Plant Traits and Production Rates from CO2 Starvation to Saturation

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Chapter 6

General Discussion

As a primary resource for plants, the availability of CO_2 has profound effects on their performance and this response is known to vary among species. Both CO_2 starvation and elevated CO_2 impact plant growth and its underlying plant traits related to biomass allocation, chemical composition and leaf morphology and physiology. However, while there is ample literature on this for elevated CO_2, the literature on low CO_2 responses is very scarce and based on very few species. There are even fewer studies in the literature that have compared low and high CO_2 responses of the same species. This is where my thesis has made a new and data-rich contribution. In light of the rapid ongoing shift to an elevated CO_2 world by the end of this century, whilst coming from a carbon starved world during the recent Pleistocene glacials, I set out to (1) better understand plant functioning at conditions of the recent geologic past and (2) to improve our understanding of the plants' role in the terrestrial carbon cycle in the past, present and future. To reach these aims I had the following general questions: (1) How are the morphological and physiological traits of plants affected by CO_2 from past Pleistocene low to future high concentrations? (2) Are there differences in response to CO_2 from past low to future high between woody, forb and grass plant types? (3) Are there interactive effects between water availability and CO_2 availability on plant functioning?

Below I will summarize and synthesise my findings, in an effort to answer these questions about interspecific variation in plant responses in terms of traits and production rates to atmospheres ranging from CO_2 starvation to saturation. I will also briefly discuss priorities for follow-up investigation.

Plant traits from low to high CO_2

Plant growth is limited by the availability of light, nutrients, water and carbon. In terms of carbon acquisition the growth rate of plants can be separated into a morphological and a physiological component (Evans 1972). Several large meta-analyses have shown that at high CO_2 both the underlying morphological traits, i.e. specific leaf area (SLA), leaf mass fraction (LMF) and root mass fraction (RMF) and the physiological traits, i.e. photosynthetic rate, nitrogen concentration and carbon concentration, are strongly influenced by CO_2 (Poorter & Navas 2003, Ainsworth & Long 2005, Norby & Zak 2011). In the meta-analysis of chapter 2 we found that the trait responses to low CO_2 were even far greater in magnitude than towards high CO_2. These results on low CO_2 were however based on a far lower number of species than the large body of work on plants response to high CO_2. An important aspect of this thesis is that the available data on plant trait responses to low CO_2 is doubled or more than doubled for many traits and as a result it is now possible to present a more comprehensive picture of how plant morphological and physiological traits are adjusted from low to high CO_2.

For the morphological traits related to carbon uptake the meta-analysis presented in chapter 2 showed that plants in low CO_2 had a greater investment in leaf biomass (higher leaf mass fraction and thus a lower root mass fraction) and a higher specific leaf area (SLA). These results were corroborated in the more extensive analysis based on new growth chamber experiments in chapter 3, where the general directions of response to low CO_2 in the experiment were similar to those in the meta-analysis of chapter 2. The SLA increase (59%)

due to low CO_2 for the 22 species tested was however far greater than for the species in the meta-analysis (17%). It should be noted that the experiments from chapter 2 were longer in duration (median 64 days) than our experiment (21 days), which could suggest that the dramatic increase in SLA found in chapter 3 holds mostly for young plants and that for larger plants at low CO_2 the adjustment diminishes. Nevertheless, from the results in chapters 2 and 3 it can be concluded that plants increase their 'leafiness' at low CO_2 concentrations and that rising CO_2 levels generally leads to species lowering their leafiness; both from low to ambient and from ambient to high CO_2.

In response to high (compared to ambient) CO_2 concentration the morphological traits related to plant growth are adjusted opposite to the adjustment to low CO_2 but only moderately so. In chapter 3 we found at elevated CO_2 only a modest decrease in SLA and no change in biomass allocation to above- versus belowground tissues. Another way of assessing patterns in biomass allocation is to determine the scaling slope between leaves and roots. When looking at the full set of species, regardless of CO_2 the scaling relationship
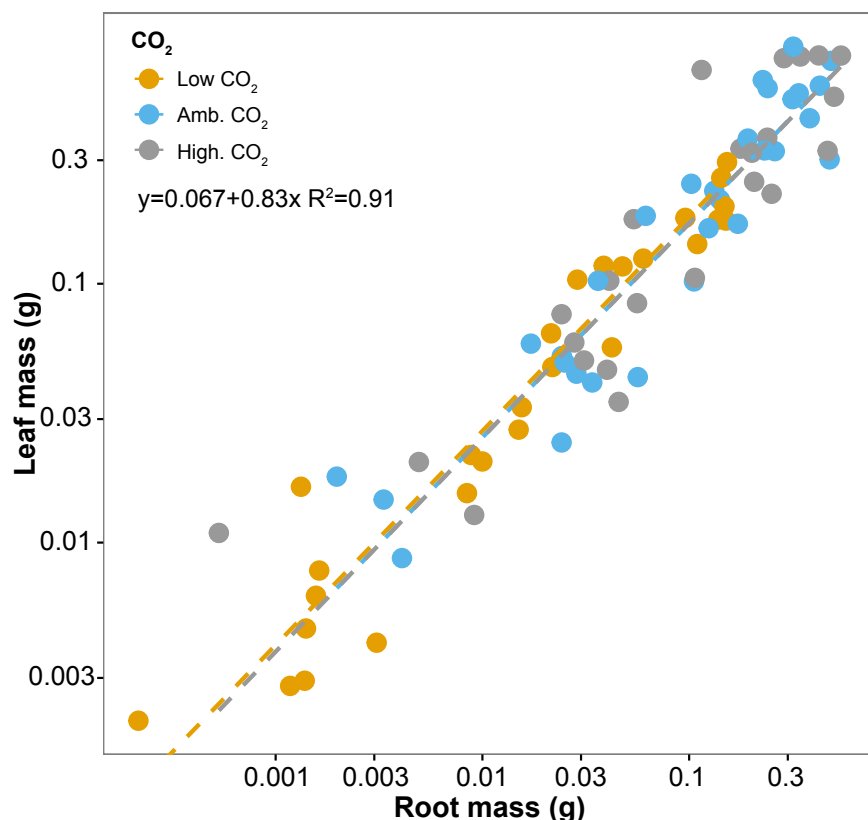
Figure 6.1 Biomass $\log_{10}$ scaling to roots and leaves at low (160ppm, blue), ambient (amb. 450ppm, orange) and high (750ppm, green) CO_2. Points and lines represent species mean leaf and root mass and the SMA regression between them (p<0.001). There was no difference in scaling slope between low, ambient and high CO_2.

between root and leaf biomass was the same (**Fig. 6.1**). When considering the 0.83 slope, as plants grew slower at low CO_2 due to carbon starvation, for a 10 fold reduction in root biomass leaf biomass was only reduced 6.7 fold. Due to this scaling relationship the effects on biomass allocation at low CO_2 (higher LMF, lower RMF) appear to be more the result of reduced plant size (Poorter *et al.* 2015) than a trait adjustment to CO_2.

In agreement with the results from the meta-analysis, I found in chapter 4 that plant physiological traits were also strongly affected by CO_2. At low CO_2 plants had a higher nitrogen content per mass and a lower photosynthesis and respiration per area. At high CO_2 plants had a lower nitrogen content per mass, a higher photosynthesis and higher respiration per area and a lower stomatal conductance per area. These physiological traits and specific leaf area interacted in such a way as to suggest that at low CO_2 plants produce thinner leaves, possibly to reduce mesophyll resistance (Loreto *et al.* 1992), whilst keeping the photosynthetic machinery (respiratory demanding) tissue intact. When considering the per mass photosynthetic rate, which can be viewed as carbon gained per carbon invested in leaf (Westoby, Reich & Wright 2013), this thinning has the effect of lowering the reduction in photosynthesis per unit mass at low CO_2. As a result the return on investment in leaf biomass is kept high (Bloom & Mooney 1985). At high CO_2 the increased photosynthetic rate per unit area allows for thicker leaves or denser leaves, which might be beneficial as a herbivory defence (Pérez-Harguindeguy *et al.* 2003), while keeping photosynthesis per mass high. Thus leaf morphology and physiology act in concert to maintain carbon acquisition as high as possible under CO_2 starvation and allow for tougher leaves at higher CO_2.

The interplay between trait levels is an important concept in plant ecology where, depending on the availability of environmental resources, plant traits shift in a concerted manner. From a resource economic perspective it is not beneficial to have excess capacity to acquire one resource when others are limiting (Bloom & Mooney 1985). Because of this there is a strong correlation between plant traits depending on environmental resource conditions. This worldwide spectrum of traits is known as the leaf or plant economic spectrum. In this spectrum, plants exhibit a concerted set of traits where traits fall in the 'slow' spectrum (as expressed by low leaf mass fraction, low specific leaf area, low nitrogen content, low photosynthetic rate, poor litter decomposability) for species adapted for growth at poor resource conditions and in the 'fast' spectrum (as expressed by high leaf mass fraction, high specific leaf area, high nitrogen content, high photosynthetic rate, high decomposability) for species adapted to environments with resources at plentiful supply (Wright *et al.* 2004, Freschet *et al.* 2012, Reich 2014). Even within species this same plant economic spectrum of traits is found across different habitats (Niinemets 2015). In chapter 4 I showed that plant traits move opposite to the general resource relations in the leaf or plant economic spectrum in relation to CO_2 as a limiting resource, by becoming more representative of the fast spectrum under CO_2 starvation (high LMF, high SLA, and high nitrogen content) and thus becoming more representative of the slow spectrum with increasing CO_2. Thus, while the slow-fast relationship holds for other resources when species adapted to different resource availabilities are compared at ambient CO_2, it appears that variation in CO_2 availability can modulate the plant economic spectrum by allowing for "slower" strategies.

One of the key knowledge gaps identified in the meta-analysis in chapter two was how the availability of CO_2 affects the links between traits. Only few studies measured sufficient

traits to allow a robust analysis of how the connections between traits were affected. In this thesis I measured a substantial set of traits on a sizable number of species. A method to visualise the relationships between traits is to plot them in an unstructured correlation network as common in systems biology (Villa-Vialaneix *et al.* 2013). For plant traits the relationships between traits related to carbon economy, in particular the plant economic spectrum, can be visualised in this way both by using mass- and area based traits (Poorter, Anten & Marcelis 2013, Poorter *et al.* 2014). Figure 6.2 shows such correlation networks for low, ambient and high CO_2. It can be seen that the networks at ambient and high CO_2 are highly comparable whereas the network at low CO_2 has far more connections. Additionally, at low CO_2 the connection between SLA and RGR has disappeared but the connection between nitrogen concentration and RGR remains, as described in chapters 3 and 4. Thus, the advantage of a fast growth morphology disappears at low CO_2 whereas nitrogen remains linked to RGR at all three CO_2 levels. Moreover, at low CO_2 stomatal conductance (*g*) is clearly much more strongly linked to other traits, especially those associated with water use. Incorporating these trait correlation networks and the effect resource availability has on them into trait-based dynamic global vegetation models would allow for a better representation of correlations and trade-offs between traits in these models (Poorter *et al.* 2013).

Plant types and CO_2

With over 350000 plant species in the Plant Kingdom ("The plant list") it is necessary to cluster groups of species on order to make general predictions on plants' responses to environmental conditions. Based on comparable categorical morphological and physiological traits (e.g. woodiness, life cycle, photosynthesis pathway.) at current conditions species can be grouped into plant functional types (Chapin *et al.* 1996). In this thesis the responses of C_3 woody plants, forbs and grasses to CO_2 have been analyzed. In response to CO_2 we found only few significant differences between these plant types and, when present, then only at low CO_2. However, it should be noted that low species numbers and large variation in the responses of woody and grass species could have contributed to the low number of significant differences. Thus, while there were strong effects of CO_2 on plant traits, the direction and magnitude of responses were broadly comparable between types. It would be interesting to investigate how the CO_2 responses of these plant types would compare at a more advanced stage of their life cycle; for woody species the proportional biomass (and presumably thereby C) allocation to stem tissue increases with plant age (Poorter *et al.* 2012b) and this could also affect their growth performance in different CO_2 atmospheres relative to those of herbaceous species.

While I found some difference in response to CO_2 between plant types this was only for a few traits. Moreover, in my experiments I found that at high CO_2 the responses between plant types were not significantly different for any of the morphological and physiological traits measured. Previous studies have also found only limited differences between these types at elevated CO_2 (Poorter & Navas 2003, Ainsworth & Long 2005). At conditions of CO_2 starvation woody types had a lower reduction in biomass, both in my experiments and in the meta-analysis of chapter 2. Their greater investment in non-photosynthetic stem tissue (Poorter *et al.* 2012b) thus appears to be no hindrance during this seedling stage. Furthermore at low CO_2 woody plants and forbs showed a dramatic increase in SLA yet

(a) Low CO$_2$

(b) Amb. CO$_2$

(c) High CO$_2$
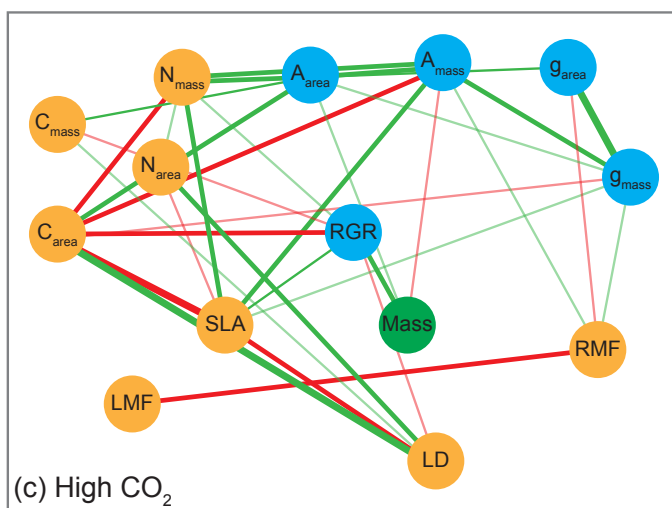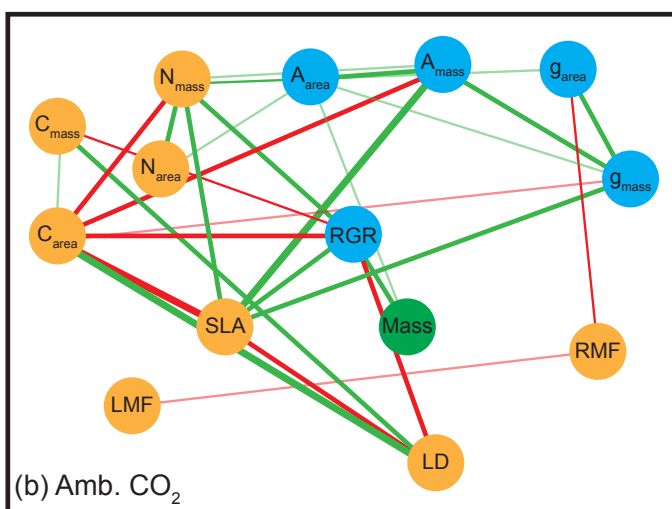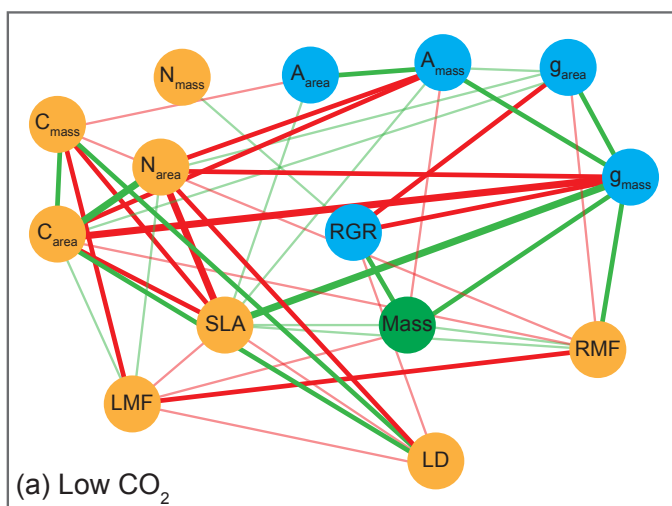
(left) Figure 6.2 Trait correlation networks at **(a)** low (160ppm), **(b)** ambient (450ppm) and **(c)** high (750ppm) CO_2. Lines show the standardized major axis regression between two traits when significant. Green lines: positive relationship, red lines: negative relationship. Faint thin lines: $0.1 < R^2 < 0.2$, intermediate lines: $0.2 < R^2 < 0.5$, thick lines: $0.5 < R^2 < 0.8$. R^2 less than 0.1 are not displayed for sake of clarity. Orange traits: ratios, Blue traits: rates. C_{mass}: leaf carbon concentration per mass (gC g^{-1}), C_{area}: leaf carbon content per area (gC m^{-2}), N_{area}: leaf nitrogen content (gN g^{-1}), N_{mass}: leaf nitrogen concentration per mass (gN g^{-1}), SLA: specific leaf area (m^2 g^{-1}) LMF: leaf mass fraction (g g^{-1}), RMF: root mass fraction (g g^{-1}), LD: leaf dry matter content (g g^{-1}), A_{area}: net photosynthetic rate per area (umol m^{-2} s^{-1}), A_{mass}: net photosynthetic rate per mass (umol g^{-1} s^{-1}), g_{area}: stomatal conductance per area (mmol m^{-2} s^{-1}), g_{mass}: stomatal conductance per mass (mmol g^{-1} s^{-1}), RGR: relative growth rate (g g^{-1} d^{-1}) Mass: total plant biomass (g)

grass species had only a minor increase in SLA. Because of this the increase in nitrogen concentration at low CO_2 was also lower for grasses. Plant types thus showed comparable response to CO_2.

Rather than between plant functional types the main difference in responses was between slow and fast growing species across these plant types. Even though the general ranking of species based on biomass and relative growth rate was not altered by CO_2 (i.e. faster growing species were always the faster growing regardless of CO_2), it was the faster growing species that lost the most in terms of relative growth rate at low CO_2, mirroring the response in RGR previously reported at high CO_2 (Cornelissen *et al.* 1999, Poorter & Navas 2003). In absolute terms the faster growing larger species had a greater reduction in biomass at low CO_2, with the woody species being among the slowest growing species in the experiment. This explains the smaller decrease in biomass accumulation they exhibited at low CO_2. Furthermore, while photosynthesis per unit mass (A_{mass}) was not significantly different between plant types, there was a clear relationship between SLA increase and reduction in A_{mass}. Species that had a larger increase in SLA had a lower reduction in A_{mass}. Thus, while clustering species into functional types has merit for certain general predictions about plant response to environmental drivers, the classification of species based on their plasticity in traits and their performance yields greater predictive value in plants' responses to changing CO_2.

Interactions between drought and CO_2

Through the stomata plants take up CO_2 and lose water via transpiration; thus there is a large potential for interactions in plants' response to the availability of these resources. At higher CO_2 concentrations plants can close their stomata more whilst keeping a high photosynthetic rate. Thus elevated CO_2 can lead to plants becoming more water conservative (Voelker *et al.* 2015). At low CO_2 plants have to open their stomata more to allow for sufficient photosynthesis leading to greater water loss per unit carbon gained (Franks *et al.* 2013). In response to drought and CO_2 availability an obvious hypothesis would thus be that plants are more vulnerable to drought at past low CO_2 and increasingly drought resistant at elevated CO_2. Experiments have shown, however, that the full story is more complex with herbaceous species showing no increased vulnerability to drought at low CO_2 (Ward *et al.* 1999) whilst a woody species does (Quirk *et al.* 2013). At elevated CO_2 drought tolerance is lower for some species (Medeiros & Ward 2013) or higher levels of CO_2 do not translate to

greater biomass in dry environments (Newingham *et al.* 2013). However, water use efficiency of photosynthesis is generally higher at elevated CO_2 levels (Franks *et al.* 2013). Thus, the interactions between CO_2 and water appear to be complex, difficult to scale up from leaf to plant and variable among species.

In chapter 5 the interaction of CO_2 and reduced precipitation was therefore tested for seven C_3 annuals. Plants were subjected to high, intermediate and low levels of soil water availability, which was achieved by watering the soil to a set soil weight several times per week. This had the effect that plants that could grow larger due to elevated CO_2 likely depleted soil water levels sooner than the small plants at low CO_2. Differences in plant mass due to drought were thus greater at elevated CO_2 than at low CO_2. However the relative effect of drought was the same across CO_2 levels. Plant size thus modulates the effect of drought (Liu et al. in press). When plants can grow large at well-watered conditions the absolute effect of a sudden drought will be greatest. Thus the extent to which CO_2 affects the response to drought appears to depend on the magnitude of the fertilization effect of CO_2. However, as the effect of drought can depend on the drought scenario (Tardieu 2012, Hartmann *et al.* 2013), exploring this plant size effect on drought stress with older plants and also grown for a longer duration seems worthwhile.

Plant traits and the terrestrial carbon cycle

Due to the feedbacks between plants and the terrestrial carbon cycle, understanding the interaction between plant traits and the CO_2 concentration in the atmosphere is important for (back-)predicting past and future climate (IPCC 2015). In chapter three and four I showed that several above- and belowground traits involved in the terrestrial carbon cycle are directly affected by the availability of CO_2. These results are in agreement with the modelled effects of CO_2 on vegetation in different biomes and global C storage (Prentice *et al.* 2011). In my experiments reduced CO_2 resulted in reduced carbon fluxes in the leaf (lower photosynthesis and lower respiration) and a reduced amount of carbon stored in biomass. Elevated CO_2 led to larger carbon fluxes and greater carbon storage in biomass. Belowground, plants had lower root mass allocation at low CO_2. In the past this lower allocation together with reduced photosynthesis likely led to lower mycorrhizal carbon exchange and to lower chemical weathering of the soil (Beerling *et al.* 2012). Based on the dramatic SLA increase at low CO_2 in my experiment decomposition of leaf material could be faster (Freschet *et al.* 2012) and material could have highly increased palatability (Pérez-Harguindeguy *et al.* 2003). However, at elevated CO_2 decomposition and soil C are not affected (Norby *et al.* 2001, Hungate *et al.* 2013). Thus CO_2 availability has a direct effect on the traits involved in C gain (through productivity) and C loss (through weathering rates, herbivory loss and flammability).

Next to the direct effect of CO_2 on plant traits involved in carbon cycling, the effect of CO_2 on community composition can also play a role in altering the carbon cycle. In ecosystems subjected to frequent burnings a community of grasses is often maintained due to seedlings of woody plant species not growing quickly enough to escape the effects of fire. Due to reduced growth at low CO_2 woody plant species in savanna ecosystems remain in this 'fire-trap' for longer, increasing the chance of seedlings dying due to surface fire. Furthermore

reduced biomass allocation belowground, i.e. reduced carbon storage, may limit the re-growth potential after a fire. (Kgope *et al.* 2010, Bond & Midgley 2012) CO_2 increases from the past to the present have thus likely contributed to increased forest cover which has had large impacts on the carbon cycle (Prentice *et al.* 2011).

At elevated CO_2 the greater stimulation of faster growing species (Cornelissen *et al.* 1999, Poorter & Navas 2003) and the general 'slowing' of traits on the plant economic spectrum could lead to shifts in community composition. Shifts in plant traits due to CO_2 could lead to altered competitive interactions when plants compete for resources. However, instead of the "slowing" of traits as found in this thesis leading to increased competition of more resource conservative plants modelling predicts reduced competition and increased evenness within communities at elevated CO_2 (Ali *et al.* 2015). In Free Air CO_2 Enrichment (FACE) plots elevated CO_2 sometimes influences community composition but not always (Norby & Zak 2011) possibly due to the low availability of other limiting resources (Smith *et al.* 2014). Scaling up the effects of trait shifts from models and growth chambers to the real world FACE sites is thus a challenge. Different species can have different traits related to carbon storage (woodiness, growth rate) and carbon fluxes (photosynthesis, respiration, decomposability). Understanding the effect of CO_2 on community composition is therefore an important but still poorly understood aspect in predicting the terrestrial carbon cycle.

Evolution and plastic responses to changing CO_2

Plants' responses to CO_2 occur at different timescales; over short time scales plants will show plastic responses in traits whereas over longer time periods adaptive evolutionary changes can occur (Medlyn & McMurtrie 2005). Given the generally short time scale of experiments, scaling up from short term plastic responses in traits to how plants will respond on a longer time scale is a key and contentious issue. Comparing the response of plants from today at conditions of the past to observable traits in fossil or subfossil materials is a way to test whether the plastic short term responses found in experiments are comparable to plants' actual response to conditions of the past. When comparing experimental results to paleo materials, only those traits that can be measured on dead or fossilized plant material can be compared. When leaves are fossilized stomatal traits can be measured (McElwain 1999) via petiole width and even specific leaf area can be estimated (Royer *et al.* 2007). The exceptional preservation qualities of pack rat urine in pack rat mildens allows for the measurement of isotopic fractionation and nitrogen composition of conifer needles >30 Kyr (Becklin *et al.* 2014). For some traits it is thus possible to compare trait plasticity during experiments to shifts in traits over evolutionary time.

In plant material preserved in pack rat middens conifer needles show no change in stomatal traits over the past 30Kyr but do show an increase in nitrogen concentration (Becklin *et al.* 2014). Plants plastic response to CO_2 in chapter 4 thus compares well to these trait shifts over evolutionary time. However, results from many other fossil and subfossil materials have shown that stomatal traits correlate quite well with CO_2 over geologic time scales (Franks & Beerling 2009) and even over the recent increases since the Industrial Revolution (Lammertsma *et al.* 2011). Species identity could play a role in this mismatch as the species measured for stomatal traits in relation to CO_2 were faster growing species, which generally

show only limited plasticity in stomatal traits (Haworth *et al.* 2013). Proper comparison of plant trait responses from controlled environments to conditions of the past requires therefore careful consideration of (1) evolutionary versus plastic response and (2) species identity, while (3) changes in other environmental factors should be taken into account as well.

For a long period in plants' recent evolutionary history atmospheric CO_2 levels have been far lower than today (Royer 2006, Lüthi *et al.* 2008, Hönisch *et al.* 2009, Franks *et al.* 2014). This period of carbon starvation has selected for plants capable of surviving these conditions. With CO_2 rapidly increasing adaptations to these conditions are no longer beneficial. However, experimental work has shown that low CO_2 is a far stronger selective agent than elevated CO_2 (Ward *et al.* 2000, Mohan *et al.* 2004) and that the enzyme kinetics of Rubisco seem to be fine-tuned to ~200 ppm CO_2 (Zhu *et al.* 2004). Plants growing at natural CO_2 vents show no consistent changes in traits compared to non-vent populations (Onoda *et al.* 2009). Thus, plants could still be adapted to conditions of the past potentially constraining their plastic response to an elevated CO_2 world in the near future (Sage & Coleman 2001, Tissue & Lewis 2012). Testing this empirically, however, will require great new intellectual and logistic effort (see below).

Future outlook for research on plant responses to CO_2

With the future atmosphere and climate becoming increasingly different from what plants have experienced for a long time during their evolutionary history, understanding how plants will respond to this altered situation is important for understanding and predicting shifts in vegetation type and productivity (Parmesan & Hanley 2015). The role of plants in the terrestrial carbon cycle makes it even more important to understand how plants are affected by the CO_2 concentration in the air and how they in turn affect the level of CO_2.

Future research on plants' responses to CO_2 should focus on the effect that a long evolutionary history at low CO_2 has on the response to elevated CO_2 and other environmental resources. Trait correlation networks can be used to find trade-offs in response to multiple environmental factors. More long term field experiments should be done to test the combined effects of availability of CO_2 and other resources on community composition, ideally also in highly under-sampled areas such as the tropics to further minimise the mismatch between models and reality (Brienen *et al.* 2015). Experimental study on evolution of plants in response to low CO_2 (e.g multi generation studies at very low CO_2) can shed further light on the relative contributions of adaptation and plasticity to rising CO_2. Exploring shifts in traits from fossil materials can give a timeframe for adaptive response to occur.

Fossil fuel emissions and land use change are resulting in a rapidly rising atmospheric CO_2 concentration. This rapid rise is leading to potentially large shifts in climate. Given plants impact on the environment understanding how they respond and adapt to this changing environment is crucial. In understanding their response to rising CO_2 plants evolutionary history at past low CO_2 should be taken into account. Plant traits at conditions of the past were markedly different than those today, indicating altered palatability, decomposability and flammability of fresh leaves and litter. Understanding the effect of trait shifts from the past to the present on the wider environment can shed light on the effect of trait shifts to

conditions of the future. By studying plant functioning and evolutionary history at conditions already different from today due to ongoing climate change, the extent to which plants are capable of adjusting to changing conditions can become apparent. With this knowledge long term predictions can be made that can aid in mitigation and adaptation to climate change. Understanding the past thus aids in predicting the future.

Chapter 6