MASTER THESIS

Effects of three different atmospheric CO_2 concentrations and two light intensities on plant physiology.

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Declaration of Authorship

I hereby declare that I am the sole author of this work. No assistance other than that which is permitted has been used. Ideas and quotes taken directly or indirectly from other sources are identified as such. This written work has not yet been submitted in any part.

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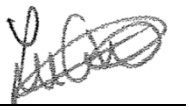

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Preface

This thesis has been written to fulfill the requirements of the Master of Science in Environmental Science from the University of Natural Resources and Life Sciences, Vienna, Austria and the University of Copenhagen, Copenhagen, Denmark. This thesis stands on its own and is not part of a larger project. I approached my supervisor, Prof. Dr. Peter Hietz, after attending one of his classes as I was interested in further studying the adaptations of plants to climate change. He suggested the specific topic and we developed the experimental approach accordingly.

Most importantly, I would like to thank my supervisor, Prof. Dr. Peter Hietz, for his constant help throughout all steps of the research project and despite the difficulties caused by COVID-19. Thank you as well to Prof. Dr. Fulai Liu, my co-supervisor at the University of Copenhagen, for his insightful remarks and suggestions despite the distance. I kindly thank Dr. Daniel Tholen for his invaluable help with the A/Ci curves; he took the time to explain the theoretical background, assisted me in the use of the instrument and provided me with the tools for the statistical analysis. I am very grateful to have also received assistance from everyone at the Institute for Botany, in particular Susanne Scheffknecht for her help with any technical issues I faced and the gardeners who helped me keep the plants healthy. Finally, I would like to thank all my family and friends who were present during the writing of my thesis.

Abstract

Human activities are modifying the carbon cycle by releasing carbon dioxide into the atmosphere through fossil fuel combustion and land use changes. As a result, the atmospheric carbon dioxide concentration, [CO_2], has increased from about 280 ppm to over 400 ppm currently and is expected to continue increasing during the 21st century. As CO_2 is the substrate of photosynthesis, this increase of [CO_2] leads to physiological changes in plants. This study aimed to investigate the physiological effects of different [CO_2] on plants grown under different light intensities. It included six C_3 species, *Phaseolus vulgaris, Pisum sativum L., Raphanus sativus*, *Rumex acetosa*, *Spinacia oleracea* and *Tropaeolum majus*, one C_4 species, *Zea mays* and one CAM species, *Kalanchoe daigremontiana.* The plants were grown in three growth chambers, each at a different [CO_2] (200, 400 and 800 ppm) and under two light intensities (400 and 100 µmol m^{-2} s^{-1} at the base of the pots). The effects of [CO_2] and light were measured through biomass, leaf traits and photosynthetic activity. The majority of species displayed an increased shoot biomass and leaf dry matter content as [CO_2] increased. Overall, the [CO_2] effect was much less pronounced than the light effect. For most species, no clear trends were identified for stomatal density, light response curves and photosynthetic activity of plants grown under the three [CO_2] environments. Very few parameters and species were influenced by the interaction between [CO_2] and light. This suggests that the light conditions do not greatly influence the physiological responses of plants to variations in [CO_2]. Finally, varied responses among the different species also highlighted the species-specific changes brought upon by changing [CO_2] growth environment.

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1.Introduction

Rising concentrations of atmospheric carbon dioxide ($[CO_2]$) is one of the main impacts of human activities on the biosphere. The interaction between $[CO_2]$ and plants is of paramount importance in the Earth's climate and ecosystem functions and therefore an important transdisciplinary research topic (Dow and Bergmann, 2014). As $[CO_2]$ continues to rise, concerns grow regarding food security and other essential ecosystem functions performed by plants (Myers et al., 2015, Pugh et al., 2016). This study aimed to understand the effect of different $[CO_2]$ coupled with different light intensities on plant leaf traits across several plant species. It would contribute to a better understanding of the range of adaptation in plant physiology in future climates.

A large body of research has been looking at the effect that increased $[CO_2]$ has on plants for several decades (Gray et al., 2016, Long et al., 2004, Pritchard et al., 1999). The limitations of experimental design as well as the multitude of potential adaptations depending on the species explain the variety of results found (Xu et al., 2016). Some overall trends have, however, appeared across different species grown in controlled environments, in Free-Air Concentration Enrichment (FACE) environments or from herbarium samples. Most studied species have responded to an increase in $[CO_2]$ by an increase in biomass production (Li et al., 2004, Ma et al., 2018, Bourgault et al., 2016). This increase is often linked to a higher net CO_2 intake despite a decrease in stomatal conductance (Berry et al., 2010). Finally, these physiological changes of plants may improve their water use efficiency (WUE) and change their nutrient content (Chun et al., 2011, Temme et al., 2017). $[CO_2]$ impacts different photosynthetic mechanisms differently. Firstly, C_3 species have been found to react to increased $[CO_2]$ through an initial increased photosynthetic productivity and reduced stomatal density and conductance (Gray et al., 2016, Xu et al., 2016). These changes are however, diminished through time or when other factors such as temperature or water stress are involved (Long et al., 2004). C_4 species do not exhibit changes as significant as C_3 plants in their photosynthetic activity and associated growth (Chun et al., 2011). On the other hand, they also do not acclimate as much or as quickly as C_3 species and could in the long-term have their biomass increased due to elevated $[CO_2]$ (Long et al., 2004, Reich et al., 2018). It has been hypothesised that long-term (several decades) effect of increased $[CO_2]$ may differ from the short-term experiments (from weeks or months to a couple years) for both C_3 and C_4 species (Reich et al., 2018). Studies looking at CAM species have also found that, on average, increased $[CO_2]$ led to higher biomass, a decrease in rubisco activity and little evidence of acclimation (Drennan and Nobel, 2000).

It is unlikely, that a single model can be identified and used to describe exactly how plants react to changes in specific concentrations of $[CO_2]$. However, knowing the range of potential adaptations given different environmental parameters and different plant species can lead to more accurate climate change mitigation strategies of biomass resources. Controlled environments do not reflect the complexity of real ecosystems (Long et al., 2004). Furthermore, the combined effect of all factors can differ from the sum of effect of those

factors analysed individually (Kadam et al., 2014). Hence, FACE experiments offer long term studies with ecosystems that are closer, in complexity and processes, to natural ecosystems. However, experiments under controlled environment can provide useful assessments of the scope of plant physiological adaptations. It is also a useful way to study how different climatic factors might interact. This in turn, helps to compare and analyse results across different studies or to interpret physiological changes in more complex settings. Finally, these types of experiments under controlled environment can help improving the experimental design of the long-term FACE experiments.

In this study, the interaction between variations in [CO_2] and light intensity was investigated. As another essential resource for photosynthesis, the light availability is highly variable across and within different ecosystems. It is thus possible that the effect of [CO_2] on plant physiology may also interact with the effect of light. Increased growth due to higher [CO_2] could change the competition for light in ecosystems and the shading of individual leaves. Studying the interaction between these two environmental factors is important to better assess the effect of an increase in [CO_2] on plant physiology and extrapolate the results of studies performed in specific light climates to other species or areas.

Leaf traits are a result of genetic expression and environmental adaptation of an individual plant. Through evolution, [CO_2] has played a significant role in shaping leaf traits (Roth-Nebelsick and Konrad, 2019). It is therefore relevant to study leaf traits in order to understand how a plant is impacted by the [CO_2] in which it grows. The modifications in leaf traits that have been documented to respond to [CO_2] are a change in stomatal density and conductance, modified leaf areas and changes in the gas exchange and photosynthetic rate of the leaves (Xu et al. 2016, Yan et al., 2017). Leaf traits also give some interpretation keys on how other plant mechanism might be impacted and why. Finally, the plasticity of leaf traits is species-specific and can inform on the plants' potential competitive advantages and disadvantages in future climatic conditions.

2.Literature review

Natural and anthropogenic [CO_2] variations

CO_2 is one the main gases contributing to climate change (IPCC, 2013). It has increased from about 280 ppm at the start of the Industrial Revolution to 412.89 ppm in November 2020 (Global Monitoring Laboratory, 2020). Its current evolution is an increase of about 1 to 2 ppm per year (Lindsey, 2020). [CO_2] has varied through history and participated in different feedback effects that have shaped the biosphere. Variations in [CO_2] is therefore normal and expected. Carbon in the form of atmospheric CO_2 is a part of the short- and long-term carbon cycles. Its current rise is due to the release of carbon stored in the long term carbon cycle over a very short period of time (Lindsey, 2020). The majority of the CO_2 emissions are originating mainly from the energy generation processes that rely on fossil fuel combustion, but also from land use changes (Global Carbon Project, 2020). A part of the additional CO_2 emitted by human activities is taken up by biomass and oceans and a part stays in the atmosphere. Thus, the rapid increase in [CO_2] since the beginning of the industrial revolution can be attributed to the anthropogenic disruption of these carbon cycles. This rise is therefore occurring much faster than the natural variations of [CO_2]. The current [CO_2] is higher than in the past 800 000 years (IPCC, 2013). At that time the Earth biosphere was highly different. The American National Oceanographic and Atmospheric Administration estimates that when [CO_2] was as high as today, over 3 million years ago, temperatures were also 2-3°C higher and the sea level 15-25m higher compared to when the [CO_2] was around 280 ppm (Lindsey, 2020). As levels of CO_2 emissions are currently still increasing, the future concentration is expected to cause significant changes in natural processes. Changes in vegetation growth is one of the main expected impacts. To be able to minimise these changes caused by [CO_2], their amplitude and the associated consequences, it is essential to develop better practices and mitigate the consequences that such a rise would have on ecosystems. Several projections have been developed for the impacts of the expected [CO_2] by the end of the century, 2100. However, incertitude remains as models are not yet able to explain all the complex mechanisms of the interactions between [CO_2] and the biosphere. A main area of incertitude is the feedback mechanisms between plant growth and [CO_2] (Leakey et al., 2012). As a resource required by plants for photosynthesis, the increase in [CO_2] also depends on the plants' capacity to absorb more carbon or on the release of carbon stored in plants through land use changes (IPCC, 2013, Pugh et al., 2016). Plant activity is thus a key actor of the Earth's climate. Studies have been looking for several decades at the interactions between [CO_2], climate and land use changes, and plant growth (Pritchard et al., 1999). Besides a potential for climate mitigation and more accurate model predictions, understanding plant adaptations to changing [CO_2] is also important to assess food security and resource management in the near future (Leakey et al., 2012).

Methodological approach to understanding the [CO_2] effect on plant physiology

Within this context, the study aims to look at the plant physiological responses to a combination of different [CO_2] and light conditions. The topic of elevated [CO_2] effect on plant physiology has been intensively researched since the 1980s and has produced a wide variety of results due to the different experimental designs and species studied (Pritchard et al., 1999). Research on the topic has been conducted with different techniques, each with their own limitations. Experiments aim to grow plants in environments representing what the future climatic conditions could be, based on predictions by various climate change scenarios. However, there is not a unique and unilateral effect of [CO_2] on plants (Palit et al., 2020). This explains why the uncertainty of how plants will develop in future climates remains. The direct and indirect effects of climate change on temperature, water availability, nutrients and so on affect plant growth in different ways (Gray et al., 2016, Leakey et al., 2012). It is experimentally complex to create an environment in which all these parameters can be manipulated. The environments created are then likely to be overly simplified compared to the reality and consequently overestimate or underestimate some effects (Long et al., 2004, Pritchard et al., 1999). Studying how each climatic parameter may impact plant growth does not allow to directly extrapolate such results to the whole biosphere but may inform on potential trends or common physiological processes (Palit et al., 2020, Pugh et al., 2016, Leakey et al., 2012).

Most of the early research was conducted in controlled environments such as growth chambers, greenhouses or open top chambers (Long et al., 2004, Pugh et al., 2016). In such experimental setups, it is possible to control the climate parameters to a high level of accuracy, but it poorly represents the complexity of interactions in an ecosystem. Often plants are grown in environments where they are provided with all the resources that they need, except for the parameters tested. It is visible from the methods chosen by several studies where optimal nutrient availability, soil moisture, temperature, light conditions and protection against pests are favoured (Li et al., 2019, Kumari et al., 2013). Moreover, many experiments are conducted over only a few months (Long et al., 2004). This provides limited information on the potential long-term adaptation of plants. It also reduces the potential of species analysed or the life stage in which they are. For instance, Aranda et al. (2020) studied the sub-Mediterranean oak species *Quercus pyrenaica*. As it is a tree species, the experiment was conducted on seedlings and for a few months only. The results can then not be extrapolated to the effect of [CO_2] on adult individuals as the physiological processes are different at different plant life stages. These experiments are however useful to understand the processes through which [CO_2] influences plant growth via precise measurements. It can then be used for more precise modelling as complex mechanisms are more fully understood. It can also help highlighting differences amongst species or subspecies in their reaction to [CO_2] as well as the main physiological adaptation to better prepare agricultural production in the future (Palit et al., 2020).

Free Air CO_2 Enrichment (FACE) experiments have been developed to address these obstacles. They are increasing [CO_2] in existing, open ecosystems (Long et al., 2004). This allows to monitor changes in plants and ecosystems over several years. It also includes natural variation of parameters affecting plant physiology, such as competition, predation, diseases, access to light, precipitation and so on. There are still limitations for this experimental technique as well. FACE experiments may increase the [CO_2] but as the exact extent of climate change remains unknown, the extrapolation from the results must be considered within these uncertainties. A study from Allen et al. (2020) has also highlighted that [CO_2] was fluctuating sometimes more significantly in FACE experiments than in natural conditions and that this fluctuation affects the results.

Finally, there are other limitations to the extrapolation of these experiments, regardless of the methodology chosen. Leakey et al. (2012) showed that the bias in the species or ecosystems studied has created a gap in the knowledge of the global biosphere changes caused by the increasing [CO_2]. Most studies looked at either temperate ecosystems or key species that play a central role in agriculture and food security. Therefore, less information is available on tropical and subtropical or polar ecosystems even though they have a high potential to have an impact on the carbon cycle. The information obtained can thus not only be attributed to the changes in [CO_2] or other parameters tested. The choice of the growing environment and the species are also affecting the results. Consequently, comparisons between studies is more complicated (Long et al., 2004).

Increased biomass caused by elevated [CO_2]

The main common impact of increased [CO_2] from the literature is an increase in plant growth. This is a trend visible across different experimental designs or species. It has also been observed when other stress factors are taken into account such as drought, temperature, and nutrient availability, for instance. This effect has been extensively researched in C_3 plants. Palit et al. (2020) reviewed studies about the impact of elevated [CO_2] on legumes, all of which are C_3 plants. They found an overall increase in the biomass of the plants across the studies included. Aranda et al. (2020) studied the effects of [CO_2] at 400 and 800 ppm through different drought treatment for the sub-Mediterranean oak species *Q. pyrenaica*. The results indicate that the biomass of the plants at higher [CO_2] was systematically higher than those grown at lower [CO_2] under all drought treatments, but biomass increase was lower as the drought intensity was increased. The [CO_2]-induced higher biomass buffered the detrimental effect of drought stress but became less important as the stress increased. Ma et al. (2018) found an increase in biomass as [CO_2] increased in beans (*Phaseolus vulgaris*) with low or high phosphorus input. These authors also found that, besides the limitations from P impacting the overall biomass, the plants grown in 650 ppm had a higher overall biomass in both P treatments than those grown in 377 ppm.

A similar impact was found in studies researching the impact of [CO_2] on CAM species. Indeed, as C_3, C_4 and CAM species have different carbon uptake and assimilation mechanisms, the same effect of [CO_2] on C_3 plants would not be expected in C_4 or CAM species. A review by Drennan and Nobel (2000) highlighted this

stimulated effect on biomass found by several studies looking specifically at CAM plants, where an increase in biomass on average was found across all studies. They also found that despite the experiment time being quite short in comparison to the life span of CAM species, an increase of about 35% in dry biomass was achieved after 3 months for plants grown in [CO_2] comprised between 650 and 750 ppm.

By contrast, for C_4 species a stimulating effect of [CO_2] on plant biomass was not found in all experiments (Chun et al., 2011, van der Kooi et al., 2016). Short-term experiments in controlled environments such as growth chambers have monitored some changes in plant physiology. Yet, these changes were not always reflected in the same way in overall biomass changes. For instance, Chun et al. (2011) found no changes in the total above-ground biomass of maize grown at either 400 or 800 ppm and at different water availability treatments. However, studies have suggested that the effect of [CO_2] under field conditions might be higher than those seen in growth chamber experiments. For instance, Wijewardana et al. (2016) studied the impact of [CO_2] along with drought and UV-B radiation on maize growth. The aim of the experiment was to identify the maize species most likely to better cope with climate change in future agricultural systems. However, the results showed that though drought and UV-B radiation drastically reduced plant growth, this decrease in growth was buffered by the effect of elevated [CO_2] for most of the maize hybrids studied. In other words, the result indicates that increasing [CO_2] could also stimulate overall biomass growth for C_4 species and that, the effect might be more visible when other stress factors are presented. Consistent with this, a review by van der Kooi et al. (2016) analysed the effect of [CO_2] and drought across several experimental designs. They found that [CO_2] increase led to a biomass increase in C_4 crop species only when the plants were also exposed to drought conditions.

Finally, the duration of the experiment appears to be very significant in understanding the [CO_2] effect on both C_3 and C_4 species. Some studies have highlighted the potential for C_4 species to have a higher increase in biomass in the long term than C_3 species (Palit et al., 2020). These data therefore suggest that the effects of [CO_2] both for C_3 and C_4 species found in experimental conditions might not correspond to the reality of what plant growth in future climates will resemble. It has been hypothesised that the effect of increase biomass for C_3 species could be overestimated whilst the effect on C_4 species underestimated (van der Kooi et al., 2016).

Effects of [CO_2] on plant structure

The increased overall biomass under higher [CO_2] is not the only result of increased carbon assimilation. Changes in [CO_2] also result in different carbon allocation in plants, resulting in different plant structures (Pritchard et al., 1999). Plants grown in higher [CO_2] display an increased height and changes in their leaf as well as root structure. Several studies have found an increase in the root/shoot ratio under elevated [CO_2] (Fan et al., 2020, Gray et al., 2016, Ma et al., 2018). Plants growing at higher [CO_2] also tend to have a more

elongated stem (Pritchard et al., 1999). As a primordial organ of photosynthesis activity, leaves are also affected by [CO_2] in ways that both adapt to and influence the overall carbon assimilation.

Leaf trait changes

The area of individual leaves and the number of leaves per plant have been found to increase in response to [CO_2] elevation in several studies resulting in an increased total leaf area at the plant level (Pritchard et al., 1999). An experiment by Temme et al. (2017) investigated other changes in leaves caused by an increase in [CO_2] in seventeen different C_3 species. They have found that the specific leaf area (SLA) decreased as [CO_2] increased from 160 to 750 ppm. Thus, at lower [CO_2] leaves were thinner and larger whereas at higher [CO_2] leaves were thicker and therefore more robust. At low [CO_2] this increase in SLA is necessary to mitigate the decrease in photosynthesis that comes with less available CO_2. They found that as the nitrogen content per area remained unchanged and the SLA was increased, then the photosynthetic decrease was partially mitigated (Temme et al., 2017). At higher [CO_2] the leaves get enough CO_2 from the environment and favour the development of thicker sturdier leaves. Interestingly, Pritchard et al. (1999) found that in crop plants the decrease of SLA with increasing [CO_2] is smaller than in tree and wild non-tree species. As the majority of experiments in growth chamber was conducted on crop or small-sized species, there are potential biases when focusing only on a few selected species.

Stomata are the most important and researched leaf trait because they interact strongly with the [CO_2] and display high physiological plasticity (Casson and Hetherington, 2010). Indeed, as stomata regulate the CO_2 uptake by the leaves for photosynthesis, they play a determining role in photosynthesis and plant adaptation to [CO_2] (Berry et al., 2010). They also regulate water loss through a trade-off between maximising carbon intake and minimising water loss (Xu et al., 2016). In that regard, their plasticity and capacity to adapt to the environment informs about the potential physiological adaption of plants under a future climate. Finally, their role in regulating gas exchange is also important to take into account in both carbon and water cycle models and need to be understood as precisely as possible for accurate predictions of future climate effects (Yan et al., 2017).

A meta-analysis by Long et al. (2004) revealed that in C_3 plants exposed to [CO_2] between 550 and 600 ppm, stomatal conductance had decreased by 20% on average across different FACE experiments. Similar results were found in studies conducted in controlled environments. Li et al. (2004) found that stomatal conductance of wheat was reduced at a [CO_2] of 700 ppm compared to their stomatal conductance at the current [CO_2]. This decrease was more pronounced for plants exposed to severer water stress. This allows plants to retain more water and is a result expected for plants exposed to drought. The same study, however, highlighted that the decrease in transpiration per area was counterbalanced by a higher total leaf area due to the increased growth under higher [CO_2]. Therefore, the overall water loss was the same across different [CO_2]. Another

study by Temme et al. (2017) looked at the stomatal conductance of seventeen C_3 species and found no changes from a lower, 160 ppm, [CO_2] to the current one. They did find a decrease at [CO_2] higher than the current concentration, as did other studies looking exclusively at higher [CO_2]. Stomatal regulation can be done in three different ways: their aperture, their size and their density. Their aperture is mainly reactive to the direct current environment. Size and density display important plasticity in between individuals but are fixed once the leaf is developed (Fan et al., 2020). Moreover, the development of stomata is species dependent and a product of their evolution. Some species are more sensitive to environmental changes whereas others do not exhibit high changes in stomatal characteristics (Yan et al., 2017). Therefore, the results of experiments strongly depend on the experimental design and the species included. This explains the diversity of findings regarding the impact of [CO_2] on stomatal characteristics.

Stomatal density is a key leaf trait used to assess the impact of [CO_2] on stomatal development both in experimental conditions and through historic changes in [CO_2]. Several reviews have highlighted that a majority of species decrease in stomatal density as [CO_2] increases (Xu et al., 2016, Casson and Hetherington, 2010). This is, however, not always the case and many studies found no changes in stomatal density, despite a change in stomatal conductance. This is the case for a study by Ogaya et al. (2011) on five "living fossil" tree species of Cretaceous and early Tertiary polar forests. Over an experimental period of four years stomatal density did not differ in plants grown at 400 or 800 ppm. Yan et al. (2017) reviewed stomatal characteristics of more than 900 species in response to experimental variation of [CO_2] or from plant samples up to over one hundred thousand years old. They concluded that though climate change, including changes in [CO_2], does alter stomatal development, it does so in a way that depends on the species and their unique environments. This highlights the difficulties to extrapolate how stomatal density, and generally speaking conductance, will change at a global scale in future ecosystems. As stomata have a decisive role on other feedback mechanisms, their plasticity will likely cause other changes in climate that are important to take into account in models (Dow and Bergmann, 2014).

Indeed, stomata are an essential part of the link between plants and the water cycle (Schlesinger and Jasechko, 2014). As leaves reduce their conductance, this allows for reduced water loss through transpiration. Many studies found that increasing [CO_2] improved the plant's water use efficiency (WUE) (Aranda et al., 2020, Long et al., 2004, Gray et al., 2016). This is considered to be a way through which increasing [CO_2] might help buffering the negative effects of climate change such as more frequent drought events (Gray et al., 2016). However, there is a threshold to that potential. Gray et al. (2016) and Aranda et al. (2020) found that beyond a certain point, a decrease in stomatal density did not help plants cope with droughts. The role of stomata for regulating plant water loss likely impacts the water cycle in the future as plant transpiration - water loss through stomata - accounts for the majority of evapotranspiration to the atmosphere (Schlesinger and Jasechko, 2014). For instance, Palit et al. (2020) reported an increase in canopy temperature as the water

evaporation rate from plants decreased due to higher [CO_2]. This, in turn, could increase the vapour pressure deficit between the leaf and the air causing increased transpiration rate.

Potential interaction between [CO_2] and light

Plants are exposed to different light intensities which have significant impacts on their physiology. Information about the interaction between [CO_2] and light availability is thus useful. Indeed, as many experiments in controlled environments expose plants to high light intensities, the information provided by such experiments are biased and the effect of [CO_2] when light availability is changed remains unclear. Furthermore, Keutgen and Chen (2001) found in their experiment on Calamondin (*Citrus madurensis*) that [CO_2] had indirect effects on the leaves. As the new leaves were bigger under higher [CO_2], the leaves present underneath were more shaded which further changed the leaf traits compared to the plants grown at current [CO_2]. This highlights the potential interactions that changed plant physiology can then have on a whole ecosystem level. It is also interesting to note that simultaneously with climate change, land use change is going to affect plant growth and ecosystem structure in the future (IPBES, 2018). It is therefore expected that plants will be exposed to different light conditions and different competition mechanisms for light access compared to current [CO_2]. As both of these factors, light and [CO_2], are likely to impact ecosystem structure and plant physiology it is important to study the potential interactions between them. Gray et al. (2016) noted that the impact of climate change is not the sum of the impact of all environmental entities that are likely to change. It is also expected that their interactions might influence plant physiology in a unique way, leading to different outcomes than those obtained from experiments looking solely at the effect of [CO_2]. The interaction between light and [CO_2] has not been extensively researched so far and could provide some interesting insights on ecosystems shifts under climate change.

[CO_2]-induced changes on photosynthesis

Overall, [CO_2] influences plant physiology in various, complex manners. Changes caused by variations of [CO_2] are usually in the form of a stimulated growth, an increase in leaf thickness and a decrease in stomatal conductance. These effects are however very dependent on the plant phenotypic plasticity and the precise environmental conditions in which they are growing. Studying the gas exchange and the modifications in photosynthetic processes leading to such changes is thus useful to identify some potential common mechanisms.

A/Ci curves provide useful information on the net amount of carbon assimilated by the plant depending on the CO_2 concentration in the leaf airspaces. Figure 1 provides an example of an A/Ci curves and the different limitations on A depending on Ci (Bernacchi et al., 2013, p. 1643). A refers to the net carbon assimilation, that is the carbon taken in during photosynthesis minus the carbon released, including through respiration. Ci is

the intercellular CO_2 concentration in the air spaces inside the leaf. The final concentration at the site of rubisco is then also limited by the rate of diffusion through the mesophyll (Warren, 2008). From this curve it is possible to obtain more precise information about the changes in photosynthesis caused by a change in [CO_2]. The rate of photosynthesis displayed in the A/Ci curves is impacted by several parameters susceptible to change based on environmental conditions or plant species. These are, the maximum carboxylation rate, Vcmax, the electron transport rate, ETR, the respiration and the mesophyll resistance to CO_2 diffusion. These parameters are often used in studies looking at the effect of [CO_2] on plant physiology as they show not only the result of such changes but also the processes through which they are affecting CO_2 uptake (Gray et al., 2016, Aranda et al., 2020).

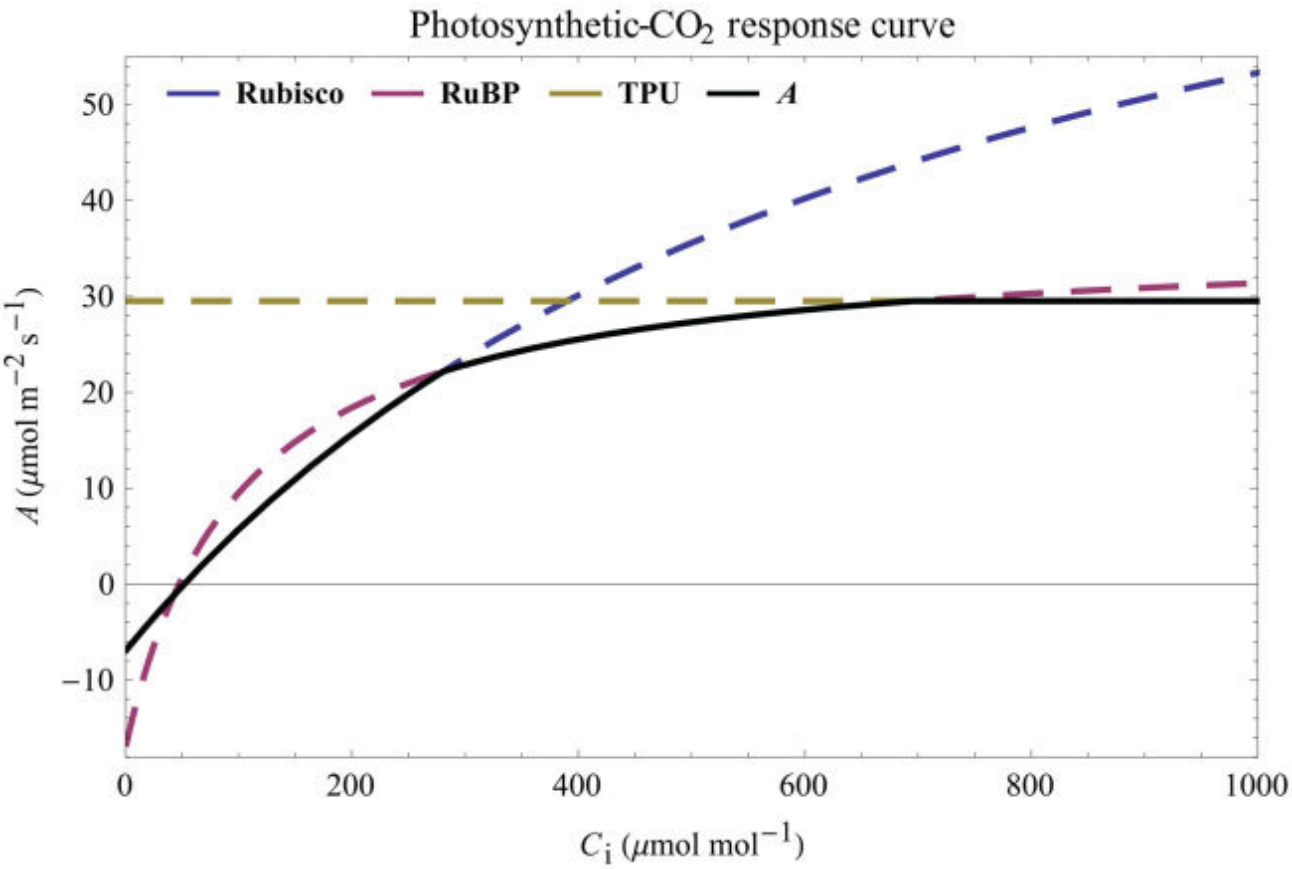

Figure 1: Example of an A/Ci curve from Bernacchi et al. (2013, p.1643). The dotted lines indicate the limitations on A. First, it is rubisco limited, then RuBP limited and finally limited by the triose-phosphate utilisation (TPU).

Several studies have found the net carbon assimilation per leaf area to increase, that is A was higher, for plants grown at higher [CO_2] (Aranda et al., 2020, Long et al., 2004, Fan et al., 2020). As [CO_2] is not the only parameter impacting photosynthesis, studies looking at the interaction between different factors of climate change have shown different changes in A. Temme et al. (2017) also found a decrease in photosynthesis at lower [CO_2] which suggests a link between [CO_2] and photosynthesis rate throughout a wide range of [CO_2]. Studies that looked at the impact of [CO_2] and drought on leaf photosynthesis showed different outcomes. Fan et al. (2020) found that increased [CO_2] led to a significant increase in carbon assimilation in bell pepper even for plants

grown under moderate and severe drought, though to a lesser extent. Other studies, such as Gray et al. (2016), have however concluded that the effects of drought on plant physiology prevented the carbon assimilation rate to increase under increased [CO_2].

The key process determining the rate of carbon assimilation and susceptible to change under changing [CO_2] is the rubisco activity. In C_3 plants, the carboxylation rate in rubisco is not fully efficient under current [CO_2]. Photosynthesis at 395 ppm is therefore rubisco-limited (Rosenthal et al., 2014). It is limited by its slow catalytic rate and the fact that it reacts both to O_2 and CO_2 (Galmés et al., 2014). Several studies have found that an increase in [CO_2] led to a higher increase of CO_2 concentration in the leaf and therefore, a higher concentration of CO_2 at the site of rubisco. Through this increase in CO_2 concentration in the leaf, increased [CO_2] improves rubisco efficiency and increases CO_2 uptake (Galmés et al., 2014, Rosenthal et al., 2014, Gray et al., 2016). Galmés et al. (2014) suggested that rubisco properties are the product of plant evolution and are therefore species dependent. As there is a negative correlation between the rate of carboxylation and CO_2 affinity, the overall efficiency of the RuBP is a product of such trade-off through evolution. In some areas, a better efficiency is favoured, however, it does not reach its maximum efficiency at current [CO_2].

The maximum carboxylation rate, Vcmax, was not always found to increase with increasing [CO_2]. The research from Gray et al. (2016) concluded that despite the [CO_2] of 500-585 ppm, the CO_2 concentration in the leaf under drought conditions was not increased and therefore the CO_2 concentration at the site of rubisco was also unchanged. Long et al. (2004) showed a decrease in rubisco activity, down-regulation, under elevated [CO_2] in the long term. They link this decrease to a higher sucrose content in leaves due to the initial high rate of photosynthesis. The accumulation of carbohydrates in the leaves was also found by Fan et al. (2020). Yet, they found that the photosynthetic activity was increased during the period of the experiment, 90 days. Nutrient deficiency is also shown to decrease rubisco activity in a study from Keutgen and Chen (2001) and in a review from Long et al. (2004). Finally, light intensity also modifies the rate of RuBP regeneration (Galmés et al., 2014). It appears that the role of rubisco in the physiological changes under higher [CO_2] is central and the knowledge of the species-specific rubisco properties could help mitigate the impact of climate change on agricultural productivity. The variations in carboxylation rate measurements highlight the uncertainty that subsists regarding the impact of [CO_2] on rubisco activity and its interaction with other stress factors impacting photosynthesis.

The electron transport chain also plays an important role in the rate of photosynthesis. The review from Palit et al. (2020) has shown that a change in this section of the photosynthetic reaction under elevated [CO_2] also caused a stimulation of leaf photosynthesis. Aranda et al. (2020) found an increase in ETR for plants grown in 800 ppm [CO_2] compared to those grown in current [CO_2]. This increase was used in photosynthesis to increase the uptake of carbon. In well-lit environments, the electron transport chain is not considered a limiting part

of the photosynthetic reaction. However, the ETR can be modified as an adaptation to different light or [CO_2] conditions. A study from Lambreva et al. (2005) also found the ETR to be initially increased under 1300 ppm [CO_2] but this effect was then down-regulated by the end of the experiment. Under unfavourable light conditions, they found no change in ETR even under high [CO_2]. As Keutgen and Chen (2001) noted, the change of light conditions for plants grown in different [CO_2] might therefore further impact the changes in the electron transport chain caused by the [CO_2].

The concentration of chlorophyll pigments in a leaf also informs about the photosynthetic capacity as it is a major component of the light reaction. Song et al. (2020) found a link between higher [CO_2] and higher chlorophyll content in leaves of cucumber grown in greenhouses with extra CO_2 fertilisation. Similarly, Ma et al. (2018) found an increase in the chlorophyll content of bean leaves grown at higher [CO_2] for both primary and secondary leaves. By contrast, a study from Keutgen and Chen (2001) on calamondin found no change in chlorophyll content throughout an [CO_2] range from 300 to 900 ppm. A meta-analysis on wheat from Wang et al. (2013) revealed that the chlorophyll content of plants grown in [CO_2] between 450 and 800 ppm decreased and that this decrease was linked to the decreased N concentration in the leaves. An increase in chlorophyll content could therefore be partly responsible for the increase of the carbon assimilation rate but it has not always been found to change.

Increased carbon uptake from the extra CO_2 available in their environment is not the only way by which net carbon assimilation increases. Indeed, increased [CO_2] also decreases carbon loss through a decrease in both dark respiration, both in the night and during the day, and photorespiration (Fan et al., 2020). Photorespiration is CO_2 lost due to RuPB oxygenation instead of carboxylation. As under high [CO_2], the CO_2/O_2 ratio is increased, carboxylation is promoted over oxygenation leading to a decrease in photorespiration (Marçal et al., 2021). A decrease in photorespiration increases rubisco efficiency (Long, 2004). Several articles have highlighted this phenomenon as a common reaction to changes in [CO_2] and playing an important role in explaining the increase in A (Palit et al., 2020, Long et al., 2004, Cowling and Sykes, 1999).

3.Objectives

In light of the above, the present study aimed to further the understanding of the impact that [CO_2] and light can have on plant physiology. These changes will be measured using biomass and physiological measurements; leaf traits such as specific leaf area, leaf dry matter content and stomatal density and; measurements of the photosynthetic activity. It is expected that plants grown in higher light and higher [CO_2] will have higher biomass resulting in taller plants with bigger and more robust leaves.

4. Materials and Methods

Plant materials

Eight different plant species were selected for this experiment. Six of them were C_3 species: bean (*Phaseolus vulgaris*), pea (*Pisum sativum L.*), radish (*Raphanus sativus*), sorrel (*Rumex acetosa*), spinach (*Spinacia oleracea*) and nasturtium (*Tropaeolum majus*). One was a C_4 species, maize (*Zea mays*) and one a CAM species, kalanchoe (*Kalanchoe daigremontiana*). They were grown from seeds obtained from Bellaflor (Vienna, Austria).

Experimental set up

Three Percival E-75L1 growth chambers (CLF Plant climatics) located in Vienna, Austria were used for this experiment. The climate settings were as follows: a 14 hours day and 10 hours night, a temperature of 26°C and 22°C respectively, and a constant relative humidity of 65%. During the day time period, the lights were fully on, at about 400 µmol m^{-2} s^{-1} (high light) at the level of the pots. A half of each chamber was shaded so that the plants on that side received about 75% less light than the other side, about 100 µmol m^{-2} s^{-1} (low light) at the base of the pots (Figure 2). The three chambers were set at 200, 400 and 800 ppm [CO_2]. Overall, there were six different treatments combining the three [CO_2] and two light intensities. Within each treatment a tray contained twenty-four 11 by 11 cm large and 15 cm deep pots.

Figure 2: Set up inside one growth chamber.

The pots were filled with soil made up of organic peat free potting soil from Frux and quartz sand (0.3 – 2.0 mm). Five parts of potting soil were mixed with two parts of sand to make up the soil solution. On May 22, 2020, seeds were sown for germination. For bean, pea, radish, nasturtium, maize and kalanchoe, three seeds were sown directly in the pots (Figure 3). Sorrel and spinach were germinated in trays, as well as some more spinach to use as control during the experiment. The seeds were then left to germinate for 10 days in a greenhouse at ambient [CO_2] and light conditions. On June 2, seedlings of sorrel and spinach were transferred to the pots along with one spinach seedling per pot to act as control (Figure 3). The trays were then placed in the chambers set at the different [CO_2] but without the shade cloth limiting light availability. The shade was added on June 5, 14 days after sowing.

Figure 3: Plants moved to the growth chamber after germination.

Overall, each species had nine replicates per treatment in three different pots (Figure 3). Each pot was positioned randomly within the chamber to minimise the potential effect of small variation of light or other factors (Figure 4). All chambers had the same pot position. The plants were watered daily to maintain sufficient water availability. The chambers were open less than thirty minutes a day during growth to limit [CO_2] fluctuation. To avoid additional stress affecting plant growth anti-rust was sprayed on the bean replicates growing under 400 µmol m^{-2} s^{-1} light as some leaves presented damages. Additional fertiliser and iron were also added to each pot 35 days after sowing as some plants presented discoloration in the older leaves. Aphids appeared in the 200 ppm growth chamber about 30 days after sowing. They were manually removed and two ladybugs and two ladybugs larvae were added in the chamber to control their spread.

Figure 4: Pot placement within each treatment.

Harvests

The plants were harvested at three different times (Table 1). The time of harvest was chosen depending on the growth of each species, some grew faster than others, and to reduce the shading of the smaller species by the bigger ones. At each harvest, one individual per pot was selected.

Table 1: Time of harvest in days after sowing.

Species	**Harvest 1**	**Harvest 2**	**Harvest 3**
Bean	27	32	46
Pea	27	32	45
Radish	27	40	49
Sorrel	35	41	66
Spinach	27	40	67
Nasturtium	27	39	62
Maize	27	38	69
Kalanchoe	34	41	71

For harvest 1 and 3 plants were measured beforehand. Their height (in mm), and length of the biggest leaf (in mm) were measured at the nearest millimetre using a tape. The number of leaves per plant was also recorded.

The chlorophyll content of five leaves per plant were obtained with a SPAD-meter (Walz). The average per plant was recorded.

Plants were harvested at the base of the shoot and the leaves separated from the stem. They were then weighted using a precision scale to the nearest mg and scanned using a desktop scanner at 300 dpi resolution. For stomatal density estimation, three of the most recently fully developed leaves were selected per plant. Stomatal imprints were obtained by softening acrylic glass slides with 2-butanone and pressing the leaf onto the slide. The same position of the abaxial side was imprinted for each species. At harvest 3 and for radish only, roots were also pulled out from the pot. The soil attached was removed under running water and their weight was recorded using a precision scale, to the nearest mg. The leaves, stem and roots (for radish only) were finally placed in an oven at 80 ± 5 °C for at least three days to dry. The dry weight of the leaves, shoots and roots was then measured to the nearest mg using a precision scale.

The specific leaf area (SLA) is a leaf trait obtained by dividing the leaf area, in mm^2, by the leaf dry biomass, in mg. The leaf area was obtained from the scanned leaves using ImageJ. The total leaf area per plant was added and converted from pixels to mm^2.

The leaf dry matter content (LDMC) is a ratio of the leaf dry weight divided by the leaf wet weight. This was obtained from the measured leaf weight at harvest and after drying, both in g.

The stomatal density (SD) is the number of stomata counted per leaf area. The leaf imprints were observed under a DM5500B transmission light microscope with a DMC2900 camera (Leica, Germany) at a 20x magnification for sorrel, spinach, maize, kalanchoe and pea. The number of stomata of each leaf was counted in four frames of 595.35 µm by 446.44 µm. The 40x magnification was used for bean, radish and nasturtium where the stomata number in four 297.67 µm by 223.22 µm frames were counted. The stomatal density was obtained by averaging the counted four frames of the three plants of each treatment in order to obtain one average value per treatment.

Collectively, the following biomass and leaf trait measurements were recorded for each plant: height (mm), length of biggest leaf (mm), number of leaves, chlorophyll content, total dry biomass (g), leaf dry biomass (g), radish root dry biomass (g), radish root/shoot ratio, SLA, LDMC and stomatal density.

Light-response curves

Light-response curves were measured for one leaf per plant for each plant at the third harvest. The leaf was clipped to a miniPAM (Walz) instrument set on a program of eleven 20s intervals at 50, 75, 100, 150, 200, 450, 600, 850, 1100, 1400, 1700 and 2000 µmol m^{-2} s^{-1}. The light-response curves were then obtained by plotting the electron transport rate (ETR) on the y-axis and the photosynthetic photon flux density (PPFD) in µmol m^{-2} s^{-1} on the x-axis, using R 4.0.3 (R Core Team, 2020). From the measured data, several parameters were derived

by fitting the function: ETR = $a*(1-exp^{-b*PPFD})$ according to Rascher et al. (2000) with non-linear squares optimisation in R. From these fitted curves, ETR@1500 was defined as the ETR at PPFD of 1500 µmol m^{-2} s^{-1}.

A/Ci curves

The gas-exchange of an 8 cm^2 leaf area of one leaf per plant of the third harvest for bean, radish and nasturtium were measured with a GFS-3000 (Walz) gas analyser. The light was set at 1000 µmol m^{-2} s^{-1}. The light intensity was previously tested to be high enough for the photosynthetic rate to not be light-limited but low enough as to not result in photoinhibition. The CO_2 concentration in the chamber was first set to 400 ppm and then reduced in intervals of 300 seconds to 250, 100, 70 and 40 ppm. It was then brought back to 400 ppm for 450 s and then increased in intervals of 300 s to 600, 900, 1200, 1450, 1700 and 2000 ppm. During each interval the gas-exchange was measured and recorded.

The A/Ci curves were fitted from these measurements using a model based on Farquhar et al. (1980) and a curve-fitting approach using the R package Saemix (Tholen, 2019, unpublished, Comets et al., 2017). The model derived four parameters from the A/Ci curve: Vcmax, the maximum carboxylation rate, RL, the respiration in light, rm, the mesophyll resistance to diffusion and Tp, the triose-phosphate utilisation.

Statistical Analysis

Two-factor ANOVAs were conducted in R 4.0.3 (R Core Team, 2020) with light intensity, 100 or 400 µmol m^{-2} s^{-1}, and CO_2 concentration, 200, 400 or 800 ppm as factors for each species separately. For some of the parameters, data from the three harvests were taken into account with date added as a factor, for others only data from harvest 3. The different parameters for which a two-factor ANOVA was conducted were: shoot biomass, root biomass (for radish only), root/shoot ratio (for radish only, harvest 3), leaf biomass, height (harvest 3), number of leaves (harvest 3), length of the biggest leaf (harvest 3), SLA, LDMC, SD (harvest 3), chlorophyll content, ETR at 1500 µmol m^{-2} s^{-1} (obtained from the light-response curves) and, Vcmax, RL, rm and Tp (obtained from the A/Ci curves). Since initially three plants per species were planted in each pot, pot number was treated as a random factor in the ANOVA. The mean values and standard deviations per treatment and species are shown as bar charts.

The height, number of leaves and length of the biggest leaf at harvest were correlated with the plant shoot biomass for plants from harvest 1 and 3. Several linear models were tested in R to obtain the one that best explained biomass.

5.Results

Biomass model proxy

Table 2: Best proxy formula to each species between biomass and morphological measurements at harvest.

Species	**Formula**	**R^2**
Bean	log(Total_dry_weight) ~ Number_leaves * Height	0.841
Pea	Total_dry_weight ~ Number_leaves * Height	0.835
Radish	log(Total_dry_weight) ~ log(Length_leaf) * Number_leaves * Height	0.933
Sorrel	log(Total_dry_weight) ~ Length_leaf * log(Number_leaves+1) * log(Height)	0.883
Spinach	log(Total_dry_weight) ~ Length_leaf * log(Number_leaves+1) * Height	0.971
Nasturtium	log(Total_dry_weight) ~ log(Length_leaf) * log(Number_leaves+1) * log(Height)	0.963
Maize	log(Total_dry_weight) ~ Number_leaves * Height	0.846
Kalanchoe	Total_dry_weight ~ Number_leaves * log(Height)	0.485

Table 2 shows the linear model that fits best the relationship between the biomass and the morphological measurements (height, length of the biggest leaf and number of leaves) for each species. From the eight species, the linear model is most accurate for the radish, spinach and nasturtium ($R^2 > 0.9$). Bean, pea, sorrel and maize all have a reasonable fit with $R^2 > 0.8$, but no suitable linear model was found for kalanchoe ($R^2 < 0.5$).

Plant growth and biomass production

Table 3: P-values for the effects of CO_2, light and their interaction on the shoot biomass, leaf biomass, height, number and length of leaf for each species. Yellow, light green and dark green represent marginally significant (p = 0.05 - 0.1), significant (p = 0.001 - 0.05) and highly significant (p < 0.001) p-values, respectively (n = 9).

	Bean	Pea	Radish	Sorrel	Spinach	Nasturtium	Maize	Kalanchoe
Shoot dry weight								
CO2	3.8E-07	0.049	0.594	0.001	0.043	0.105	0.825	0.002
Light	0.054	2.1E-04	0.041	3E-05	1.6E-06	0.837	0.003	3.3E-07
CO2 x Light	0.062	0.404	0.836	0.193	0.138	0.016	0.046	0.025
Leaf dry weight								
CO2	1.2E-06	0.106	0.674	1.83E-04	0.056	0.126	0.623	0.001
Light	0.001	0.011	0.005	5.6E-07	1E-06	0.304	0.006	1.6E-07
CO2 x Light	0.014	0.944	0.870	0.093	0.102	0.035	0.046	0.016
Height (harvest 3)								
CO2	2.8E-04	0.362	0.238	0.018	0.910	0.271	0.332	0.264
Light	0.223	0.454	0.013	0.325	4.2E-04	1.2E-05	1.4E-07	0.284
CO2 x Light	0.727	0.571	0.440	0.286	0.556	0.872	0.098	0.327
Number of leaves (Harvest 3)								
CO2	7.8E-05	0.075	0.129	0.015	0.071	0.623	0.003	0.071
Light	0.052	0.394	0.044	0.587	8.0E-06	0.502	0.121	0.140
CO2 x Light	0.711	0.560	0.404	0.813	0.231	0.453	0.393	0.417
Length of longest leaf (Harvest 3)								
CO2	0.001	0.733	0.245	0.019	0.831	0.166	0.380	0.286
Light	6.5E-05	0.448	0.357	0.427	0.307	0.009	1.91E-10	0.331
CO2 x Light	0.247	0.257	0.185	0.372	0.980	0.007	5.1E-05	0.428

Plant biomass

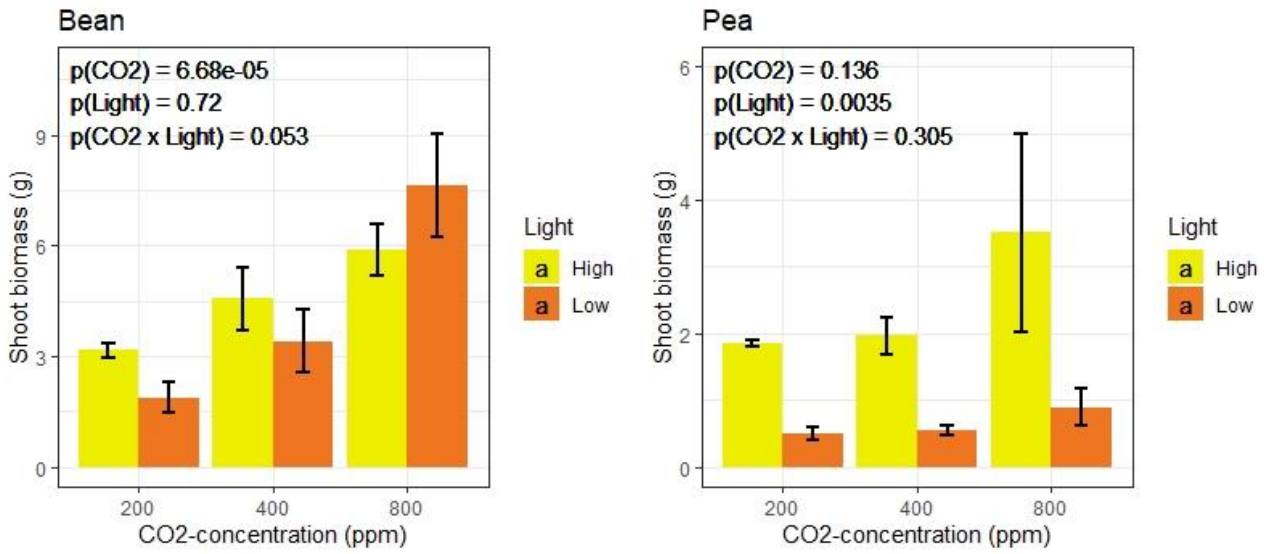

Figure 5: Shoot biomass of bean (left) and pea (right) per treatment for the last harvest. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction.

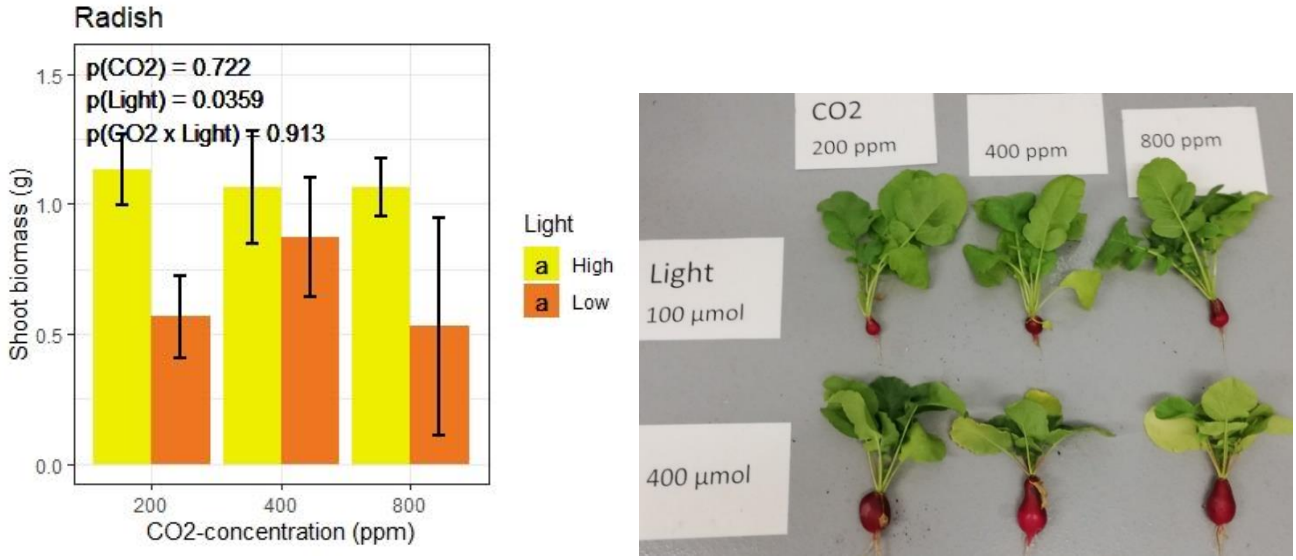

Figure 6: Shoot biomass of radish per treatment (left) at the last harvest. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction. Photo of one radish plant per treatment at the end of the experiment (right).

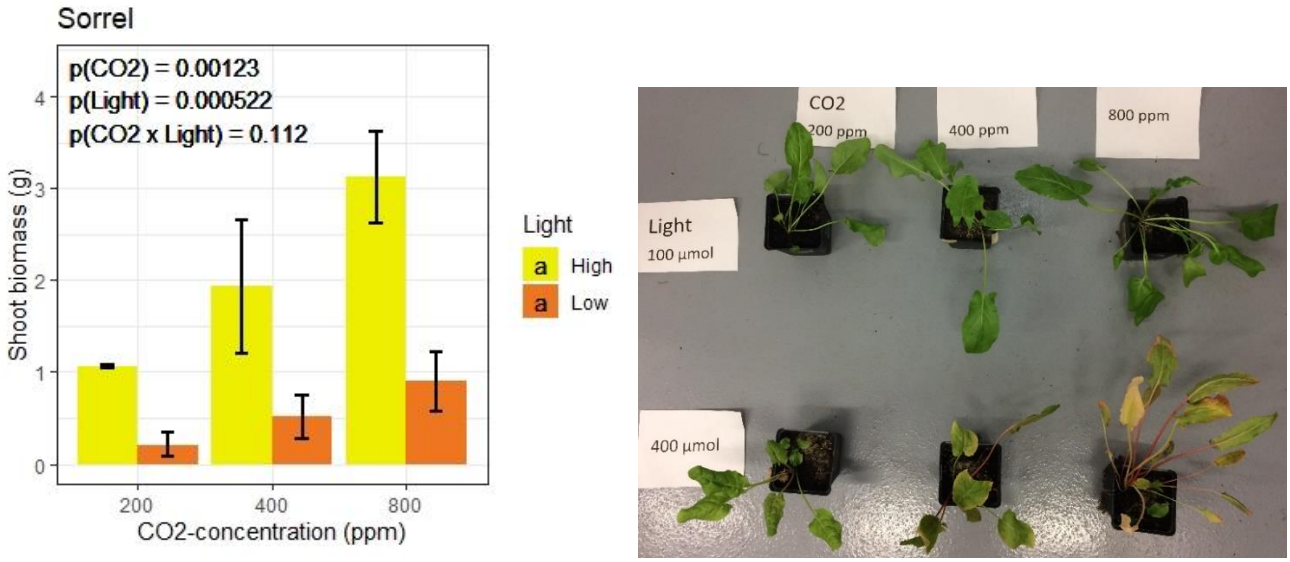

Figure 7: Shoot biomass of sorrel per treatment (left) at the last harvest. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction. Photo of one sorrel plant per treatment at the end of the experiment (right).

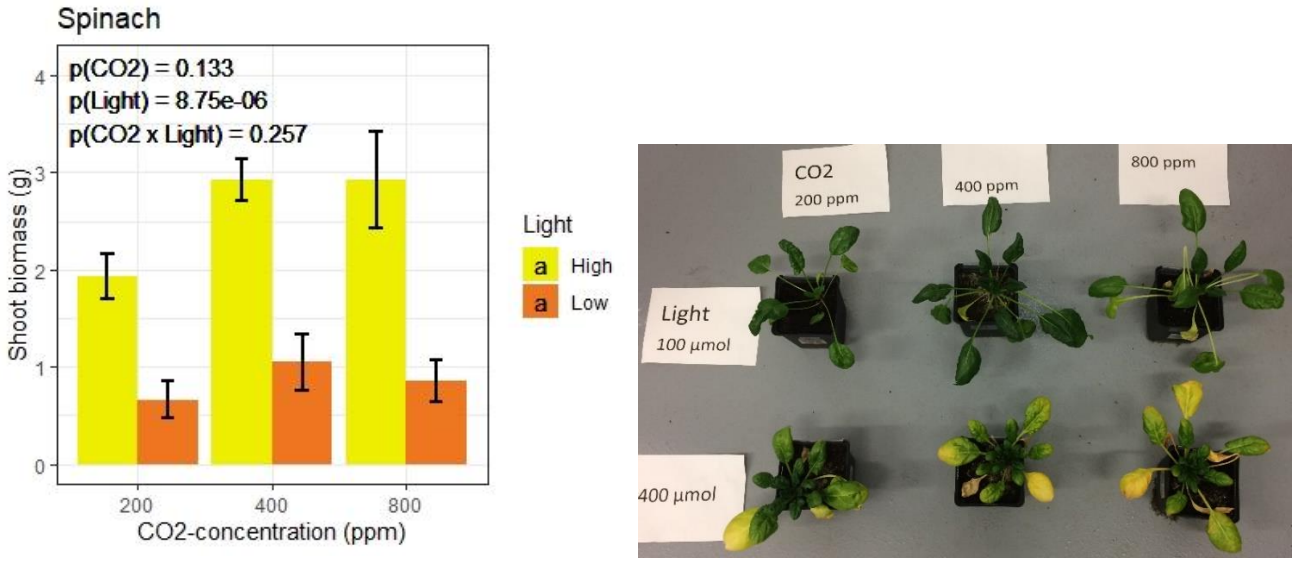

Figure 8: Shoot biomass of spinach per treatment (left) at the last harvest. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction. Photo of one spinach plant per treatment at the end of the experiment (right).

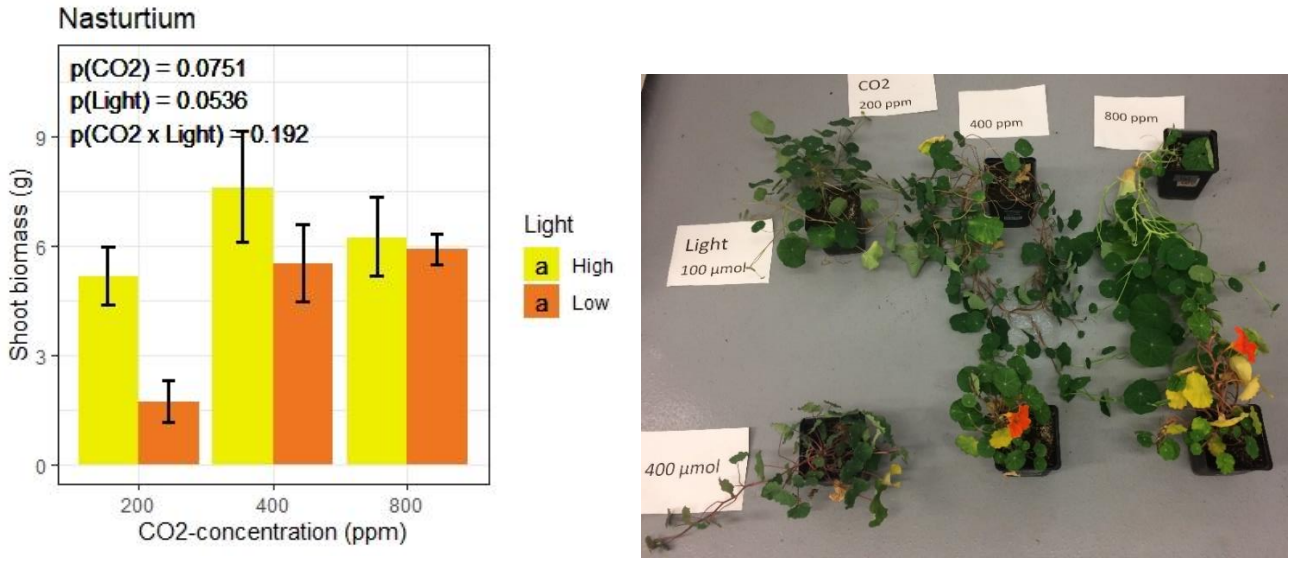

Figure 9: Shoot biomass of nasturtium per treatment (left) at the last harvest. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction. Photo of one nasturtium plant per treatment at the end of the experiment (right).

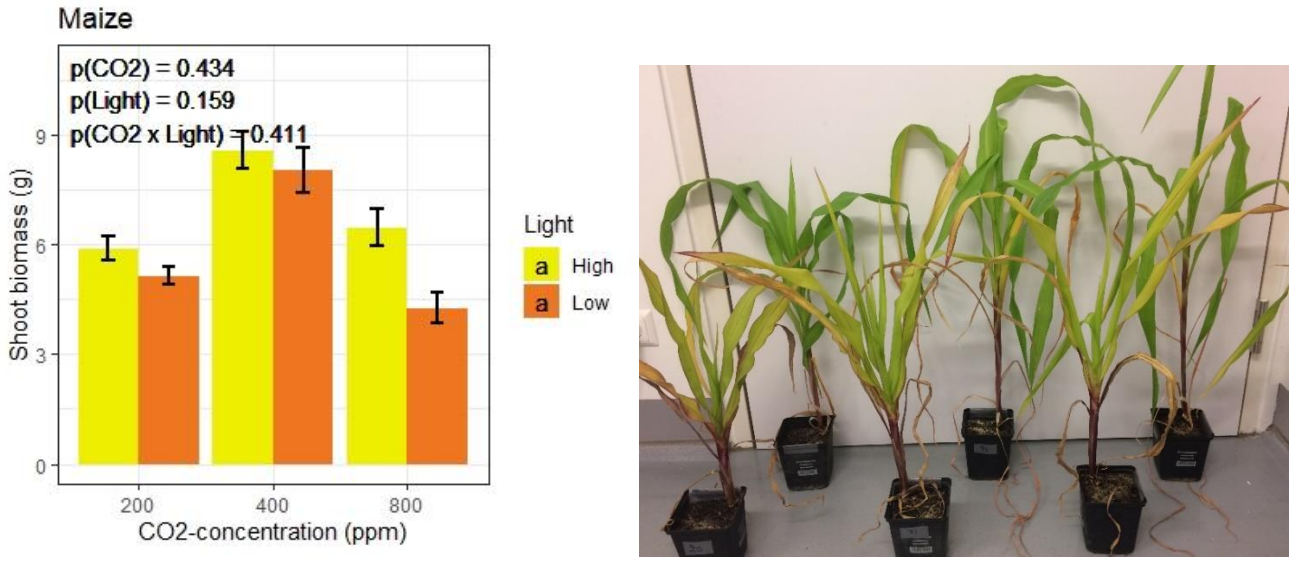

Figure 10: Shoot biomass of maize per treatment (left) at the last harvest. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction. Photo of one maize plant per treatment at the end of the experiment (right). From back to front; light treatments, and from left to right CO_2 treatments, in increasing order.

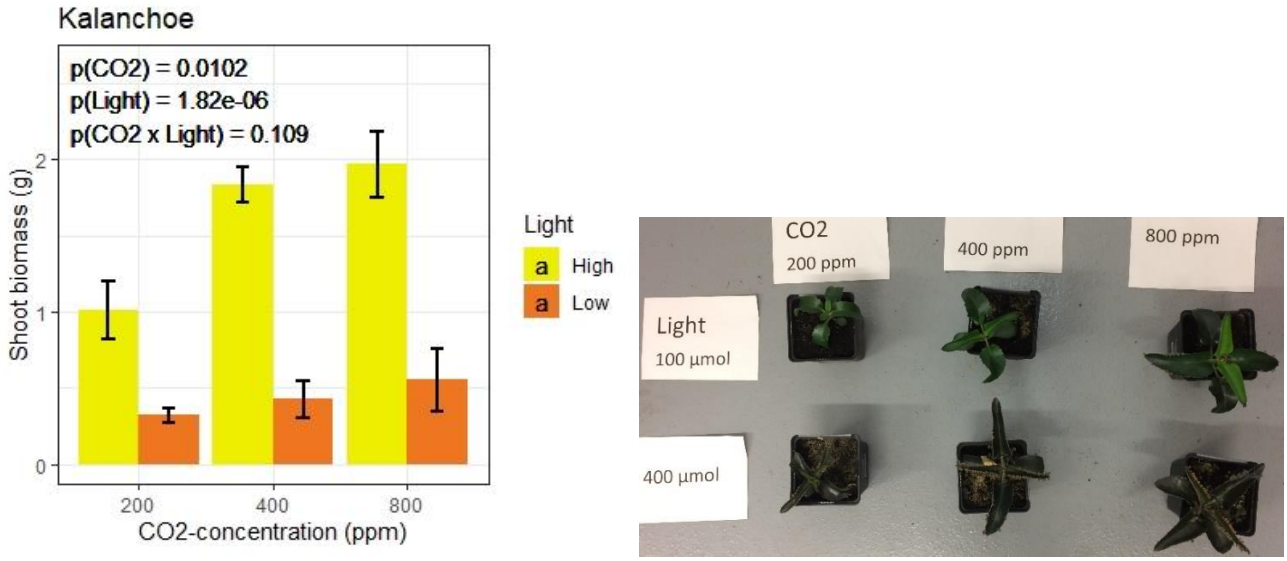

Figure 11: Shoot biomass of kalanchoe per treatment (left) at the last harvest. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction. Photo of one kalanchoe plant per treatment at the end of the experiment (right).

[CO_2] significantly ($p < 0.05$) affected the shoot biomass of five species, bean, pea, sorrel, spinach and kalanchoe (Table 3). For the five species the biomass increased as [CO_2] increased under both light conditions. It doubled from 200 to 800 ppm for bean and kalanchoe and tripled for sorrel (Figures 5, 7 and 11). The increase for spinach was substantial between 200 and 400 ppm and then plateaued between 400 and 800 ppm (Figure 8). Finally, the increase for pea was less pronounced, notably due to the higher variation in biomass for plants grown under 800 ppm (Figure 5).

Light significantly affected the growth of all species except nasturtium (Table 3). Bean was only marginally affected ($p = 0.054$) and the difference between the light treatments reverts for plants grown under 800 ppm [CO_2] where the biomass of plants grown under lower light was higher (Figure 5). For all the other significantly affected species, the biomass of plants grown under higher light was greater. For radish and maize, the difference was not as pronounced as for pea, sorrel, spinach and kalanchoe where the biomass of plants grown under high light was approximately four times greater than when grown under low light (Figures 5, 6, 7, 8, 10 and 11). The interaction between light and [CO_2] was significant for nasturtium, maize and kalanchoe (Table 3). No clear trends could be identified for nasturtium or maize (Figures 9 and 10). For kalanchoe, the biomass increase of plants grown under high light was greater than those of plants grown under low light. Thus, the difference between the biomass of the different light treatments increased as [CO_2] increased (Figure 11). The biomass of nasturtium was not significantly affected by either [CO_2] nor light but the plants grown in 400 and 800 ppm [CO_2] under high light had flowered by the end of the experiment (Figure 9).

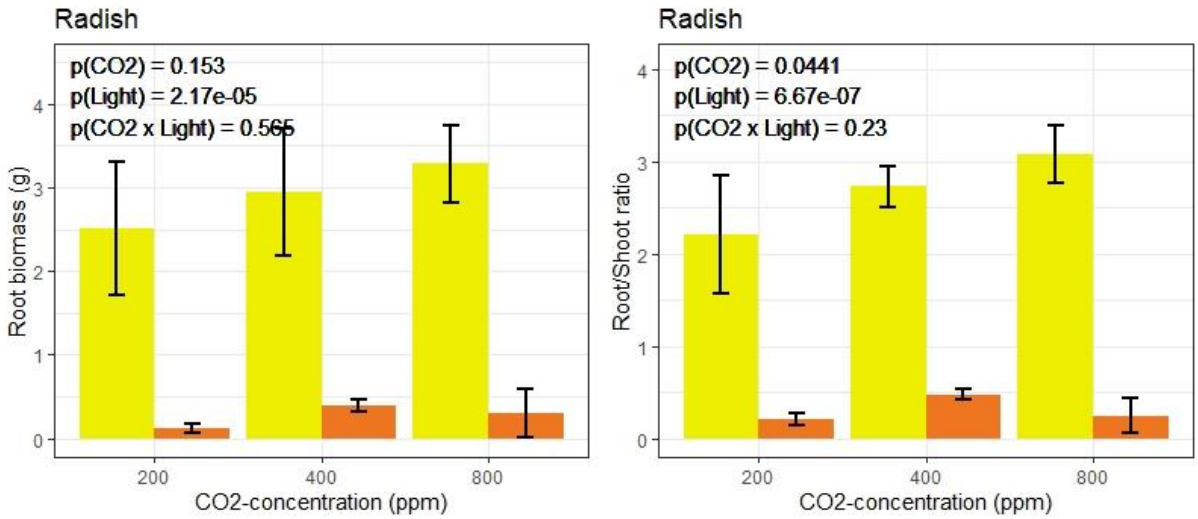

Figure 12: Root biomass of radish per treatment (left) and root/shoot ratio of radish per treatment (right). Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction.

Light conditions significantly affected the root biomass and the R/S of radish (Figure 12). Both increased greatly under higher light conditions. The interaction between [CO_2] and light was not significant for both ($p > 0.05$) but [CO_2] did significantly affect the R/S. From Figure 12, it appears that the R/S of radish increased from 200 to 800 ppm under high light. The increase under low light less visible.

Leaf biomass

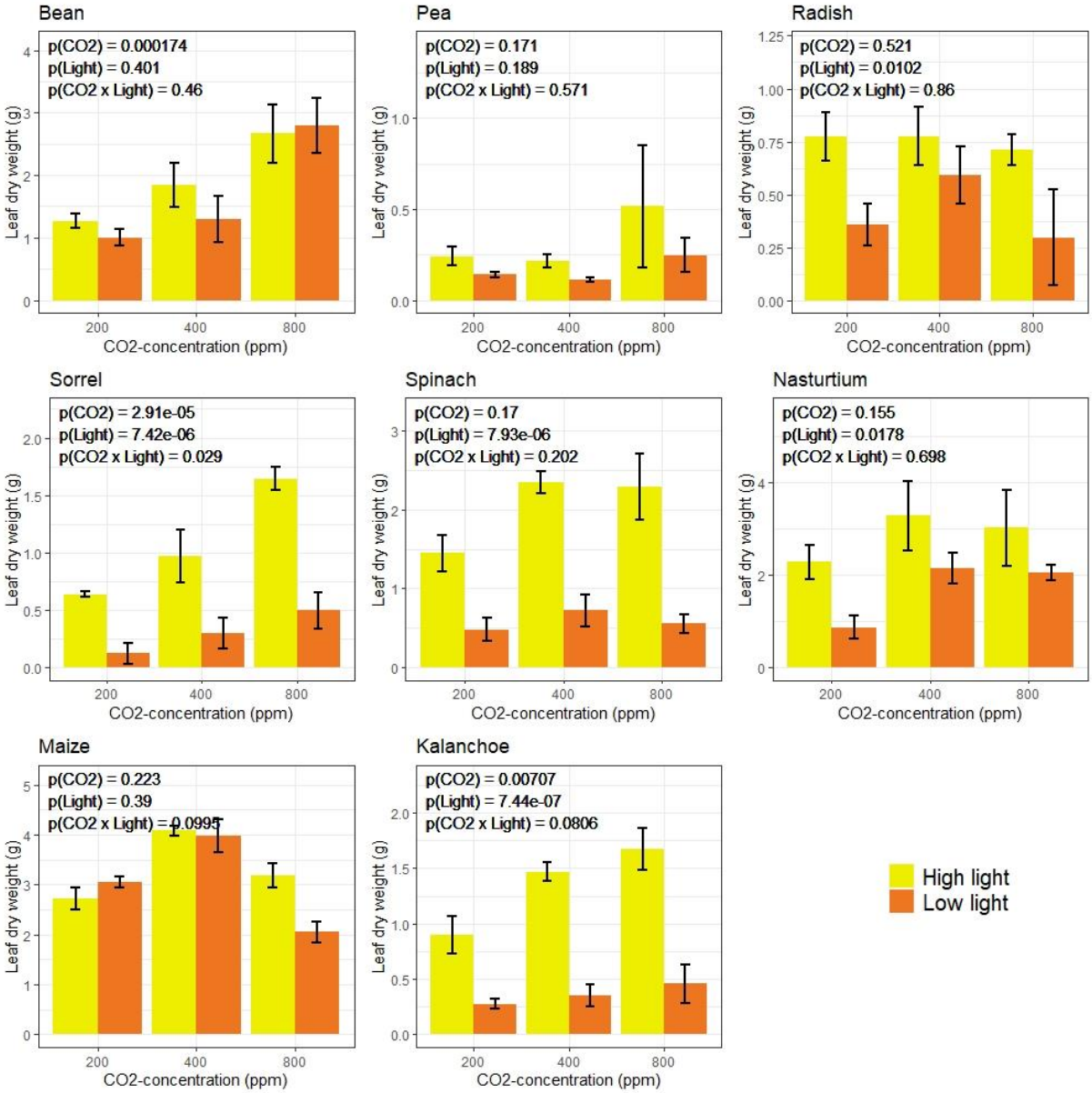

Figure 13: Leaf biomass per treatment for all species at the last harvest. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction.

The [CO_2] only significantly affected the leaf biomass of three species of the experiment, bean, sorrel and kalanchoe (Table 3). For these three species an increase in [CO_2] led to an increase in leaf biomass under both light conditions. This increase was greater for sorrel, and bean grown under low light, where it approximately tripled from 200 to 800 ppm (Figure 13). The leaf biomass of spinach was also marginally affected (p = 0.056, Table 3). This increase was more pronounced between 200 and 400 ppm and then plateaued between 400 and 800 ppm (Figure 13).

Light significantly affected the leaf biomass of all species except nasturtium (Table 3). Most of the species showed an increase in biomass as light increased. The magnitude of the difference was species dependent.

Pea, sorrel, spinach and kalanchoe showed a substantial difference in the biomass of the plants grown in low and high light conditions. The leaf biomass of plants gown under high light was about two times, for pea, and at least four times, for sorrel, spinach and kalanchoe, greater than those of the plants grown under low light (Figure 13). The difference was less pronounced for bean, radish and maize, where the leaf biomass of bean in 800 ppm and maize in 200 ppm [CO_2] was greater for plants grown under low light.

Finally, the interaction between [CO_2] and light was significant for bean, nasturtium, maize and kalanchoe (Table 3). All four species displayed different trends. The difference between light treatments appeared to decrease with increasing [CO_2] for bean and nasturtium whereas it increased for kalanchoe. No clear trend could be seen for maize (Figure 13).

Morphological measurements

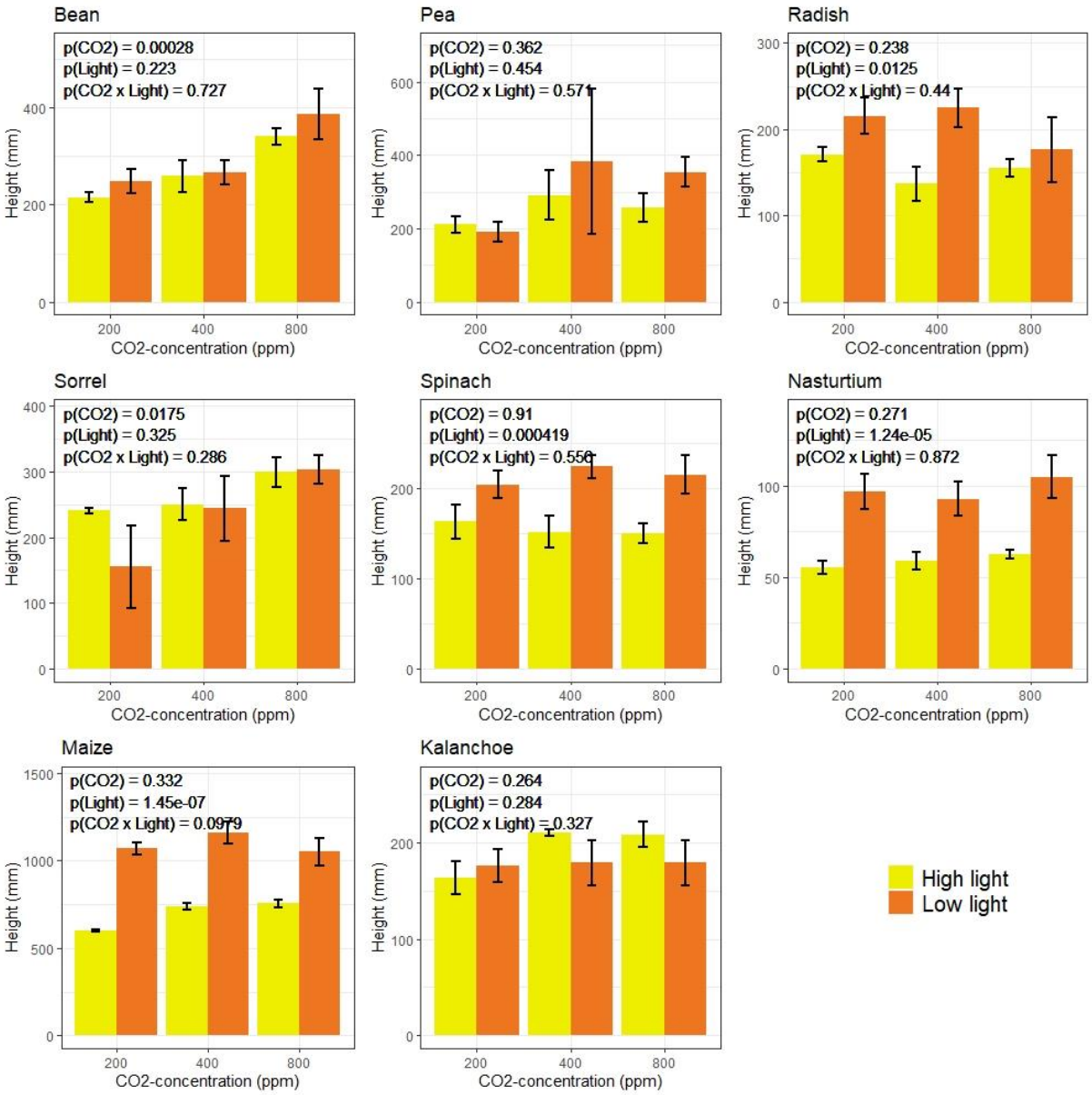

Figure 14: Plant height at the end of the experiment per treatment for all species. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction.

Plant height at the end of the experiment significantly increased with [CO_2] in bean and sorrel (p = 0.00028 and p = 0.0175 respectively, Figure 14). Height significantly decreased as light increased in half of the species, radish, spinach, nasturtium and maize ($p < 0.05$, Table 3). Overall, there was no significant interactive effect between [CO_2] and light on plant height.

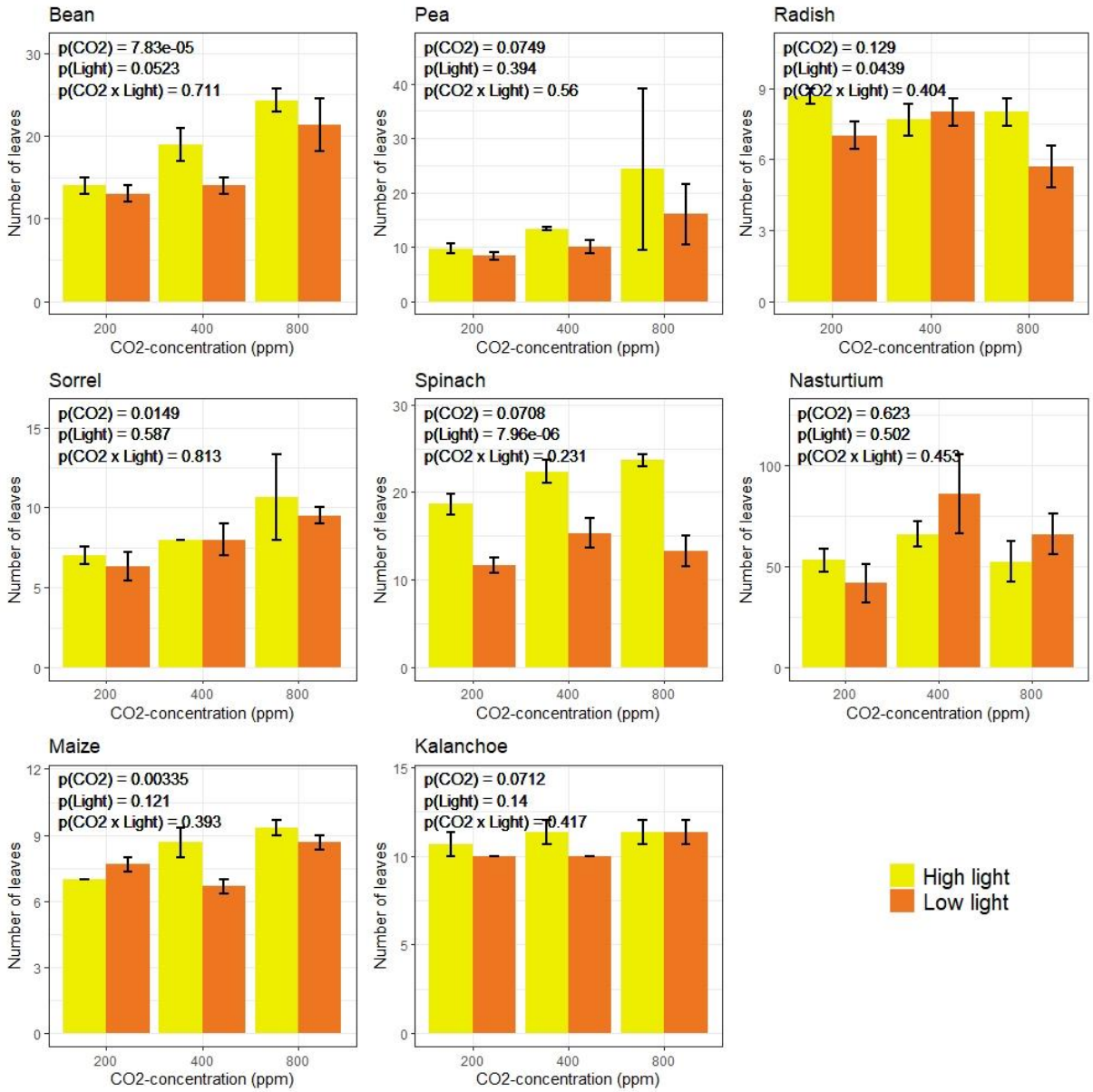

Figure 15: Number of leaves per plant at the end of the experiment per treatment for all species. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction.

The number of leaves per plant was significantly affected by [CO_2] for bean, sorrel and maize (Table 3) For bean and sorrel, the number of leaves increased as [CO_2] increased (Figure 15). The number of leaves in maize also tended to increase with increasing [CO_2] under high light but not under low light.

Light also significantly affected the number of leaves of radish, spinach and marginally affected bean (p = 0.0523, Table 3). For these three species, the number of leaves per plant increased as light increased with a more pronounced difference between the two light treatments for spinach. The number of leaves of the other species was unaffected by light and there was also no interaction between light and [CO_2].

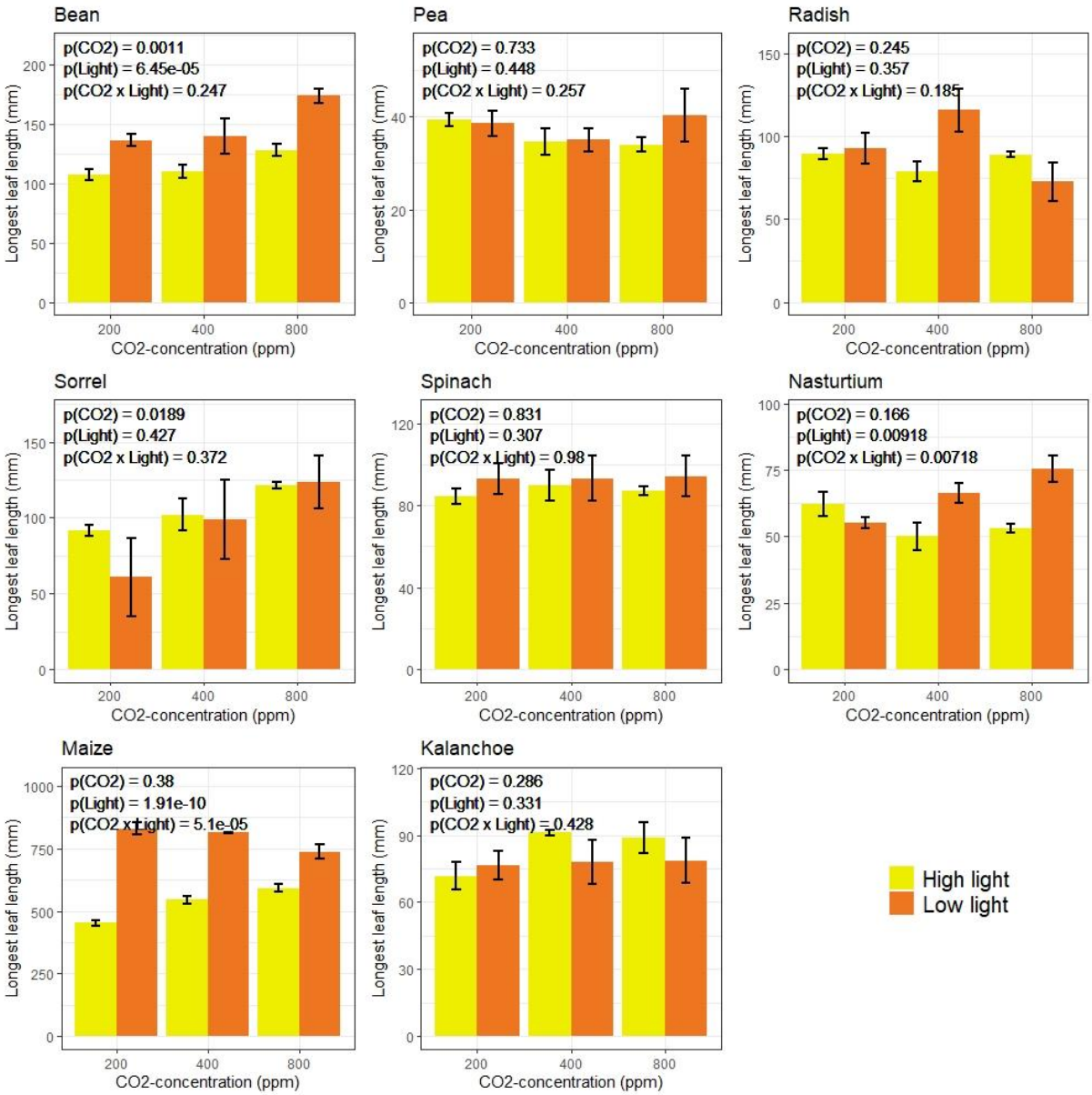

Figure 16: Biggest leaf length per plant at the end of the experiment per treatment for all species. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction.

Only the length of the leaves of bean and sorrel were significantly affected by [CO_2] (Table 3). The length of the biggest leaf of plants grown at higher [CO_2] was longer for both species and under both light conditions (Figure 16).

The leaf length of bean was also significantly affected by light with leaves grown under low light being longer than those grown under higher light (Figure 16). Finally, the leaf length of both nasturtium and maize were significantly affected by both light and the interaction between light and [CO_2]. Similar to bean, their leaves tended to be longer as the light conditions declined. Moreover, the leaf length of maize increased with increasing [CO_2] under high light but decreased under low light while the opposite trend was observed for nasturtium (Figure 16).

The differences among treatments were more reflected in the biomass than in the morphological measurements at harvest. Indeed, the [CO_2] and light significantly affected the shoot and leaves biomass of most species (Table 3). On the other hand, few differences were significant for the height of plants, their number of leaves or the length of the biggest leaf. The differences in biomass was poorly reflected by the morphological measurements at harvest (Figures 5 to 16).

Leaf morphological traits

Table 4: P-values for the effects of CO_2, light and their interaction on the SLA, LDMC and stomatal density. Yellow, light green and dark green represent marginally significant (p = 0.05 0.1), significant (p = 0.001 - 0.05) and highly significant (p < 0.001) p-values, respectively (n = 9).

	Bean	Pea	Radish	Sorrel	Spinach	Nasturtium	Maize	Kalanchoe
SLA								
CO2	0.351	0.642	0.096	0.227	0.002	0.003	0.639	0.065
Light	1.4E-04	0.074	3.8E-07	5.6E-08	5.7E-09	4.1E-08	0.002	4.0E-08
CO2 x Light	0.883	0.298	0.219	0.832	0.756	0.833	0.735	1.2E-05
LDMC								
CO2	0.003	0.044	0.168	0.017	0.014	0.003	0.669	1.9E-04
Light	3.9E-06	0.006	1.6E-07	1.2E-05	4.7E-09	3.1E-06	9.9E-11	3.6E-10
CO2 x Light	0.638	0.178	0.878	0.599	0.009	0.556	0.026	0.076
Stomatal density								
CO2	0.090	0.605	0.059	0.216	0.007	0.107	0.421	0.607
Light	1.2E-10	0.607	3.0E-09	0.023	4.1E-08	5.6E-05	5.9E-09	2.4E-04
CO2 x Light	0.144	0.332	0.292	0.005	0.612	0.718	0.421	0.136

Specific Leaf Area (SLA)

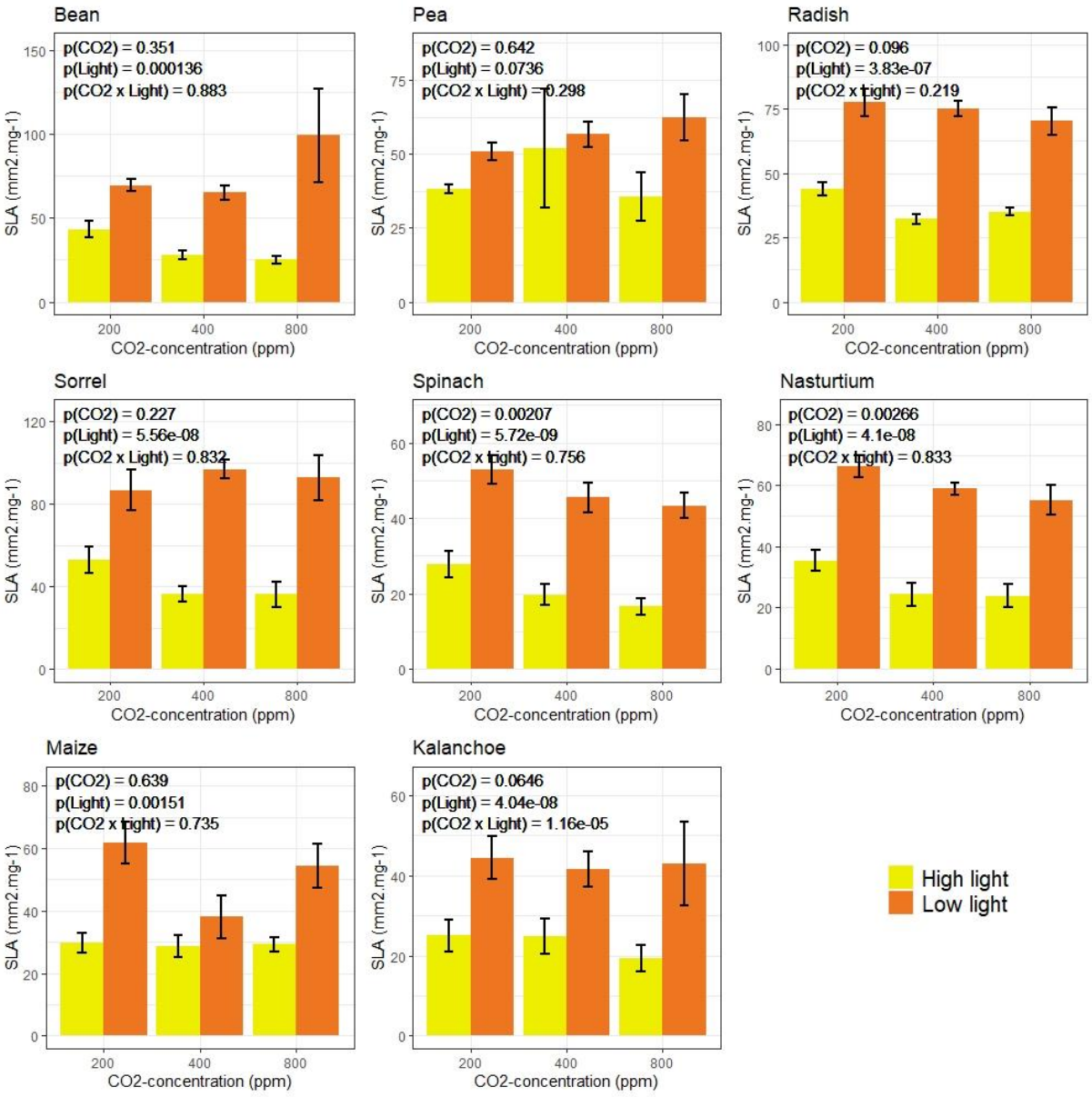

Figure 17: SLA per treatment for all species. Error bars are 1 SE (n = 9). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction.

Only the SLA of spinach and nasturtium were significantly decreased with increasing [CO_2] (Table 4). Both species presented a similar decrease in magnitude and under both light treatment (Figure 17). The interaction of [CO_2] and light significantly affected the SLA of kalanchoe where it decreased under high light more than under low light as [CO_2] increased (Figure 17).

A significant decrease in SLA with increasing light was found in all eight species in the experiment (Table 4). The SLA of plants grown under high light was on average half or less of the SLA of plants grown under low

light, except for pea where the differences was less pronounced (Figure 17). The light effect was thus much greater in magnitude and in the number of species affected than the effect of [CO_2].

Leaf dry matter content (LDMC)

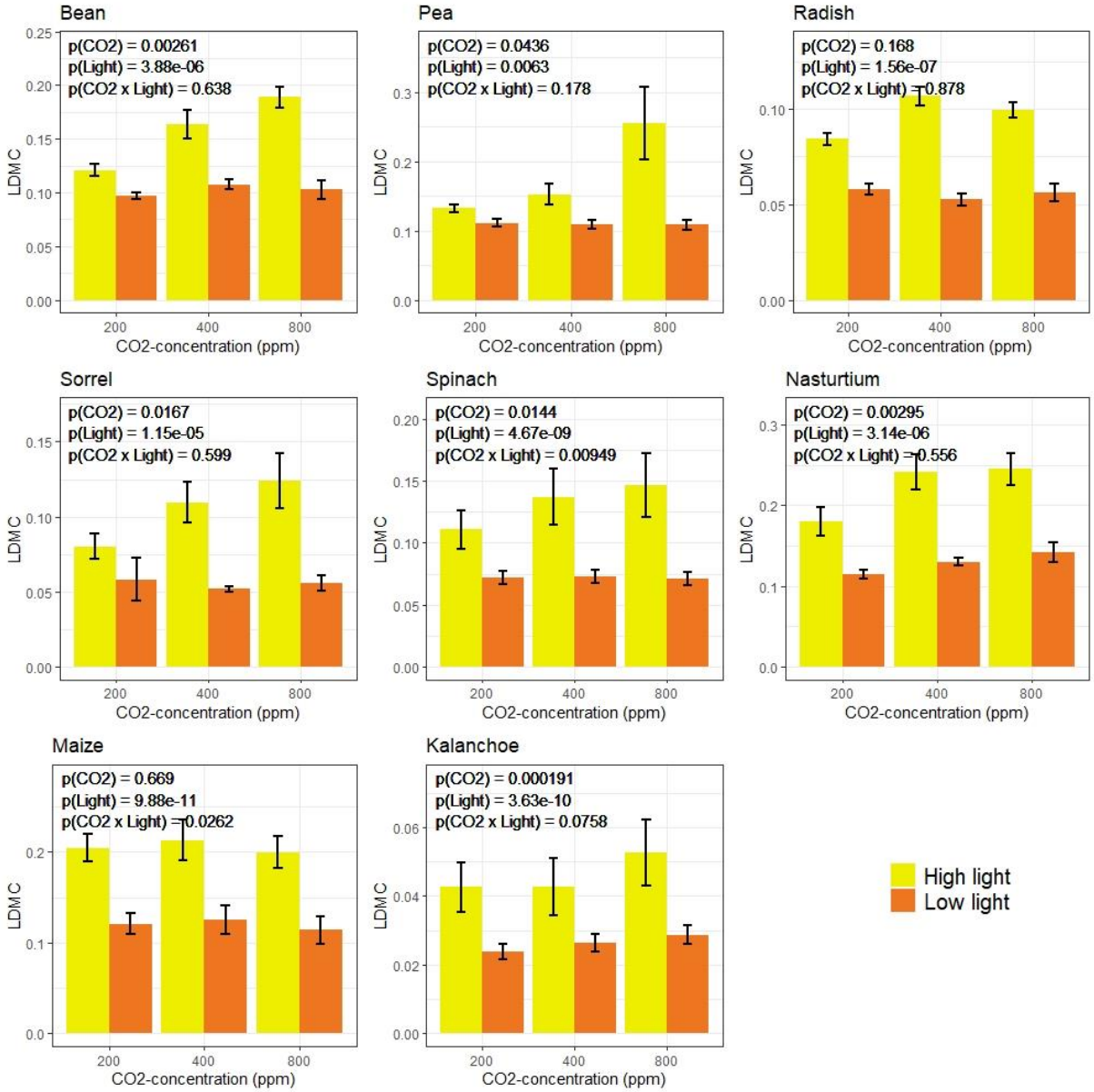

Figure 18: LDMC per treatment per species. Error bars are 1 SE (n = 9). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction.

The LDMC was significantly affected by [CO_2] for six species used in the experiment, bean, pea, sorrel, spinach, nasturtium and kalanchoe (Table 4). For all of them, the LDMC increased with increasing [CO_2] when grown under high light. However, it remained more stable under low light (Figure 18). Hence, the interaction of [CO_2] and light was significant for spinach (Table 4). As previously stated, the increase in LDMC was much more pronounced under high light which increased the difference between light treatment as [CO_2] increased

(Figure 18). The interaction was also significant for maize where variations were much less pronounced through both [CO_2] and light treatments.

Light significantly affected the LDMC of all species and in the same manner. LMDC was higher for leaves grown under high light than under low light (Figure 18).

Stomatal density (SD)

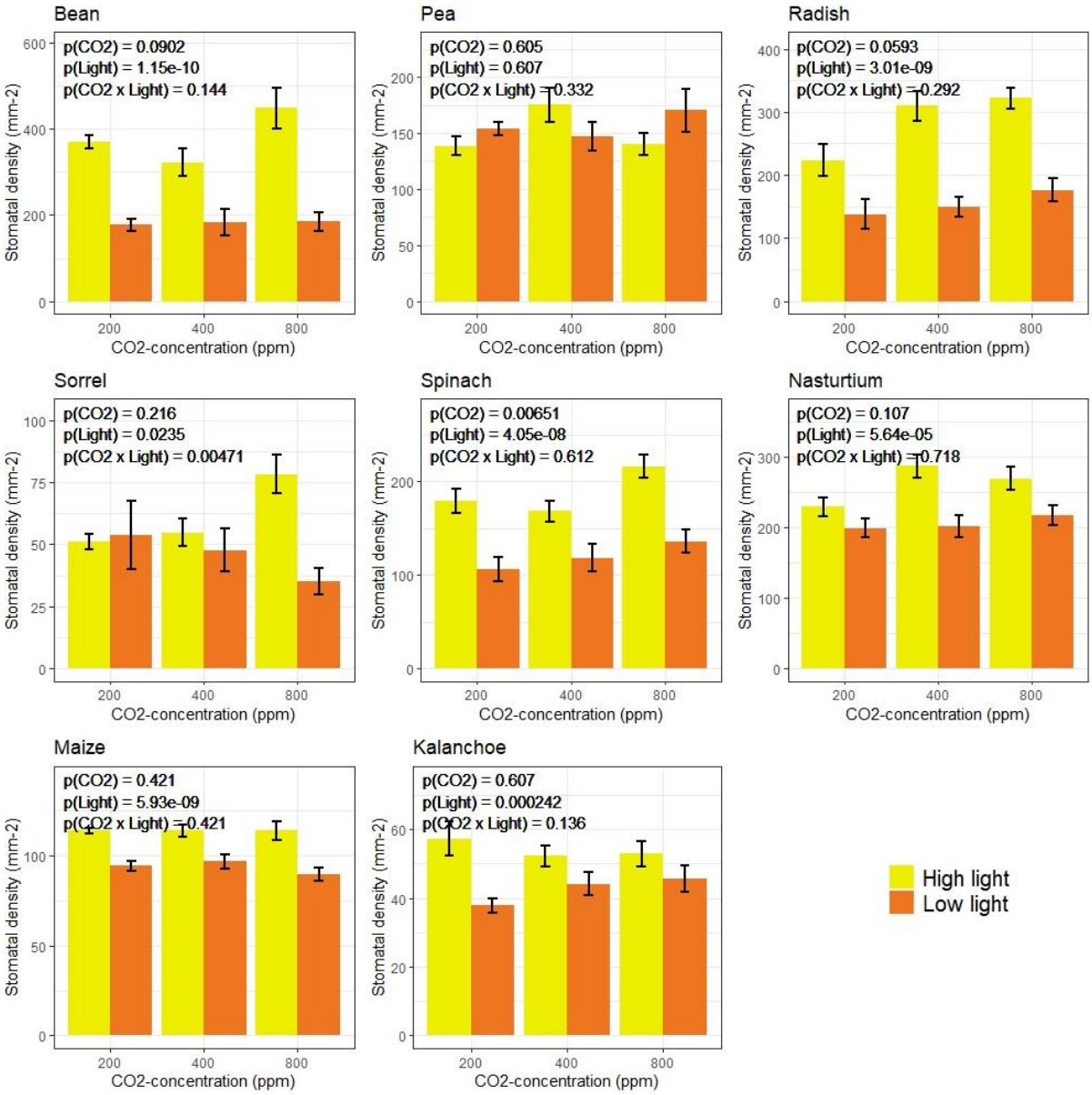

Figure 19: SD per treatment per species for the last harvest. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction.

Stomatal density was mostly unaffected by the [CO_2] in this experiment. Spinach only displayed a significant but slight increase in SD as [CO_2] increased (Figure 19). The SD of radish was only marginally significantly affected as well and display a similar increasing trend.

Higher light led to a significantly higher SD in all species but pea (Figure 19). The increase was more pronounced for bean and radish where the SD of plants grown under high light was approximately double the SD of the plants grown under low light. The SD of sorrel was significantly affected by the interaction between [CO_2] and light as it increased under high light but decreased under low light leading to a greater difference as the [CO_2] increased (Figure 19).

Light-response curves

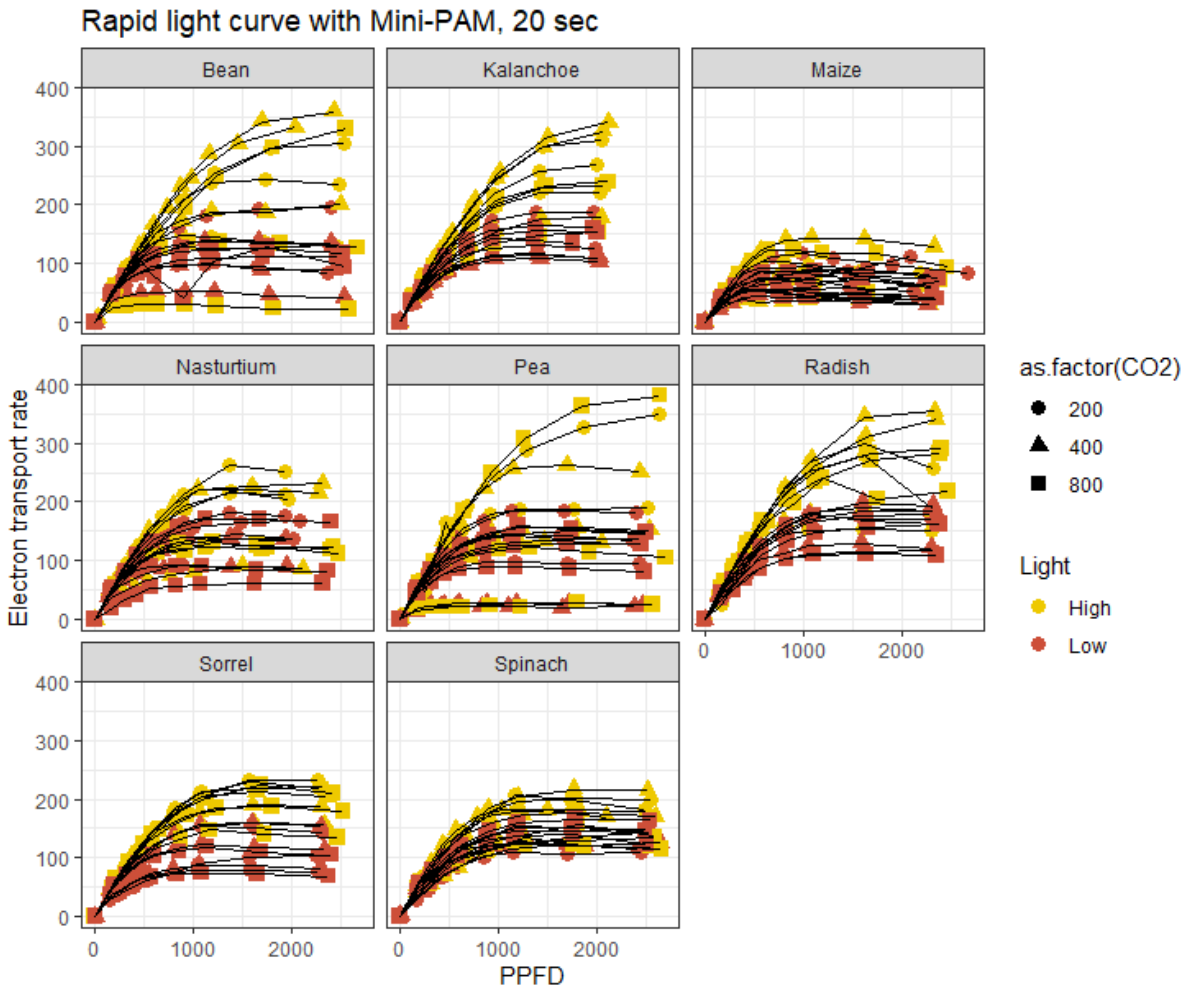

Figure 20: Light curves of individual plants per species and per treatments.

From Figure 20, it is possible to see that the light-response of the different species of the experiment differ between the different environments. Species such as bean, pea, radish, nasturtium and kalanchoe display a greater variation in their light response curve than species such as maize, sorrel and spinach for which the changes in growing conditions did not result in a pronounced change in their light response curves. The plants grown under low light appear to display, on the whole, lower ETR than those grown under high light.

Table 5: P-values of the effect of CO_2, light and their interaction on chlorophyll content and electron transport rate at saturating light. Yellow, light green and dark green represent marginally significant (0.05 to 0.1), significant (0.001 to 0.05) and very significant (lower than 0.001) p-values respectively (n = 9 for chlorophyll content and 3 for ETR at 1500 µmol m^{-2} s^{-}).

	Bean	Pea	Radish	Sorrel	Spinach	Nasturtium	Maize	Kalanchoe
Chlorophyll content (SPAD)								
CO2	0.025	0.029	0.521	0.151	0.449	0.873	1.6E-04	0.170
Light	0.130	0.910	0.002	0.021	0.001	0.847	1.6E-08	0.001
CO2 x Light	0.575	0.180	0.970	0.032	0.135	0.861	0.006	0.355
ETR at 1500 µmol m^{-2} s^{-1}								
CO2	0.309	0.529	0.712	0.289	0.067	0.006	0.802	0.414
Light	0.018	0.079	0.001	4.4E-05	0.004	0.035	0.320	1.6E-04
CO2 x Light	0.417	0.573	0.560	0.611	0.001	0.327	0.791	0.135

Chlorophyll content (SPAD reading)

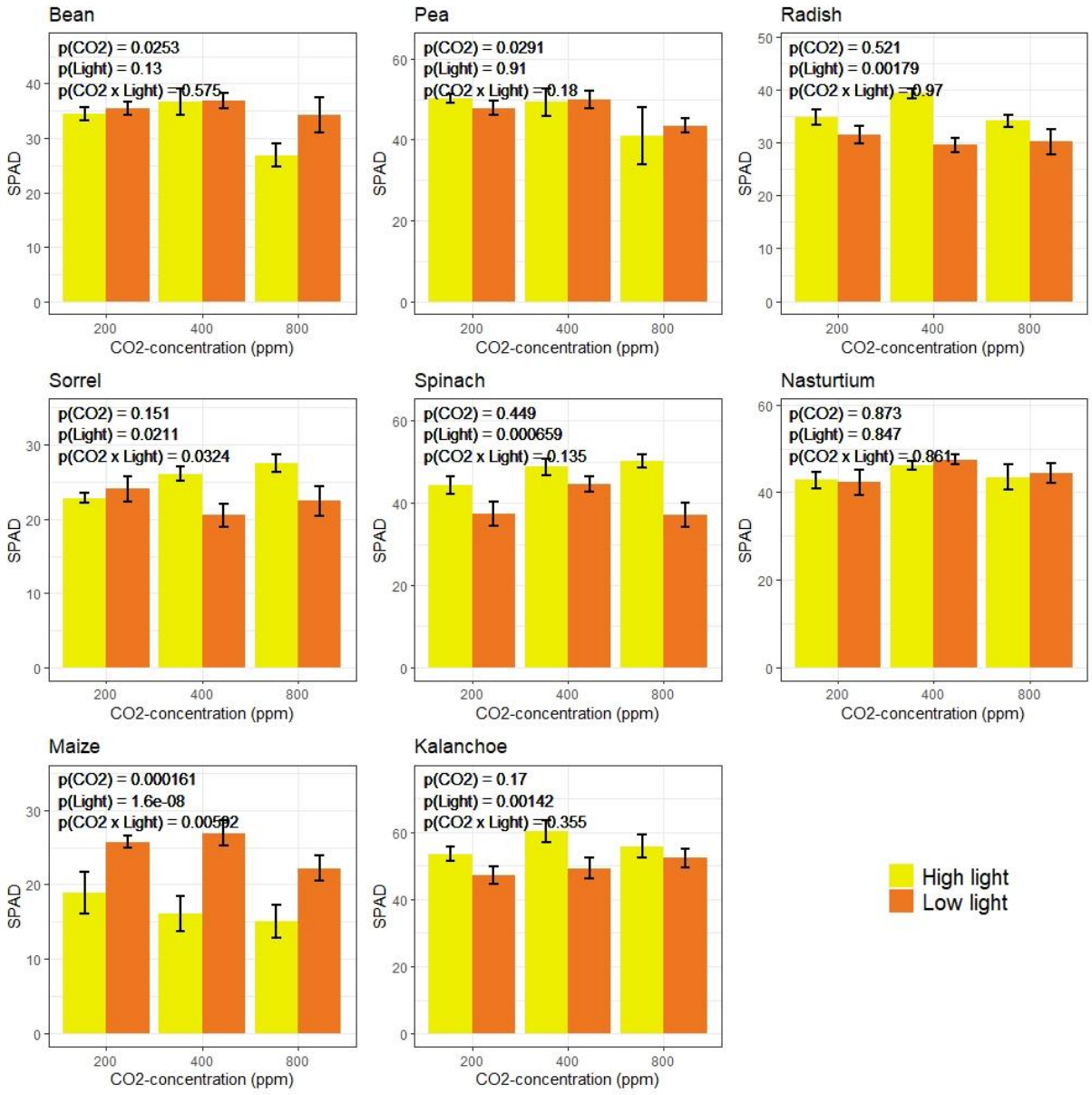

Figure 21: Chlorophyll content per treatment per species. Error bars are 1 SE (n = 9). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction.

The chlorophyll content of leaves was only significantly affected by the [CO_2] for bean, pea and maize where it decreased slightly with increasing [CO_2] under both light treatments (Figure 21). Light had a greater impact than [CO_2] on chlorophyll content with radish, sorrel, spinach, maize and kalanchoe all significantly affected by it (Table 5). The chlorophyll content of their leaves was slightly higher when grown under high light except for maize where it was much lower (Figure 21).

Electron transport rate at light saturation (ETR)

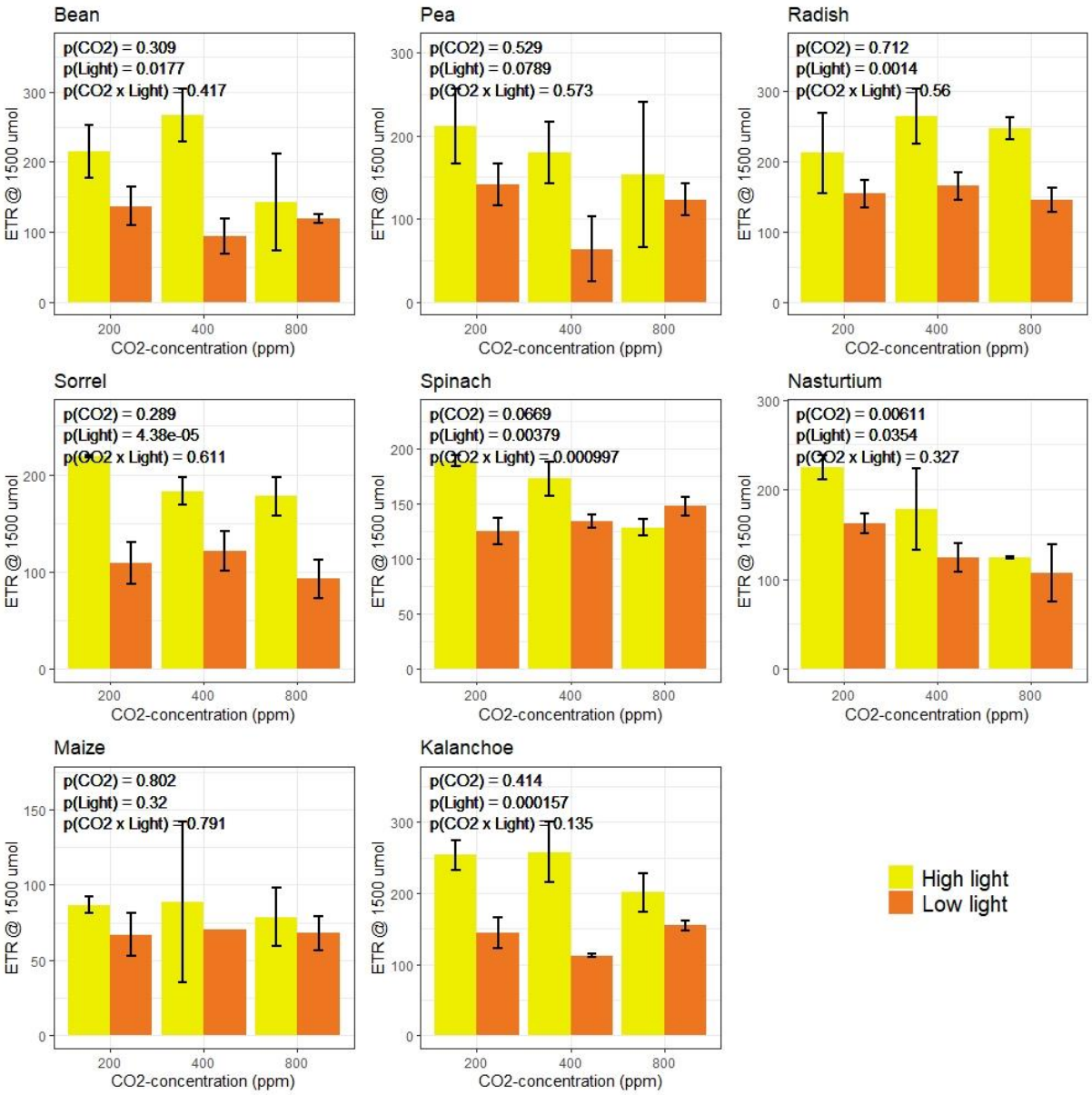

Figure 22: ETR measured at 1500 µmol m^{-2} s^{-1} per treatment per species. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2, light and the CO_2 x light interaction.

The [CO_2] only significantly affected the electron transport rate at saturating light in nasturtium (p = 0.006, Table 5). Under both light conditions, the ETR decreased with increasing [CO_2] (Figure 22). Spinach also exhibited a decrease in ETR with increasing [CO_2] when grown under high light but increased when grown under low light. Thus, the interaction of light and [CO_2] on ETR was significant with a greater difference between the light treatments at low [CO_2] (Figure 22).

The light treatment has a more common impact on the ETR at 1500 µmol m^{-2} s^{-1} with six species being significantly affected by it, bean, radish, sorrel, spinach, nasturtium and kalanchoe. Pea was also marginally

significantly affected by light (Table 5). The ETR for those species was higher for plants grown under high light even though the variability within treatments was large (Figure 22).

A/Ci curves

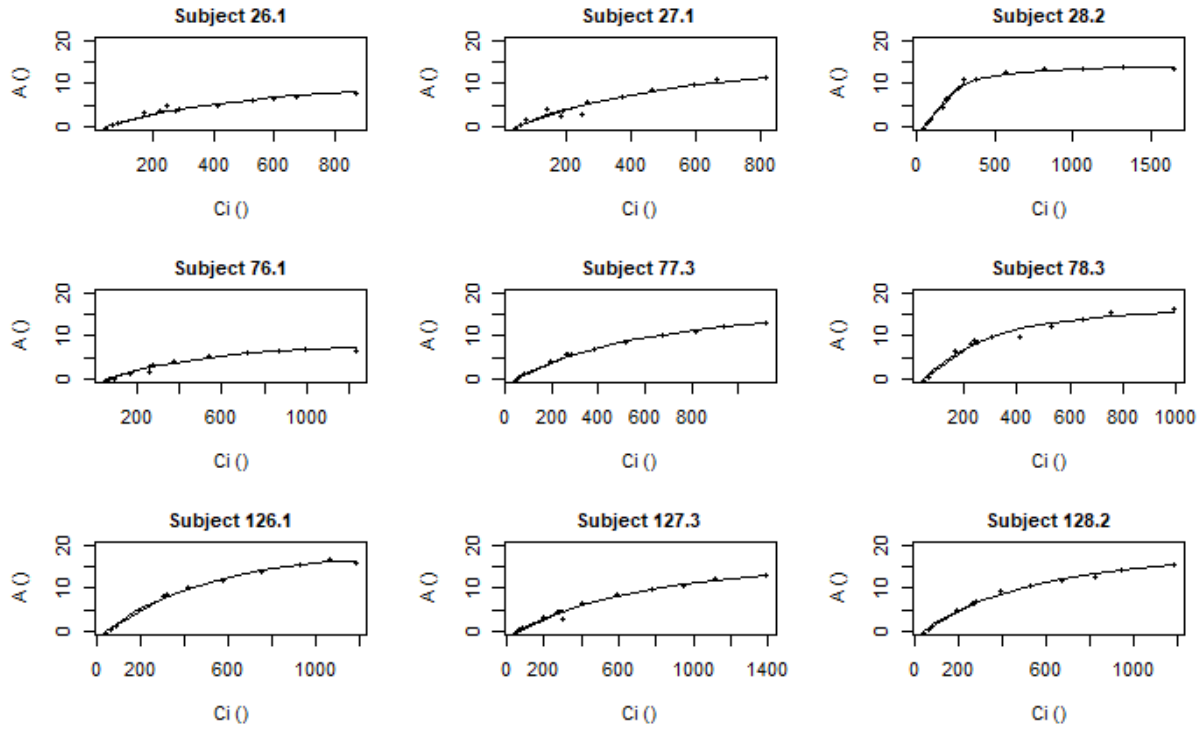

Figure 23: Individual A/Ci curves of bean grown under 100 µmol m^{-2} s^{-1} light. A is in µmol m^{-2} s^{-1} and Ci is in ppm. From top to bottom, plants grown at 200, 400 and 800 ppm [CO_2].

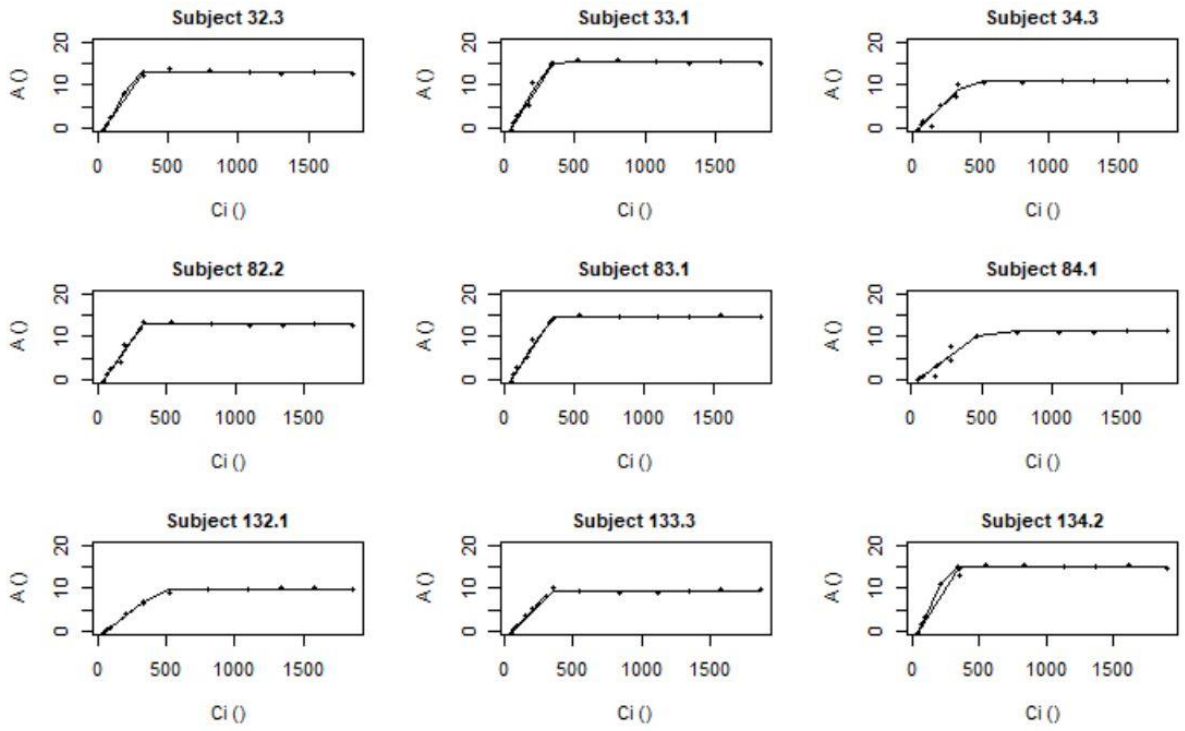

Figure 24: Individual A/Ci curves of radish grown under 100 µmol m^{-2} s^{-1} light. A is in µmol m^{-2} s^{-1} and Ci is in ppm. From top to bottom, plants grown at 200, 400 and 800 ppm [CO_2].

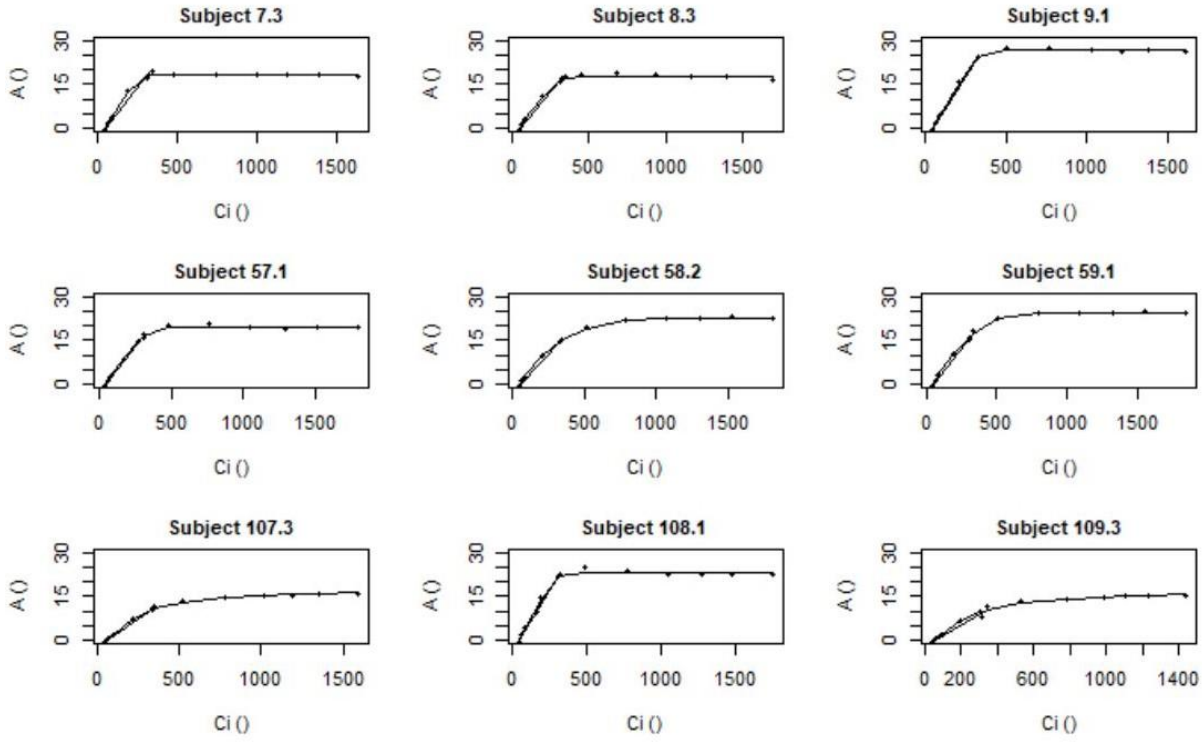

Figure 25: Individual A/Ci curves of radish grown under 400 µmol m^{-2} s^{-1} light. A is in µmol m^{-2} s^{-1} and Ci is in ppm. From top to bottom, plants grown at 200, 400 and 800 ppm [CO_2].

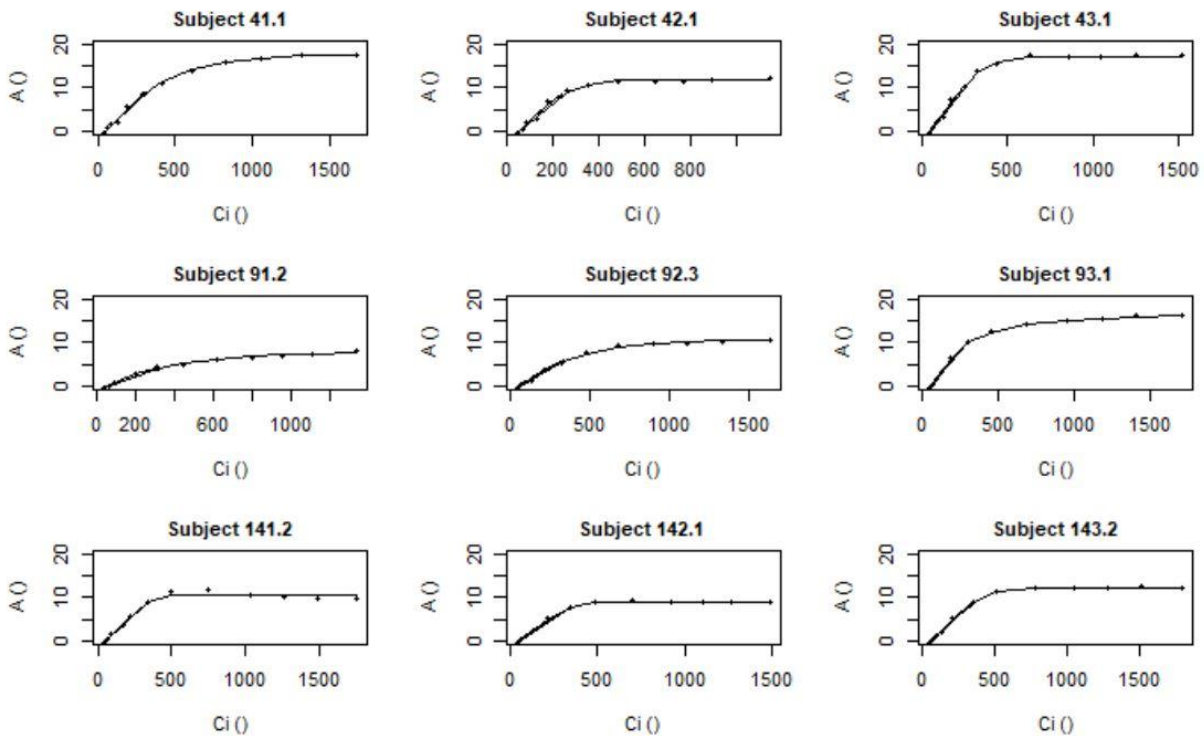

Figure 26: Individual A/Ci curves of nasturtium grown under 100 µmol m^{-2} s^{-1} light. A is in µmol m^{-2} s^{-1} and Ci is in ppm. From top to bottom, plants grown at 200, 400 and 800 ppm [CO_2].

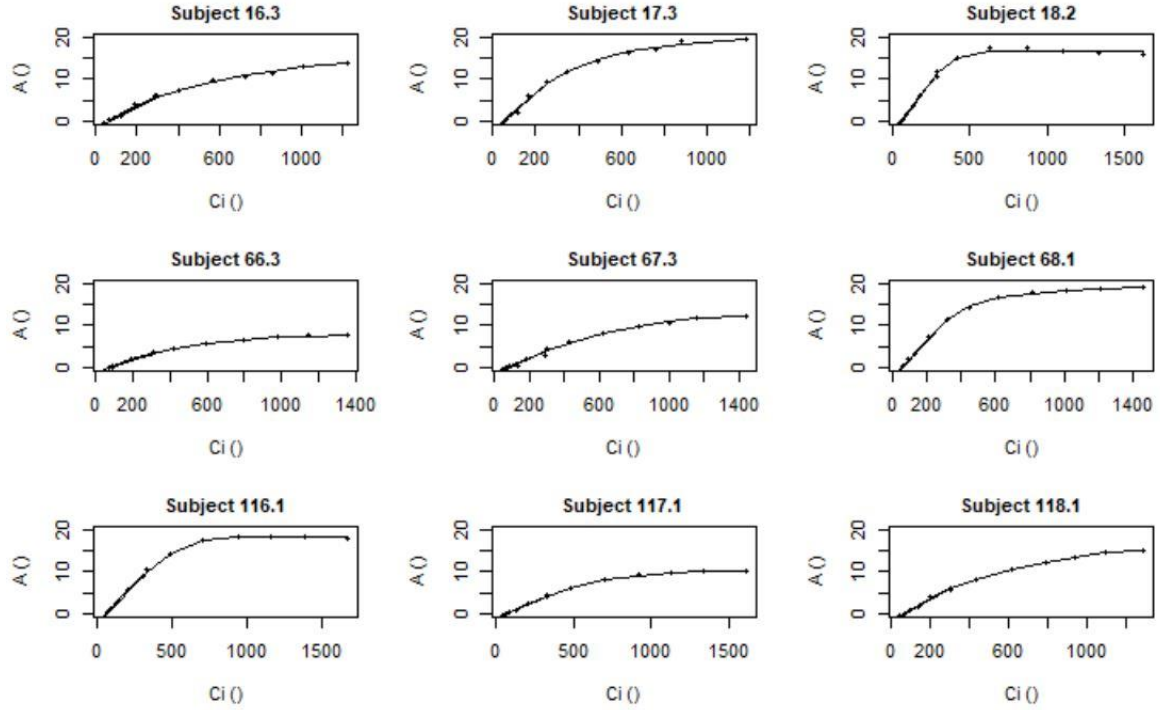

Figure 27: Individual A/Ci curves of nasturtium grown under 400 µmol m^{-2} s^{-1} light. A is in µmol m^{-2} s^{-1} and Ci is in ppm. From top to bottom, plants grown at 200, 400 and 800 ppm [CO_2].

The curves from Figures 23 to 27 all represent individual plants. They are the curves obtained from fitting the data according to the model from Tholen (2019, unpublished), not from plotting the data directly. There are no clear trends across different treatments and species as the individual curves vary even within one treatment.

Table 6: P-values of the CO_2 effect on the parameters derived from the A/Ci curves. The results are displayed per species and per light treatment (100 or 400 µmol m^{-2} s^{-1}, low and high respectively). Yellow, light green and dark green represent marginally significant (0.05 to 0.1), significant (0.001 to 0.05) and very significant (lower than 0.001) p-values respectively. The data for Vcmax for radish and for Tp for bean were not fitting to the model correctly and could not be used (NA).

	Bean	Radish	Radish	Nasturtium	Nasturtium
Light treatment	**Low**	**High**	**Low**	**High**	**Low**
Maximum carboxylation rate (Vcmax)					
CO2	0.796	NA	NA	0.249	3.7E-05
Respiration in the light (RL)					
CO2	0.249	8.5E-06	8.8E-05	0.935	2E-05
Mesophyll conductance (rm)					
CO2	0.529	0.0976	0.249	0.629	0.931
Triose-phosphate utilisation (Tp)					
CO2	NA	0.531	0.393	0.912	0.0208

Maximum carboxylation rate (Vcmax)

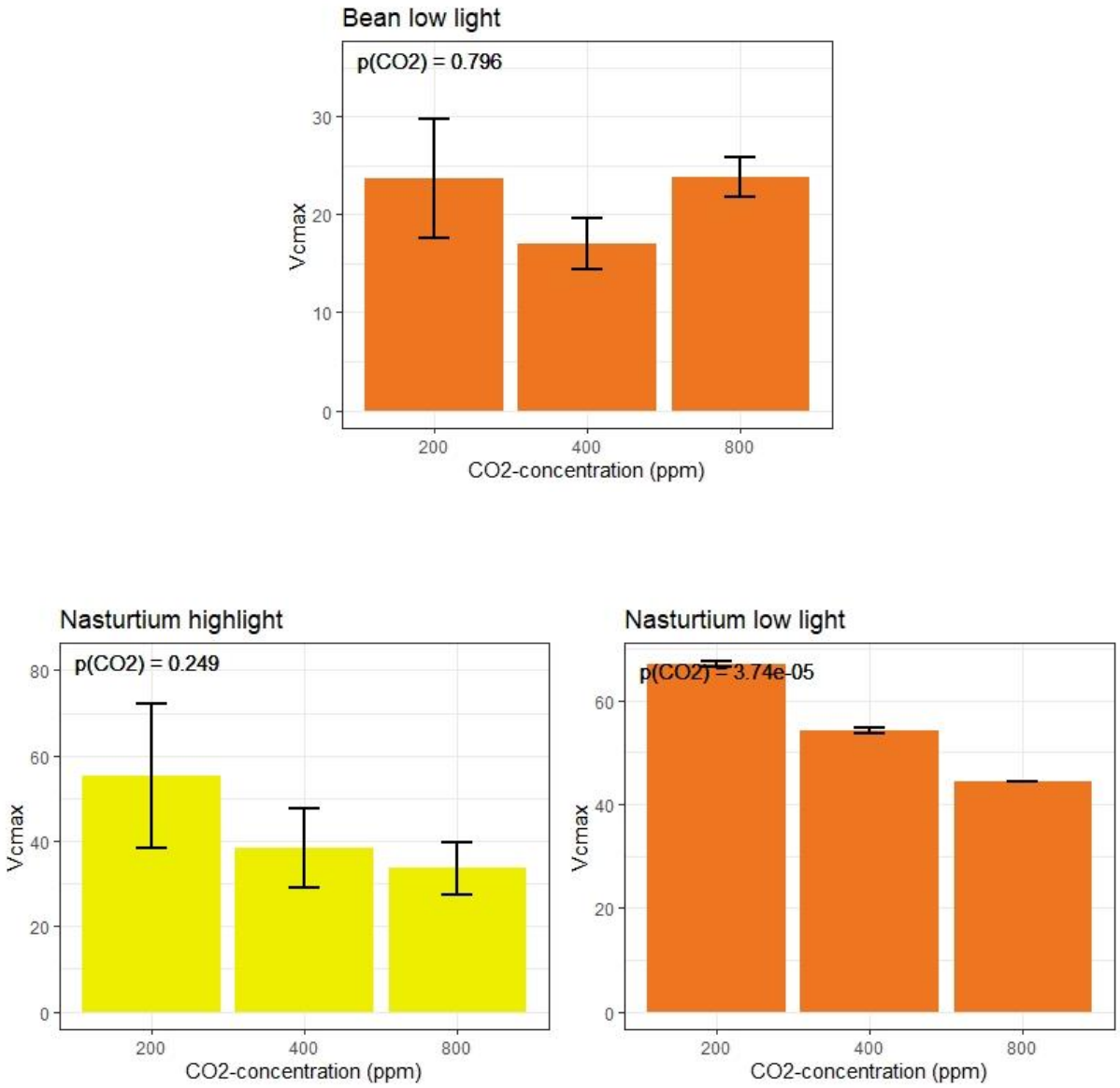

Figure 28: Maximum carboxylation rate, Vcmax, per treatment and per species. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2.

The carboxylation rate derived from the A/Ci curve was significantly decreased with increasing [CO_2] for nasturtium grown under low light only (Figure 28). A similar decrease can be observed for the nasturtium grown under high light but the difference was found not significant. The Vcmax values of radish were not fitting the model correctly and could thus not be used.

Respiration in the light (RL)

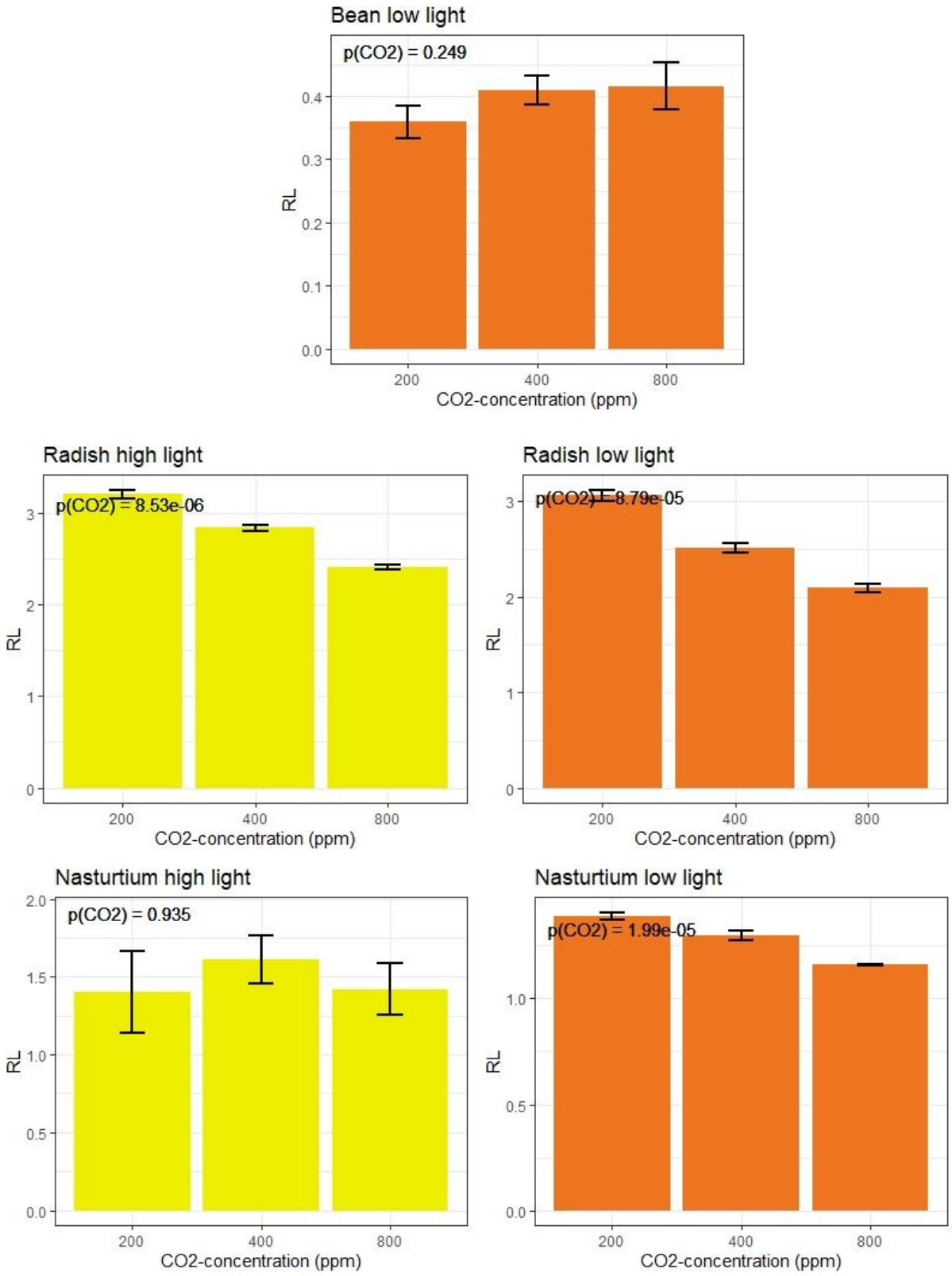

Figure 29: Respiration in the light, RL, per treatment and per species. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2.

The respiration of radish under both lights and nasturtium under low light were significantly decreased with increasing [CO_2] (Figure 29). This decrease was similar in magnitude across the different species and light treatments.

Mesophyll conductance (rm)

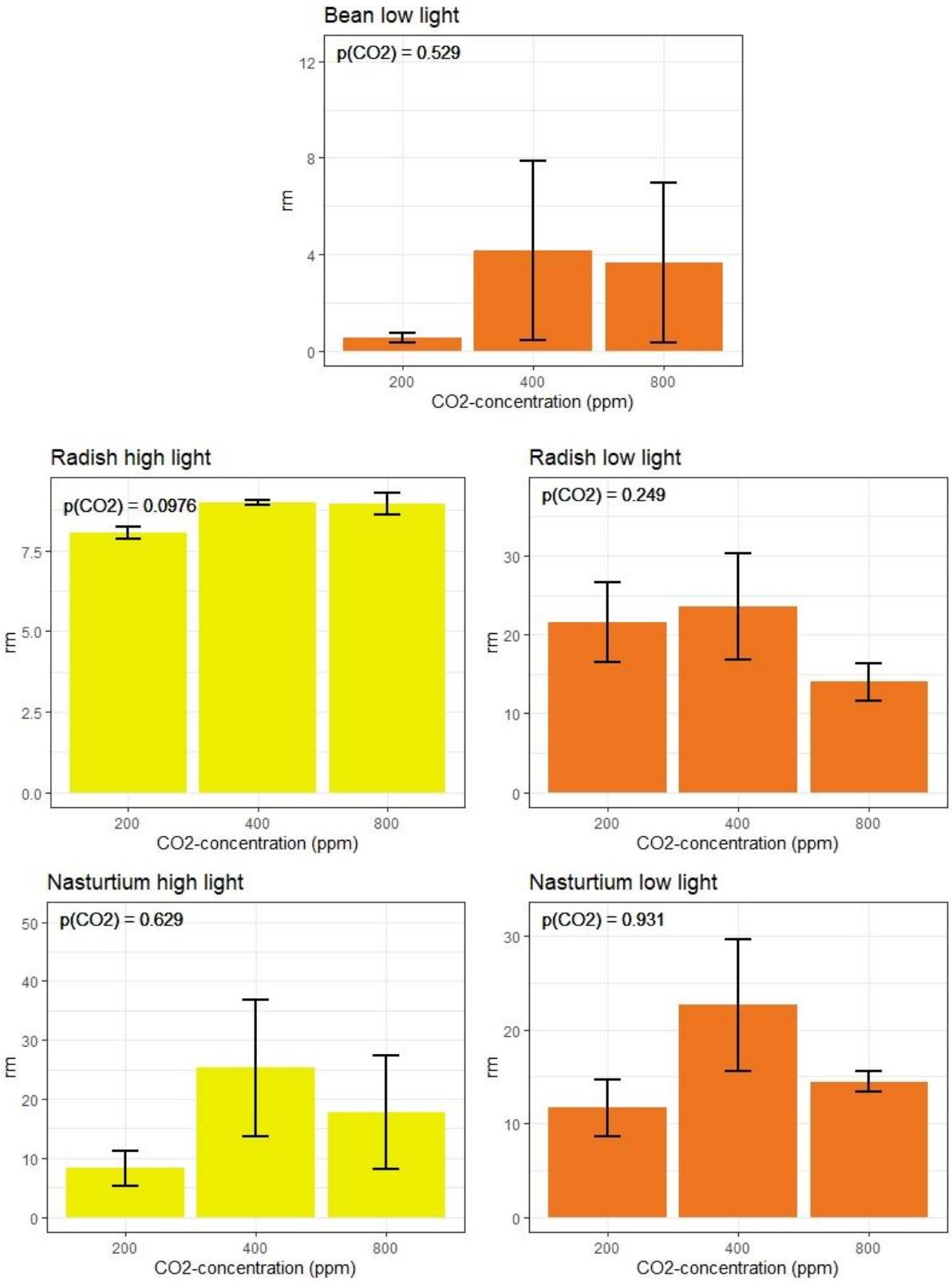

Figure 30: Mesophyll resistance to CO_2 diffusion, rm, per treatment and per species. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2.

The mesophyll resistance was only marginally affected by [CO_2] for radish grown under high light (Table 6). From Figure 30, it seems that rm increased with increasing [CO_2] but there was no common trend across the three species and two light treatments.

Triose-phosphate utilisation (Tp)

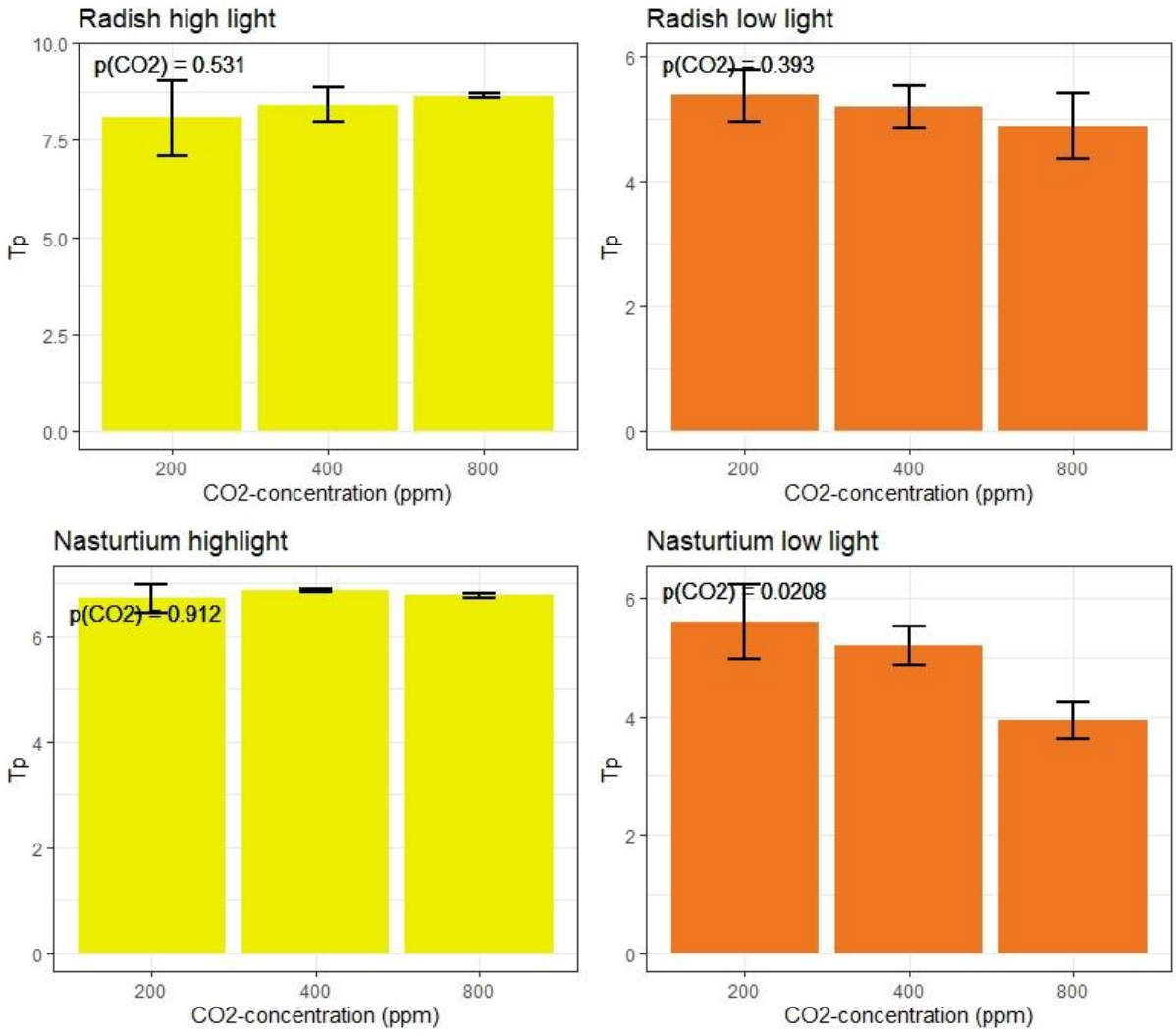

Figure 31: Triose-phosphate utilisation, Tp, per treatment and per species. Error bars are 1 SE (n = 3). Values in the graphs indicate p-values for the effect of CO_2.

From Figure 31, it appears that the triose-phosphate utilisation for nasturtium grown under low light only was significantly affected by [CO_2], where it decreased with increasing [CO_2]. The values for this parameter for beans were not fitting the model correctly and thus were removed.

6. Discussion

I. Limitations of the experiment

The findings of this experiment must be understood within the limits that the experimental design created. Plants grown in growth chambers are exposed to a unique growing environment that is likely to impact the results. Despite setting the same parameters, beside light intensity and [CO_2], equal, these probably varied slightly from chamber to chamber. Moreover, three main issues have been identified to impact the results presented here. Firstly, the limited number of replicates, secondly, the competition between plants in a closed environment and thirdly, nutrient limitation.

Limited number of replicates

Due to the space limit of each treatment and the number of species included in the study, a limited number of replicates could be used. For each treatment only nine plants of the same species were grown in total. Out of these just three were kept to the end of the experiment and were available for gas-exchange measurements. Due to the high variability that plant physiology display within one treatment the low sample size reduces the probability of detecting significant differences between treatments.

Plant competition

As the space within each treatment was limited, plant competition increased as plants grew. The bigger or faster growing species have consequently shaded the smaller plants. This has impacted the plants' access to light and consequently is likely to have also impacted the growth rate. Moreover, there were three plants per pot. Experiments in growth chamber are often impacted by the need to use pots with limited soil volume. The presence of several plants within one pot reduced the available soil space for each. For that reason, plants were sequentially harvested so that for the final harvest only one plant per pot remained.

Nutrient limitation

Finally, the finite soil available also led to nutrient deficiencies by the end of the experiment. All species, apart from kalanchoe, displayed leaf discoloration in some individuals, mostly under high light when grown in 400 and 800 ppm [CO_2] (Figure 6 to 10). This deficiency is likely to have impacted the magnitude of the measurements conducted. As this nutrient deficiency impacted first the treatments displaying greater growth, it is also possible that it reduced the difference between the treatments.

II. Biomass

Biomass proxy

Plant height, leaf number and/or length of the biggest leaf at harvest were positively correlated with the total shoot biomass in four of the species used, namely radish, sorrel, spinach and nasturtium ($R^2 > 0.85$, Table 2). Hence, in these species the parameters are good proxy measures of the biomass and its variation through different [CO_2] and different light availabilities, which is useful as biomass per se can only be obtained destructively. It also provides information on how biomass is allocated during growth and in different treatments. A documented effect of [CO_2] on plant growth is an increase in shoot length due to the increased biomass (Palit et al., 2020). It thus appears that measuring plants' size and leaf characteristics is a good way to estimate biomass of these species during growth without having to harvest replicates and allowing for a greater number of individuals being kept until the end of the experiment. Better estimation of the correlation between morphological measurements and biomass in this experimental setting could be obtained by increasing the number of measurements, i.e. measure the size of several leaves or increasing the number of replicates.

In bean, pea and maize the correlation between total biomass and plant height and the number of leaves was relatively lower (R^2 between 0.8 and 0.85, Table 2). The R^2 value is still high enough that it can be said that there is a correlation between these parameters, but the uncertainty is increased. The increased uncertainty indicates that other morphological measurements might be better suited to estimate biomass during growth for these species. Moreover, this correlation was not relevant for kalanchoe with the best possible correlation not exceeding a resulting R^2 of 0.485 (Table 2). Kalanchoe is a slow growing species with leaves slowly growing and in pairs which may explain why the variations in biomass found in the results were not reflected in the plants' morphological measurements.

Overall, estimation of correlation between biomass and plants' morphological characteristics is useful for a better apprehension of future ecosystems. Experimental data could potentially indicate the range of added biomass that an increase in [CO_2] could generate for various plant species or plant type. Furthermore, if we know how this biomass is related to how tall plants become or how the leaves are developed then it could help to better model the composition of future ecosystems and how these might enhance further changes.

Light effect on shoot biomass

The biomass was mostly significantly affected by the light treatment. Plants grown under high light had significantly higher shoot biomass in six out of eight species ($p < 0.05$, Table 3). It appears that plants grown under high light have greater biomass than plants grown under low light (Figures 5 to 11). This indicates that light was a limiting factor for growth. A similar increase under higher light intensity was also found on coffee

plants (Marçal et al., 2021). The difference in bean was also marginally significant ($p = 0.054$, Table 3) where the biomass of plants grown under high light was greater only when grown in either 200 or 400 ppm [CO_2]. It is interesting to note that bean and nasturtium growing under high light had started flowering, and producing fruits for bean, in the 800 and 400 ppm chambers (Figure 9). Therefore, the lack of significant difference in biomass for bean in 800 ppm and nasturtium could be explained by a difference in resource allocation at different developmental stages. Marchiori et al. (2014) found that the shaded part of sugarcane were morphologically different to the well-lit part with, for instance, elongated stalks. Hence, in our study, for plants growing under low light, the products of photosynthesis were invested in a taller plant with larger leaves. By contrast, plants grown under high light reached maturity faster and used the photosynthesis products for reproductive development.

[CO_2] effect on shoot biomass

The CO_2 effect on biomass was less pronounced as compared with light. Out of the eight species included in this study, five showed a significant increase in above ground biomass as the [CO_2] increased ($p < 0.05$, Table 3 and Figures 5, 7, 8 and 11). The increase was clear for bean, pea, sorrel and kalanchoe. The shoot biomass of spinach plateaued between 400 and 800 ppm which may be due to the limitations of space and nutrients. The [CO_2] effect was found not significant for radish, nasturtium and maize where trends towards an increase were also not visible from the data (Figures 6, 9 and 10). There were large variations within treatments, which might explain the lack of trends. This indicates that under the conditions of the experiment, the increase in [CO_2] led to an increase in shoot biomass for most of the plants while for some others; the variations in sizes within treatments masked the potential effect of [CO_2].

The same effect is corroborated by equivalent findings in several studies looking at the effect of [CO_2] on plants. Legumes, such as the common bean and the pea species included in the study, were found in a review from Palit et al. (2020) to have increased biomass and growth when exposed to elevated [CO_2]. Most of these studies compare current levels of atmospheric CO_2 (around 400 ppm) to the range of increased [CO_2] that can be expected in the future depending on different climate model predictions, often between 600 and 1000 ppm (Gray et al., 2016, Long et al., 2004). The present study also encompassed lower levels of [CO_2] so the physiological adaptation of plants throughout a wide range of [CO_2] can be better understood. This also helps in comparing the results of the studies from paleontological records of plants grown in a lower [CO_2] with the findings of experiments conducted in controlled environments (Yan et al., 2017). The fact that the increase is visible throughout the three levels of [CO_2] indicates that the extra CO_2 available is used by the plants to produce more biomass. Therefore, a 'fertilising effect' of CO_2 was also found for most species in this experiment (Fan et al., 2020, p.12). This increased biomass only impacted the plant's height for bean and sorrel where increasing [CO_2] led to an increase in height ($p < 0.05$, Figure 14).

Bean, pea, radish, sorrel, spinach and nasturtium are C_3 species. Studies have shown that they react stronger to the increase in [CO_2] because their rubisco capacity at the current [CO_2] is not optimised to its maximum (Rosenthal, 2014, Galmés, 2014). This indicates that the C_3 plants are able to take in more CO_2 molecules if [CO_2] increases, leading to higher photosynthesis (Fan et al., 2020). Higher photosynthesis means higher carbon assimilation which could directly result in higher biomass production. This extra biomass can then be potentially allocated to stems, leaves, roots and fruits (Pugh et al., 2016).

Maize is a C_4 species, for which the positive effect of an increase in [CO_2] is lower than in C_3 plants (Lee, 2011, Vu and Allen Jr., 2009). Even though this study only included one C_4 species, similar results were found in other experiments (Chun et al., 2011). C_4 species are affected by variations in [CO_2], but not in the same way because they have evolved a more efficient photosynthetic carbon dioxide fixation (Wijewardana et al., 2016). In C_4 mesophyll tissues, the carboxylation of phosphoenolpyruvate (PEP) results in organic acids. They are then transported to the bundle sheath cells, where CO_2 is released and fixed by rubisco, resulting in a concentration at least ten times higher than in mesophyll cells. This makes C_4 photosynthesis highly efficient even at low [CO_2] (Sage and Sage, 2013). Therefore, even under current [CO_2], the CO_2 concentration at rubisco are close to the highest potential concentration in C_4 plants (Chun et al., 2011). An increase in [CO_2] thus does not benefit C_4 plants as much as C_3 plants. Our study only encompassed one C_4 species and showed no significant variation in biomass among the three [CO_2] treatments (Figure 10). It could be because the effect on biomass from [CO_2] was too small to be significant. It could also be due to the nutrient limitation that appeared strongest in maize and may have limited growth. Finally, it could also be that the conditions of this experiment led to less difference in growth in C_4 than in C_3 species. Indeed, a study by van der Kooi et al. (2016) documented the increased biomass of both C_3 and C_4 crop species. This meta-analysis found that C_4 species may in the long-term benefit similarly to C_3 plants from an increase in [CO_2] when other effects of climate change, such as droughts, are taken into account.

Kalanchoe is the only CAM plant included in the study. CAM plants display a similar photosynthetic mechanism as C_4 plants but separate carbon uptake during night-time from the light reaction and rubisco activity during the day. The PEP carboxylation concentrates CO_2 at the site of rubisco through the transport of the organic acid malate. This uptake is done at night which limits the water lost through the stomata. During day time the CO_2 concentration at the site of rubisco is much higher than in ambient air and photosynthesis can occur with the stomata closed to limit water loss (Drennan and Nobel, 2000). The present study shows an increase in shoot biomass with increasing [CO_2] ($p= 0.002$, Table 3, Figure 11). Fewer research has been conducted on the impact of [CO_2] on CAM species compared to C_3 and C_4 species. A review from Drennan and Nobel (2000) summarised that most experiments showed an increase in biomass with increasing [CO_2]. A similar increase in biomass with increasing [CO_2] was also found by Weiss et al. (2010). Thus, the published studies as well as the

presented results suggest that CAM species may benefit from increased [CO_2], resulting in increased plant growth. Just as for C_3 and C_4 species, this effect is not linear and is also dependent on several other factors.

Interaction between [CO_2] and light

There was a significant light x [CO_2] interaction for nasturtium, maize and kalanchoe in this study but only kalanchoe showed a clear trend (Table 3, Figures 9, 10 and 11). The result indicated that the difference between the two light treatments was increased as the [CO_2] increases. The biomass of plants growing under high light increased faster than those grown under low light (Figure 11). Yet, for the five other species, the interaction was found not significant, meaning that light was not affecting the CO_2 effect. This indicates that despite lower biomass due to lower light availability, the proportional increase in biomass due to increased [CO_2] was not significantly different across treatments. One study on coffee also found that both higher light and [CO_2] led to a higher biomass but that these two factors affected growth independently (Marçal et al., 2021). There is little other research on the interaction between light and [CO_2] so the results of the present study are complicated to compare. However, other factors have been shown to limit the impact of higher [CO_2] on plant growth (Leakey et al., 2012). This impact of another factor influencing plant growth has been documented in studies looking at the effect of [CO_2] and water or nutrient input or soil type or salinity etc. Several studies have found that these factors were reducing the CO_2 fertilising effect in several ways: 1) In case of water limitation, plants may tend to favour a reduction of water loss instead of increasing biomass (Allen Jr. et al., 2011). Both C_3 and C_4 species display a greater water use efficiency in higher [CO_2] which has sometimes masked the effects of drought on plant growth (Wijewardana et al., 2016, Liu et al., 2019). However, Gray et al. (2016) found that for soybeans in a FACE experiment, the effect of elevated [CO_2] did not mitigate the effect of drought as drought conditions intensified. Thus, it highlights a potential threshold to the [CO_2] mitigation of water stress. 2) Increased biomass is possible because the plant's photosynthetic rate is higher. Thus, the plant is speeding up its growth compared to when growing at lower [CO_2]. As plants require a wide range of nutrients and elements for photosynthesis, this means that they also consume these resources faster than at lower [CO_2]. This has been found to create faster depletion of nutrients in soil, such as phosphorus (Ma et al., 2018). It also means that competition between species or individuals will be highly impacted by the differential rate at which each plant is increasing its use of resources compared to its competitors. Nutrients such as nitrogen are also likely to undergo increased mobilisation by plants under growth stimulated by increasing [CO_2]. Under nutrient limitation, plants would still be able to grow but with detrimental effects in their metabolisms such as earlier leaf or plant senescence or altered nutritional quality (Palit et al., 2020, p. 2). The non-exhaustive selection of stress impacting plant growth in experimental conditions, here a lack of light only, may lead to a potential biased estimation of the impact that an increase in [CO_2] would have on plant growth and size in nature (Long et al., 2004).

Root biomass

It is also interesting to note that several studies have recorded a change in root/shoot ratio due to increased [CO_2]. Roots tend to be stimulated more from the extra CO_2 than the shoot. This results in a higher increase in root biomass than shoot biomass and an increase in root/shoot ratio (Fan et al., 2020). Our study did not include the root growth and biomass of the plants except radish. As three plants were growing per pot and were not harvested at the same time, it was then not possible to extract the root at the time of harvest. The radish root biomass alone was not significantly affected by the [CO_2] which, like for the shoot biomass, may be due to the high variability within treatments (Figure 12). It is important to note that radish was partially shaded in some treatments by bigger plants due to the restrained space explaining, in part, the variation within one treatment. For the root/shoot ratio, the results agree with other findings as it increased with increasing [CO_2] ($p = 0.044$, Figure 12). There was also a strong effect from light, with a root biomass and the root/shoot ratio drastically lower for plants grown under low light ($p = 2x10^{-5}$ and $7x10^{-7}$, Figure 12). The limited results from the present study consequently provides evidence that the difference in the overall plant biomass (shoot + root) is potentially greater between CO_2 treatments than the difference between the biomass of the shoot exclusively. Changes in the rhizosphere conditions may have further indirect impact on plant growth by modifying resource mobilisation and their biotic and abiotic environment.

Leaf biomass

Similar to the findings of the total shoot biomass, the leaf biomass of bean, sorrel, kalanchoe and marginally spinach, increased as [CO_2] increased (Figure 13). The difference in leaf biomass for pea, radish, nasturtium and maize was however not significant across the three [CO_2] (Table 3). For the species displaying a significant change in leaf biomass, the total weight of the leaves of each plant was higher as a direct effect of CO_2 fertilisation (Fan et al., 2020). The difference in the overall leaf biomass may be linked to two different physiological changes, first an increased number of leaves and second, larger leaves. The number of leaves per plant by the end of the experiment was significantly impacted by [CO_2] in bean, sorrel and maize ($p < 0.05$, Table 3) and marginally in pea, spinach and kalanchoe (Figure 15). The number of leaves increased with increasing [CO_2] (Figure 15), whereas leaf length was significantly affected only in bean and sorrel ($p = 0.001$ and 0.019 respectively, Table 3). Though these two species showed an increase in the length of their biggest leaf as [CO_2] increases, most of the species of the experiment did not display any significant changes (Figure 16).

There was also a significant impact of light on the leaf biomass for seven species except nasturtium and a significant interaction of [CO_2] and light on four of the species, bean, nasturtium, maize and kalanchoe (Table

3). Plants grown under high light had a higher leaf biomass than under low light (Figure 13). Light also significantly affected the number of leaves for two species, radish and spinach and marginally for bean ($p = 0.052$, Table 3). The number of leaves per plant increased with increase of light except for radish (Figure 15). Leaves under low light were significantly longer in bean, nasturtium and maize (Figure 16). Apart from changes in total biomass and biomass allocation, [CO_2] and light availability also resulted in leaf trait modifications.

III. Leaf traits

How leaves develop in their environments is unique for each plant and depends on the resources and stresses that it encounters as it grows. The rate of photosynthesis is regulated by leaf traits. Understanding how light and [CO_2] impact them can help to understand more precisely how plant growth is affected by different parameters of the environment.

SLA

Firstly, the specific leaf area can differ depending on the environment in which the plants grew. The [CO_2] effect was marginally significant for radish and kalanchoe and significant for spinach and nasturtium (Table 4). The radish, spinach, nasturtium and kalanchoe plants all showed a decrease in SLA under both light treatments as [CO_2] increases (Figure 17). This difference was however stronger in species such as nasturtium and spinach and less pronounced, almost equal for radish and kalanchoe. SLA is the area of a leaf per leaf biomass. In higher [CO_2], the extra carbohydrates produced are stored in the leaf or allocated to thicker and hence, more robust, leaves. The detrimental effect that thicker leaves can have on CO_2 diffusion is mitigated by more CO_2 being available. Thus, there is more biomass per area of the leaf. This results in a decrease in SLA (Kimball et al., 2002). A decrease in SLA as [CO_2] increases was also found in other studies or reviews and is generally accepted as an effect of elevated [CO_2] (Palit et al., 2020, Temme et al., 2017, De Temmerman et al., 2007). High [CO_2] was found to impact CAM leaf thickness in other studies (Drennan and Nobel, 2000). An increase in leaf thickness would lead to a decrease in SLA. The results here do present a slight decrease in SLA. As Kalanchoe is a slow growing species, the difference between treatment is perhaps not as strongly reflected as those of faster growing species.

As the second factor studied in this experiment, light influenced the SLA very significantly, except for pea, affected only marginally (Table 4). It was consistently higher for plants grown under low light than under high light (Figure 17). This reflects a strategic trade-off for leaves growing in the shade, where light is the limiting factor for the photosynthetic reaction. These plants favour a larger leaf area for each unit of added leaf biomass so they can maximise light interception and in turn, photosynthesis (Temme et al., 2017). Thus, the leaves are thinner which also means that they are more sensitive to damages from environmental impacts. By contrast, plants grown under high light do not need to maximise area. The biomass allocated in leaves is thus

leading to thicker, more robust leaves (Temme et al., 2017). The interaction between [CO_2] and light was significant for kalanchoe only with no clear trend visible (Figure 17). It would be useful to repeat the experiment with similar conditions on a higher number of replicates to gain a better understanding of the interaction of light and [CO_2] on the SLA of kalanchoe.

LDMC

LDMC increased significantly with [CO_2] increase for six species. Radish and maize were not significantly affected (Figure 18). This indicates that as [CO_2] increases there is a smaller proportion of water compared to leaf biomass. This is further evidence of a change in leaf structure rather than a simple increased growth proportional to an increased overall biomass. Finally, LDMC is a measure of leaf density and structural complexity which indicates that this change in leaf structural arrangement will have impacts on photosynthesis (Eckert et al., 2020).

Light also influenced the leaf dry matter content with a higher LDMC in plants grown under high light (Figure 18). This increase is significant for all species (Table 4). LMDC is negatively correlated with SLA (Tasset et al., 2019). Hence, such results were expected. It indicates that under high light the leaves are thicker and more dense leading to a higher LDMC.

The interaction between light and CO_2 factors played a role, in the overall differences in LDMC of spinach, maize and marginally in kalanchoe (Table 4). For spinach and kalanchoe, the increase in LDMC was clearly visible under high light but flatter if not absent under low light as [CO_2] increased. Similarly to the effect of interaction on biomass, the difference between the high and low light treatment becomes increasingly larger as the [CO_2] increases (Figure 18). Therefore, it shows that as [CO_2] continues to increase there is a potential for plants in different light conditions to demonstrate enhanced differences in their physiology. The impact of light on the CO_2 effect is not only due to the light intercepted by the leaf. A leaf in a shaded area will have different air humidity or stress levels than one in full light hence leading to a different LDMC.

Stomatal density

The stomatal characteristics of a plant play a central role in controlling the rate of photosynthesis because there is a direct link between stomatal development and the gas-exchange capacities of a leaf (Dow and Bergmann, 2014). The main trade-off that stomata regulate is to maximize CO_2 intake while minimizing water loss. To achieve this, stomatal development can be adapted in many ways in reaction to its direct environment. This adaptation can be either through their aperture, their size or their density (Yan et al., 2017). In this study, only the density was studied as it is considered an important indicator of the stomatal impact on photosynthesis. Stomatal density (SD) may reflect the long-term physiological response of an individual plant whereas the reduction in conductance through stomata closing is a short-term reaction to a rapid change in

its environment. It is therefore useful to highlight the impact of [CO_2] on stomatal development and better apprehend its consequences for leaf photosynthesis (Dow and Bergmann, 2014).

In the present study, SD was higher under high light in all species except pea (Figure 19). As stomata regulate the exchange of CO_2 and H_2O, a decrease in SD indicates that the plants grown under low light probably had a lower water loss compared to those grown under high light (Fan et al., 2020). Under light limitation, Ci will be higher than in plants under high light, so the effect of external CO_2 might be reduced. It is therefore a logical physiological adaptation to have a lower stomatal density in lower light settings as the diffusion of CO_2 through the stomata and in the mesophyll is less limiting.

The [CO_2] was found to significantly impact the stomatal density only in one of the eight species, namely spinach (Table 4), where stomatal density increases with [CO_2] increase (Figure 19). Overall, this study found very little impact of [CO_2] on stomatal density. One of the most widely accepted consequences of a rise in [CO_2] is a decrease in stomatal density (Chater et al., 2015, Yan et al., 2017). It is partly explained by an adjustment by plant in order to limit water loss whilst making use of the increased CO_2, which increases water use efficiency (WUE) (Palit et al., 2020). In this experiment, plants were abundantly watered, thus there was no need to reduce water loss. This means that the stomatal regulation between CO_2 intake and water loss was less visible. In experiments where plants were put in some forms of water stress, the decrease in stomatal density was stronger for the treatments experiencing very limited water availability (Xu et al., 2016).

Yet, research has also shown that not all plants had the same variations in stomatal development in response to elevated [CO_2]. For instance, an increased stomatal number was found in capsicum grown at 800 ppm compared to 400 ppm (Fan et al., 2020). This was in turn linked to the increased biomass as the higher number of stomata enable faster CO_2 diffusion from the atmosphere to the mesophyll tissues. Some other studies found no change in stomatal density in response to rising [CO_2] (Rosenthal et al., 2014). A meta-analysis of a combination of experiments and past plant records also showed a decrease in stomatal density with increasing [CO_2] but with important limitations (Yan et al., 2017). The experimental and environmental conditions, the duration of the experiment or the species studied all impacted the stomatal density resulting in a wide range of seemingly contradictory results.

From the literature it is therefore possible to identify three factors that can explain the insignificant CO_2 effect on SD found in this study: 1) The response of SD to [CO_2] in experiments has been highly dependent on its duration, the experimental design and the species. It is a very adaptable trait and will change depending on the combination of environmental factors included in the study ([CO_2], temperature, nutrient availability etc) (Yan et al., 2017). In this experimental setting it is possible that the environment in which the plants grew, for instance, the unlimited water availability, lead to an unchanged SD. 2) The seeds were all left to germinate at the same [CO_2] and this may have impacted the phenological adaptability of the plant even for the newer

leaves that had grown after the pots were moved to the chambers and, 3) given the high variation in SD within and among plants, the number of stomata counted per leaf and the number of leaves or plants may have been too low to significantly detect small effects of CO_2.

Overall, this experiment confirms that [CO_2] impacts leaf traits by affecting how the produced biomass is allocated in the leaves. The light effect was much more pronounced on SLA, LDMC and SD than the CO_2 effect. The CO_2 effect observed for some species were expected, such as a decrease in SLA and increase in LDMC as [CO_2] increases. There were substantial differences between species; pea, radish and maize for instance, being mostly not significantly affected by the [CO_2] whilst others such as spinach and nasturtium displayed greater differences between the treatments. Interaction between the two factors was low except on LDMC where its increase with increasing [CO_2] was stronger under high light than under low light (Table 4). As [CO_2] increases leaves may become larger and thicker as more biomass is allocated to them. There is not enough evidence to suggest that an interaction with light or a change in stomatal density are expected as these were not commonly found. The changes in leaf traits are expected to have an impact on the leaf photosynthetic rate by impacting both the gas exchange capacities and the electron transport chain.

IV. Photosynthesis

An increase in [CO_2] can be accompanied by an increase in leaf photosynthesis (Fan et al., 2020). The effects of [CO_2] and light outlined above can be seen as causes or results of a change in the photosynthetic activity of the plant. According to De Temmerman et al. (2007), photosynthetic activity is dependent on chlorophyll concentration, stomatal conductance and the CO_2 fixation – rubisco activity. In the present experiment, chlorophyll content, the electron transport rate as well as gas exchange capacities at three CO_2 concentrations were quantified. The electron transport rate is a direct effect of light intensity and gas exchange are impacted by a multitude of factors including [CO_2] and light (Long et al., 2004). Thus, by looking at these parameters, the impact of both treatment variables should be highlighted. This information would contribute to a better understanding about how light and [CO_2] influence photosynthesis in the eight different plant species studied.

Electron transport rate and chlorophyll content

[CO_2] significantly affected leaf chlorophyll content in bean, pea and maize (Table 5). From these plants, a decline in chlorophyll variation can be identified through the three increasing [CO_2] (Figure 21). In some plants, nitrogen limitation may have limited the response in chlorophyll concentrations by the end of the experiment. Therefore, it is a likely explanation of why the chlorophyll content was decreasing due to dilution by an increased plant biomass. Yet, chlorophyll content exclusively from plants grown under low light also decreased even though no leaf discoloration was observed (Figures 10). Chlorophyll content was significantly changed at higher [CO_2] in several studies. Ma et al. (2018) found that bean grown in 650 ppm [CO_2] had a higher

chlorophyll content than those grown in ambient air. On the contrary, a meta-analysis on wheat found that on average chlorophyll content decreased under higher [CO_2] (Wang et al., 2013). In agreement with this, in the present study, chlorophyll content was significantly affected in three of the species for which it showed a subtle decrease as [CO_2] increased.

Light significantly affected the chlorophyll content of radish, sorrel, spinach, maize and kalanchoe (Table 5). The chlorophyll content was higher for plants grown under high light for all species, except maize (Figure 21). Chlorophyll content is strongly linked to the rate of photosynthetic activity. Hence a leaf increasing its chlorophyll content is aiming to increase its photosynthetic activity (Marchiori et al., 2014). The higher chlorophyll content found in the leaves grown under high light could indicate a higher photosynthetic activity due to more light being available. The chlorophyll content of maize leaves grown under high light was much lower than those grown under low light. This may be due to the leaf discoloration observed in maize under high light caused by nutrient limitations (Figure 21).

The electron transport rate provides information on how a leaf reacts to and utilise light. Plants adapted to a higher light availability will have a higher rate of light reaction and thus, the ETR will be higher (Figure 20, Pérez-López et al., 2015). This was seen here in the results for six species where the ETR at saturating light, 1500 µmol m^{-2} s^{-1}, was significantly lower for the plants grown under low light than under high light ($p < 0.05$, Figure 22). The difference in ETR for pea grown under low vs. high light was only marginally significant and not significant for maize (Table 5). When the electron transport rate is increased, it contributes to the overall stimulation of photosynthesis leading to increased growth (Palit et al., 2020). Indeed, a higher ETR provides more energy in the form of ATP and NADPH for the dark reaction (Pérez-López et al., 2015). In the species of the present experiment where ETR increased with high light, their biomass generally also increased (Figures 5, 6, 7, 8, 9, 11 and 22).

The results of this study only showed a significant decrease in ETR at saturating light as [CO_2] increased in nasturtium. The interaction between CO_2 and light was significant for spinach and showed a decrease of the ETR with increasing [CO_2] for the plants grown under high light and an increase for the plants grown under low light (Figure 22). The decrease of ETR as [CO_2] increased suggests a downregulation of electron transport. As photosynthesis is made easier by the extra CO_2 available, plants downregulate their photosynthetic activity. This contrasts with some published results where the light reaction of photosynthesis was increased with increasing [CO_2] (Pérez-López et al., 2015, Lambreva et al., 2005). For instance, the ETR in a study from Aranda et al. (2020) was 20% higher in 800 ppm [CO_2] than in 400 ppm. Yet, Lambreva et al. (2005) also identified that the higher starch accumulation due to both high light and [CO_2] could explain the less pronounced stimulation of electron transport capacity. This could explain the significant interaction between light and [CO_2] for the maximum ETR of spinach. It is plausible that higher starch accumulation in high light led to a decrease in ETR.

On the contrary, plants grown under low light could have increased their light reaction, to balance with the increased CO_2 available, without the starch accumulation down-regulating this effect. From these data, it can be said that light affects the ETR much more substantially than [CO_2] and can be linked to the increase in biomass as light increases. However, [CO_2] mostly does not affect the ETR and variations in the light reaction, besides the exception of a couple of species, and can therefore not explain the differences in biomass obtained from different [CO_2].

Gas-exchange measurements

The gas exchange measurements conducted in this study were used to obtain A/Ci curves for bean, nasturtium and radish (Figures 23 to 27). The expected effect of higher [CO_2] is a stimulation of the gas exchange in the form of a greater CO_2 net intake (Fan et al., 2020). However, several studies have also found a downregulation of A at higher [CO_2] due to an increased carbohydrate content in leaves (Long et al., 2004, Keutgen and Chen, 2001). Nitrogen-deficiency is also contributing to a decrease in A (Pritchard et al., 1999). The lack of clear differences in the A/Ci curves in the present study can be due to a downregulation of the species in reaction to [CO_2]. It is also likely that some limitations of the experiment impacted the measurements. Plants growing in the 800 ppm [CO_2] and to a lesser extent also those growing in a 400 ppm [CO_2] experienced some nutrient deficiency by the end of the experiment. Thus, the fact that some plants were experiencing an added stress from this resource deficiency may have impacted the photosynthetic activity, even if the leaves measured were younger and less likely to be impacted by nutrient limitations.

From these A/Ci curves, four additional parameters were derived to better analyse individual components contributing to net CO_2 uptake. The first parameter is Vcmax, the maximum rate of rubisco carboxylase activity. Several studies have found that an increase in [CO_2] led to a higher Vcmax (Long et al., 2004, Lambreva et al., 2005, Rosenthal et al., 2014). Others have found a decrease in Vcmax as plants acclimate to higher [CO_2] or when other factors are considered (Salazar-Parra et al., 2015). The impact of [CO_2] on this parameter was found significant for nasturtium under low light ($p < 0.05$, Table 6). In nasturtium, Vcmax is decreased with increasing [CO_2] (Figure 28). This indicates a downregulation of rubisco activity. Current kinetics of rubisco of many C_3 plants are not optimal and depend on environmental conditions. A study from Galmés et al. (2014) found that under moderately increased [CO_2], 550 ppm maximum, there was an increase in rubisco performance only when water availability was decreased. This indicates that the increase in Vcmax expected with increased [CO_2] is in reality dependent on the complete future environmental conditions. This also provides some insights into the results obtained in this study, as neither the water availability nor the temperature changed between treatments. Rubisco is expected to remain a limiting step for photosynthesis (Galmés et al., 2014).

The other parameters derived from the A/Ci curves are the respiration, RL, the triose-phosphate utilisation, Tp, and the mesophyll resistance to CO_2 diffusion, rm. For C_3 plants, increasing [CO_2] leads to an increase in photosynthetic rate both through more carbon uptake but also less respiration (Fan et al., 2020). The RL values for nasturtium grown under low light and radish grown under both lights all show a significant decrease with high [CO_2] (Figure 29). Therefore, the plants grown at higher [CO_2] have a lower respiration in the light. For a similar amount of CO_2 assimilated by photosynthesis, the net CO_2 intake is consequently higher. The variations in triose-phosphate utilisation were found in this study dominantly not significant, except for nasturtiums grown in low light (Table 6). The utilisation appears to be decreasing within increasing [CO_2] (Figure 31). Therefore, from the measurements done in this experiment, it is complicated to interpret these results in terms of the effect that [CO_2] had on them. The last parameter is the mesophyll resistance, rm. For this parameter, [CO_2] was found not significant for all species measured (Table 6). It is marginally significant for radish grown under high light in which it appears to be increasing ($p = 0.0976$, Figure 30). Mesophyll resistance is dependent on the complexity of the leaf structure and its density (Eckert et al., 2020). As LDMC and SLA are affected by [CO_2] and light, it is likely that mesophyll resistance would vary accordingly as they reflect changes in leaf thickness and tissue density. Thus, the increase found in radish is logical but this [CO_2] effect on rm is small and only marginally significant in one species in the present study.

Overall, gas exchange at the leaf level is impacted by many environmental factors. Experimental conditions aim to reduce the influence of these factors to only those studied. However, there are limits to the experimental design of this study. The variation in measurements due to unwanted variations in the environment could be better take into account with a higher number of measured leaves per treatment. More accurate results could also have been obtained by providing plants with more space so that all required resources were in abundance. Yet, as this is not likely to be the case for ecosystems in the future, there are still some useful insights that this study have highlighted. From the limited experimental set obtained, both the carboxylation rate and the respiration appear to be downregulated as [CO_2] increases.

V. Insights for future climate and ecosystems

The future increase in [CO_2] results in climate change and thus also to higher temperature and regional increases in drought (Gray et al., 2016). Moreover, the increased growth will create higher pressure on plant resources leading to potential shifts in ecosystem composition or agricultural stability. Nutrients use is increased under elevated [CO_2] and is expected to potentially limit growth or the nutritional quality of crops (Ma et al., 2018, Pérez-López et al., 2015). Though, global light is not expected to change, an increase in the [CO_2] could also impact the plants' use of light. Higher growth may affect Leaf Area Index (LAI) and thus, increase competition and shading for individual leaves. In the present study, very few interactions between CO_2 and light were found. This suggests that the changes brought upon by the increased [CO_2] are not likely

to be impacted by the light conditions of the individual plants. Yet, the changes in plant size in the chambers led to some differences in the competition between plants. As each treatment had a limited space available, some plants shaded others as they grew. The competition was stronger where the [CO_2] was higher. The faster growing and bigger species shaded some other plants in the 800 ppm chamber whereas very few shading was noticed in the 200 ppm chamber. This competition for light was an involuntary effect of the experimental design and the effects were species-specific. It highlights the potential indirect [CO_2] and light effect on ecosystem composition.

Using the data obtained in controlled environments in order to model the expected changes that will occur during this century is a complex task (Pugh et al., 2016, Fatichi and Leuzinger, 2013). The CO_2 fertilisation effect is included in models of future ecosystems and agricultural crops but is likely to be overestimated (Fan et al., 2020). All the individual environmental parameters may interact together and impact plant growth in a unique way (Palit et al., 2020). Moreover, the effect of increased [CO_2] is species-specific and the experiments have studied a bias selection of species (Leakey et al., 2012). Researching the impact of [CO_2] in combination with the other impacts of climate change on a wide range of species would therefore allow for better predictions. It would also allow for the selection of better suited species for the adaptation of agricultural systems to future climates (Bourgault et al., 2016, Galmés et al., 2014).

7.References

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