ainsworth and Rogers, 2007; Norby and Zak, 2011). At low and high CO₂, relative to ambient CO₂, plants' leaf area ratio (leaf area per plant mass, LAR) is strongly adjusted, following the response of one of its components, SLA (Temme et al., 2013, 2015). Plants drastically increase LAR at low CO₂ and decrease it at high CO₂. Under unchanged unit leaf rate (plant biomass production rate per unit leaf area, ULR) this would be expected to lead to faster growth at low CO₂ and slower growth at high CO₂, as relative growth rate (RGR) is the product of LAR and ULR (Lambers and Poorter, 1992). However, in reality quite the opposite happens because ULR, which is related to photosynthetic rates, is itself also affected by CO₂ availability (Poorter and Navas, 2003). Thus CO₂ starvation leads to reduced growth (Temme et al., 2015) and

excess CO₂ enhances growth (Poorter and Navas, 2003; Ainsworth and Rogers, 2007; Norby and Zak, 2011). This would imply stronger shifts in ULR in response to CO₂ that are only partially compensated for by altered leaf morphology and/or allocation (SLA, LAR).

Gas exchange and chemical composition are the key traits underlying biomass production per unit leaf rate (Evans and Poorter, 2001; Poorter et al., 2014). Important in plants' physiological response to CO₂ are the stomata as the entry point of CO₂ into the leaf. Over past geological cycles of shifts in CO₂, stomata have been relatively small and numerous during periods of low CO₂ (promoting high maximum stomatal conductance) and large but low in density during periods of high CO₂ (low maximum stomatal conductance) (Woodward, 1987; Franks and Beerling, 2009; de Boer et al., 2012). A higher stomatal conductance allows for greater carbon uptake at low CO₂ levels whereas at high CO₂ a developmentally lower maximum stomatal conductance allows for greater fine-tuning of stomatal conductance (Drake et al., 2013).

Besides by gas exchange leaf chemical composition is strongly altered by CO₂, with greater nitrogen content at low CO₂ (Temme et al., 2013). This may reflect greater investment into RuBisCO in order to facilitate the drawdown of CO₂ in photosynthesis via a stronger diffusion gradient. On the other hand the increase of nitrogen at low CO₂ might also be due to luxury consumption of nitrogen by small carbon-starved plants (Chapin, 1980). At higher CO₂, generally, nitrogen content is reduced (Ainsworth and Rogers, 2007). This reduction could be due to increased carbohydrate accumulation and/or redistribution of nitrogen towards other growth limiting steps (Díaz et al., 1993). Alternatively, closure of stomata to reduce transpiration at higher CO₂ limits the water flux through the roots, thereby possibly reducing N uptake (Taub and Wang, 2008). A comprehensive understanding of the linkages between gas exchange, stomatal traits, chemical composition and growth performance across the range from low to high CO₂ is still missing however, especially because empirical data on plant responses to low CO₂ are still scarce (Gerhart and Ward, 2010; Franks et al., 2013; Temme et al., 2013).

How the concerted trait shifts of individual species in response to CO₂ levels affects community composition requires broad generalizations. Scaling up individual species' responses to ecosystems is generally done by classifying species by functional type (Chapin et al., 1996). Whether and how different plant functional types (PFT), notably grasses, forbs and woody species, respond to CO₂ will provide a basis for (back-)predicting potential shifts in community composition from the low CO₂ past to the high CO₂ near future (Prentice and Harrison, 2009). Importantly in this context, dynamic global vegetation models that are coupled to global circulation models to predict biogeochemical cycling and climate at different CO₂ regimes, use similar functional types and traits (Sitch et al., 2008; Verheijen et al., 2015a,b). In relation to carbon availability different PFTs show different responses in traits to low CO₂ (Temme et al., 2013, 2015) versus high CO₂ (Poorter and Navas, 2003; Ainsworth and Rogers, 2007; Norby and Zak, 2011) but, again, information on physiological responses to low CO₂ is still minimal and based on a very small number of species (Temme et al., 2013).

With humanity increasingly altering the carbon cycle at an unprecedented rate through fossil fuel emissions and land use change, understanding plants' response to future conditions becomes increasingly important. Given plants' >>> 10Ma year evolution in a low CO₂ atmosphere, understanding how plants function at low CO₂ could shed light on how plants will respond to future high CO₂ environments. We therefore asked ourselves the following questions:

- How are leaf physiological traits related to plant carbon uptake affected by CO₂ from past low to future high levels and are there consistent differences in the response among plant functional types?
- How do the changes in leaf morphological traits in response to low versus high CO₂ serve to underpin the changes in plant physiology

in order to maintain sufficient levels of carbon uptake?

- As there is an apparent decoupling of plant morphology from growth rate across the range from low to high CO₂, is there a similar decoupling for plant physiological traits or does a greater investment in photosynthetic machinery still lead to higher growth rates regardless of CO₂?

We sought to answer these questions by studying the physiological response of 28 temperate C3 plant species to low and high CO₂, and its effect on plant growth using an experimental approach. This study complements a previous study we had conducted on the effect of CO₂ on plant morphology and its effect on growth (Temme et al., 2015).

2. Methods

Plants of up to 28 different species (Appendix Table A.1 in Supplementary material), ranging broadly in phylogeny and functional type, which consisted of 6 woody species (2 trees, 4 shrubs), 16 forb species, and 6 grasses were grown at 160 ppm (low; range 150–180 ppm), 450 ppm (near-ambient) and 750 ppm (high) CO₂ at 70% relative humidity and a 19–22 °C day-night with a 10 h photoperiod at ~350 μmol PAR. Total daily photon flux was comparable to that of an average March day in the Netherlands, which is when several of the species would have naturally germinated and started to grow (Temme et al., 2015). Seeds of these species were germinated on wet filter paper and shortly after germination transplanted to 400 ml pots filled with coarse sand, watered daily and given 50 ml of Hoagland's solutions every two days (as described in Temme et al., 2015).

Growth measurements were carried out as described in Temme et al. (2015). In brief, a representative subsample of individuals was harvested for biomass measurement after the expansion of the first leaf. Three weeks later the remaining individuals were harvested and plant biomass and RGR determined according to Hoffmann and Poorter (2002). Replication varied between 4 and 10 (mean 7). SLA was determined from a representative leaf of each plant at final harvest. To determine nitrogen and carbon content all leaves were separated from stems after harvest and oven-dried and ground in a ball mill (Retch MM200, Hahn, Germany). Three to four μg of leaf powder was then analysed for C&N content using a dry combustion element analyser (Carlo Erba NA1500, Rodana, Italy).

Photosynthesis was measured within the last 2 days prior to final harvest and from at least one hour after “daylight”. One fully developed leaf per individual (4 individuals per species/treatment) was placed in a LiCor 6400 infrared gas analyser and allowed to acclimate to the cuvette, set similar to growth conditions, for two minutes in the light after which three measurements were taken and averaged to take into account any error introduced by the analyser. Subsequently the red-blue light source (LI-6400-02B) was turned off and the leaf was allowed to acclimate for three minutes in the dark after which three measurements were taken again to measure respiration. The area inside the leaf cuvette was marked and removed at final harvest. When leaves did not fill the total cuvette area (6 cm²) the portion of the leaf that could be placed inside the cuvette was scanned using a Cannon LiDe 110 scanner. Leaf area was then measured using ImageJ v1.47. Net area-based photosynthesis (A_{net}) and respiration rates (R) and stomatal conductance (g_s) were then calculated by using the correct area in the gas analyser equations.

On a subset of 8 species stomatal sizes and densities were measured on a nail polish imprint taken from a single typical full-grown leaf per individual (4 individuals per species per CO₂ treatment) prior to harvest. Clear nail polish was applied directly on the leaf and allowed to dry for 45 min. After drying nail polish films were removed by sticking on clear tape and peeling from the leaf. Nail polish films of abaxial and adaxial leaf sides were mounted on a glass slide and an image was taken using CellSens Entry v1.7.1 via an Olympus SC30 camera attached to an Olympus CX41 microscope at 10× optical magnification. Per leaf side

up to five (fewer when quality of nail polish film was low, generally not so) 640 μm by 320 μm (204800 μm²) viewing frames were saved at a resolution of 0.1024 μm² per pixel. Numbers of epidermal cells and stomata were subsequently counted using Adobe Photoshop CS5.5 and ImageJ v1.47 using the CellCounter plugin. Occasionally poor image quality prevented counting of the full frame and a smaller area of good quality was counted. Per leaf side 15 stomata were measured for stomatal pore size, guard cell length and guard cell width. This was measured live using CellSens Entry v1.7.1 at a resolution of 80 nm per pixel length (0.0064 μm² pixel⁻¹) at 40× optical magnification. Stomatal size traits (pore size, guardcell width/length) as well as densities of all frames were averaged per individual plant. Using stomatal density, maximum pore aperture and guard cell width as a proxy for pore depth we calculated maximum stomatal conductance (Parlange and Waggoner, 1970; Dow et al., 2014).

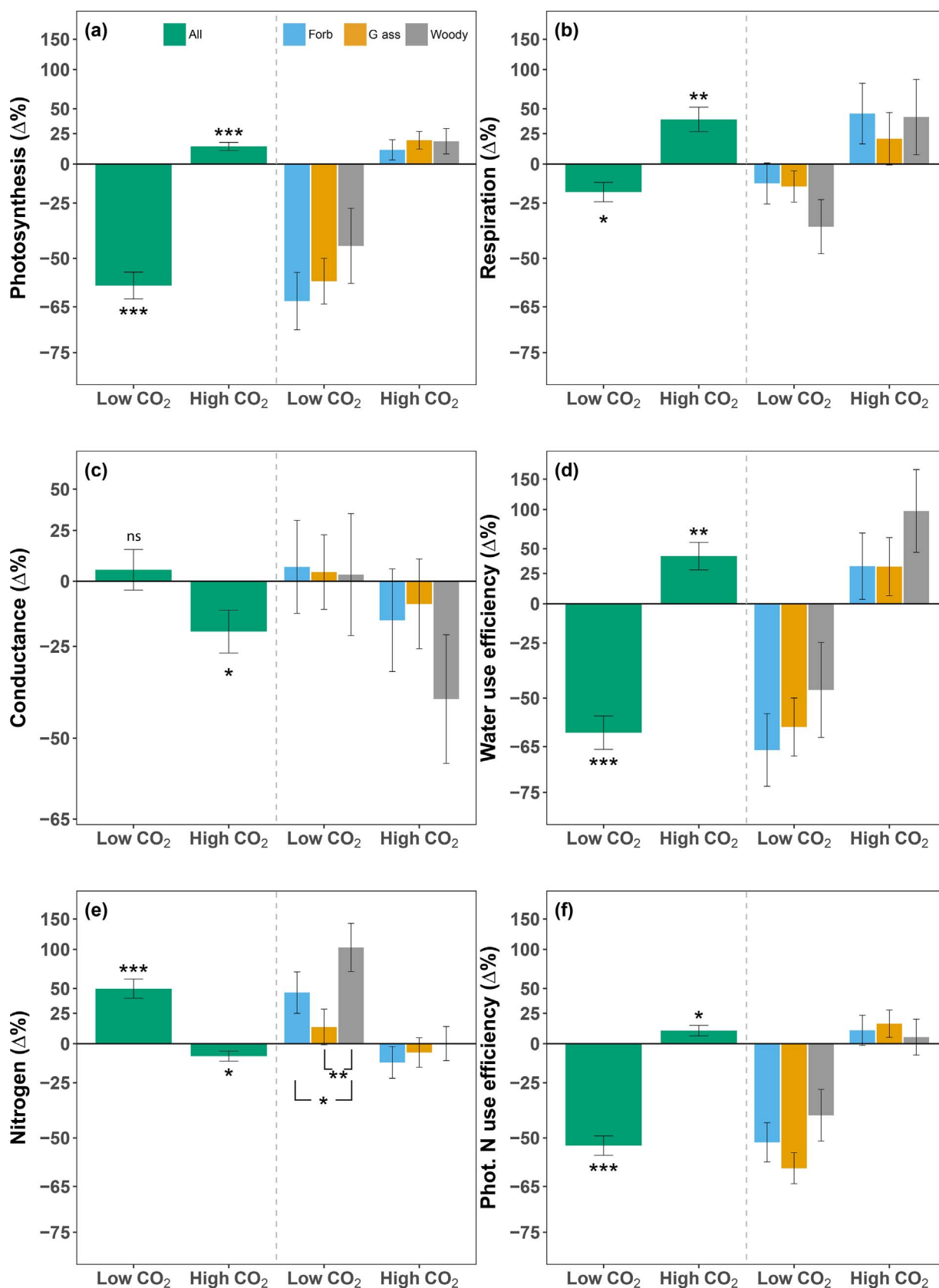
Statistics were carried out using R version 3.4.0 running in RStudio version 1.0.143. As in Temme et al. (2015), CO₂ effects on traits and species differences in traits were tested by comparing the shift in trait value to the trait value at ambient CO₂. To improve normality and minimise skew, trait values were natural log (ln) transformed prior to analysis. The difference in ln-transformed trait level from ln-transformed ambient level was then the relative shift in trait level via $e^{\ln\text{transformed difference}} - 1$. This approach had the added benefit that a halving or a doubling in trait value from ambient had the same ln-transformed difference. Rather than fit a slope of trait value to CO₂ level we tested whether the relative shifts in trait levels were significant and whether plant functional types (PFT) responded differently. Per CO₂ shift (ambient to low and ambient to high) we tested the relative shift in trait level via a linear mixed model approach using the package LME4 v1.1-13 with PFT, as a fixed factor and species as a random effect. Using model selection we tested for a significant CO₂ effect (significant intercept) and for a difference between PFT. Post-hoc, PFTs were contrasted using a Tukey test with Holm-Bonferroni correction. We used standard major axis (SMA) regression in R using the package SMATR v3.4 to test the relationship between shifts in SLA and shifts in photosynthesis and respiration per unit mass. In addition we also used SMA regression to test if the interspecific relationship between relative growth rate and nitrogen content changed with CO₂ concentration.

3. Results

The plants generally responded strongly to the CO₂ treatments with all species having lower photosynthetic rates per area at low CO₂ and higher at high CO₂ (when compared to ambient CO₂), combined with strong adjustments in nitrogen content per unit mass and photosynthetic nitrogen use efficiency. Leaf size occasionally precluded measurements of gas exchange when leaves did not fit the LiCor 6400 cuvette. Nevertheless we could measure gas exchange for 17 species and nitrogen content for the full species set (see Appendix Table A.1 for details in Supplementary material)

3.1. Physiological traits and CO₂

CO₂ concentration strongly influenced photosynthesis and respiration rates. Plant photosynthesis per area at growth conditions (A_{net} , μmol m⁻² s⁻¹) was strongly affected by CO₂ (Fig. 1a) with species at low CO₂ on average having a 59% ± 4 (17 species, $p \ll 0.001$) lower A_{net} compared to that at ambient CO₂. Plant functional types (PFTs) did not respond significantly differently (possibly due to small sample size). Per type the reduction for forb species was 64% ± 8 (9 species) followed by grasses (58% ± 7; 5 species) and woody species 45% ± 15; 3 species). At high CO₂ A_{net} was stimulated far less than it was reduced at low CO₂ with species on average having increased A_{net} by 14% ± 3 (16 species, $p \ll 0.001$). Again different PFTs did not respond differently. Respiration per unit leaf area (r_{net} , μmol m⁻² s⁻¹) followed a similar pattern to A_{net} (Fig. 1b), although the reduction at low CO₂ was



(caption on next page)

Fig. 1. Relative shifts in trait values at low or high CO₂ compared to ambient CO₂ for forb, grass and woody species. Bars indicate percentage shift in trait value at low CO₂ (160 ppm) and high (750 ppm) CO₂ compared to trait value at ambient (450 ppm) CO₂. Axes are natural log transformed so that the size of the bars at a 50% decrease or a 100% increase is the same (reflecting a factor 2 adjustment). Green bars: all species, blue bars: forb species, orange bars: grass species, grey bars: woody species. Error bars give standard error of the mean. *s near error bars indicates significant difference from zero. For nitrogen content at low CO₂ there was a significant difference (after Bonferroni correction) between grass and woody species. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. **a.** Photosynthetic rate at growth conditions (A_{net} , $\mu\text{mol m}^{-2} \text{s}^{-1}$). **b.** Respiration at growth conditions (r_{net} , $\mu\text{mol m}^{-2} \text{s}^{-1}$). **c.** Stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$), note the different axis scaling. **d.** Intrinsic water use efficiency, A_{net}/g_s (iWUE, $\text{mmol H}_2\text{O } \mu\text{mol CO}_2^{-1}$). **e.** Nitrogen content, (N_g , g N g leaf^{-1}). **f.** Photosynthetic nitrogen use efficiency (PNUE, $\mu\text{mol CO}_2 \mu\text{mol N}^{-1} \text{s}^{-1}$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

less strong ($19\% \pm 6$; 15 species, $p < 0.05$). At high CO₂ r_{net} was increased by $39\% \pm 12$ (15 species, $p < 0.01$). Again, likely due to small sample sizes and large spread we **did not find** significant difference between PFTs.

Surprisingly stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) was not affected by low compared to ambient CO₂ (Fig. 1c). Across species there was a varied response with some species having higher g_s and others with lower g_s (Appendix Fig. A.1 in Supplementary material), resulting in average g_s remaining the same across species. At high CO₂ g_s was significantly lower across all species, ($20\% \pm 8$; 16 species, $p < 0.05$). The combined effect of CO₂ on A_{net} and g_s resulted in a shift in intrinsic water use efficiency, A_{net} over g_s (iWUE, $\mu\text{mol CO}_2 \text{mmol H}_2\text{O}^{-1} \text{s}^{-1}$), which at low CO₂ closely followed the effect on A_{net} at low CO₂ but exceeded the effect on A_{net} at high CO₂ (Fig. 1d). iWUE was reduced at low CO₂ by $61\% \pm 5$ (17 species, $p < 0.001$) across species and increased by $42\% \pm 14$ (16 species, $p < 0.01$) at high CO₂. This suggests increased water demand proportional to decreased photosynthesis at low CO₂ but a greater than proportional decrease in water demand at high CO₂ due to the combined effect of CO₂ increasing A_{net} and decreasing g_s .

Stomatal traits were surprisingly unresponsive to CO₂. In a subset of 8C₃ forbs from the total species set we found that species showed a clear scaling relation from a limited number of large stomata to a large number of small stomata (Fig. 2a). However, the effect of CO₂ on this scaling relation was very limited. Guard cell size was largely unresponsive to CO₂ with only minor variation with growth CO₂ and no consistent direction across species (Fig. 2b). Stomatal density of both leaf sides taken together (only *Geranium* and *Clinopodium* were hypostomatous) was also largely unresponsive to CO₂ (Fig. 2c). However, there was a slight trend of lower stomatal densities at low CO₂. When viewed separately the abaxial leaf side stomatal density was significantly lower ($19\% \pm 7$; 8 species, $p < 0.05$) at low CO₂ across all 8 species. Changes in epidermal cell density (mm^{-1}), pore size, guard cell width (μm) and maximum stomatal conductance calculated from stomatal density and stomatal pore traits varied inconsistently with CO₂ across species (Appendix Fig. A.2 in Supplementary material). At low CO₂ however there was a correlation between maximum stomatal conductance and actual measured stomatal conductance (Appendix Fig. A.3 in Supplementary material) suggesting pores were more constrained.

Plant leaf nitrogen content per unit mass (N_g , g N g leaf^{-1}) increased sharply at low (as compared to ambient) CO₂ (Fig. 1e) with species having on average a $50\% \pm 11$ (25 species, $p < 0.001$) higher N_g . This response was different among PFTs, with woody plants showing a significantly larger increase than grasses and forbs at $103\% \pm 36$ (6 species, $p < 0.01$), although with large spread among species. At high CO₂ species on average had a $9\% \pm 4$ reduction in N_g (20 species, $p < 0.05$) with no significant difference between PFTs. Photosynthetic nitrogen use efficiency (PNUE, $\mu\text{mol CO}_2 \mu\text{mol N}^{-1} \text{s}^{-1}$) followed the shifts in A_{net} with shifts in CO₂ (Fig. 1f) and was reduced by $53\% \pm 3$ (17 species, $p < 0.001$) at low CO₂ and increased by $10\% \pm 4$ (10 species, $p < 0.05$) at high CO₂.

3.2. Morphological traits complement physiology

Standard major axis regression revealed how species shifts in SLA influenced their photosynthetic carbon gain and respiratory carbon loss

per unit mass (Fig. 3). At low CO₂ plant photosynthetic rate per area was reduced (Fig. 1a). However, many plant species concurrently drastically increased their SLA (see Temme et al., 2015). Thus, with a greater area capable of carbon uptake per unit mass invested in leaf tissue, photosynthetic rate per unit mass (A_{mass} $\mu\text{mol g}^{-1} \text{s}^{-1}$) was reduced by $35\% \pm 8$ (17 species, $p < 0.01$) (Appendix Fig. A.4 in Supplementary material), less than per area photosynthesis, which across all species was reduced by $59\% \pm 4$ (Fig. 1a). Furthermore, there was a clear relationship between the extent of SLA increase and the reduction in A_{mass} (Fig. 3a). Species that had a greater increase in their SLA had a smaller reduction in A_{mass} ($r^2 = 0.54$, $p < 0.001$) even going so far as to have a higher A_{mass} than at ambient for the forb *Atropa belladonna*. While plants increased their SLA at low CO₂, thus incorporating less mass per area, the thinning of the leaf did not appear to come at the expense of energy demanding leaf tissue as there was an almost proportional increase in dark respiration ($r^2 = 0.81$, $p < 0.001$) with increasing SLA (Fig. 3c). Energy demanding leaf tissue appeared to have remained stable whereas less demanding tissue was reduced as evidenced by the large increase in SLA and only slight decrease in respiration per area (Fig. 1b).

At high CO₂ plant photosynthesis per area was increased (Fig. 1a). However, SLA was decreased for most species which resulted in no net change in A_{mass} across all species at high CO₂, though there was strong variation between species (Appendix Fig. A.4 in Supplementary material). The relationship between shifts in SLA and A_{mass} at high CO₂ was such that species that had a greater reduction in SLA (thicker or denser leaves) also had a greater reduction in A_{mass} ($r^2 = 0.52$, $p < 0.01$) (Fig. 3b). However, across species increased carbon availability appears to offset the relationship in such a manner that plants can afford to decrease their SLA while maintaining a similar A_{mass} as at ambient CO₂ (Fig. 3b). Interestingly the decrease in SLA at higher CO₂ appears to not be the result of increased energy demanding tissue as there is no clear relationship between decreases in SLA and respiration per unit mass (Fig. 3d).

3.3. Physiological traits and growth

Nitrogen content per unit mass remained positively coupled to RGR at all three CO₂ levels (Fig. 4). When analysed together using SMA regression we found no significant difference in the slope of the relationship between nitrogen content (N_g , g N g leaf^{-1}) and relative growth rate (RGR, $\text{g g}^{-1} \text{day}^{-1}$) with different CO₂ levels. Rather the intercept of the relationship was significantly altered by CO₂ ($p < 0.001$). Pairwise comparison among CO₂ treatments showed that the elevation at ambient and high CO₂ were not significantly different but that the elevation at low CO₂ was significantly lower than at ambient and high CO₂ ($p < 0.001$). Thus, regardless of CO₂ concentration species interspecific variation in growth rate was related to variation in nitrogen content. CO₂ starvation offset this relationship across species reducing growth rate at all nitrogen levels and requiring a greater investment in nitrogen to maintain growth rates.

4. Discussion

In this study we sought to understand how plants' physiological traits are affected by CO₂ availability going from being carbon starved at a level representative of the Pleistocene past to plentiful carbon

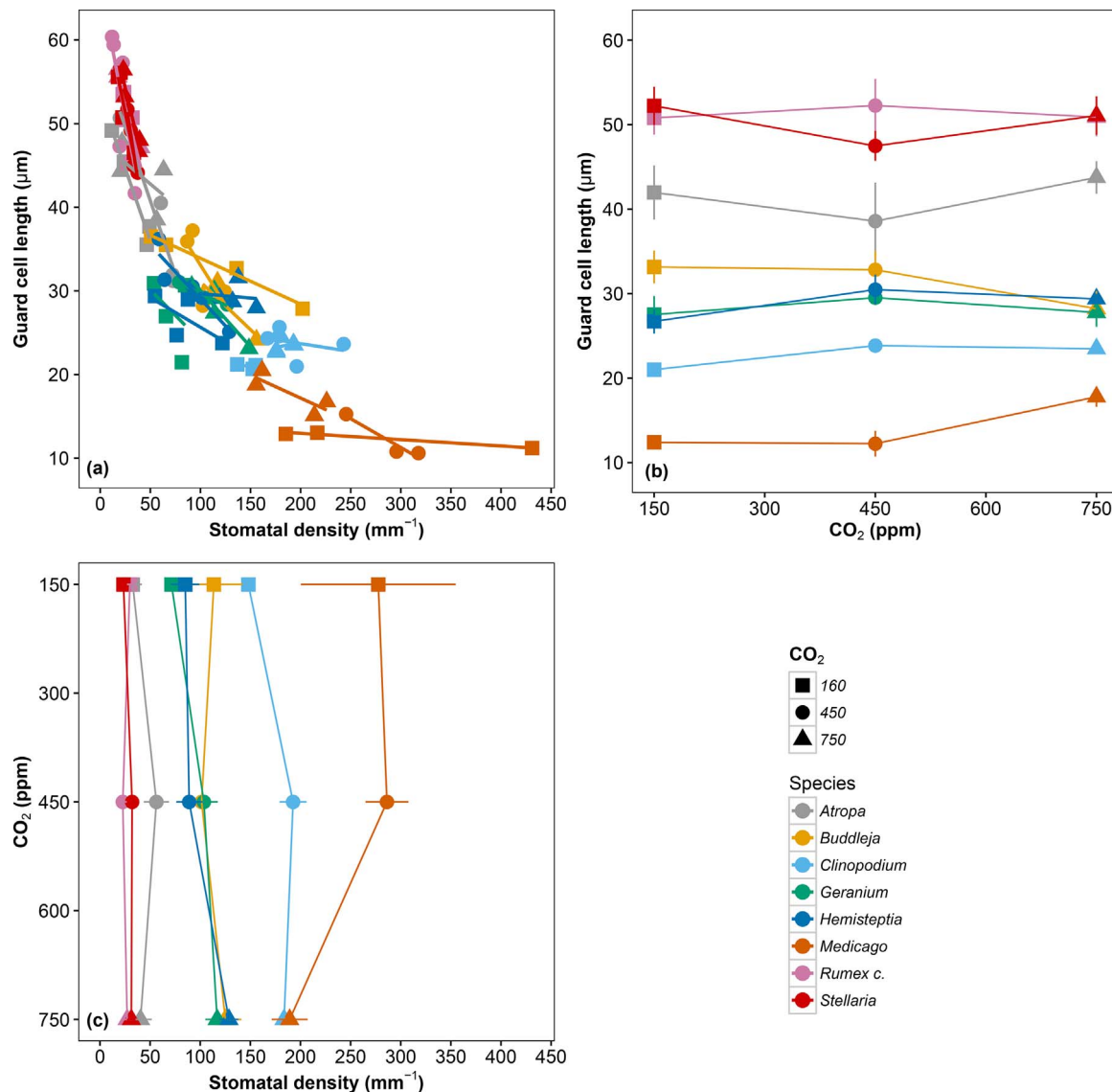


Fig. 2. Effect of CO₂ on the relationship between stomatal guard cell size and stomatal density for a subset of 8 C3 forbs. (a) Average stomatal density (mm⁻¹) of adaxial and abaxial leaf sides and length of abaxial stomatal guard cells (μm) in relation to growth at low (160 ppm) ambient (450 ppm) and high (750 ppm) CO₂ of 8C3 forbs. Each point represents the average density of 10 counting frames and 30 stomata of a single leaf (5/15 abaxial and adaxial) of an individual. Colours represent different species with symbols representing CO₂ treatment. Squares: Low CO₂, circles: ambient CO₂, triangles: High CO₂. (b) Species average (n = 3–4) guard cell length at low, ambient and high CO₂. (c) Species average (n = 3–4) stomatal density at low, ambient and high CO₂. Note panels (b) and (c) unfold panel (a) to describe change of stomatal density and guard cell size with CO₂.

(750 ppm) representative of the end of the present century. In a previous study we found that plants' morphological traits were greatly affected by CO₂ but that this variation was decoupled from interspecific differences in growth rate (Temme et al., 2015). In contrast, we found in this study that interspecific variation in growth rate at past, present and future CO₂ can be explained satisfactorily by responses of plant physiological traits. The great adjustment we found in morphological traits such as specific leaf area serves to offset changes in photosynthesis due to carbon availability by reducing the carbon cost for growth at low CO₂ while increased photosynthesis at higher CO₂ allows for more robust (lower SLA) leaves at high CO₂. Our multispecies findings, together with previous studies on small species numbers, demonstrate that, going from past low CO₂ to future high CO₂ plants *generally* reduce both their specific leaf area and their leaf nitrogen content. This strongly suggests that increasing CO₂ has allowed plant species to grow faster while at the same time adopting a more resource conservative ("slow") strategy on the leaf economic spectrum, as expressed by lower nitrogen content, higher SLA and lower photosynthesis per unit mass.

4.1. Plant physiological traits and CO₂

Plant physiological traits were strongly affected by CO₂. Across 17 species we found that low CO₂ decreased photosynthesis and respiration rates but stomatal conductance remained surprisingly unchanged. High CO₂ increased photosynthesis and respiration rates and, contrasting to low CO₂, stomatal conductance was reduced, albeit modestly. Intrinsic water use efficiency thus followed the response of photosynthesis and respiration being lower at low CO₂ and higher at high CO₂. Combined with results from previous experiments showing that specific leaf area is greatly increased at low CO₂ (i.e. thinner or less dense leaves) and reduced at high CO₂ (thicker or denser leaves) (Temme et al., 2015), the results for respiration and iWUE provide an interesting picture. At low CO₂, while growing less, the carbon cost for growth is reduced (lower respiration, more leaf area per unit mass) next to a greater water cost (lower iWUE). In high CO₂, while growing more, the carbon cost for growth is increased (greater respiration, less leaf area per unit mass) yet the water cost is reduced (higher iWUE). Plant nitrogen content per mass was greatly increased at low CO₂ and

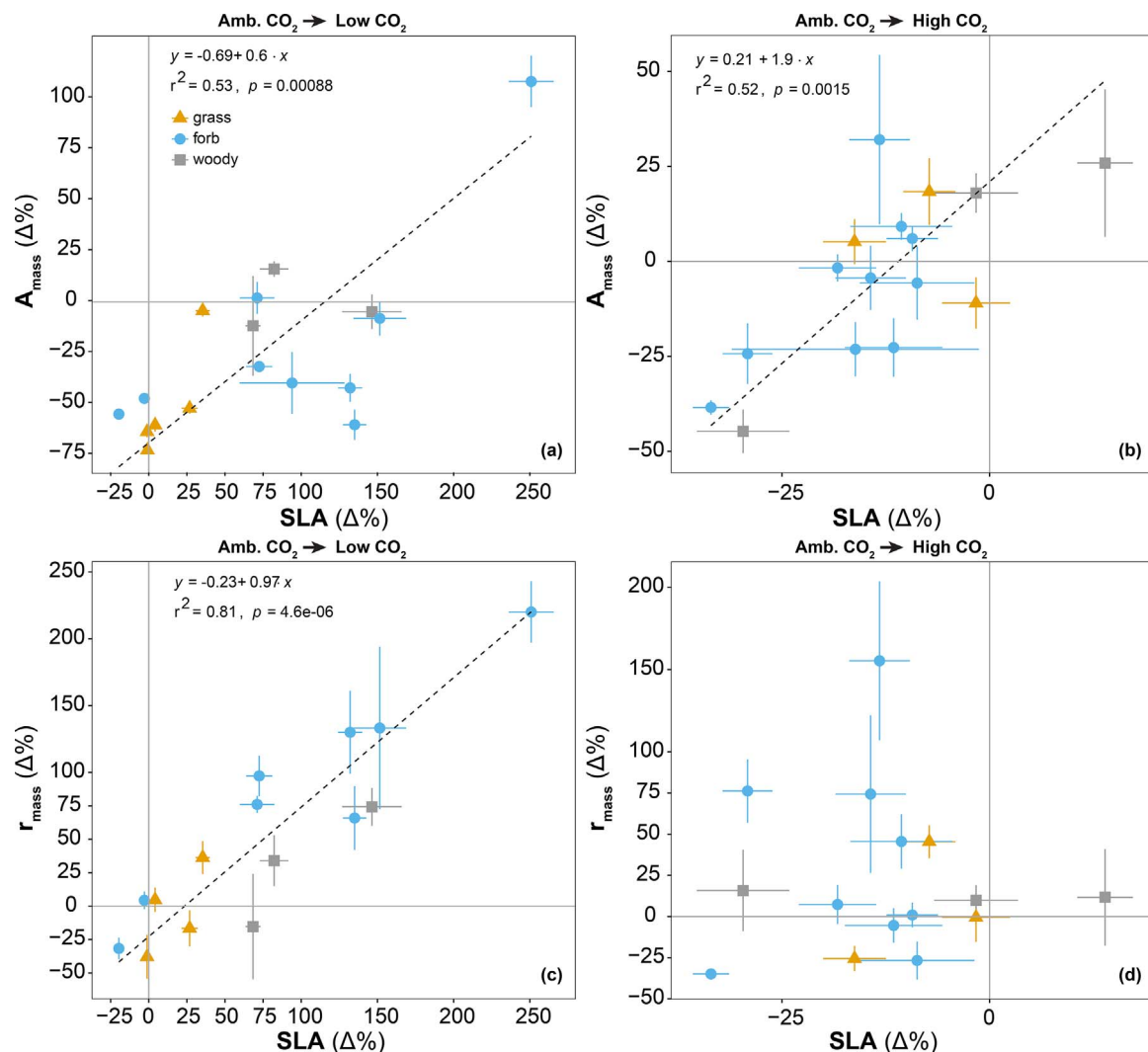


Fig. 3. Shifts in mass-based photosynthesis (A_{mass}) and respiration (r_{mass}) versus shifts in SLA. Left panels indicate shifts between ambient (450 ppm) CO₂ and low (150–180 ppm) CO₂, right panels shifts between ambient and high CO₂. Each point represents a species with bars denoting SE, orange triangles: grasses, blue circles: forbs, grey squares: woody species. Dashed line is the standard major axis regression of shift in specific leaf area (SLA, m² leaf g⁻¹) versus photosynthetic rate and dark respiration per leaf mass (μmol g⁻¹ s⁻¹). Dark grey lines indicate 0% change in trait. (SLA data from Temme et al., 2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reduced at high CO₂. In combination with changes in photosynthesis this resulted in photosynthetic nitrogen use efficiency moving in tandem with CO₂ availability. It should be noted however that nitrogen content per area was not affected by CO₂ (Appendix Fig. A.5 in Supplementary material).

Interestingly and contrary to expectations from our meta-analysis (Temme et al., 2013), we found no change in stomatal conductance (g_s) from ambient to low CO₂. Also at the short time scale of our experiment, i.e. within a single generation, species showed no plasticity in stomatal size and density. As the species measured for stomatal traits were relatively fast growing forbs, it is more likely they would respond plastically by regulating stomatal aperture than developmentally by changing stomatal frequency to CO₂ (Haworth et al., 2013). Only in variable environments (e.g. drought-prone), where fine control of stomata is necessary, might there be stronger selection on stomatal size and densities resulting in lower numbers of bigger stomata (Raven, 2014).

The striking lack of responsiveness of stomata to our CO₂ treatments points to limitations in generalizing our data to past conditions due to differences in adaptive (i.e. evolutionary) versus plastic response to CO₂. As we grew plants from seed and harvested them in the seedling stage, only plastic responses to CO₂ were measured. We did not allow

our plant species the time to adjust evolutionarily to CO₂ over multiple generations. Generally there is a clear relationship between CO₂ and number and size of stomata (Woodward, 1987; Royer, 2001). Already over a comparatively short 150 year period a decreasing number of stomata were found across most species in a Florida bog (Lammertsma et al., 2011). Contrastingly conifer needles from packrat middens gathered during the last glacial maximum showed no strong increase in stomatal density but did show a large increase in nitrogen content as we found as well (Becklin et al., 2014). We know from the very few available studies that CO₂ stress can act as a selective agent in *Arabidopsis* (Ward et al., 2000) and *Acer rubrum* (Mohan et al., 2004). The extent and rate at which plants adapt to CO₂ as a selective agent, and how this relates to phenotypic responsiveness to CO₂ in the present, is an area warranting in-depth research (Medlyn and McMurtrie, 2005).

4.2. Morphological leaf traits and changes in physiology

The unchanged nitrogen content per area with a higher SLA suggests that the same amount of protein, including RuBisCO, is now embedded in less leaf mass. Consequently, while photosynthesis per area is reduced, photosynthetic gain per unit mass is less affected, thereby somewhat counteracting strong reductions in photosynthetic

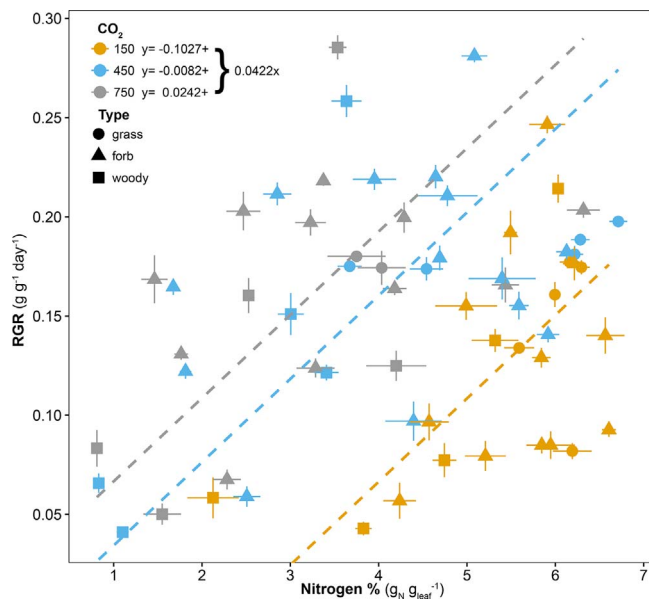


Fig. 4. Relationship between leaf nitrogen content (g N g leaf^{-1}) and relative growth rate (RGR) at past low (160 ppm), current ambient (450 ppm) and future high (750 ppm) CO_2 . Points indicate species mean RGR and N content with SE with different symbols noting different types and different colours for the different CO_2 treatments; circles: grass species, triangles: forb species, squares: woody species. Lines represent the standard major axis regression of nitrogen% and RGR, which showed no significant difference in slope but did show significant difference in elevation.

rate. Thus the return on invested C in leaf is diminished less for species that increase their SLA more. However, from a mechanical and defensive point of view there are substantial costs involved in having such a high SLA leaf: they should be mechanically weak, highly palatable and prone to wilting (Poorter et al., 2009). Increasing CO_2 leads to increased photosynthetic rates which thus allows for a reduction in SLA with the additional benefit of a mechanically stronger and less palatable leaf with a longer life span. We recommend further study to directly test for leaf toughness, palatability and lifespan (Pérez-Harguindeguy et al., 2003) of leaves of different species grown at CO_2 levels ranging from low to high.

For herbivores this nitrogen dilution with increasing CO_2 may have had a large effect going from glacial to present levels (Cowling, 2001) and may have an effect with CO_2 increasing in the future (Bazzaz, 1990). Per gram of foliage consumed (at least for herbs and woody species) in a low CO_2 atmosphere a lot more nitrogen is consumed. Thus, while leaf quantity may have been lower due to reduced growth in low CO_2 (Temme et al., 2015), food quality may have been substantially higher than from leaves at ambient CO_2 . Moreover, a changed C/N ratio could show a shift in the allocation of resources to secondary metabolites for defence. Altogether, as CO_2 increases from past to future levels it appears food quality decreases but food quantity increases.

4.3. CO_2 , the leaf economic spectrum and relative growth rate

The Leaf Economic Spectrum (LES) is a set of coordinated traits along the slow-fast growth axis. In deep shade species with slow traits that conserve energy (low respiratory losses and slow leaf turnover) are successful (Baltzer and Thomas, 2007; Lusk et al., 2008). In drought-prone habitats species that conserve water and have relatively large root systems are successful (Poorter et al., 2012). Similarly in environments of low nutrient availability species adopt slow strategies (Grime and Hunt, 1975; Aerts and Chapin, 2000). Our multi-species experiment has shown that, unlike other resources, CO_2 causes traits (SLA, N concentration, A_{mass}) to move in the opposite direction within given species, pushing plants towards the resource acquisitive (“fast”) side at resource-poor conditions in terms of CO_2 and towards the

resource-conservative (“slow”) side at resource-rich conditions (i.e. higher CO_2).

Thus CO_2 serves to provide a negative feedback to the commonly observed positive relationships of the LES with RGR (Lambers and Poorter, 1992; Cornelissen et al., 1997; Reich, 2014). The question remains whether the envelope of trait levels is moved in its entirety or whether CO_2 adjusts only the boundaries of viable strategies. The mechanical limits precluding further increases in SLA at low CO_2 (Milla and Reich, 2007) seem to suggest that at low CO_2 species’ possible range of trait values is constrained. Moreover, the results of this study show that the relationship between N content and RGR has a common slope shifted in elevation (higher RGR and lower N) due to CO_2 increase. Thus the increase in photosynthetic rate due to higher carbon availability makes even more resource-conservative traits in terms of nutrient and water economy a viable possibility.

If increases in CO_2 do lead to slower strategies, has this led and will this lead to changing inter- and intraspecific competition? Individual plants can survive at poorer conditions with “slower” traits. As increased CO_2 make slower traits a viable strategy, are specialists already exhibiting slow traits now crowded out by generalists encroaching on their range? Are the specialists now capable of expanding to even poorer conditions due to even slower traits? Thus while the ranking of species for traits and RGR remains broadly the same regardless of CO_2 (Albert et al., 2011; Temme et al., 2015), the winners and losers at the edges of a resource niche could shift (Alberto et al., 2013) due to changes in economically viable leaf strategies. However, changing climate due to changes in CO_2 should also be taken into account. Climatic models based on increased temperature and shifts in precipitation have suggested that tropical forest communities will move towards more resource acquisitive traits. (Chain-Guadarrama et al., 2017; Dubuis et al., 2013). Thus shifts in community composition will be the resultant of the effect of changing climate and changing atmospheric composition. Shifts in community composition could lead to changes in the carbon cycling in a broad area. With carbon storage in soils and plants becoming increasingly important for climate change models (McMahon et al., 2011) understanding the impact of plant trait adjustments due to CO_2 increase on community composition becomes increasingly important.

5. Conclusion

CO_2 has strong effects on plant physiology with photosynthesis and respiration closely following CO_2 availability. Across all 28 species measured we found a concerted response to CO_2 where plants adjust their leaf morphology by thinning the leaf and incorporating less mass per area but keeping the nitrogen content per area constant. From a resource economics perspective plants thus adjust the cost for growth towards the availability of carbon and the rate of assimilation: lower respiration at low CO_2 and a lower carbon cost to grow a unit leaf area. At higher CO_2 the carbon costs increase due to increased respiration and higher leaf mass per area. Increasing CO_2 from past low to future high serves to make leaves more robust along the leaf economic spectrum across all species. CO_2 starvation, which has been common for the past 10 million years, forces plants to adopt more resource-acquisitive trait levels at high nutrients and water conditions. The question how plant response to CO_2 interacts with other limiting conditions is an area warranting further research.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ppees.2017.11.003>.

References

- Aerts, R., Chapin, F.S., 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns (ed. D Fitter, AH and Raffaelli). *Adv. Ecol. Res.* 30, 1–67.
- Ainsworth, E.A., Rogers, A., 2007. The response of photosynthesis and stomatal conductance to rising $[CO_2]$: mechanisms and environmental interactions. *Plant Cell Environ.* 30, 258–270.
- Albert, C.H., Grassein, F., Schurr, F.M., Vieilledent, G., Violle, C., 2011. When and how should intraspecific variability be considered in trait-based plant ecology? *Perspect. Plant Ecol. Evol. Syst.* 13, 217–225.
- Alberto, F.J., Aitken, S.N., Alfà, R., González-Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., Savolainen, O., 2013. Potential for evolutionary responses to climate change – evidence from tree populations. *Global Change Biol.* 19, 1645–1661.
- Baltzer, J.L., Thomas, S.C., 2007. Determinants of whole-plant light requirements in Bornean rain forest tree saplings. *J. Ecol.* 95, 1208–1221.
- Bazzaz, F., 1990. The response of natural ecosystems to the rising global CO_2 levels. *Annu. Rev. Ecol. Syst.* 21, 167–196.
- Becklin, K.M., Medeiros, J.S., Sale, K.R., Ward, J.K., 2014. Evolutionary history underlies plant physiological responses to global change since the last glacial maximum. *Ecol. Lett.* 17, 691–699.
- Bloom, A.J., Mooney, H.A., 1985. Resource limitation in plants – an economic analogy. *Ann. Rev. Ecol. Syst.* 16, 363–392.
- Chain-Guadarrama, A., Imbach, P., Vilchez-Mendoza, S., Vierling, L.A., Finegan, B., 2017. Potential trajectories of old-growth Neotropical forest functional composition under climate change. *Ecography* 40, 001–014.
- Chapin, F.S., Bloom, A.J., Field, C.B., Waring, R.H., 1987. Plant responses to multiple environmental factors. *Bioscience* 37, 49–57.
- Chapin, F.S., Bret-Harte, M.S., Hobbie, S.E., Zhong, H., 1996. Plant functional types as predictors of transient responses of arctic vegetation to global change. *J. Veg. Sci.* 7, 347–358.
- Chapin III, S.F., 1980. The mineral nutrition of wild plants. *Annu. Rev. Ecol. Syst.* 11, 233–260.
- Christin, P.-A., Osborne, C.P., 2014. The evolutionary ecology of C 4 plants. *New Phytol.* 204, 765–781.
- Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Le Quéré, C., Myneni, R.B., Piao, S., Thornton, P., 2013. Carbon and Other Biogeochemical Cycles. *Climate Change 2013: The Physical Science Basis*. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Cornelissen, J.H.C., Castro Diez, P., Hunt, R., 1996. Seedling growth, allocation and leaf attributes in a wide range of woody plant species and types. *J. Ecol.* 84, 755–765.
- Cornelissen, J.H.C., Werger, M.J.A., Castro-Díez, P., Van Rheeën, J.W.A., Rowland, A.P., 1997. Foliar nutrients in relation to growth, allocation and leaf traits in seedlings of a wide range of woody plant species and types. *Oecologia* 111, 460–469.
- Cowling, S.A., 2001. Plant carbon balance, evolutionary innovation and extinction in land plants. *Global Change Biol.* 7, 231–239.
- de Boer, H.J., Eppinga, M.B., Wassen, M.J., Dekker, S.C., 2012. A critical transition in leaf evolution facilitated the Cretaceous angiosperm revolution. *Nat. Commun.* 3, 1221.
- Díaz, S., Grime, J.P., Harris, J., McPherson, E., 1993. Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature* 364, 616–617.
- Dow, G.J., Bergmann, D.C., Berry, J., 2014. An integrated model of stomatal development and leaf physiology. *New Phytol.* 201, 1218–1226.
- Drake, P.L., Froend, R.H., Franks, P.J., 2013. Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. *J. Exp. Bot.* 64, 495–505.
- Dubuis, A., et al., 2013. Predicting current and future spatial community patterns of plant functional traits. *Ecography* 36, 1158–1168.
- Edwards, E.J., Osborne, C.P., Strömberg, C.A.E., Smith, S., Bond, W.J., Christin, P.-A., Cousins, A.B., Duvall, M.R., Fox, D.L., Freckleton, R.P., Ghannoum, O., Hartwell, J., Huang, Y., Janis, C.M., Keeley, J.E., Kellogg, E., Knapp, A.K., Leakey, A.K., Nelson, A.D.B., Saarela, D.M., Sage, J.M., Sala, R.F., Salamin, O.E., Still, C.J., Tipler, B., 2010. The origins of C4 grasslands: integrating evolutionary and ecosystem science. *Science* 328, 587–591.
- Evans, G.C., 1972. *The Quantitative Analysis of Plant Growth*. Blackwell Scientific, Oxford.
- Evans, J.R., Poorter, H., 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell Environ.* 24, 755–767.
- Franks, P.J., Beerling, D.J., 2009. Maximum leaf conductance driven by CO_2 effects on stomatal size and density over geologic time. *Proc. Natl. Acad. Sci. U. S. A.* 106, 10343–10347.
- Franks, P.J., Adams, M., Amthor, J.S., Barbour, M.M., Berry, J., Ellsworth, D.S., Farquhar, G.S., Ghannoum, O., Lloyd, J., McDowell, N., Norby, R.J., Tissue, D.T., von Caemmerer, S., 2013. Sensitivity of plants to changing atmospheric CO_2 concentration: from the geological past to the next century. *New phytol.* 197, 1077–1094.
- Gerhart, L.M., Ward, J.K., 2010. Plant responses to low $[CO_2]$ of the past. *New phytol.* 188, 674–695.
- Grime, J.P., Hunt, R., 1975. Relative growth-rate: its range and adaptive significance in a local flora. *J. Ecol.* 63, 393.
- Hönisch, B., Hemming, N.G., Archer, D., Siddall, M., McManus, J.F., 2009. Atmospheric carbon dioxide concentration across the mid-Pleistocene transition. *Science* 324, 1551–1554.
- Hattenschwiler, S., Körner, C., 2000. Tree seedling responses to in situ CO_2 -enrichment differ among species and depend on understorey light availability. *Global Change Biol.* 6, 213–226.
- Haworth, M., Elliott-Kingston, C., McElwain, J.C., 2013. Co-ordination of physiological and morphological responses of stomata to elevated $[CO_2]$ in vascular plants. *Oecologia* 171, 71–82.
- Hoffmann, W.A., Poorter, H., 2002. Avoiding bias in calculations of relative growth rate. *Ann. Bot.* 80, 37–42.
- Huang, Y., Shuman, B., Wang, Y., Webb, T., Grimm, E.C., Jacobson, G.L., 2006. Climatic and environmental controls on the variation of C3 and C4 plant abundances in central Florida for the past 62,000 years. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 237, 428–435.
- Lambers, H., Poorter, H., 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Adv. Ecol. Res.* 23, 187–261.
- Lammertsma, E.I., de Boer, H.J., Dekker, S.C., Dilcher, D.L., Lotter, A.F., Wagner-Cremer, F., 2011. Global CO_2 rise leads to reduced maximum stomatal conductance in Florida vegetation. *Proc. Natl. Acad. Sci. U. S. A.* 108, 4035–4040.
- Lusk, C.H., Reich, P.B., Montgomery, R.A., Ackerly, D.D., Cavender-Bares, J., 2008. Why are evergreen leaves so contrary about shade? *Trends Ecol. Evol.* 23, 299–303.
- McMahon, S.M., Harrison, S.P., Armbruster, W.S., Bartlein, P.J., Beale, C.M., Edwards, M.E., Kattge, J., Midgley, G., Morin, X., Prentice, I.C., 2011. Improving assessment and modelling of climate change impacts on global terrestrial biodiversity. *Trends Ecol. Evol.* 26, 249–259.
- Medlyn, B.E., McMurtrie, R.E., 2005. Effects of CO_2 on plants at different timescales. In: Ehleringer, J.R., Cerling, T.E., Dearing, M.D. (Eds.), *A History of Atmospheric CO_2 and Its Effects on Plants, Animals, and Ecosystems*, pp. 441–467.
- Milla, R., Reich, P.B., 2007. The scaling of leaf area and mass: the cost of light interception increases with leaf size. *Proc. R. Soc. B: Biol. Sci.* 274, 2109–2115.
- Mohan, J., Clark, J., Schlesinger, W., 2004. Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO_2 . *Global Change Biol.* 10, 233–247.
- Niinemets, Ü., 2015. Is there a species spectrum within the world-wide leaf economics spectrum? Major variations in leaf functional traits in the Mediterranean sclerophyll *Quercus ilex*. *New Phytol.* 205, 79–96.
- Norby, R.J., Zak, D.R., 2011. Ecological lessons from free-air CO_2 enrichment (FACE) experiments. *Annu. Rev. Ecol. Syst.* 42, 181–203.
- Pérez-Harguindeguy, N., Díaz, S., Vendramini, F., Cornelissen, J.H.C., Gurvich, D.E., Cabido, M., 2003. Leaf traits and herbivore selection in the field and in cafeteria experiments. *Austral Ecol.* 28, 642–650.
- Parlange, J.Y., Waggoner, P.E., 1970. Stomatal dimensions and resistance to diffusion. *Plant Physiol.* 46, 337–342.
- Poorter, H., Navas, M.-L., 2003. Plant growth and competition at elevated CO_2 : on winners, losers and functional groups. *New Phytol.* 157, 175–198.
- Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J., Villar, R., 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytol.* 182, 565–588.
- Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P., Mommer, L., 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol.* 193, 30–50.
- Poorter, H., Lambers, H., Evans, J.R., 2014. Trait correlation networks: a whole-plant perspective on the recently criticized leaf economic spectrum. *New Phytol.* 201, 378–382.
- Prentice, I.C., Harrison, S.P., 2009. Ecosystem effects of CO_2 concentration: evidence from past climates. *Clim. Past* 5, 297–307.
- Quirk, J., McDowell, N.G., Leake, J.R., Hudson, P.J., Beerling, D.J., 2013. Increased susceptibility to drought-induced mortality in *Sequoia sempervirens* (Cupressaceae) trees under Cenozoic atmospheric carbon dioxide starvation. *Am. J. Bot.* 100, 582–591.
- Raven, J., 2014. Speedy small stomata? *J. Exp. Bot.* 65, 1415–1424.
- Reich, P.B., 2014. The world-wide fast-slow plant economics spectrum: a traits manifesto (ed. H. Cornelissen). *J. Ecol.* 102, 275–301.
- Royer, D.L., 2001. Stomatal density and stomatal index as indicators of paleoatmospheric CO_2 concentration. *Rev. Palaeobot. Palynol.* 114, 1–28.
- Sack, L., Scoffoni, C., John, G.P., Poorter, H., Mason, C.M., Mendez-Alonso, R., Donovan, L., 2013. How do leaf veins influence the worldwide leaf economic spectrum? Review and synthesis. *J. Exp. Bot.* 64, 4053–4080.
- Sage, R.F., Coleman, J.R., 2001. Effects of low atmospheric CO_2 on plants: more than a thing of the past. *Trends Plant Sci.* 6, 18–24.
- Sitch, S., Huntingford, C., Gedney, N., Levy, P.E., Lomas, M., Piao, S.L., Betts, R., Ciais, P., Cox, P., Friedlingstein, P., Jones, C.D., Prentice, I.C., Woodward, F.I., 2008. Evaluation of the terrestrial carbon cycle, future plant geography and climate-carbon cycle feedbacks using five Dynamic Global Vegetation Models (DGVMs). *Global*

- Change Biol. 14, 2015–2039.
- Smith, R.A., Lewis, J.D., Ghannoum, O., Tissue, D.T., 2012. Leaf structural responses to pre-industrial, current and elevated atmospheric [CO₂] and temperature affect leaf function in *Eucalyptus sideroxylon*. *Funct. Plant Biol.* 39, 285.
- Taub, D.R., Wang, X., 2008. Why are nitrogen concentrations in plant tissues lower under elevated CO₂? A critical examination of the hypotheses. *J. Integr. Plant Biol.* 50, 1365–1374.
- Temme, A.A., Cornwell, W.K., Cornelissen, J.H.C., Aerts, R., 2013. Meta-analysis reveals profound responses of plant traits to glacial CO₂ levels. *Ecol. Evol.* 3, 4525–4535.
- Temme, A.A., Liu, J.C., Cornwell, W.K., Cornelissen, J.H.C., Aerts, R., 2015. Winners always win: growth of a wide range of plant species from low to future high CO₂. *Ecol. Evol.* 5, 4949–4961.
- Verheijen, L.M., Aerts, R., Bönisch, G., Kattge, J., van Bodegom, P.M., 2015a. Variation in trait trade-offs allows differentiation among predefined plant functional types: implications for predictive ecology. *New Phytol.* 209, 563–575.
- Verheijen, L.M., Aerts, R., Brovkin, V., Cavender-Bares, J., Cornelissen, J.H.C., Kattge, J., van Bodegom, P.M., 2015b. Inclusion of ecologically based trait variation in plant functional types reduces the projected land carbon sink in an earth system model. *Global Change Biol.* 21, 3074–3086.
- Ward, J.K., Antonovics, J., Thomas, R.B., Strain, B.R., 2000. Is atmospheric CO₂ a selective agent on model C3 annuals? *Oecologia* 123, 330–341.
- Woodward, F.I., 1987. Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels. *Nature* 327, 617–618.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R., 2004. The worldwide leaf economics spectrum. *Nature* 428, 821–827.
- Zhu, X.G., Portis, A.R., Long, S.P., 2004. Would transformation of C3 crop plants with foreign Rubisco increase productivity? A computational analysis extrapolating from kinetic properties to canopy photosynthesis. *Plant Cell Environ.* 155–165.