Plant Traits and Production Rates from CO2 Starvation to Saturation

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Chapter 5

Hungry and Thirsty: Interactive Effects of CO_2 and Water Availability on Plant Performance in Seven C_3 Annuals

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Abstract

Carbon dioxide and water are crucial resources for plant growth. With anthropogenic fossil fuel emissions CO_2 availability is increasing whereas periods of low water availability are expected to increase in frequency in large parts of the world. How plants respond to these changes in the availability of above- and belowground resources, i.e. CO_2 and water, will help in understanding plants' responses to the future climate. How does drought affect CO_2 responses and are there trade-offs in responsiveness to CO_2 and drought? We grew seedlings of seven C_3 annuals at past low (160 ppm), ambient (450 ppm) and elevated (750 ppm) CO_2. At each concentration plants were subjected to well-watered conditions (100 % soil water availability, SWA), 40% SWA or 20% SWA. Plants were measured for biomass (allocation) and relative growth rate as well as for N and C concentration and gas exchange. Compared to well-watered conditions the relative effect of drought was the same at all CO_2 conditions. Plant size was a key element in the absolute response to SWA decrease. Thus, in absolute terms the larger, faster growing species were more affected by drought at high CO_2. Biomass allocation was not affected by drought, but plants invested relatively less in belowground tissue at low CO_2. Bigger is not always better, as smaller plants cope better with reduced SWA. As plants grow faster and larger at future high CO_2 conditions the effects of drought may become more noticeable with potentially large effects for highly productive plant species.

Introduction

Due to global climate change drought events are expected to increase in frequency and intensity during the coming century (IPCC 2015). As this will likely lead to increases in tree mortality (Choat *et al.* 2012) and severely declining grassland production (Brookshire & Weaver 2015), this will have far reaching consequences for global biodiversity and the terrestrial carbon cycle. The major cause for this are inputs into the carbon cycle from fossil fuels emissions and land use change (IPCC 2015). This will likely increase the current carbon dioxide concentration from 400ppm to an estimated ~800 ppm by the end of the century (Meinshausen *et al.* 2011). In plants' recent geologic past CO_2 levels have risen from a >2Myr period of low CO_2 (~180 ppm during the Pleistocene glacials) to today's 400 ppm after the Industrial Revolution (Hönisch *et al.* 2009). In the light of plants' evolutionary history in low CO_2 and fast shift to high CO_2, understanding plants' responses to water and carbon availability over the full range of CO_2 could aid in predicting their responses to the future climate (Tissue & Lewis 2012).

Carbon and water fluxes are tightly linked through the stomata, as carbon enters and water exits the leaf. This underpins the potential for interaction between CO_2 and water availability. At elevated CO_2, transpiration is reduced plastically due to closing stomata (Ainsworth & Rogers 2007) or developmentally by a lower number, or density, of stomata (Haworth *et al.* 2013). Elevated CO_2 also reduces the nitrogen concentration in plants (Ainsworth & Long 2005). One of several mechanisms hypothesized to cause this is a reduced flux of water across the roots due to reduced transpiration (Feng *et al.* 2015). Thus plant responses to limit water loss, by closing stomata, can affect performance at elevated CO_2 indicating the potential for trade-offs between plant responses to carbon and water availability.

Given the rapid increase to an elevated CO_2 atmosphere and a changing climate, a lot of research has been done on how plants respond to elevated CO_2 and how water availability interacts with that response. For example, in forest plots canopy water use has been found to be elevated in response to elevated CO_2 in developing stands, but reduced in established stands (Warren *et al.* 2011). In climate chambers the CO_2 fertilization effect has been found to be relatively higher under dry conditions (Poorter & Pérez-Soba 2001). Drought tolerance is however not necessarily improved as, while stomatal conductance and transpiration is lower at elevated CO_2 (Poorter & Navas 2003), elevated CO_2 increases the risk of embolism during drought (Medeiros & Ward 2013). In a desert environment elevated CO_2 was thought to increase productivity due to increased water use efficiency. However after 10 years of elevated CO_2 productivity and community composition remained unaltered (Newingham *et al.* 2013, Smith *et al.* 2014). Here the strong water limitation led to no stimulation by elevated CO_2.

While the majority of research has, justifiably, been done on predicting plants' response to future environmental conditions, understanding plants' response to conditions of the past might provide clues about possible constraints in response to future conditions. Plants' recent, >2 Myr, evolutionary history has been at a lower CO_2 concentration than today (Franks *et al.* 2014). Low CO_2 has profound impacts on plant traits and plant performance (Gerhart & Ward 2010, Temme *et al.* 2013, 2015), including a strong reduction in biomass and growth rate, higher specific leaf area (SLA, i.e. thinner or less dense leaves), larger leaf mass fraction (leaf mass per plant mass); (Temme *et al.* 2015) and strongly increased nitrogen concentration (Becklin *et al.* 2014)(chapter 3). Leaf traits are adjusted in such a way as to move towards the resource acquisitive end of the across-species leaf economic spectrum (Wright *et al.* 2004, Reich 2014) as expressed by higher leaf mass fraction, higher SLA and higher nitrogen content. However, this suite of traits is associated with lesser drought tolerance (Hallik, Niinemets & Wright 2009, Ouédraogo *et al.* 2013). This strongly suggests that there is a trade-off between adaptations to high CO_2 and those to drought.

Owing to the technical difficulties associated with growing plants at low CO_2 only a few studies have assessed drought effects on plant functioning at past low CO_2 (Gerhart & Ward 2010). While across all species instantaneous water use efficiency is reduced at low CO_2 (Polley, Johnson & Mayeux 1995, Temme *et al.* 2015)(Temme ch3), plant performance at dry conditions shows contrasting responses. In *Sequoia* trees low CO_2 exacerbated the effects of drought stress. Plants had greater xylem hydraulic failure, increased mortality and reduced defensive compounds (Quirk *et al.* 2013). In a study comparing C_3 and C_4 types, only C_4 species showed enhanced recovery rates after drought with increased CO_2 (Ward *et al.* 1999). Another study surprisingly found that at low CO_2 drought tolerance was increased due to improved xylem functioning (Medeiros & Ward 2013). The smaller size of plants at low CO_2 might even benefit them during periods of reduced precipitation as they take up less water and may deplete water more slowly within a limited rooting volume (Liu et al., in press).

As the effects of increased drought can be far reaching, capturing this in climate models is important for projections to the future. Recent modelling efforts could reproduce 66% of forest mortality events found in reality but drought was only a factor in 30% of those cases (Steinkamp & Hickler 2015). This highlights both the importance of improving the

representation of drought effects in climate change models and to improve our understanding of drought effects on plants to properly link drought to mortality events instead of other factors such as disease and herbivory.

Plants balance resource uptake such that growth is equally limited by all resources (Bloom & Mooney 1985, Chapin *et al.* 1987). At CO_2 increasing experimentally from past to present levels some plant species can adjust certain traits to improve drought tolerance (Quirk *et al.* 2013) while other species cannot (Ward *et al.* 1999, Medeiros & Ward 2013). Here we aim to reveal overall patterns of plant trait and performance response to CO_2 and water availability regimes, addressing: (1) how CO_2 concentration from past to future and soil water availability interact to affect plant performance and (2) whether there are trade-offs in the growth responsiveness to CO_2 and to drought.

We sought to answer these questions by growing seedlings of seven annual C_3 forbs broadly ranging in responsiveness to CO_2 and differing in specific leaf area and leaf mass fraction at past low, ambient and future high CO_2 and at a broad range of soil water availability (SWA).

Methods

In order to assess the responsiveness of plants to CO_2 and water availability we grew seedlings of 7 different C_3 herbaceous species, including grasses (G), forbs (F) and N_2-fixers (NF), at three levels of carbon and water supply in a full factorial design. Species grown were *Agrostis capillaris* (G), *Clinopodum chinense* (F)*, Hemisteptia lyrata* (F), *Medicago lupulina* (NF), *Rumex chalepensis* (F), *Stellaria media* (F) and *Vicia sepium* (NF), a subset of the species in Temme *et al.* (2015). These species were selected based on the broad range in allocation, N uptake strategy and leaf traits they represented.

Plants were grown in three controlled-environment walk-in chambers (Reftech bv, Sassenheim) at the Phytotron labs at Utrecht University at which we kept CO_2 at low, ambient and high level respectively following Temme *et al.* (2015). CO_2 in the low chamber was kept at a low 160 ppm by scrubbing ambient air of CO_2 with a molecular sieve (PG 1500L, CMC Instruments GmbH, Eschborn). The ambient CO_2 chamber was not directly controlled for CO_2. Concentration there was found to be 450 ppm, likely due to the chambers being situated inside an office building and near to a major road. The elevated CO_2 chamber was kept at 750 ppm by adding fossil fuel derived CO_2 from pressurized canisters to ambient air ventilating the chamber. CO_2 levels were continuously monitored (GMP343, Vaisala GmbH, Bonn) with scrubber capacity and CO_2 supply adjusted accordingly. While handling plants in the low chamber exhaled breath was captured using a gas mask in an airtight bag in order to limit CO_2 levels rising.

Individuals were germinated from field collected seeds on wet filter paper or sand and transplanted to 400 ml pots containing course sand and growing conditions shortly after germination. We did not expect pot size to play a substantial role in plant response to CO_2 and drought as plants were expected to remain below 1g L^{-1} during the experiment (Poorter *et al.* 2012a) and the soil water content at which drought effects become apparent is not related to pot size (Ray & Sinclair 1998).

Growth conditions were set to ~350 μmol light, during a 10-h photoperiod, at 21°C during day, 18°C at night and 70% relative air humidity. Up to the development of the first leaf, pots were watered three times per day automatically from below. After that individuals were separated into three equal size groups, one of which was kept at full water supply and two of which were subjected to drought. To prevent nutrient limitation 50mL of full Hoagland solution was added three times per week. Damage to freshly germinated individuals was prevented by slowly increasing the concentration from 25% to full Hoagland as plants grew until full development of the first leaf.

After full opening of the first leaf individuals were separated into three equal groups. Control plants were kept at the same water treatment as before (100 % soil water availability, SWA) while droughted plants were elevated slightly to prevent water coming up from below. Three times per week pots were weighed and water was added to achieve 40% SWA in the moderately droughted plants and 20% SWA in the severely droughted plants.

After the opening of the first leaf a baseline harvest was performed in order to calculate relative growth rate during the experiment (Hoffmann & Poorter 2002). Plants were then grown for three more weeks after which a final harvest was done. At final harvest we measured root and shoot fresh weight and leaf area and fresh weight of a single representative leaf. Plant material was oven dried for >48 hrs at 70°C after which root, shoot and leaf dry mass was determined. Leaves were subsequently removed from stems, or stem-like tough tissue, and stem dry mass determined. Leaf material was ground up in a bead shaker (type) and leaf C and N concentration determined using a flash combustion method (type). For a smaller subset of species leaves were large enough to allow that a day prior to harvest we could measure gas exchange using a LI-6400 (LICOR, Nevada, USA).

Data analysis and statistics were carried out using R version 3.12 (R core team, Vienna, Austria) and RStudio (version 0.99, RStudio, Inc., Boston, MA) as in Temme *et al.* 2015. To limit the effect of pseudoreplication we took the individual species mean response as replicates in all analyses on trait responses to CO_2 and SWA. Trait responses to CO_2 and soil water content (SWA) were viewed both in absolute terms and relative to ambient CO_2 or control full water. For responses relative trait values were ^{e}log transformed prior to analysis. This approach has the benefit that a halving or a doubling in trait value from ambient CO_2 has the same transformed difference. Trait responses to CO_2 and SWA were tested via ANCOVA with CO_2 and SWA as covariates. Within each CO_2 or SWA level the effect of SWA or CO_2 respectively was tested using linear regression. Association between traits and trait responses were tested via Standardized major axis regression (SMATR package in R).

Results

Plants were strongly impacted by drought at all CO_2 levels. At the lowest level of soil water availability (20% SWA) some individuals of most species died, except in *Agrostis capillaris* and *Vicia sepium*. For *Clinopodium* and *Medicago* this only happened at low CO_2, for *Rumex* only at high and low CO_2, for *Stellaria* only at ambient CO_2 and for *Hemisteptia* at all levels.

Drought and CO_2 effects on plant performance and traits

Increasing carbon availability from 160ppm CO_2 to 750 ppm CO_2 resulted in larger plant biomass at all three water levels (p<0.001, **Fig. 5.1a**). CO_2 concentration and SWA interacted in such a manner that water availability modulated the effect of increasing CO_2 by having the highest stimulation (greatest slope) at 100% SWA (**Fig. 5.1a$_3$**) and that at very low CO_2 concentration (160ppm) increased drought had no significant effect on biomass (**Fig. 5.1b$_1$**).

Relative growth rate (RGR, $g\,g^{-1}\,d^{-1}$) was affected by CO_2 comparably to plant biomass. With increasing CO_2 RGR was significantly increased (**Appendix 4.1a**). The relationship with SWA was weaker, only at high CO_2 was there a significant decrease in RGR at lower SWA (**Appendix 4.1b**). SWA and CO_2 did not show a significant interaction.

Relatively to non-limiting water availability the effect of reducing SWA from 100% to 20% was similar between all three CO_2 levels and there was no interaction between CO_2 and
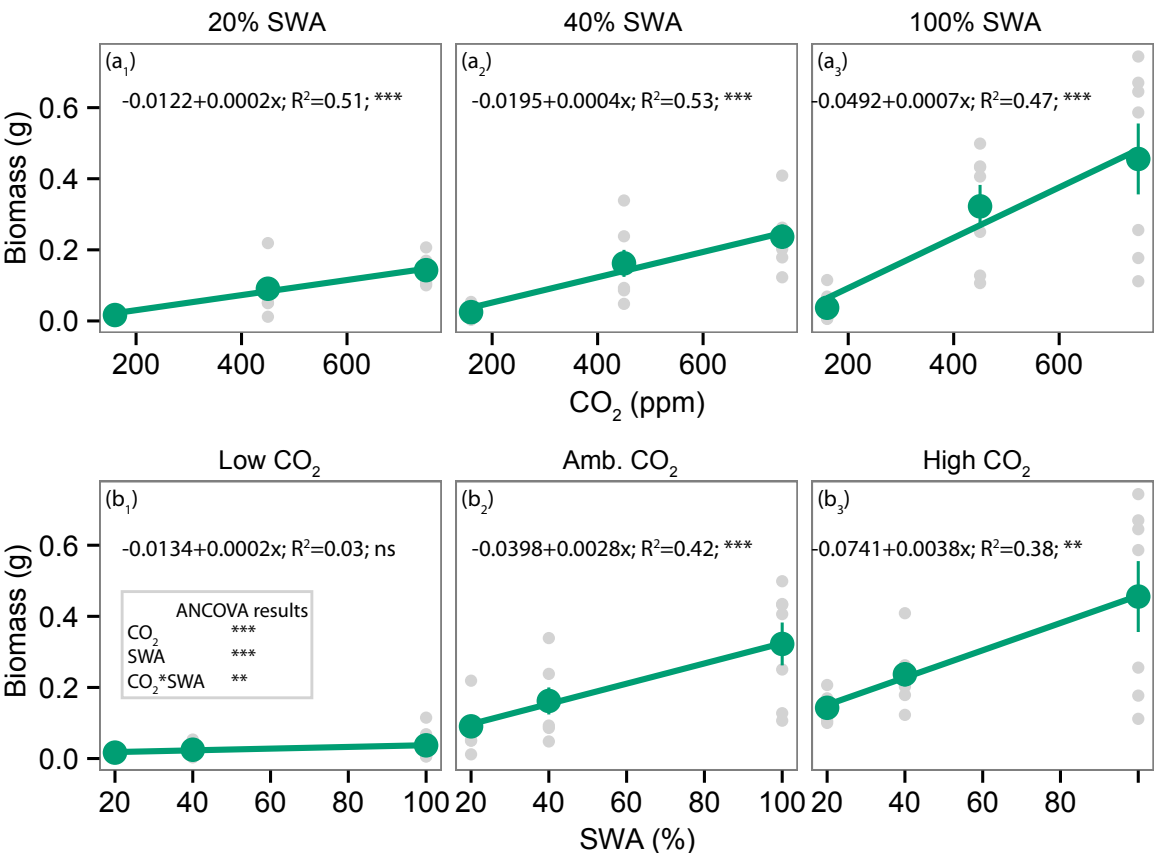

Figure 5.1 Plant biomass in relation to **(a)** CO_2 concentration and **(b)** soil water availability (SWA). Grey dots indicate species average biomass (n=4-6 per species), green lines give linear regression of biomass to CO_2 or SWA across all species points (equation given in panel). Box inset notes ANCOVA result main effects of CO_2 and SWA and their interaction. ns: not significant, **:p<0.01,***:p<0.001

SWA. Thus, while in absolute terms the biomass reduction due to drought at low CO_2 did not show a significant decrease, the relative effect of drought was significant at low CO_2 leading to relatively smaller plants at droughted conditions (**Fig. 5.2a**). For relative growth rate the difference (RGR being an exponent) due to reduced SWA was similar between CO_2 levels (**Fig. 5.2b**). Thus at each CO_2 concentration the relative effect of drought was the same.
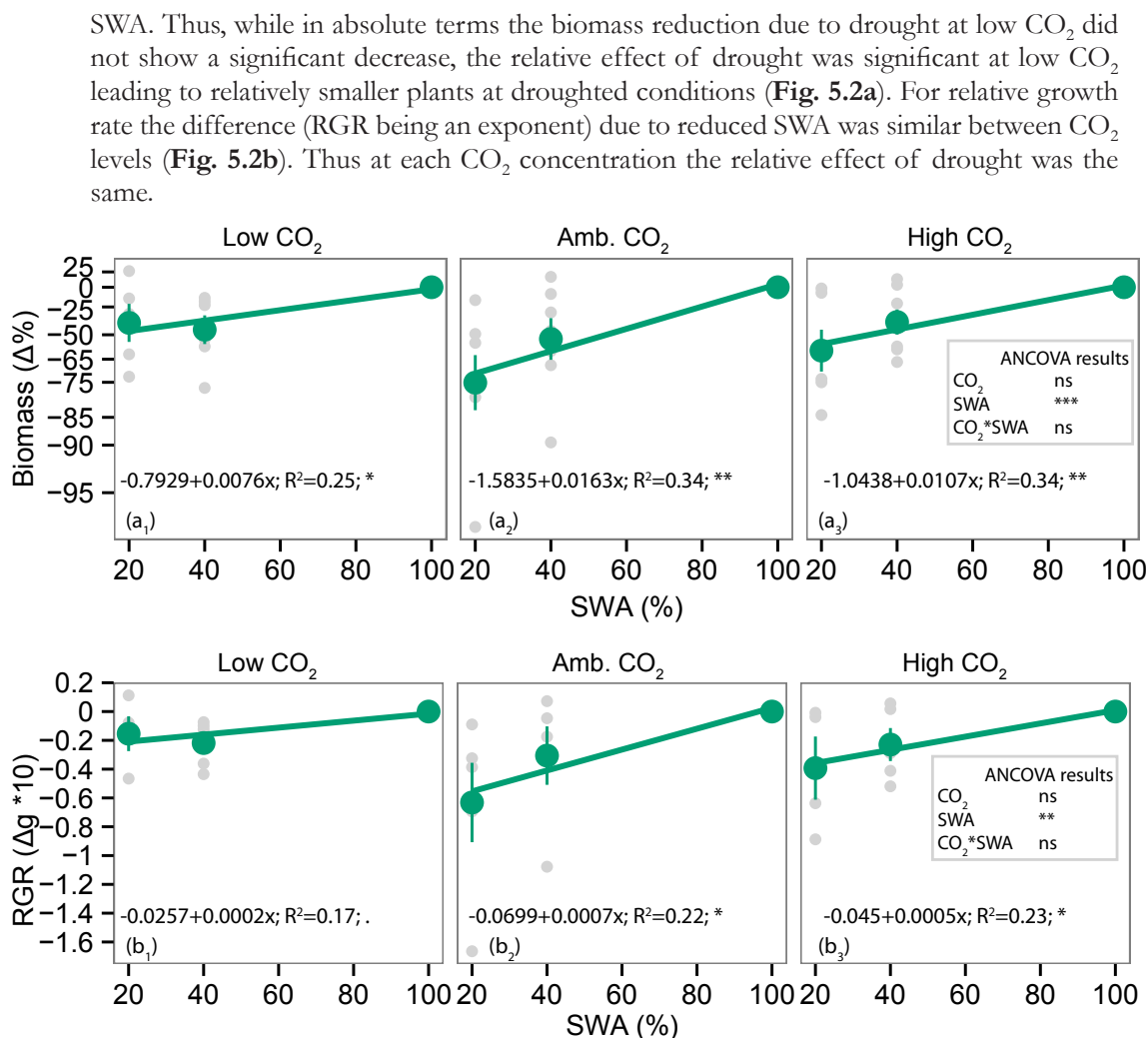

Figure 5.2 Relative effect of soil water availability (SWA), normalized to 100%SWA, on **(a)** biomass and **(b)** relative growth rate at three CO_2 levels. Grey dots indicate species average biomass (n=4-6 per species), green lines give linear regression of biomass to CO_2 or SWA across all species points (equation given at top of panel). Box inset notes ANCOVA result main effects of CO_2 and SWA and their interaction. ns: not significant, .:$p<0.1$,*:$p<0.5$, **:$p<0.01$

Biomass allocation to roots and leaves was affected by both CO_2 and SWA. Lower SWA led to increased root mass fraction (RMF) at ambient CO_2 ($p<0.01$) but not at low or high CO_2 ($p<0.1$, **Appendix 4.2b**). CO_2 increase from low to ambient concentration substantially increased RMF, although the increase from ambient to high did not result in an equally high increase in RMF (**Appendix 4.2a**). Relative to 100% SWA a decrease in SWA led at all three CO_2 levels to strongly increased RMF and CO_2 availability did not modulate this effect (**Appendix 4.3**). Leaf mass fraction was neither significantly affected by SWA nor by CO_2 (**Appendix 4.4**).

The scaling relationship between log-transformed leaf biomass and root biomass did not differ between water treatments, but there was a difference in elevation between CO_2 levels (**Fig. 5.3**). SMA regression showed a similar slope between CO_2 levels when all water treatments were pooled. We found that the relationship between leaf and root biomass at ambient and high CO_2 was the same, with plants at high CO_2 being moved along the common axis indicating the stimulating effect of CO_2 on plant biomass. However, the scaling slope at low CO_2 had a higher intercept than at ambient and high CO_2 ($p<0.01$). (**Fig. 5.3**). Thus for a given leaf biomass plants grown at low CO_2 had a lower root biomass.
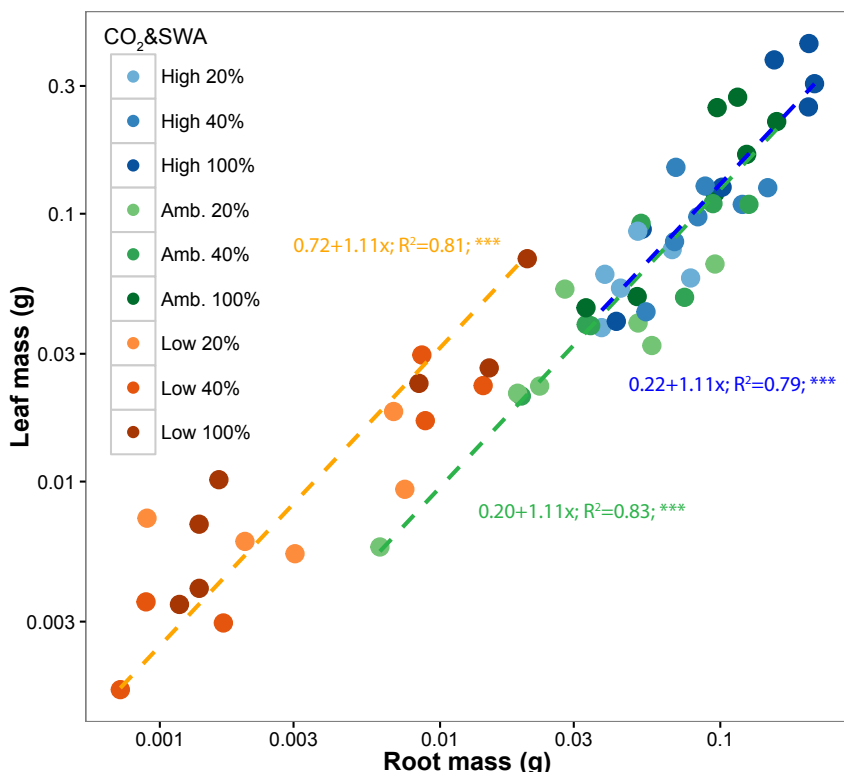

Figure 5.3 Allometric relationship between leaf and root biomass at low 160ppm, ambient 450 ppm, and high 750 ppm CO_2. Lines indicate proportional (log_{10}) scaling slope of leaf biomass to root biomass at each CO_2 concentration based on SMA regression. ***:$p<0.001$

Leaf traits were affected by CO_2 and SWA as well. Specific leaf area (SLA) was strongly affected by CO_2 with species increasing their SLA at low CO_2 and decreasing SLA at high CO_2 (**Appendix 4.5**). SWA only affected SLA at low CO_2 where drought led to plants having a lower SLA ($p<0.05$). Thus drought caused plants to adopt a lower SLA at low CO_2. Nitrogen concentration per unit mass (N%) was strongly affected by CO_2 with higher levels at lower CO_2 ($p<0.001$), while it was not affected by SWA (**Appendix 4.6**).

Leaf gas exchange (photosynthesis rate and stomatal conductance) could only be obtained from plants grown at ambient and high CO_2. Leaves of individuals at low CO_2 and 20 % SWA were too small to fit the LiCOR 6400 sensor head, so the interaction of CO_2 and SWA
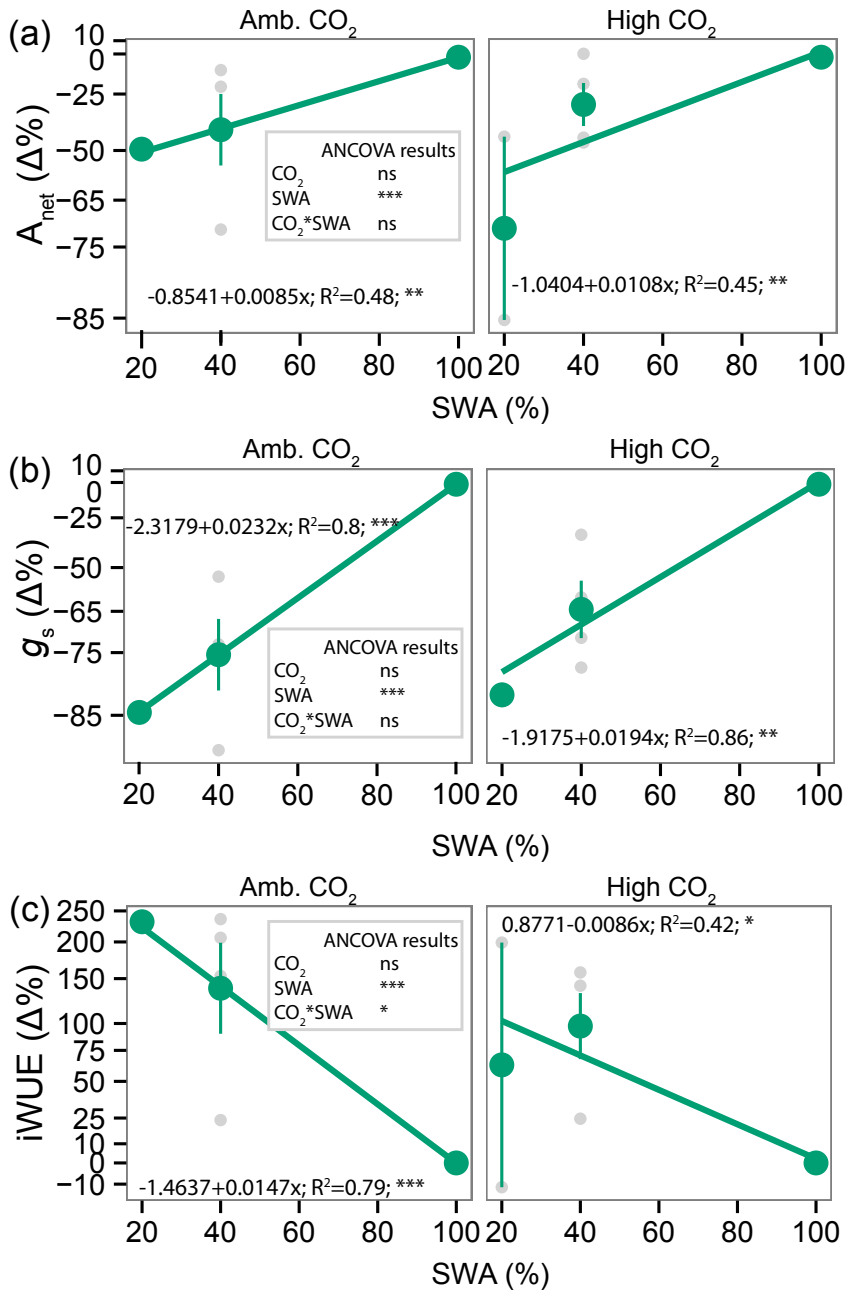
Figure 5.4 Relative effect of soil water availability (SWA), normalized to 100%SWA, on **(a)** net photosynthesis (A_{net}), **(b)** stomatal conductance (g_s) and **(c)** intrinsic water use efficiency (iWUE) at ambient (450 ppm) and elevated CO_2 (750 ppm). Grey dots indicate species average trait level (n=4-6 per species), green lines give linear regression of trait to SWA across all species points (equation given in panel). Box inset notes ANCOVA result main effects of CO_2 and SWA and their interaction. ns: not significant,*:p<0.5, **:p<0.01,***:p<0.001

could not be tested at low CO_2. Photosynthesis (A_{net}) was low at low CO_2 and high at high CO_2 (**Appendix 4.7a₂**). At ambient and high CO_2 reducing SWA led to lower A_{net} ($p<0.05$) (**Appendix 4.7b**). Relatively to 100% SWA the reduction in A_{net} was not significantly different between ambient and high CO_2 (**Fig. 5.4a**). Stomatal conductance (g_s) was not affected by CO_2 though plants did show a strong decrease in g_s at lower SWA. Relative to 100% SWA the reduction in g_s was similar between ambient and high CO_2 and greater than the reduction in A_{net} (**Fig. 5.4b**). This resulted in intrinsic water use efficiency (iWUE, A_{net}/g_s) being significantly higher at reduced water availability, though significantly more so at ambient than high CO_2 (**Fig. 5.4c**).
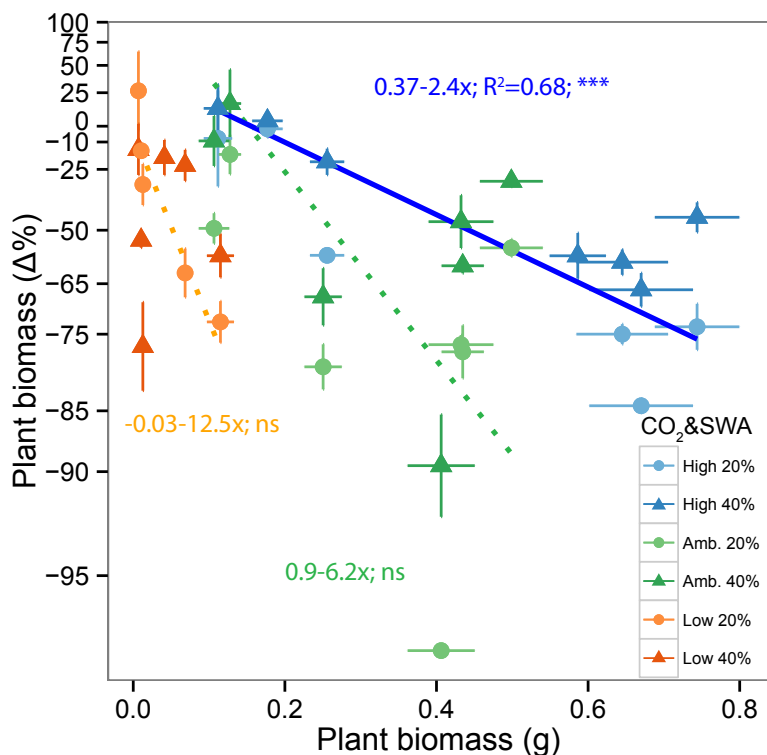

Figure 5.5 Effect of plant size at well-watered conditions on the level of biomass reduction due to soil water availability (SWA) at low (160ppm), ambient (450ppm) and high (750ppm) CO_2. Lines indicate SMA regression between biomass and biomass effect at reduced SWA at (red) low CO_2, (green) ambient CO_2 and (blue) high CO_2. Dashed lines: non-significant slope. ***:$p<0.001$

Trade-offs in biomass response to CO_2 and water

Trade-offs in response to CO_2 and soil water availability were not readily apparent. At low CO_2 there was no relationship between the extent at which carbon starvation decreased biomass compared to ambient CO_2 and the effect of reduced soil water availability on biomass compared to that at100% SWA at low CO_2. Thus, plants that could cope well with drought stress were not affected differently by reduced carbon concentration. (**Appendix 4.8a**) Species that were stimulated more by elevated CO_2 also tended to be more affected by reduced SWA (**Appendix 4.8b**). There was a significant relationship between plant biomass and biomass reduction due to drought (**Fig. 5.5**, $R^2=0.68$, $p<0.001$). Thus, in absolute

terms larger plants were more affected by drought than smaller plants. Taken together this suggests that the large plants that were stimulated most by elevated CO_2 were the most negatively affected by drought.

Discussion

There is a large potential for interactive effects between carbon gain and water loss as the control of these rates are linked via the leaf, with a key role for the stomata. We investigated for 7 C_3 annuals how CO_2 affected plant performance from well-watered to severely droughted conditions and if there were trade-offs in the growth responsiveness to CO_2 versus to drought. In agreement with earlier work we found that plant growth and biomass were strongly reduced at low CO_2 (Gerhart & Ward 2010) and stimulated by high CO_2 (Poorter & Navas 2003). Drought led to a similar relative reduction of plant biomass at all CO_2 levels. However, due to the carbon fertilisation effect of increasing CO_2, plants accumulated more biomass in absolute terms at higher CO_2 levels. Thus, the absolute effect of drought was in fact greater at higher CO_2 because plants could grow larger at well-watered conditions. This shows that plant biomass appears to be a key element in the responsiveness to soil water availability (SWA) at past low, ambient and future high CO_2.

Interactive effects of carbon and water
We found an interactive effect between CO_2 and water availability. Compared to ambient CO_2 we found no increase in CO_2 stimulation at dry conditions, which contrasts with an earlier study (Poorter & Pérez-Soba 2001). However we did find that plants that grew largest at high CO_2 and ample water were more affected by SWA (**Fig. 5.5**). Thus lower SWA at elevated CO_2 reduced the effect of CO_2 fertilization on biomass production. When SWA is low it seems that excess available carbon cannot be used and the stimulating effect of elevated CO_2 decreases. Furthermore, we found that the allometric relationship between leaf and root biomass was different at low CO_2 from that at ambient and high CO_2 (**Fig. 5.3**). For a given leaf mass plants had less root mass at low CO_2. Longer drought than in this study could exacerbate negative effects on growth at low CO_2 as low root mass allocation is linked to poor drought tolerance (Zwicke *et al.* 2015).

The combination of drought and higher biomass accumulation at high CO_2 could have interesting implications for ecosystem functioning. We found that the relative effect of drought stress was similar at all CO_2 levels, thus the absolute effect of drought on biomass was greater when plants grew larger in response to elevated CO_2. In an elevated CO_2 world a period of drought would thus lead to a larger absolute reduction in biomass than at ambient CO_2. As the absolute biomass production is relevant to the overall C budget in an area, one can envisage that a community of herbivores accustomed to a large amount of biomass production would experience a larger effect of drought than today (Frank & Mcnaughton 1992).

Trade-offs in responsiveness to CO_2 and drought
In this short-term study we found no clear evidence for trade-offs in the responses to water

versus to CO_2 (**Appendix 4.8**). Species that could tolerate lower SWA did not respond differently to elevated or reduced CO_2. However with more species or longer drought duration these trade-offs, if present, may become apparent. Plant species with high growth rates are stimulated more by elevated CO_2 (Cornelissen, Carnelli & Callaghan 1999, Poorter & Navas 2003) and more affected by reduced CO_2 (Temme *et al.* 2015). Traits associated with high drought tolerance (high RMF, low SLA) are negatively related to growth rates (Reich 2014). Thus their lower growth rates due to adaptations to poor water conditions could result in the reduced stimulation by elevated CO_2 at well-watered conditions.

From a competition point of view, the greater stimulation of fast growers with traits at low levels of drought tolerance could help them in outcompeting drought tolerant slow growers. However, in a model that incorporates nutrient use and capture it was found that elevated CO_2 led to increased coexistence between species due to reduced competitive ability and increased evenness because resources were more evenly distributed (Ali *et al.* 2015). Field experiments combining CO_2 and drought would be an excellent way to see if plants exhibit the same response to water as modelled for nutrients; and how, as in our study, plant size modulation of drought effects affects this response.

Recommendations and experimental considerations

The role plant size had in modulating the effect of SWA is likely influenced by our drought scenario. Drought effects can be investigated using many different scenarios (Tardieu 2012, O'Grady *et al.* 2013, He & Dijkstra 2014). Here we achieved drought stress experimentally by subjecting plants to reduced precipitation. SWA was kept at 20% and 40% of fully watered conditions by adding water to the desired level three times a week. Locally in the soil SWA was then inevitably higher than the average SWA. For small plants these short time periods where they had access water may have been enough to maintain functioning. As larger plants need more water they draw down soil water supply more quickly and as such are more affected by reduced precipitation (Liu *et al.* in press).

Longer term drought combined with carbon starvation as in the past could have had more detrimental effects on plant growth and performance (Hartmann *et al.* 2013). At high CO_2 changes in plant morphology and physiology could also modulate the effects of longer term drought (Sperry & Love 2015). CO_2 starvation leads to plants with high SLA and low root mass fraction (Temme *et al.* 2015) whereas in dry conditions plants with low SLA and high root mass fraction perform best (Hallik *et al.* 2009, Ouédraogo *et al.* 2013). Thus longer dry periods could show trade-offs as, in the long term, there could be opposite responses to CO_2 and drought. Moreover, long term periods of elevated CO_2 and environmental stress could lead to evolutionary adaptations different from the plastic response to rapid shifts in CO_2, as was shown for natural CO_2 vents (Onoda *et al.* 2009).

Our results corroborate earlier findings that herbaceous C_3 species, when grown at elevated CO_2, are not more drought tolerant (Medeiros & Ward 2013) and that the relative effect of drought is not greater at low or high CO_2 (Ward *et al.* 1999). However, the results from an earlier study with the tree *Sequioa sempervirens* (Quirk *et al.* 2013) are markedly different from our and previous results based on herbaceous species. These studies found that low CO_2 led to increased drought stress in the form of greater mortality and slower growth. This raises

the question if availability of water and carbon affects (slow growing) woody plants species differentially. Further experiments on woody species are especially important as drought induced mortality in forests has potentially profound effects on the terrestrial carbon cycle (Peng *et al.* 2011).

Conclusion

This study shows that small plants cope better with drought due to their lower water demand for transpiration. On the other hand, compared to fully watered conditions, the relative effect of drought is similar from low to high CO_2. However, as plants are stimulated by elevated CO_2 the absolute reduction in biomass production is far greater at higher CO_2. As plants will grow faster and larger in a future atmosphere the effects of drought will thus become more noticeable, with potentially large effects on highly productive plant species. While our results contribute to our knowledge on the general responses of herbaceous species to combined drought and CO_2 regimes, longer term experiments are needed, involving more species and including also the response of woody plants.

Chapter 6

General Discussion