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 glacial) 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 plasticly 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 CO2 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 CO2 and water availability regimes, addressing: (1) how CO2 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 CO2 and to drought.

We sought to answer these questions by growing seedlings of seven annual C3 forbs broadly ranging in responsiveness to CO2 and differing in specific leaf area and leaf mass fraction at past low, ambient and future high CO2 and at a broad range of soil water availability (SWA).

Methods

In order to assess the responsiveness of plants to CO2 and water availability we grew seedlings of 7 different C3 herbaceous species, including grasses (G), forbs (F) and N2-fixers (NF), at three levels of carbon and water supply in a full factorial design. Species grown were Agrostis capillaris (G), Clinopodium 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 CO2 at low, ambient and high level respectively following Temme et al. (2015). CO2 in the low chamber was kept at a low 160 ppm by scrubbing ambient air of CO2 with a molecular sieve (PG 1500L, CMC Instruments GmbH, Eschborn). The ambient CO2 chamber was not directly controlled for CO2. 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 CO2 chamber was kept at 750 ppm by adding fossil fuel derived CO2 from pressurized canisters to ambient air ventilating the chamber. CO2 levels were continuously monitored (GMP343, Vaisala GmbH, Bonn) with scrubber capacity and CO2 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 CO2 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 CO2 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 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\textsubscript{2} effects on plant performance and traits**

Increasing carbon availability from 160 ppm CO\textsubscript{2} to 750 ppm CO\textsubscript{2} resulted in larger plant biomass at all three water levels (p<0.001, Fig. 5.1a). CO\textsubscript{2} concentration and SWA interacted in such a manner that water availability modulated the effect of increasing CO\textsubscript{2} by having the highest stimulation (greatest slope) at 100% SWA (Fig. 5.1a) and that at very low CO\textsubscript{2} concentration (160 ppm) increased drought had no significant effect on biomass (Fig. 5.1b).

Relative growth rate (RGR, g g\textsuperscript{-1} d\textsuperscript{-1}) was affected by CO\textsubscript{2} comparably to plant biomass. With increasing CO\textsubscript{2} RGR was significantly increased (Appendix 4.1a). The relationship with SWA was weaker, only at high CO\textsubscript{2} was there a significant decrease in RGR at lower SWA (Appendix 4.1b). SWA and CO\textsubscript{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\textsubscript{2} levels and there was no interaction between CO\textsubscript{2} and

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**Figure 5.1** Plant biomass in relation to (a) CO\textsubscript{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\textsubscript{2} or SWA across all species points (equation given in panel). Box inset notes ANCOVA result main effects of CO\textsubscript{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\textsubscript{2} did not show a significant decrease, the relative effect of drought was significant at low CO\textsubscript{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\textsubscript{2} levels (Fig. 5.2b). Thus at each CO\textsubscript{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\textsubscript{2} levels. Grey dots indicate species average biomass (n=4-6 per species), green lines give linear regression of biomass to CO\textsubscript{2} or SWA across all species points (equation given at top of panel). Box inset notes ANCOVA result main effects of CO\textsubscript{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\textsubscript{2} and SWA. Lower SWA led to increased root mass fraction (RMF) at ambient CO\textsubscript{2} (p<0.01) but not at low or high CO\textsubscript{2} (p<0.1, Appendix 4.2b). CO\textsubscript{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\textsubscript{2} levels to strongly increased RMF and CO\textsubscript{2} availability did not modulate this effect (Appendix 4.3). Leaf mass fraction was neither significantly affected by SWA nor by CO\textsubscript{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 CO₂ levels (Fig. 5.3). SMA regression showed a similar slope between CO₂ levels when all water treatments were pooled. We found that the relationship between leaf and root biomass at ambient and high CO₂ was the same, with plants at high CO₂ being moved along the common axis indicating the stimulating effect of CO₂ on plant biomass. However, the scaling slope at low CO₂ had a higher intercept than at ambient and high CO₂ (p<0.01). (Fig. 5.3). Thus for a given leaf biomass plants grown at low CO₂ had a lower root biomass.

![Allometric relationship between leaf and root biomass at low 160ppm, ambient 450 ppm, and high 750 ppm CO₂. Lines indicate proportional (log₆) scaling slope of leaf biomass to root biomass at each CO₂ concentration based on SMA regression. ***: p<0.001](Figure 5.3)

Leaf traits were affected by CO₂ and SWA as well. Specific leaf area (SLA) was strongly affected by CO₂ with species increasing their SLA at low CO₂ and decreasing SLA at high CO₂ (Appendix 4.5). SWA only affected SLA at low CO₂ where drought led to plants having a lower SLA (p<0.05). Thus drought caused plants to adopt a lower SLA at low CO₂. Nitrogen concentration per unit mass (N%) was strongly affected by CO₂ with higher levels at lower CO₂ (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 CO₂. Leaves of individuals at low CO₂ and 20% SWA were too small to fit the LiCOR 6400 sensor head, so the interaction of CO₂ and SWA...
Figure 5.4 Relative effect of soil water availability (SWA), normalized to 100%SWA, on (a) net photosynthesis ($A_{\text{net}}$), (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 4.7a). 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 ($g$) was not affected by CO$_2$ though plants did show a strong decrease in $g$ at lower SWA. Relative to 100% SWA the reduction in $g$ 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$_{net}$/ $g$) being significantly higher at reduced water availability, though significantly more so at ambient than high CO$_2$ (Fig. 5.4c).

![Figure 5.5](image)

**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 at 100% 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\textsubscript{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\textsubscript{3} annuals how CO\textsubscript{2} affected plant performance from well-watered to severely droughted conditions and if there were trade-offs in the growth responsiveness to CO\textsubscript{2} versus to drought. In agreement with earlier work we found that plant growth and biomass were strongly reduced at low CO\textsubscript{2} (Gerhart & Ward 2010) and stimulated by high CO\textsubscript{2} (Poorter & Navas 2003). Drought led to a similar relative reduction of plant biomass at all CO\textsubscript{2} levels. However, due to the carbon fertilisation effect of increasing CO\textsubscript{2}, plants accumulated more biomass in absolute terms at higher CO\textsubscript{2} levels. Thus, the absolute effect of drought was in fact greater at higher CO\textsubscript{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\textsubscript{2}.

**Interactive effects of carbon and water**

We found an interactive effect between CO\textsubscript{2} and water availability. Compared to ambient CO\textsubscript{2} we found no increase in CO\textsubscript{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\textsubscript{2} and ample water were more affected by SWA (Fig. 5.5). Thus lower SWA at elevated CO\textsubscript{2} reduced the effect of CO\textsubscript{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\textsubscript{2} decreases. Furthermore, we found that the allometric relationship between leaf and root biomass was different at low CO\textsubscript{2} from that at ambient and high CO\textsubscript{2} (Fig. 5.3). For a given leaf mass plants had less root mass at low CO\textsubscript{2}. Longer drought than in this study could exacerbate negative effects on growth at low CO\textsubscript{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\textsubscript{2} could have interesting implications for ecosystem functioning. We found that the relative effect of drought stress was similar at all CO\textsubscript{2} levels, thus the absolute effect of drought on biomass was greater when plants grew larger in response to elevated CO\textsubscript{2}. In an elevated CO\textsubscript{2} world a period of drought would thus lead to a larger absolute reduction in biomass than at ambient CO\textsubscript{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 & Menaunghton 1992).

**Trade-offs in responsiveness to CO\textsubscript{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 Sequoia 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