# Impacts of elevated $CO_2$ and light on growth and stoichiometry in *Arabidopsis*

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# ABSTRACT

The effect of elevated $CO_2$ on plant stoichiometry has been given a lot of attention in the last years; however, extended studies on the effects of elevated light added to the increased $CO_2$ are still insufficient.

Plant growth chambers were set up to grow *Arabidopsis thaliana* under ambient (~400 ppm) and elevated (~700 ppm) $CO_2$. The chambers made it possible to monitor and adjust $CO_2$ levels precisely while also tracking the light, temperature and relative humidity. Two separate experiments were run; experiment 1 with ambient (~170 µE) light and experiment 2 with increased (~350 µE) light to test the effects of elevated $CO_2$ and light on plants. We weighed the plants and analysed the tissues for chlorophyll, C, N and P levels to test if there was a correlation between $CO_2$, light and element composition. With elevated $CO_2$, the average dry shoot biomass was reduced in both experiments. The clearest changes were between experiments; biomass and C increased, and N, P and chlorophyll decreased with elevated light. When adding elevated $CO_2$, the trends were even more profound. This resulted in a substantial increase in the C:P and C:N ratios. The results largely suggest that elevated $CO_2$ together with the corresponding changes in climate have a clear effect on the element composition of plants. The effect of elevated $CO_2$ and light on plant quality (nutrient and protein content) could have negative impacts on small and large herbivores, and even humans.

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# INTRODUCTION

The global average $CO_2$ has fluctuated between about 180 ppm during ice ages and 280 ppm during interglacial warm periods in the last 800,000 years, and when we entered the industrial revolution in the 18$^{th}$ century, the global average was about 280 ppm (NOAA/ESRL, 2013). This was when humans started to affect the atmospheric $CO_2$ levels. The industrial revolution led to increased fossil $CO_2$ emissions, and the rate of increase has accelerated more than 100 times since the last ice age. From 1970 to 2010, 78% of the total greenhouse gas (GHG) emission increase came from fossil fuels and industrial processes (IPCC, 2014a). Recent data from the National Oceanic and Atmospheric Administration (NOAA) show that the global monthly mean $CO_2$ was 412 ppm in December 2019 (NOAA/ESRL). This 45% increase in $CO_2$ concentrations, combined with the increase of other greenhouse gases, has caused mean annual global temperatures to increase by nearly 1.0°C since the industrial revolution (IPCC, 2018).
The Intergovernmental Panel on Climate Change (IPCC) has constructed future climate scenarios based on economic and population growth. Without additional mitigation, we are predicted to exceed $CO_2$ levels of 450 ppm by 2030 and reach concentration levels between 750 and 1300 ppm by 2100. This will lead to an increase in mean surface temperature from 3.7°C to 4.8°C compared to pre-industrial levels (IPCC, 2014a). A more realistic scenario is some mitigation and change from fossils to renewables however, pointing towards 2.5-3.0 °C by 2100 (Hoegh-Guldberg et al., 2019).

The increase in atmospheric $CO_2$ is expected to have direct effects on plants.
Plants are highly important to all life on earth. In addition to the conversion and storage of carbon (C), they are a major source of food, medicine and wood (Fabricant & Farnsworth, 2001; Loladze, 2002). Increased ambient $CO_2$ can lead to decreased water use; stomata of many species will reduce conductance (Drake et al., 1997), and some species have also reduced the number of stomata in young leaves as a response (Woodward et al., 2002). The reduction of stomata conductance will reduce the evapotranspiration from plants, which could increase their water use efficiently (Zhu et al., 2017). This could again reduce the flow of nutrients from the soil (Loladze, 2002).
More $CO_2$ available can increase the uptake of C in plants, which can further stimulate and accelerate photosynthesis (Leakey et al., 2009). With the right conditions this could also increase plant biomass, especially in $C_3$ species (Poorter & Navas, 2003). With increased $CO_2$ levels, plant groups with ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco)-limited photosynthesis ($C_3$ species) will have the potential to increase their rate of photosynthesis and favour the carboxylase reaction of Rubisco. This would suppress the photorespiration and lead to further increased rate and efficiency of photosynthesis (Ainsworth & Rogers, 2007), potentially causing a reduction of the Rubisco concentration in plants. Rubisco is one of the most abundant proteins on Earth, and the nitrogen (N)-pool in plants largely consists of Rubisco. The reduction would result in a reduction in leaf N concentration, as Rubisco constitutes 25% of leaf N in $C_3$ plants (Drake et al., 1997). Studies have also shown that increased $CO_2$ can further lower N concentrations by inhibiting nitrate assimilation (Bloom et al., 2010), although this is currently

under debate (Andrews et al., 2018).
Rising $CO_2$ is not predicted to directly impact photosynthesis in $C_4$ plants, but the results are highly variable. There have been found an increase in photosynthetic rate, height, and biomass in sugarcane ($C_4$) grown at elevated $CO_2$ (~720 ppm). These plants also had lower stomatal conductance and transpiration rates, higher water use efficiency and an increase of about 29% in sucrose content (De Souza et al., 2008). There has also been found changes at the transcript level of genes associated with increased cell growth and proliferation, respiratory breakdown of starch and nitrate transport, and reduced transcripts for protein synthesis and fatty acid production in soybean grown at elevated $CO_2$ (Ainsworth et al., 2006b).

The increase in C uptake can have several consequences for the plants and their consumers. An elevated plant biomass may take place at the expense of nutritional quality. Nitrogen (N) and phosphorus (P) are both important for growth, N in building proteins from amino acids, and P in nucleic acids (Sterner & Elser, 2002).

Terrestrial vascular plants obtain $CO_2$ from the atmosphere, but they get all other nutrients and elements from soil or water (Loladze, 2002). With increased C levels, the result could be a shift in plant elemental stoichiometry between C and other key elements, especially a skewed ratio of C:N. Increased light will further reinforce this and other effects of increased $CO_2$, because of an increased demand for nutrients (Sterner & Elser, 2002). This will make the C:N ratio increase even more, and the increased light has also been shown to reduce the P:C and chlorophyll:carbon ratios (Hessen et al., 2002). $CO_2$ also affects the uptake rates of N relative to P, and elevated levels of $CO_2$ could cause a significant down-regulation of tissue N:P in aboveground and belowground biomass in terrestrial plants. How elevated $CO_2$ impacts plant and soil P content is much less explored, but there has been found significant decrease in P concentration in plants even though this is disproportionate with the decline in N (Deng et al., 2015).

From the industrial revolution, a doubling of the atmospheric $CO_2$ concentrations would lead to an increase in the production of carbohydrates in $C_3$ plants by 19-46% (Leakey et al., 2009). A significant increase in sucrose and starch has also been found in soybean, a nitrogen fixating legume (Ainsworth et al., 2006a). Historical and experimental data from North America indicated a strong significant correlation between increases in $CO_2$ and increased C:N ratio, and an overall decline in pollen protein concentration by up to 1/3. Pollen is the main protein source for bees and other pollinators (Ziska et al., 2016). The increase in C:P and C:N ratios may reduce growth and survival rate in heterotrophs with frequent dietary N- or P-limitation (Sterner & Elser, 2002). P-limitation and -deficiency is more common in aquatic ecosystems (Hessen et al., 2013), but has also been found in terrestrial systems (Elser et al., 2000). In Daphnia, up to 40% of reduction in growth rate could be a result of increased C:P ratios (Hessen et al., 2002). A study done on species of deciduous trees and insect larvae showed that increased C:N ratio changed the nutritional constituents of tree foliage. This reduced the survival of insect larvae that fed on plant compounds in elevated $CO_2$ and high light with up to 62%. It also showed increased development time and reduced pupal mass. Long-term bioassay results indicate that specific combinations of

$CO_2$, light and host species have a strong potential to reduce insect population growth (Agrell et al., 2000).
76% of the global human population gets most of their protein from plants. Studies of important crops like rice and wheat predicted that if global atmospheric $CO_2$ concentrations exceed 500 ppm by 2050, 6.57% of the global population will be at risk of protein deficiency (Medek, 2017). A study on livestock in North America showed that the change in C:N ratio could further increase the DOM:CP (digestible organic matter:crude protein concentration) of cattle diet (Craine et al., 2017). This could result in a reduced cattle weight gain. The DOM:CP was 13% higher in 2005-2015 than in 1994-2004, as a result of reduced N concentration (Craine et al., 2017). Several studies have raised the concerns of micronutrient malnutrition in human diets due to the effect of elevated $CO_2$ on crops (Loladze, 2002; Zhu et al., 2018). These trends show that elevated $CO_2$ may negatively impact human nutrition, as well as domestic and wild herbivores from insects to mammals.

The interactions among $CO_2$ and light on plant growth and elemental composition are still poorly explored. It is however known that increased flux of photons may upregulate photosynthesis without corresponding uptake of nutrient elements, notably under nutrient scarcity (Hessen et al., 2002; Sterner et al., 1997). This means that light likely will interact with $CO_2$ and that high light levels may intensify the quantitative and qualitative effects of elevated $CO_2$. While there are many studies on $CO_2$ and light alone, few have addressed the interactive effects. Throughout this project, I hope to literally shed some light on the possible consequences.

In this project, I will explore the growth and elemental composition of *Arabidopsis thaliana* (Thale cress). *Arabidopsis thaliana* is a $C_3$ plant, and it was chosen because it is the standard model organism for research in plant biology. Its short genome was the first plant genome to be fully sequenced, it has a short generation time, is small in size and self-pollinated (Koornneef & Meinke, 2010).

The novelty of this project lies in an extended analysis of impacts of $CO_2$ on not only plant quantity, but also quality by analysing tissue-specific responses of elemental (C, N, P) content and their ratios. Studying $CO_2$, light and nutrients jointly is a novel approach as well, yet realistic under a factorial design in the phytotron facility with our $CO_2$-chambers. Based on stoichiometric theory I will assess the decline in nutritional value for herbivores in a broad sense.

Hypotheses:

1. Elevated $CO_2$ do promote growth, but at the expense of nutritional quality, i.e., C:N and C:P will increase, N:P will decrease and protein content will decline.
2. High light levels will add to the effects of elevated $CO_2$ and strengthen the responses even further.
3. Plant responses will be tissue-specific, with strongest responses in leaves and reproductive tissues but weakest in stems and roots.

# Materials and methods

Pots with seeds from *Arabidopsis thaliana* were set into two separate growth chambers. Two experiments were performed where each experiment lasted through the vegetative phase, and into the reproductive phase.
Both chambers had ambient air flow through a ventilation system, one chamber was also connected to a $CO_2$ gas tank.
In experiment 1, chamber 1 had ambient air flow through the chamber (~400 ppm $CO_2$), and chamber 2 had an added $CO_2$ gas tank to reach a $CO_2$ level of ~700 ppm. Above both chambers were two light-emitting diode (LED) lamps (produced by Valoya, https://www.valoya.com/spectra/), with a total light level of ~170 µE at plant height. The LEDs emitted light of a wide spectrum within the ranges 380-780 nm (Figure 1).

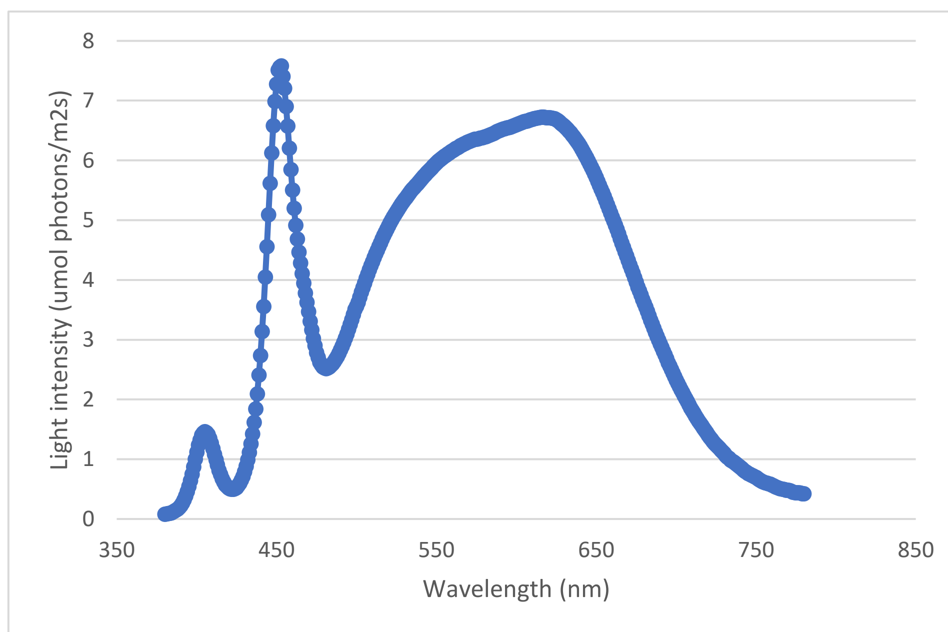



Figure 1: Spectre of one of the LED lamps, measured with a handheld spectrometer (UPRtek mmodel mk350s, Taiwan). The LED lamps had a wavelength of 380-780 nm.

In experiment 2, the chamber systems were set up in the same way, but the light level in both chambers was now adjusted; two more LED lamps were added above each chamber to reach a level of ~350 µE. The spectre was similar in all the lamps. The LEDs were places outside the chambers to avoid heat accumulation.
According to NASA scientists, LEDs are the most optimal SSL (sole-source lighting) for indoor plant cultivation. The LEDs make it possible to adjust the wavelength to simulate sunlight, to ensure that the plants have optimal growing conditions under the correct wavelengths with wide enough photosynthetically active radiation (PAR, 400-700 nm) (Mitchell & Stutte, 2015). LEDs are getting cheaper to produce, they do not contain mercury, and they have a long operating life. They also emit much less heat than other light sources, which means that they are more energy efficient and can be installed closer to the plants (Morrow, 2008).

## PLANT GROWTH CHAMBERS

The size of the chambers was 122 cm x 91 cm x 46 cm. They were set up inside a phytotron room at the Department of Biosciences (IBV) of the University of Oslo. They are air-tight, and the walls are made of 6.4 mm thick plexiglass sheets with weather stripping between the glass and the chamber frames (wood) on one side to prevent ambient air from leaking into the chamber. On this side the glass can be removed to access the plants for daily maintenance.

The chamber design was set up by William Hagopian (Hagopian et al., 2018; Hagopian et al., 2015). Each chamber was connected to a flow-through ventilation system, where ambient air was drawn into the chambers from outside the phytotron facility. The air was pumped in through a pipe using a 12-volt DC fan on one end of the chamber, and out on the other end. In one of the chambers, the system was connected to a pure $CO_2$ gas tank where a micro-control valve secured a stable amount of gas supplementing the ambient air to increase the $CO_2$ level. An example of the $CO_2$ stability in both chambers (Chamber 1 ~400 ppm and Chamber 2 ~700 ppm) is provided in Figure 2, which shows the $CO_2$ levels over nine days in Experiment 1. The few random points above or below average are a result of maintenance of the plants/chambers. This occurred before the lights went on.

In the latest Climate Change Synthesis report from the Intergovernmental Panel on Climate Change (IPCC), four Representative Concentration Pathways (RCPs) are being used to describe different 21$^{st}$ century pathways of emissions, atmospheric concentrations, and land use. Scenario RCP 4.5 is the lowest of the two intermediate scenarios, with an annual $CO_2$ emission of 580-720 ppm. In this scenario, it is unlikely (0-33%) that we stay below a global temperature increase of 2°C relative to pre-industrial levels (IPCC, 2014b).

$CO_2$ levels were measured with a LI-840A $CO_2$ gas analyser. The levels were measured inside both chambers and in the air in the vent every five minutes. The $pCO_2$ levels were logged over several weeks before start-up to ensure stable levels in both chambers.

To flush the LI-840 gas analyser, the intake flow was diverted through a $CO_2$ scrubbing canister and manually zeroed via the software interface once a week. This is standard procedure as a quality control of the analyser.

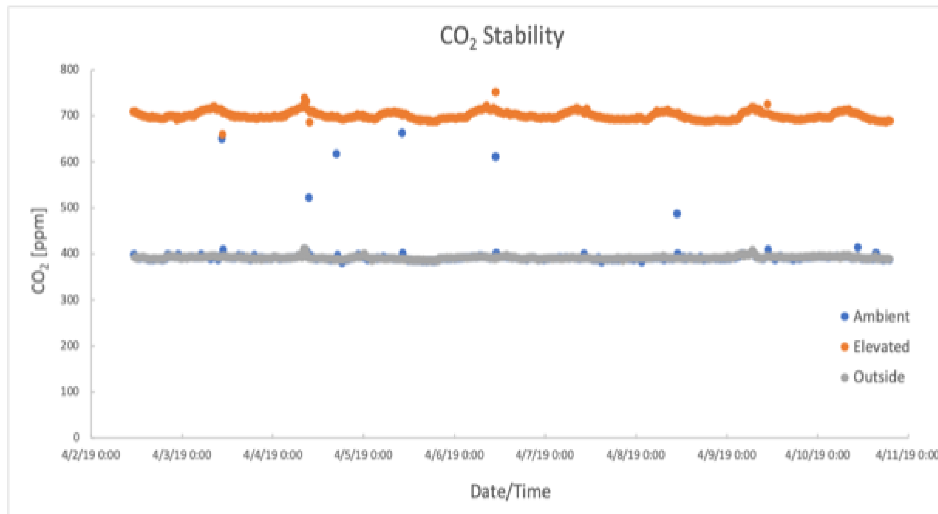



Figure 2: $CO_2$ levels in the ambient and elevated chambers, compared to the $CO_2$ level outside. The ambient level was about the same as the levels outside and is therefore hard to spot. The levels in the ambient chamber and outside were stable on ~400 ppm $CO_2$ while the level in the elevated chamber was ~700 ppm $CO_2$.

To ensure stable relative humidity (RH), one humidifier was set up inside each chamber. The humidifiers were custom made, they consisted of a Mist Maker element placed within a plastic container filled with water, with a bottle that functioned as an extended water reservoir. The humidifiers were controlled using an Arduino MEGA 2560 R3 microcontroller (Adafruit.com, New York, NY, USA), running a simple closed-loop program configured with an SHT15 humidity and temperature sensor (Sensirion AG, Stäfa, Switzerland) and set to trigger on at 50% and off at 55% RH (Figure 3). Temperature and RH were measured and logged by a HOBO U12-012 data logger (Onset Computer Corp., Bourne, MA, USA).

The LEDs were connected to a control system which ensured that they turned on and off automatically at a set hour. I ran two 16-hour daytime experiments with daytime from 10 AM to 2 AM. The maintenance was done before the lights were switched on and consisted of refilling the humidifiers and watering the plants. Experiment 1 lasted 47 days and experiment 2 lasted 36 days.

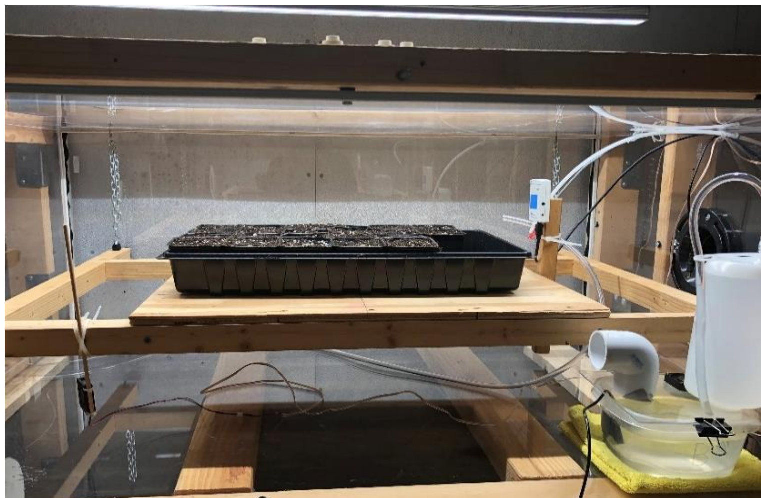


Figure 3: One of the two plant growth chambers with newly sown seeds.

## EXPERIMENT 1

In experiment 1 I tested hypothesis 1, that elevated $CO_2$ in the atmosphere will increase biomass by accumulating a higher amount of carbon (C) in the plant, and that this would affect the plant stoichiometry and the amount of other elements, here nitrogen (N) and phosphorus (P). I also tested hypothesis 3, that the plant response would be tissue specific.

The experiment lasted from 29.03.2019-14.05.2019. Pots were filled with standardized low nutrient soil from Hasselfors Garden ("Såjord, så- og priklejord"). Four *Arabidopsis thaliana* seeds were sown in each pot. 22 pots were placed together in a large plastic box in each chamber and covered with plastic that was white on the outside and black on the inside, to ensure an optimal imbibition and a synchronized germination. The plastic was removed after four days. The first seedlings were discovered after ten days.

Relative humidity (RH) and temperature (°C) measurements through the experiments showed a stable environment with very little difference between the chambers (Figure 4 and 5). RH ranged between 50 and 55 % most of the time in both chambers. There were two periods with high levels of RH (up to 65%), this was related to rainfall outside of the phytotron facility. The temperature ranged between 19 and 24°C during the day and between 14 and 18°C during the night in both chambers.

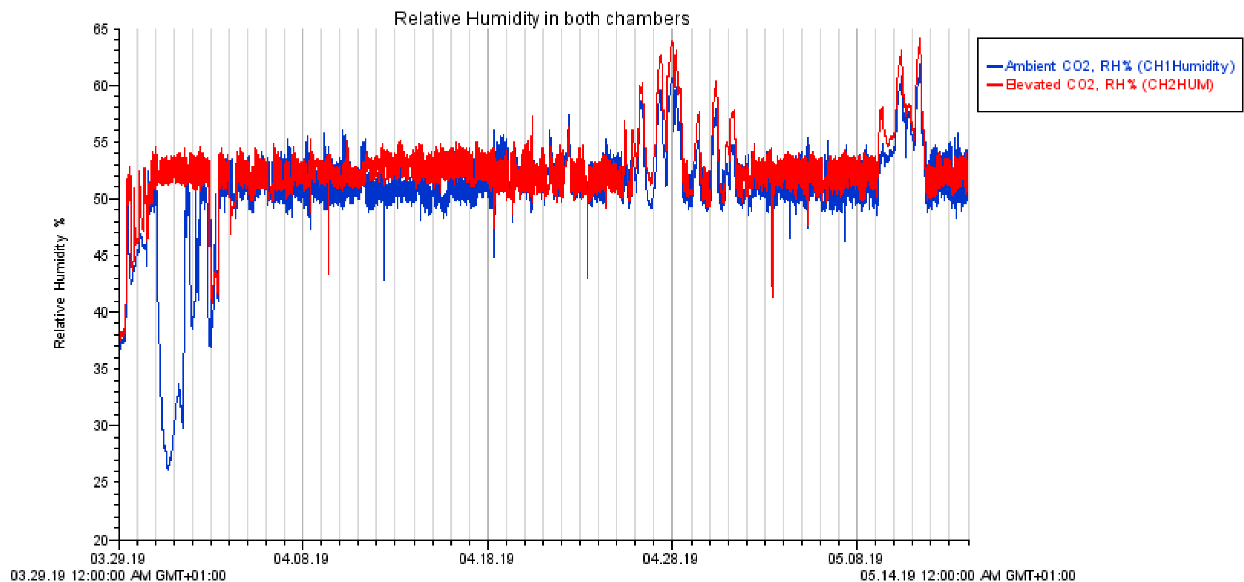



Figure 4: Relative humidity (% RH) in both chambers through the entire experiment. % RH was measured with a HOBO U-12-012 data logger (Onset Computer Corp., Bourne, MA, USA).

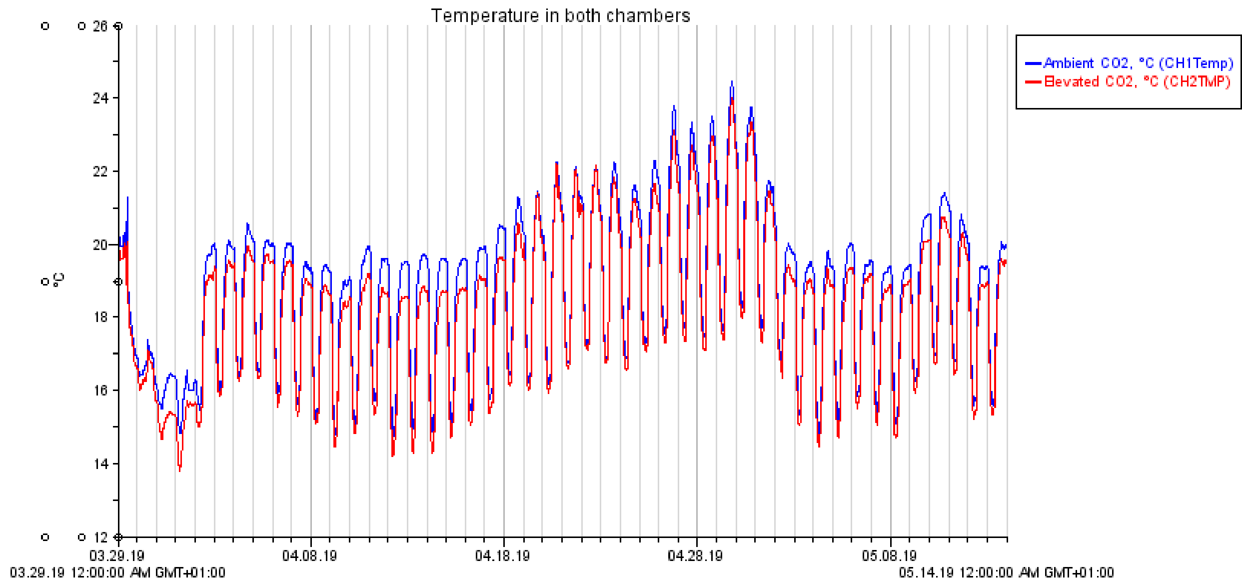



Figure 5: Temperature (°C) in both chambers through the entire experiment. Measured with a HOBO U-12-012 data logger (Onset Computer Corp., Bourne, MA, USA).

The pots were watered from below when needed, they were placed in tap water up to about two cm from the soil surface for five minutes. After the excess water had run off, they were placed back into the chambers. After the first watering, I covered the plants with transparent plastic, still allowing gas flow. After three weeks the transparent plastic was removed. There had been a few moss outbreaks due to moisture accumulation, this was reduced by breaking down the moss with a stick to make it dry out. After 26 days I removed extra seedlings in each pot and saved the largest one, making sure that the number of leaves on each plant were similar. The thinning was to ensure that one plant got enough space, so it could get access to enough water, light and nutrients. The pots with the two smallest plants from each chamber were removed. Plants per pot were counted right before thinning and the leaves on the remaining plant were counted after thinning.

In chamber 1, an average of 3.9 plants had germinated per pot and the plants kept had on average 7.9 leaves. In chamber 2, an average of 3.4 plants had germinated per pot and the plants kept had on average 7.1 leaves. After five weeks the pots were taken out from the big plastic box and placed separately on petri dishes to space them out and avoid shading of neighbours.

The light output on the leaves were measured to see how the light was spread out. The average light level in chamber 1 was 174 ± 4 µE (mean ± SD) and the average light level in chamber 2 was 169 ± 5 µE.

The plants were harvested after 47 days. They were cut just above the soil and put in small bags made of aluminium foil. Above-soil biomass was measured for all plants both before and after drying at 105 °C for 48 h.

## EXPERIMENT 2

In experiment 2 the same procedure was used, but two more LEDs were added above each chamber. The four lights were also lowered to increase the light level further to reach a light level of ˜350 µE. The light levels were increased to test hypothesis 2; that high light levels will further strenghten the responses from elevated $CO_2$.

The light output on the leaves were measured to an average of 346 ± 27 µE (mean ± SD) in chamber 1 and an average of 369 ± 26 µE in chamber 2.

The relative humidity (RH) and temperature (°C) measurements through the experiment showed that RH fluctuated a lot more than in experiment 1, because of rain outside the phytotron. I still managed to keep the RH levels similar between the chambers (Figure 6 and 7). RH ranged between 50 and 75% most of the time in both chambers. The temperature was generally higher than in experiment 1, and ranged between 24 and 27°C most of the time during the day and between 17 and 19°C most of the time at night in both chambers.

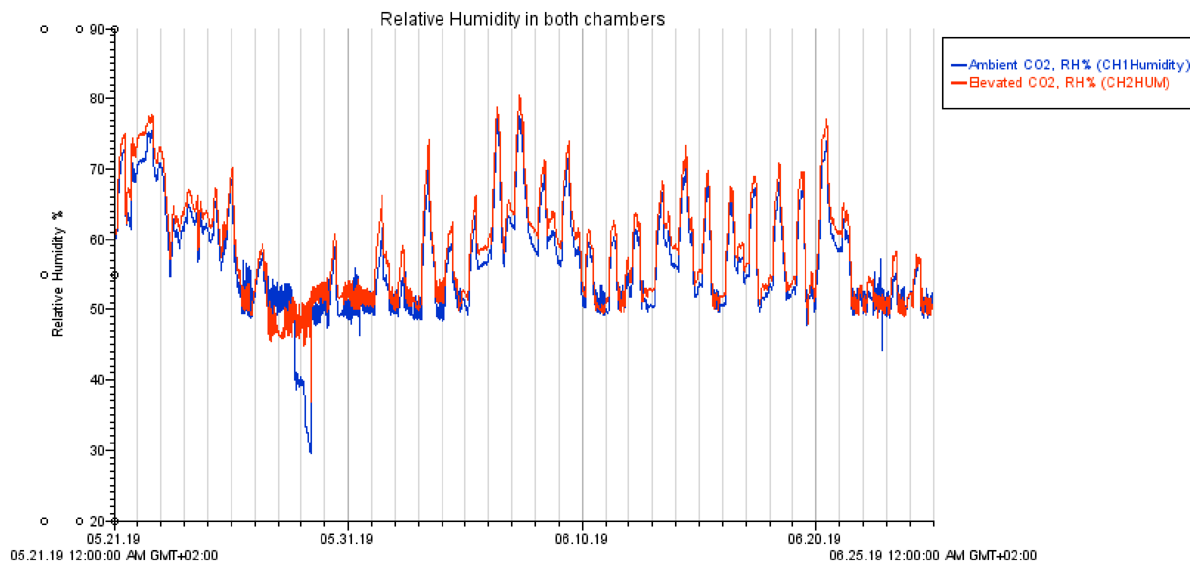



Figure 6: Relative humidity (% RH) in both chambers through the entire experiment. % RH was measured with a HOBO u12-012 data logger (Onset Computer Corp., Bourne, MA, USA).

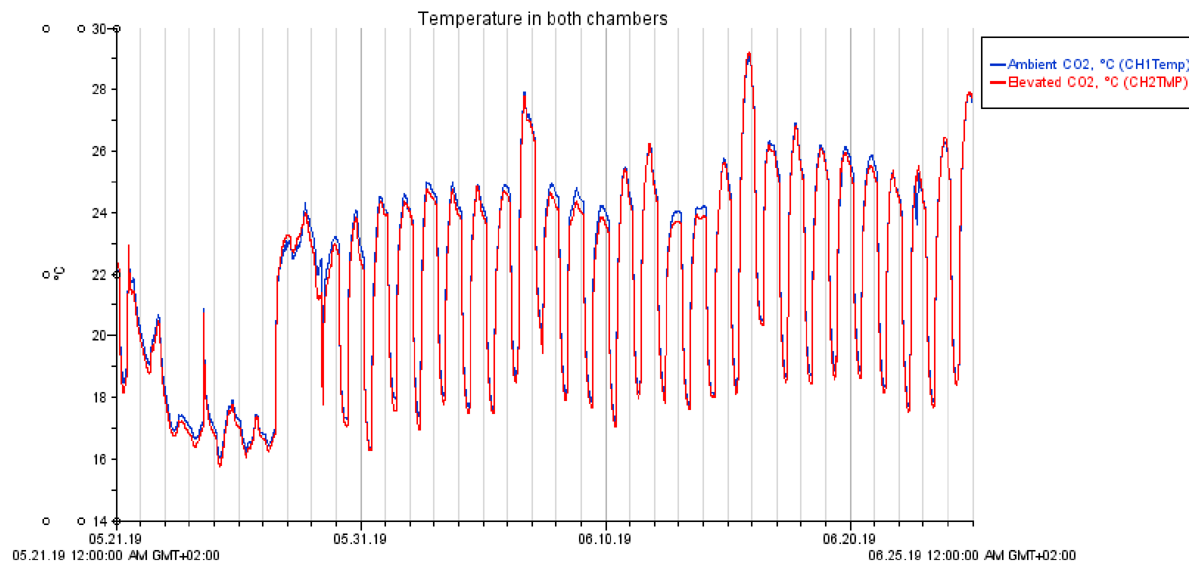



Figure 7: Temperature (°C) in both chambers through the entire experiment, measured with a HOBO U12-012 data logger (Onset Computer Corp., Bourne, MA, USA).

The experiment lasted from 21.05.2019-25.06.2019. Instead of using black plastic like last time to cover the plants, I started with the see-through plastic. The lights were kept off and the plants were kept in the dark for three days inside the chambers. The plastic was removed after two weeks. I thinned the plants after 17 days; the average number of leaves was 6.9 in chamber 1 and 7.3 in chamber 2. They were moved to petri dishes after 29 days and harvested on day 36.

## Analysis

For the carbon (C), nitrogen (N) and phosphorus (P) analysis, the plants were first dried in a chamber of 105°C. Small pieces from leaves, stem and flowers were placed in pre-weighed Zn-capsules for C and N analysis and in plastic containers for P analysis. One leaf sample was taken from each plant. In addition, flower- and stem- samples were taken from five random plants from each chamber, 120 samples were analysed in total. For C- and N-analysis, the samples were combusted in a Thermo Finnigan elemental analyser (EA) 1112 series flash system. Total P was analysed with a Technicon autoanalyzer after persulphate digestion (Figure 8).

For the chlorophyll tests, samples were taken from leaves by using a cork borer and a hammer. The samples were 11 mm in diameter and had an area of 0.95 cm². They were placed in 2.5 ml cryo tubes and stored in a freezer of -80 °C. Then 1 ml ethanol was added in experiment 1, and 1.8 ml ethanol was added in experiment 2 for extraction. Further, the samples were shaken (sonificated) and placed in a refrigerator (4°C). After 48 hours, the samples were shaken again and centrifuged (1500 rpm, 20 min). The samples were diluted 100x and 1000x (exp 1 and 2,

respectively). 250 µl diluted sample were transferred to a well plate, with three pseudoreplicates from each sample.

Internal standards were used to make a calibration curve, to determine the chlorophyll concentration of the samples. The standards were diluted 10x, 100x and 1000x, and three pseudoreplicates were transferred to the well plate, as well as several blank samples (only ethanol).

The samples were run through a microplate reader (BioTek Synergy Mx), which measured fluorescence at set wavelengths for excitation/emission at 430/670 nm.

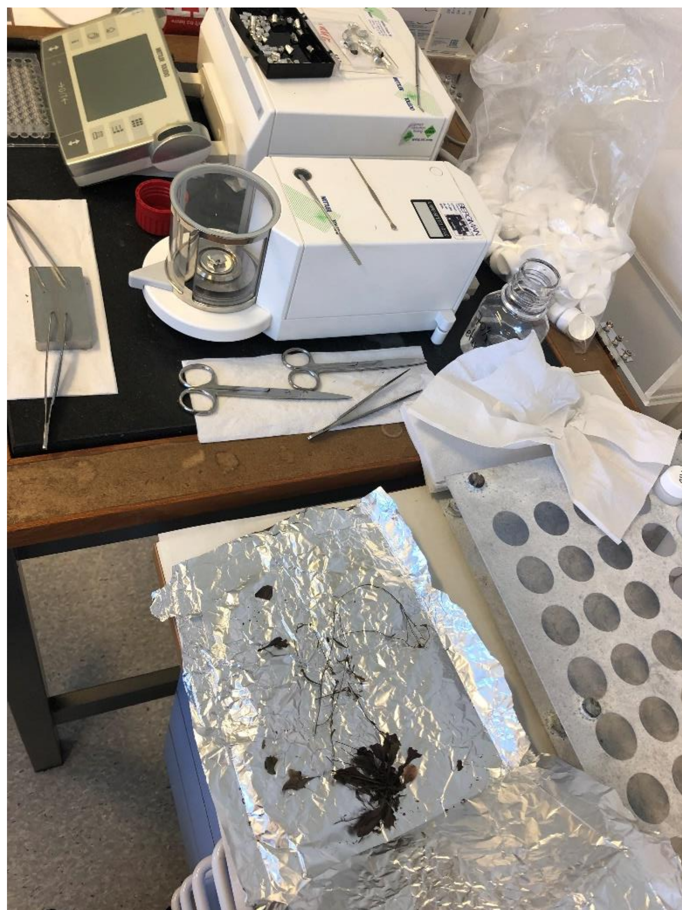


Figure 8: Pieces of dried plant material was placed in Zn capsules for analysis of C, N and P

## STATISTICAL ANALYSIS/CONSIDERATIONS

When estimating the content of elements per shoot, I assumed that the leaves counted for most of the total weight and used % concentration in the leaves as an average for % concentration in the entire shoot. This was then multiplied with the dry weight of the shoot and divided by 100 to find the content in the shoot (and then multiplied with 1000 to get mg).

Outliers that clearly differed from the other observations were removed in both experiments. The outliers removed were:
Experiment 1: 3.558 %N in flower, ch2. 0.164 %P in stem, ch1. 1.818 %P in stem, ch2
Experiment 2: 6.437 %N in flower, ch2. 68.415 %C in flower, ch2. 55.979 %C in leaf, ch2.

To test for differences between the chambers, a two-sample t-test was applied after testing for normal distribution. The t-test was applied to test for significant differences in biomass, chlorophyll concentration, tissue specific concentration and element ratios. The significance level was set at $p < 0.05$.

SPSS was used to make generalized linear models (GLM) to test for differences between the two experiments. The models were tested with AIC to decide which variables best explained the variation in biomass, chlorophyll, element content, concentration and ratios. The leaves were used when comparing elemental values. The explaining factors tested were shoot dry weight (DW), light, $CO_2$, light*$CO_2$, light*DW, $CO_2$*DW and light*$CO_2$*DW. The least significant factor was removed after each run before the model was tested again. The light and $CO_2$ factors were categorical with two levels, whereas DW was included as a covariate, and thus a linear factor.

The parameter estimates were used to find the intercept and slope of increase for each group of plants. Estimated Marginal Means were used to find the mean response for plants from each treatment level of the categorical factors. The treatments that did not have overlapping 95% confidence intervals were considered significantly different from each other. The SPSS statistics was aided by co-supervisor Ane Vollsnes.

# RESULTS

The two experiments showed significant changes in element levels, ratios, and biomass in *Arabidopsis thaliana*. In both experiments, the plants in chamber 2 were exposed to elevated $CO_2$ levels (~700 ppm). The plants in experiment 2 were also exposed to a doubled amount of light (~340 µE) in both chambers.

Biomass can be found in appendix table 1 and 2.

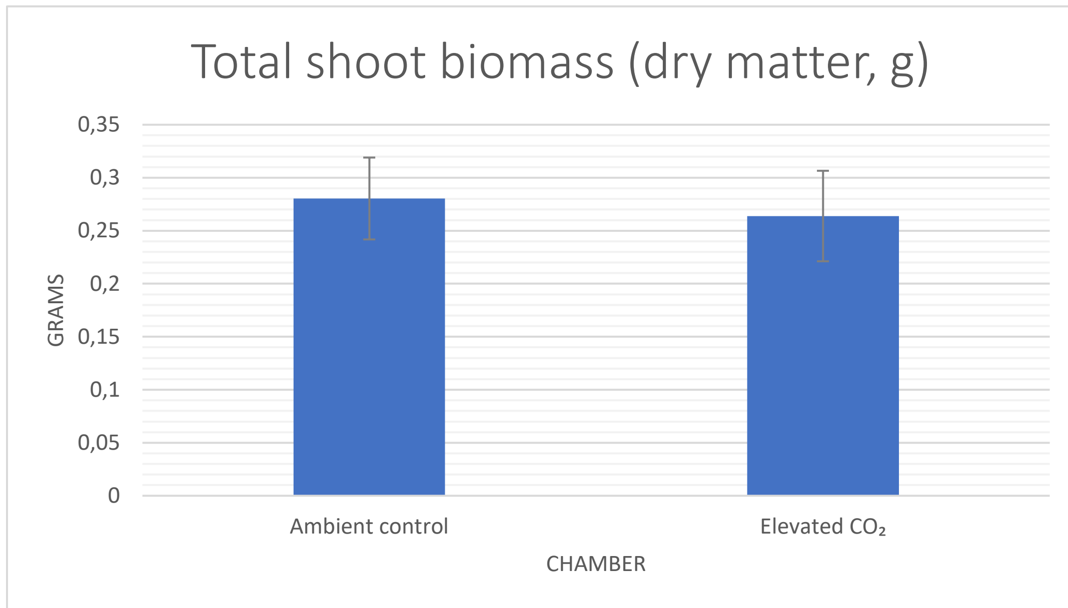
## EFFECTS OF ELEVATED $CO_2$ AND LIGHT ON BIOMASS

In experiment 1, there was no significant difference in the average dry shoot biomass between the chambers ($p = 0.206$). The shoots in chamber 1 (ambient control) weighed 0.280 ± 0.039 g dry weight (DW) (mean ± SD) and in chamber 2 (elevated $CO_2$) 0.264 ± 0.043 g (Figure 9A). In chamber 1, the shoots each had an individual biomass ranging from 0.216 g to 0.359 g DW. In chamber 2, the individual biomass ranged from 0.172 g to 0.338 g.

Similarly, in experiment 2, there was no significant difference in average dry shoot biomass between the chambers ($p = 0.281$). The shoots in chamber 1 (ambient control) weighed 0.317 ± 0.065 g and in chamber 2 (elevated $CO_2$) 0.294 ± 0.068 g (Figure 9B). In chamber 1, the shoots each had an individual biomass ranging from 0.209 g to 0.436 g. In chamber 2, the individual biomass ranged from 0.184 g to 0.416 g.

Including data from both experiments in the same analysis showed that light was the only significant factor (Table 18). This coincides with the results between chambers and indicates that $CO_2$ did not have an effect on biomass, even with increased light. Increased light caused increased biomass under elevated $CO_2$, however.

*a) Experiment 1*




*b) Experiment 2*

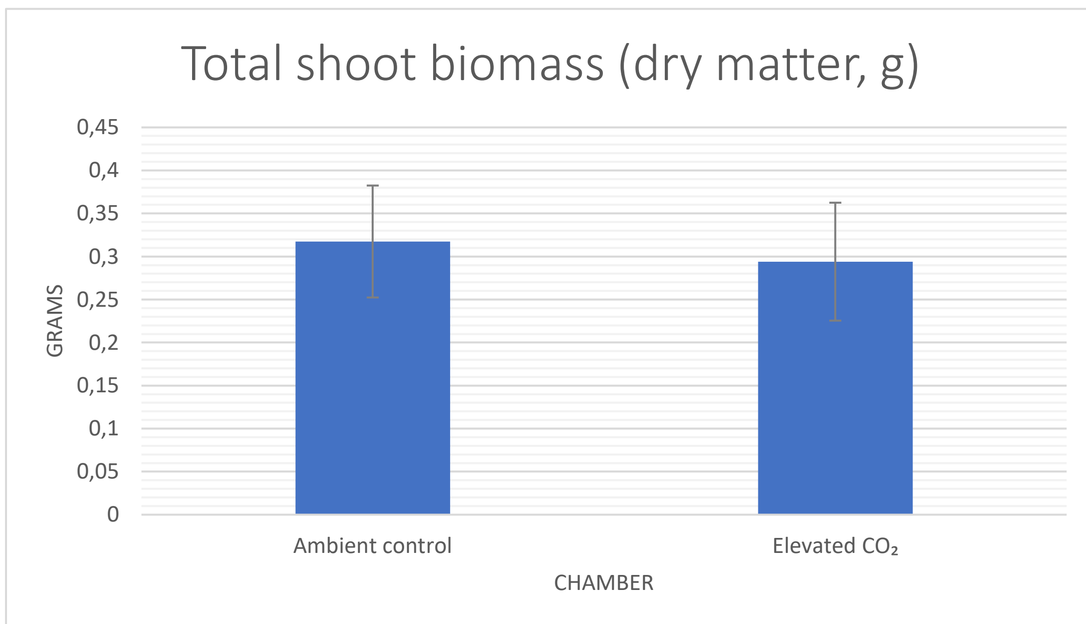



Figure 9: Average total shoot biomass (dry weight) in grams per plant in chamber 1 (ambient control) and chamber 2 (elevated $CO_2$). A) Experiment 1. B) Experiment 2 with elevated light in both chambers.

## EFFECTS OF ELEVATED $CO_2$ AND LIGHT ON CHLOROPHYLL CONCENTRATION

Results from the chlorophyll concentration analysis can be found in appendix table.

Chlorophyll concentration was analysed by extraction from a standardized area from leaves. There was a significant difference in chlorophyll concentration between the chambers in both experiments. In experiment 1, the leaves had 26.43 ± 2.7 µg (mean ± SD) chlorophyll per $cm^2$ under ambient $CO_2$ versus 28.18 ± 2.2 µg per $cm^2$ with elevated $CO_2$. In experiment 2, the corresponding concentrations were 21.52 ± 2.03 µg per $cm^2$ under ambient $CO_2$ and 28.08 ± 4.7 µg per $cm^2$ with elevated $CO_2$ (Figure 10), hence under low $CO_2$ elevated light reduced area-specific chlorophyll in line with expectations, while this light effect was nearly absent at high $CO_2$.

Including data from both experiments in the same analysis showed that light, $CO_2$, DW and light*$CO_2$ contributed significantly to the specific chlorophyll concentrations (Table 18). This indicates that the chlorophyll concentration increased similarly with shoot size in the four treatment groups across $CO_2$ and light levels (see slope of increase in Table 1). Further, the significant effect of the interaction between $CO_2$ and light levels indicates that the effect of elevated $CO_2$ differed between the two experiment runs with differing light levels.

Table 1: Table for regression lines of chlorophyll concentration (µg per $cm^2$) against shoot dry weight (g) generated from the Generalized linear model analysis with data from both experiments included (Light 170: Experiment 1, Light 350: Experiment 2). Different letters indicate significantly different intercepts.

| Light | $CO_2$ | Intercept | Slope of increase |
|---|---|---|---|
| 170 | 400 ppm | 21.53 b | 17.47 |
| 170 | 700 ppm | 23.57 b | 17.47 |
| 350 | 400 ppm | 16.09 a | 17.47 |
| 350 | 700 ppm | 22.95 b | 17.47 |

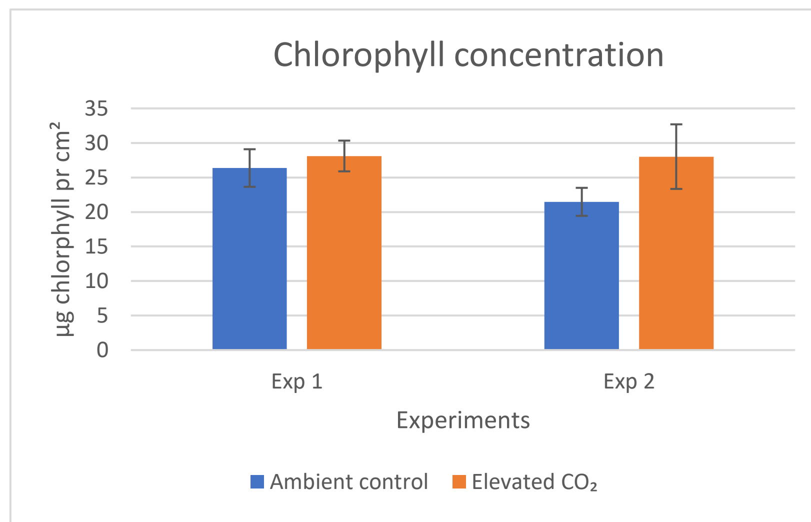
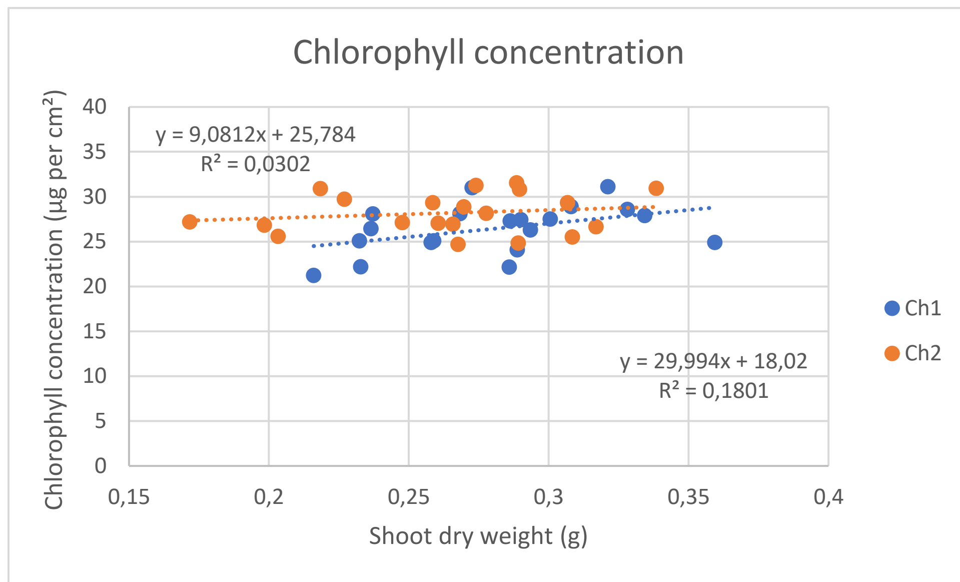



Figure 10: Chlorophyll concentration average ± SD in the leaves. µg chlorophyll per $cm^2$.

*a) Experiment 1*




*b) Experiment 2*

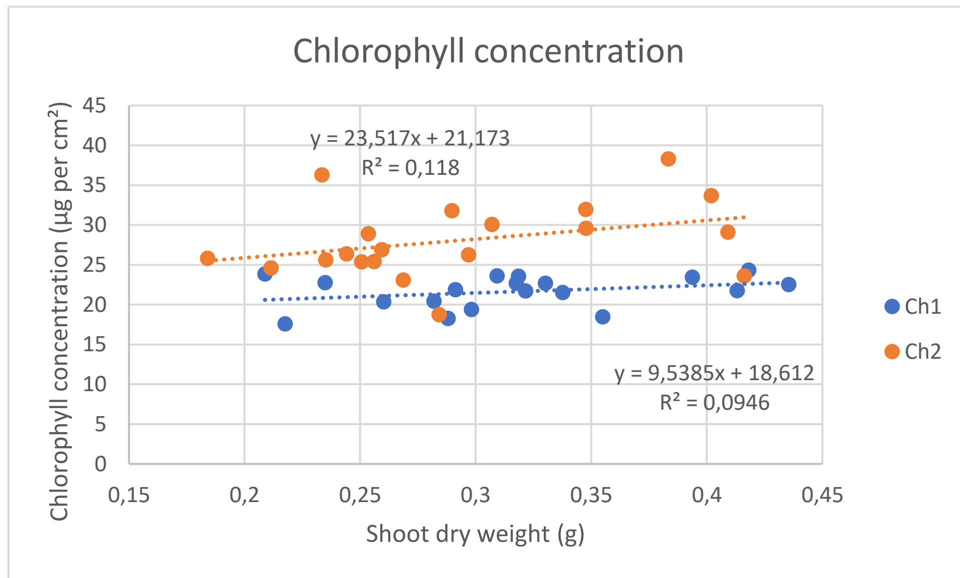



Figure 11: Chlorophyll concentration µg per cm² in the shoot (DW). Chamber 1 is ambient control and chamber 2 is elevated $CO_2$. A) Experiment 1, B) Experiment 2, increased light

## Effects of elevated $CO_2$ and light on element content

All elemental results can be found in Appendix table 1 and 2.

### Carbon content

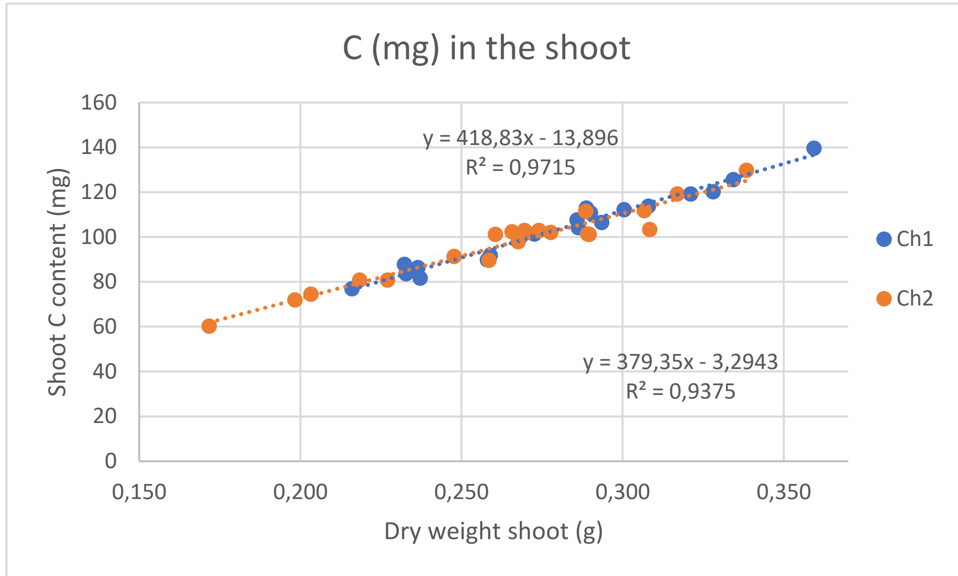
Carbon (C) in the shoot increased linearly with the dry weight of the shoot in both experiments (Figure 12). In experiment 1, total C per plant ranged from 77 mg to 140 mg in chamber 1, and from 60 mg to 130 mg in chamber 2. In experiment 2, the C content ranged from 88 mg to 193 mg in chamber 1, and from 81 mg to 225 mg in chamber 2. $R^2$ was close to 1 in all treatments, but down on 0.82 in chamber 2 in experiment 2. This implies close to linear proportion between mg C and DW of the shoot.

Including data from both experiments in the same analysis showed that light, $CO_2$, DW, light*DW and $CO_2$*DW were significant factors (Table 18). This indicates that the C content changed with the DW of the shoot, but that the effect was different between chambers and light levels. C content increased with DW, the slope of increase was highest with low $CO_2$ in experiment 1 and with high $CO_2$ in experiment 2 (See slope of increase, table 2).

Table 2: Table for regression lines of carbon content (mg) against shoot dry weight (g) generated from the Generalized linear model analysis with data from both experiments included (Light 170: Experiment 1, Light 350: Experiment 2). Different letters indicate significantly different intercepts.

| **Light** | **$CO_2$** | **Intercept** | **Slope of increase** |
|---|---|---|---|
| 170 | 400 ppm | -16.7 a | 430 |
| 170 | 700 ppm | -0.5 a | 368 |
| 350 | 400 ppm | -18.6 b | 493 |
| 350 | 700 ppm | -2.4 b | 430 |

*a) Experiment 1*




*b) Experiment 2*

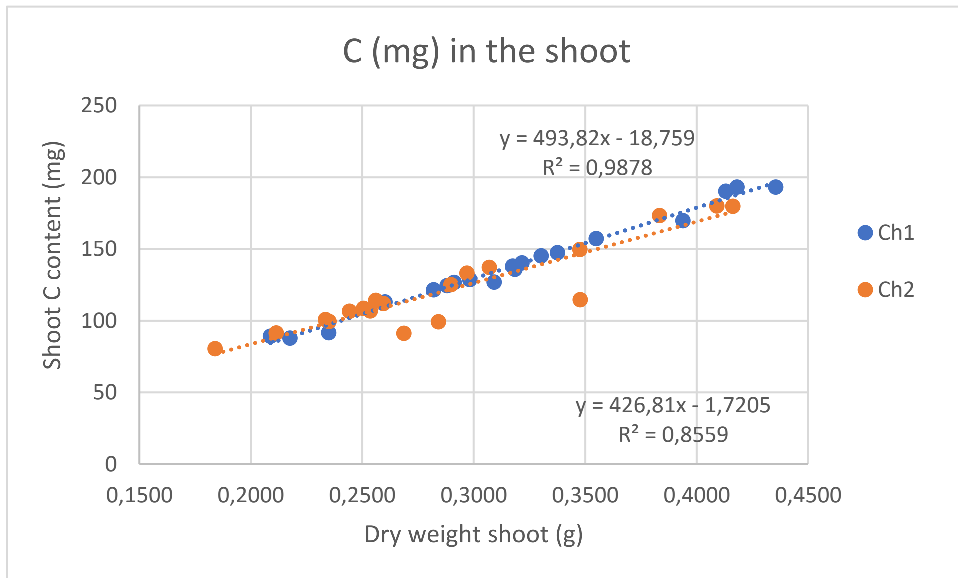



Figure 12: Carbon (C) in mg per gram dry weight of the shoot per plant (dots). Average C content in mg per gram dry weight of the shoot (lines). Chamber 1 is ambient control and chamber 2 is elevated $CO_2$. A) Experiment 1, B) Experiment 2, increased light. As there were no difference in trends, the treatments were pooled into one single regression line.

### NITROGEN CONTENT

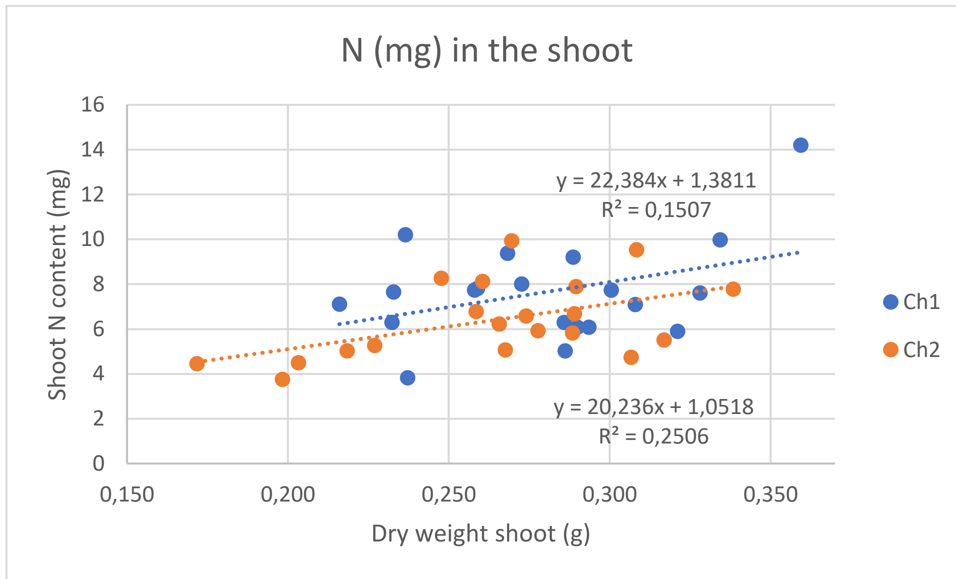
Nitrogen (N) in the shoot increased linearly with increased DW in both experiments (Figure 13). In experiment 2, there was a significant difference in total N between chambers (Table 18). Here, chamber 2 had N-content that was 3 mg (49%) lower than in chamber 1 on average. In experiment 1, the N-content ranged from 3.8 mg to 14.2 mg in chamber 1, and 3.8 mg to 9.9 mg in chamber 2. In experiment 2, the N-content ranged from 3.5 mg to 11.6 mg in chamber 1, and from 1.6 mg to 6.1 mg in chamber 2. $R^2$ for the regression lines was low in both chambers in experiment 1 and with ambient $CO_2$ in experiment 2 (<0.25), but higher with elevated $CO_2$ (0.65). This indicates that very little of the variation in N could be explained by the DW when studying each plant group separately.

Including data from both experiments in the same analysis showed that light, $CO_2$, light*$CO_2$ and DW were significant factors (Table 18). This indicates that N increased similarly with the weight of the shoot in all four treatments across $CO_2$ and light levels (see slope of increase, table 3). The higher number of data points made this pattern significant, although it was not when studying smaller parts of the data set separately, as in Figure 13. The significant effect of Light*$CO_2$ indicates that the effect of elevated $CO_2$ differed between the two experiments.

Table 3: Table for regression lines of nitrogen content (mg) against shoot dry weight (g) generated from the Generalized linear model analysis with data from both experiments included (Light 170: Experiment 1, Light 350: Experiment 2). Different letters indicate significantly different intercepts.

| **Light** | **$CO_2$** | **Intercept** | **Slope of increase** |
|---|---|---|---|
| 170 | 400 ppm | 2.93 c | 16.8 |
| 170 | 700 ppm | 1.95 bc | 16.8 |
| 350 | 400 ppm | 1.13 b | 16.8 |
| 350 | 700 ppm | -1.64 a | 16.8 |

*a) Experiment 1*




*b) Experiment 2*

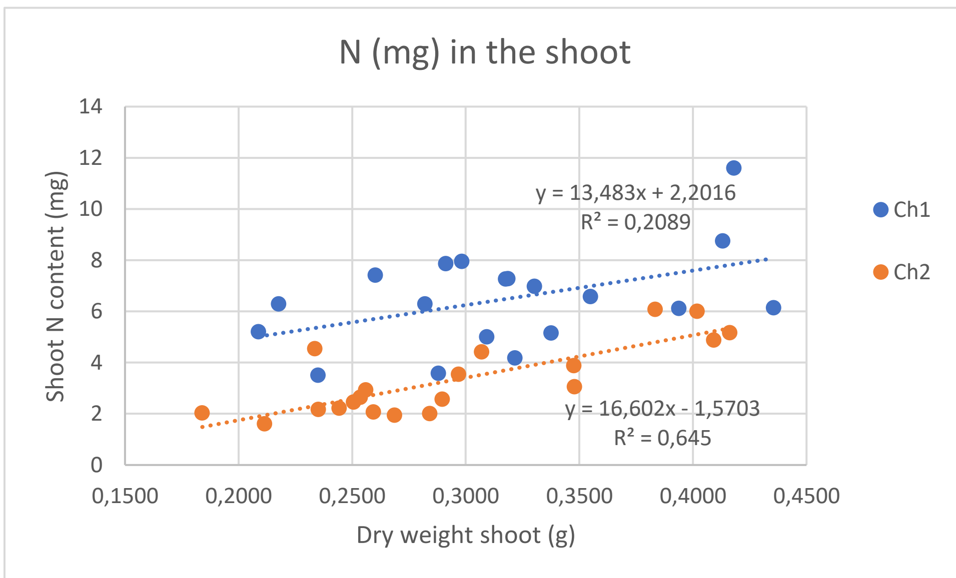



Figure 13: Nitrogen (N) in mg per gram dry weight of the shoot per plant (dots). Average content of N in mg per gram dry weight of the shoot (lines). Chamber 1 is ambient control and chamber 2 is elevated $CO_2$. A) Experiment 1. B) Experiment 2, increased light.

### Phosphorus content

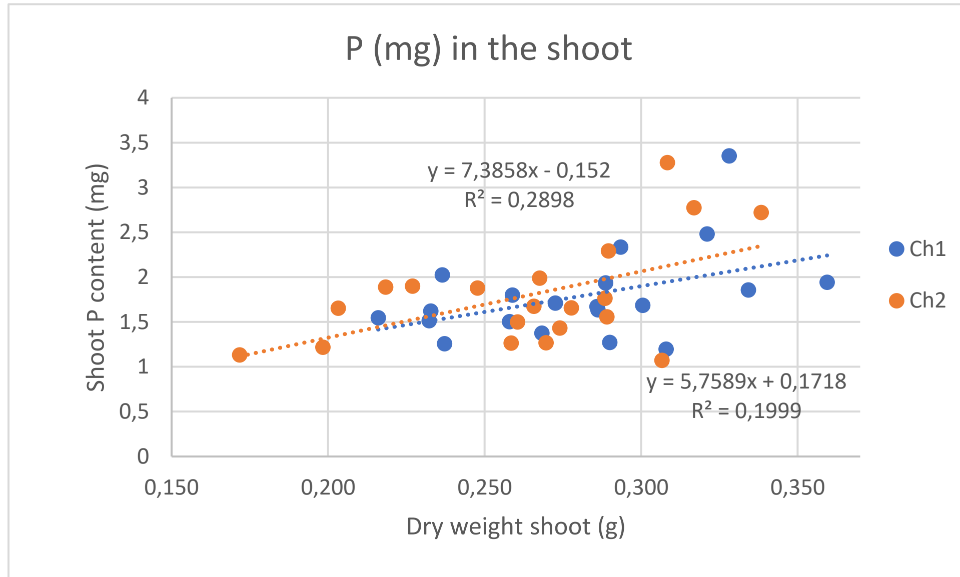
Also the phosphorus (P) content in the shoot increased linearly with the weight of the shoot in both experiments (Figure 14). In experiment 1, the increase in total P relative to DW was a little bit higher in chamber 2. The P-content ranged from 1.2 mg to 2.5 mg in chamber 1, and from 1.1 mg to 3.3 mg in chamber 2. In experiment 2, the weight of the plants was by and large similar in both chambers, but the P-content was significantly lower in chamber 2 (Table 18). The P-content ranged from 0.8 mg to 2.2 mg in chamber 1, and from 0.4 mg to 2.1 mg in chamber 2. $R^2$ for the regression lines was low in all treatments (< 0.29), indicating that very little of the variation in P could be explained by the DW when studying each plant group separately.

Including data from both experiments in the same analysis showed that light, $CO_2$, light*$CO_2$, DW and DW*light were significant factors (Table 18). This indicates that there was a significant difference between some of the treatments. Further, the significant effect of the interaction between light and DW indicates that the effect of the DW differed between the two experiments (see slope of increase, table 4).

Table 4: Table for regression lines of phosphorus content (mg) against shoot dry weight (g) generated from the Generalized linear model analysis with data from both experiments included (Light 170: Experiment 1, Light 350: Experiment 2). Different letters indicate significantly different intercepts.

| **Light** | **$CO_2$** | **Intercept** | **Slope of increase** |
|---|---|---|---|
| 170 | 400 ppm | 0.581 c | 6.65 |
| 170 | 700 ppm | 0.041 c | 6.65 |
| 350 | 400 ppm | 0.643 b | 2.33 |
| 350 | 700 ppm | 0.103 a | 2.33 |

*a) Experiment 1*




*b) Experiment 2*

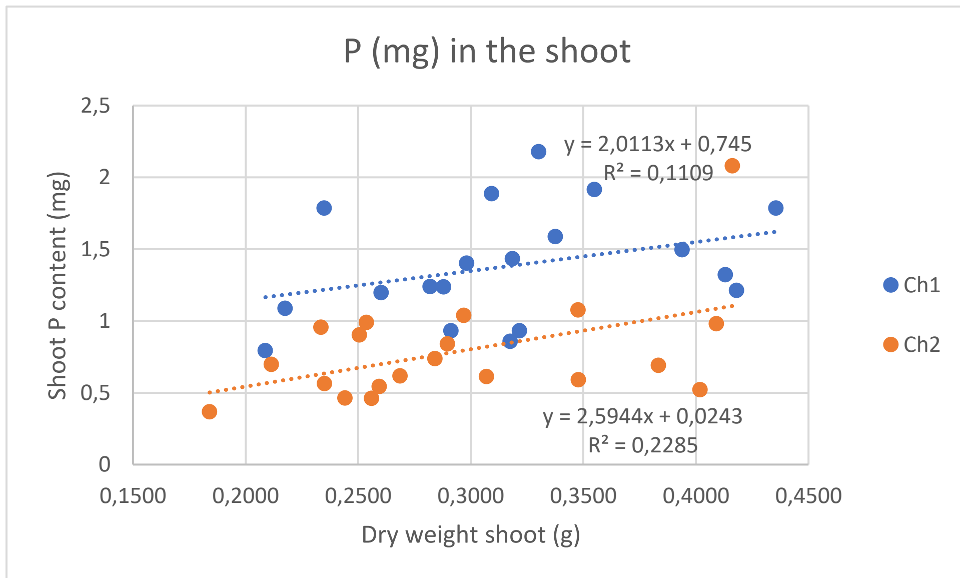



Figure 14: Phosphorus (P) in mg per gram dry weight of the shoot per plant (dots). Average content of P in mg per gram dry weight of the shoot (lines). Chamber 1 is ambient control and chamber 2 is elevated $CO_2$. A) Experiment 1. B) Experiment 2, increased light.

## Effects of elevated $CO_2$ on tissue specific concentration

### Carbon concentration

#### *Experiment 1*

Shoot C concentration (%) per DW was quite similar in the leaves and the stems, while the flowers had a significantly higher concentration than the other tissues in both chambers. Analyses of %C did not show any significant changes in the leaves between the chambers. There was a significant decrease in the flowers ($p = 0.023$) from the ambient control chamber to the elevated $CO_2$ chamber, with an average decline of 7.75% (Table 5). All the flower samples from the elevated $CO_2$ chamber showed lower levels of C than in the chamber with ambient $CO_2$ except for one. The samples from the leaves had almost the same level of C in each chamber (Figure 14). In the stem, the average was a bit lower in chamber 1 than in chamber 2 (2.12%), but the decline was not significant.

Table 5: Average and SD from the results of the %C analysis on different plant tissues after treatment with ambient and elevated $CO_2$. P-value from t-test between chambers. All significant p-values are bolded.

| %C | Leaf | Stem | Flower |
|---|---|---|---|
| **Average Ambient** | 36.838 | 36.155 | 43.469 |
| **Average Elevated** | 36.646 | 38.278 | 40.099 |
| **SD Ambient** | 1.222 | 1.913 | 0.477 |
| **SD Elevated** | 1.499 | 1.3634 | 2.637 |
| ***p*** | 0.659 | 0.078 | **0.023** |

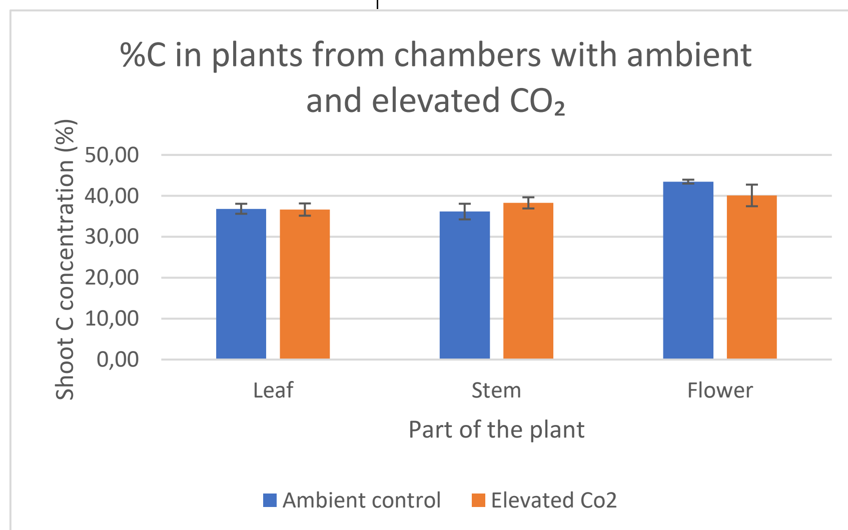



Figure 14: Shoot C concentration (%, DW) means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber 1, elevated $CO_2$ is chamber 2.

### *Experiment 2*

Shoot C concentration (%) per DW was significantly higher in the stem and flowers than in the leaves in both chambers (Table 6). There were no significant changes in %C between the chambers (Figure 15). There was a larger variation in specific C in chamber 2 compared with chamber 1, as reflected in the higher SDs.

Table 6: Average and SD from the results of the %C analysis on different plant parts after treatment with ambient and elevated $CO_2$. P-value from t-test between chambers.

| %C | Leaf | Stem | Flower |
|---|---|---|---|
| **Average Ambient** | 43.166 | 47.533 | 49.785 |
| **Average Elevated** | 42.107 | 47.602 | 49.634 |
| **SD Ambient** | 1.670 | 1.128 | 0.144 |
| **SD Elevated** | 3.709 | 2.172 | 2.901 |
| ***p*** | 0.254 | 0.952 | 0.909 |

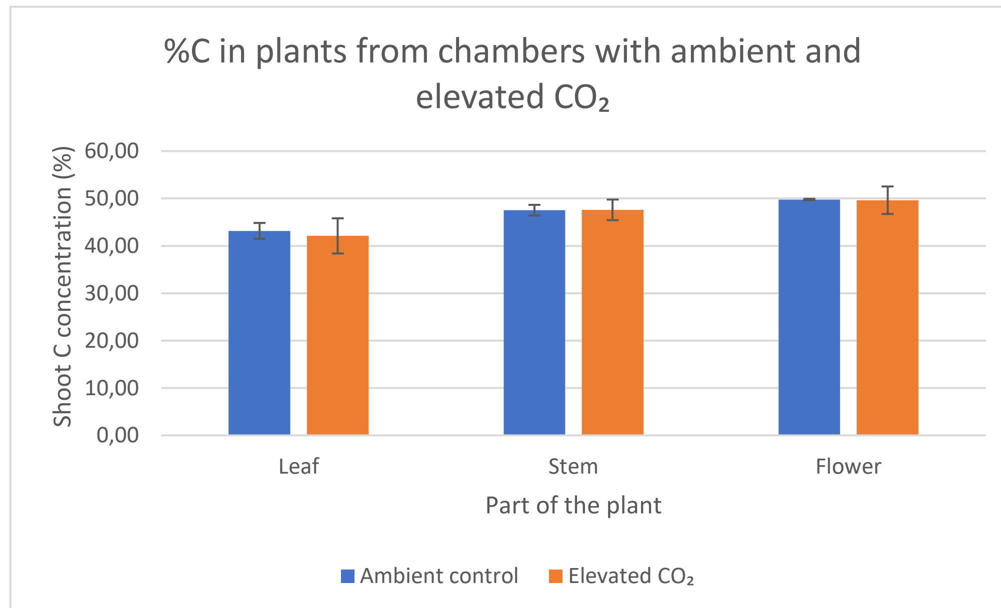



Figure 15: Shoot C concentration (%, DW) means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber 1, elevated $CO_2$ is chamber 2.

### NITROGEN CONCENTRATION

#### *EXPERIMENT 1*

Shoot N concentration (%) per DW was higher in the stem than in the leaves, with a significant difference in chamber 2 (Figure 16). An average of 3% N was substantially higher in the flower than in the other tissues, in fact 67% higher than the stem in chamber 1 and 34% higher in chamber 2 (Table 7). There was a significant decrease in the flowers ($p = 0.017$) with an average decline of 10.84% from the ambient control chamber to the elevated $CO_2$ chamber. The flowers in chamber 1 had a very stable N level, ranging from 5.1 to 5.8%. There was a decline of %N in all tissue with elevated $CO_2$, except for the stem where a high variability rendered an insignificant increase.

Table 7: Average and SD from the results of the %N analysis on different plant parts after treatment with ambient and elevated $CO_2$. P-value from t-test between chambers. All significant p-values are bolded.

| **%N** | **Leaf** | **Stem** | **Flower** |
|---|---|---|---|
| **Average Ambient** | 2.746 | 3.223 | 5.386 |
| **Average Elevated** | 2.429 | 3.583 | 4.803 |
| **SD Ambient** | 0.731 | 0.462 | 0.286 |
| **SD Elevated** | 0.546 | 0.835 | 0.267 |
| ***p*** | 0.128 | 0.424 | **0.017** |

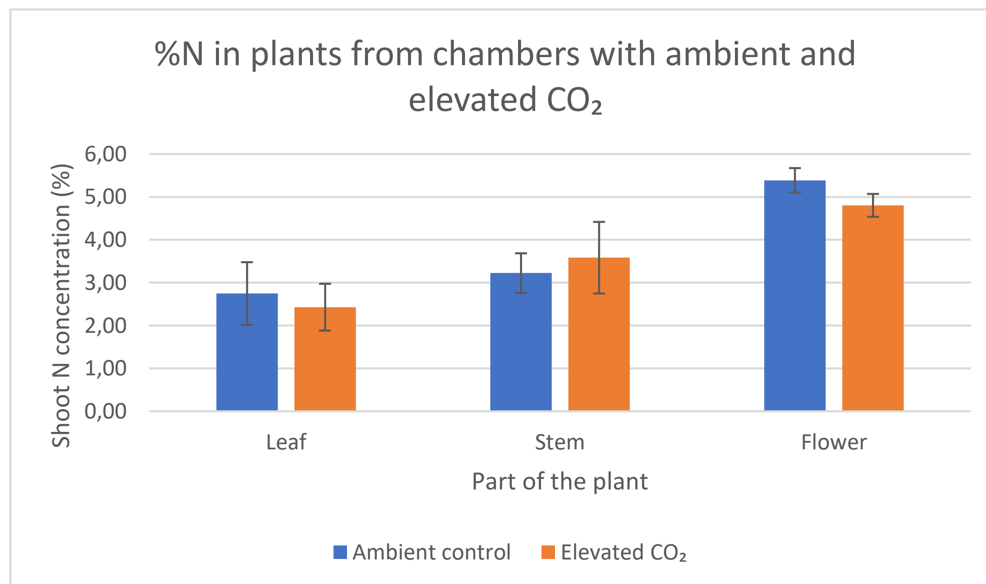



Figure 16: Shoot N concentration (%, DW) means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber 1, elevated $CO_2$ is chamber 2.

*EXPERIMENT 2*

Shoot N concentration (%) per DW was almost identical in the leaves and stems in chamber 1, but a bit higher in the stems than in the leaves in chamber 2 (Figure 17). %N in the flowers was significantly higher than in the other tissues in both chambers with almost 5% N compared to 2% in the leaves in chamber 1 and ~4% compared to ~1% in the leaves in chamber 2 (Table 8). There was a significant decline in stems (p = 0.004, 37.5%), leaves ($p << 0.001$, 45.9%) and flowers (p = 0.0397, 10.7%) with elevated $CO_2$.

Table 8: Average and SD from the results of the %N analysis on different plant parts after treatment with ambient and elevated $CO_2$. P-value from t-test between chambers. All significant p-values are bolded.

| %N | Leaf | Stem | Flower |
|---|---|---|---|
| **Average Ambient** | 2.038 | 2.027 | 4.750 |
| **Average Elevated** | 1.103 | 1.266 | 4.242 |
| **SD Ambient** | 0.571 | 0.292 | 0.353 |
| **SD Elevated** | 0.320 | 0.324 | 0.210 |
| ***p*** | **1.664E-07** | **0.005** | **0.040** |

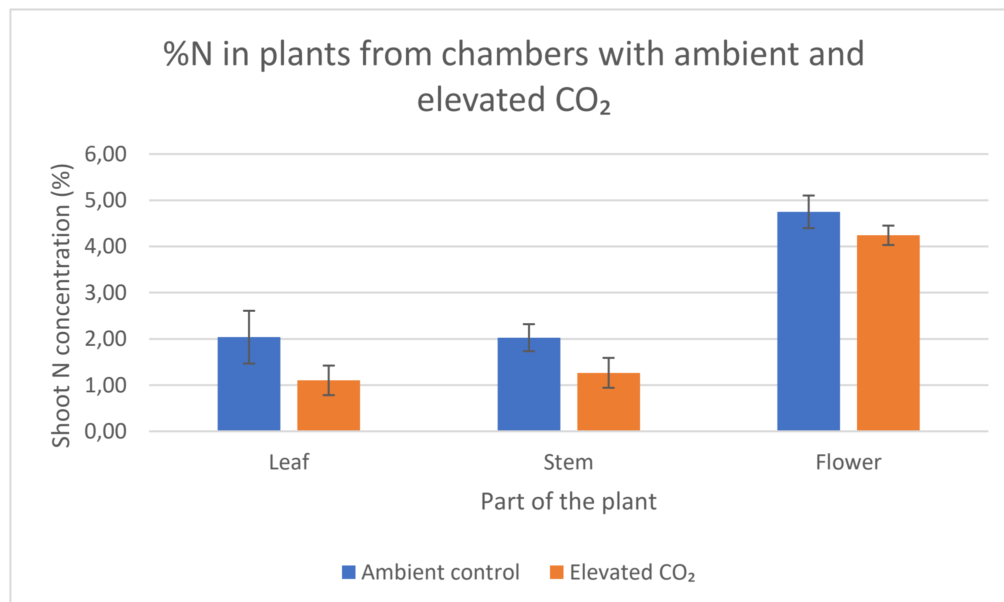



Figure 17: Shoot N concentration (%, DW) means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber 1, elevated $CO_2$ is chamber 2.

## PHOSPHORUS CONCENTRATION

### *EXPERIMENT 1*

Shoot P concentration (%) per DW was quite similar for leaves and stems in both chambers, but significantly higher (almost double) in the flowers (Figure 18). In chamber 2, %P was around 0.6% in the leaves, ~0.7% in the stem, and ~1.3% in the flowers (Table 9). There were however no significant differences between the ambient control chamber and the elevated $CO_2$ chamber, even though there were slightly elevated concentrations of P with elevated $CO_2$ in all tissues.

Table 9: Average and SD from the results of the %P analysis on different plant parts after treatment with ambient and elevated $CO_2$. P-value from t-test between chambers.

| **%P** | **Leaf** | **Stem** | **Flower** |
|---|---|---|---|
| **Average Ambient** | 0.639 | 0.676 | 1.156 |
| **Average Elevated** | 0.691 | 0.759 | 1.271 |
| **SD Ambient** | 0.148 | 0.106 | 0.225 |
| **SD Elevated** | 0.172 | 0.168 | 0.307 |
| ***p*** | 0.411 | 0.434 | 0.517 |

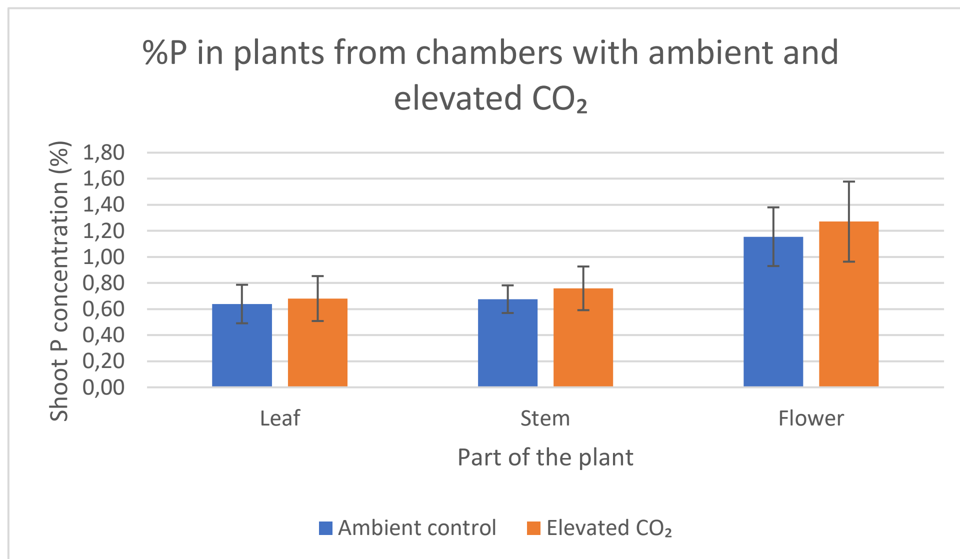



Figure 18: Shoot P concentration (%, DW) means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber 1, elevated $CO_2$ is chamber 2.

### *Experiment 2*

Shoot P concentration (%) per DW was quite similar in the leaves and stems in chamber 1, but more than twice as high in the flowers. In chamber 2, there was on average 0.27 %P in the leaves, 0.4 in the stems and 0.8 in the flowers (Figure 19). There was a significant decline in %P of 14.4% in the flowers, and a significant decline in %P of 38.5% in the leaves with elevated $CO_2$ (Table 10). In the stems the levels of P in chamber 1 were between 0.21% and 0.62%, and between 0.16% and 0.63% in chamber 2.

Table 10: Average and SD from the results of the %N analysis on different plant parts after treatment with ambient and elevated $CO_2$. P-value from t-test between chambers. All significant p-values are bolded.

| **%P** | **Leaf** | **Stem** | **Flower** |
|---|---|---|---|
| **Average Ambient** | 0.437 | 0.452 | 0.938 |
| **Average Elevated** | 0.269 | 0.398 | 0.803 |
| **SD Ambient** | 0.131 | 0.165 | 0.069 |
| **SD Elevated** | 0.096 | 0.227 | 0.054 |
| ***p*** | **4.268E-05** | 0.676 | **0.009** |

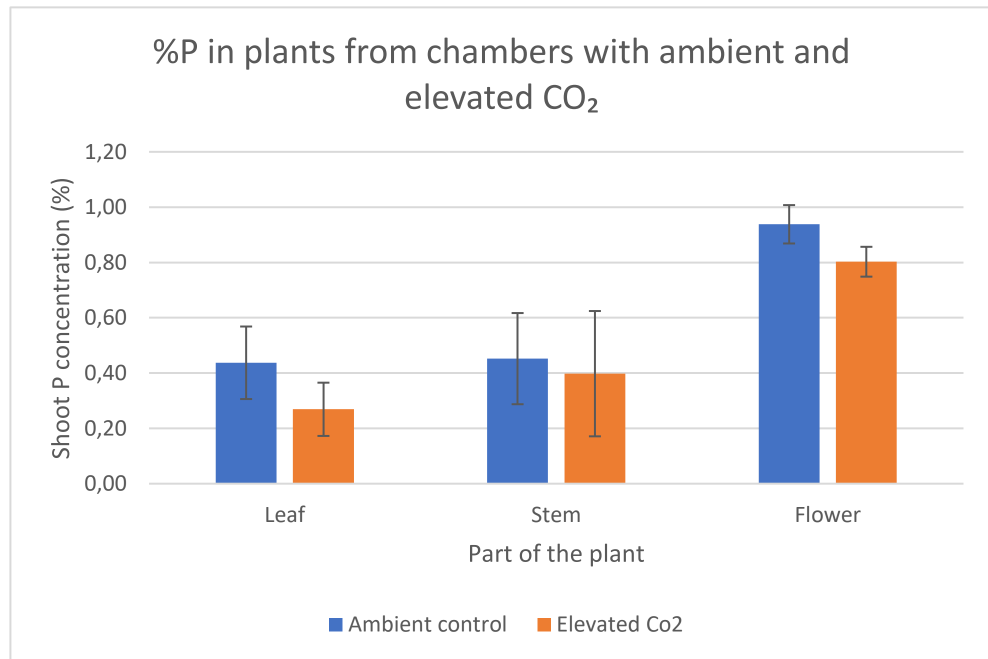



Figure 19: Shoot P concentration (%, DW) means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber 1, elevated $CO_2$ is chamber 2.

## EFFECTS OF ELEVATED $CO_2$ ON ELEMENT RATIOS

### C:P RATIOS

#### *EXPERIMENT 1*

There was a significant difference between the C:P ratios of the leaves and flowers in both chambers, with highest ratio in the leaves (Figure 20). There were no significant differences in the C:P ratios between the chambers. The ratio was slightly lower, and the variation was higher in chamber 2 (Table 11).

Table 11: The C:P ratio mean, SD, and estimated p-value from samples of plant tissue from *Arabidopsis thaliana* after one growth season. P-value from t-test between chambers.

| C:P | Leaf | Stem | Flower |
|---|---|---|---|
| **Average Ambient** | 60.638 | 55.492 | 38.819 |
| **Average Elevated** | 57.619 | 52.825 | 33.368 |
| **SD Ambient** | 14.354 | 9.830 | 7.765 |
| **SD Elevated** | 16.656 | 13.504 | 9.829 |
| ***p*** | 0.543 | 0.760 | 0.359 |

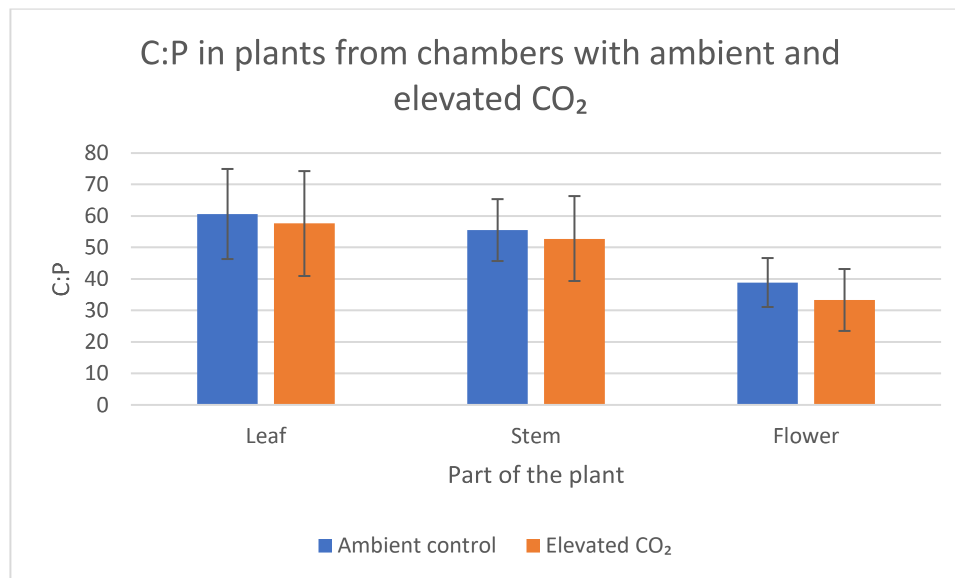



Figure 20: The C:P ratio means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber, elevated $CO_2$ is chamber 2.

*EXPERIMENT 2*

The C:P ratio in the leaves and stems were quite similar in both chambers. C:P in the flowers was significantly lower than in the leaves in both chambers (Figure 21). There was a significant increase in the C:P ratio in the leaves, even with high variation in chamber 2 (Table 12). There was also a significant increase in the flowers. The C:P ratio in the chamber with elevated $CO_2$ was higher than in the chamber with ambient $CO_2$ in all plant tissues.

Table 12: The C:P ratio mean, SD, and estimated p-value from samples of plant tissue from *Arabidopsis thaliana* after one growth season. P-value from t-test between chambers. All significant p-values are bolded.

| **C:P** | **Leaf** | **Stem** | **Flower** |
|---|---|---|---|
| **Average Ambient** | 107.489 | 123.106 | 53.320 |
| **Average Elevated** | 166.965 | 169.102 | 60.506 |
| **SD Ambient** | 32.330 | 64.373 | 4.122 |
| **SD Elevated** | 50.872 | 112.249 | 4.361 |
| ***p*** | **< 0.001** | 0.450 | **0.039** |

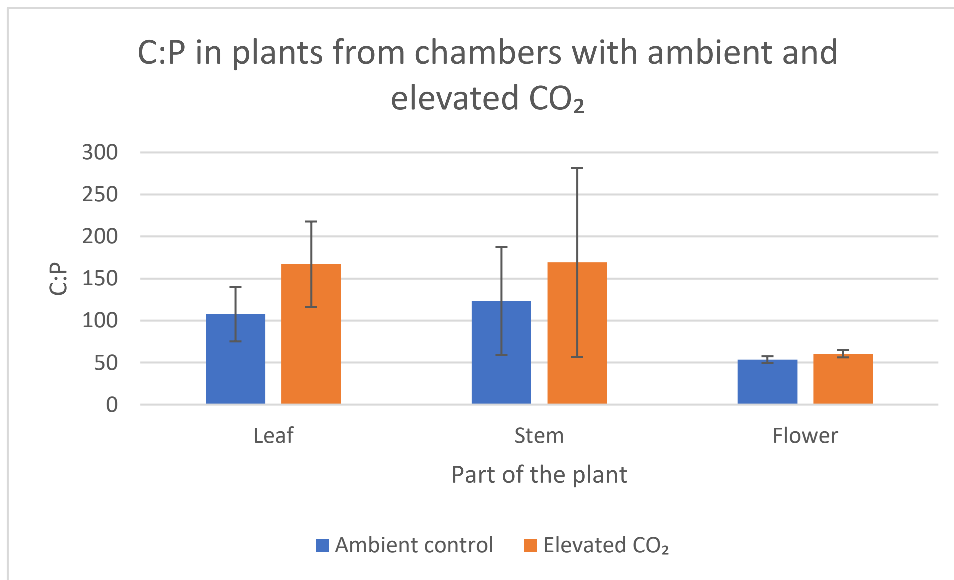



Figure 21: The C:P ratio means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber 1, elevated $CO_2$ is chamber 2.

### C:N RATIOS

#### *EXPERIMENT 1*

The C:N ratio was highest in the leaves and lowest in the flowers in both chambers. C:N was significantly lower in the flowers than in the stems and leaves in chamber 1, and significantly higher in the leaves than in the stems and flowers in chamber 2 (Figure 22). The ratio increased with elevated $CO_2$ in the leaves and the flowers, while the stem showed a slight decrease. None of the changes between the chambers were significant (Table 13).

Table 13: The C:N ratio mean, SD, and estimated p-value from samples of plant tissue from *Arabidopsis thaliana* after one growth season. P-value from t-test between chambers.

| C:N | Leaf | Stem | Flower |
|---|---|---|---|
| **Average Ambient** | 14.350 | 11.438 | 8.088 |
| **Average Elevated** | 15.810 | 11.166 | 8.562 |
| **SD Ambient** | 3.836 | 2.019 | 0.428 |
| **SD Elevated** | 3.569 | 2.632 | 0.847 |
| ***p*** | 0.220 | 0.859 | 0.307 |

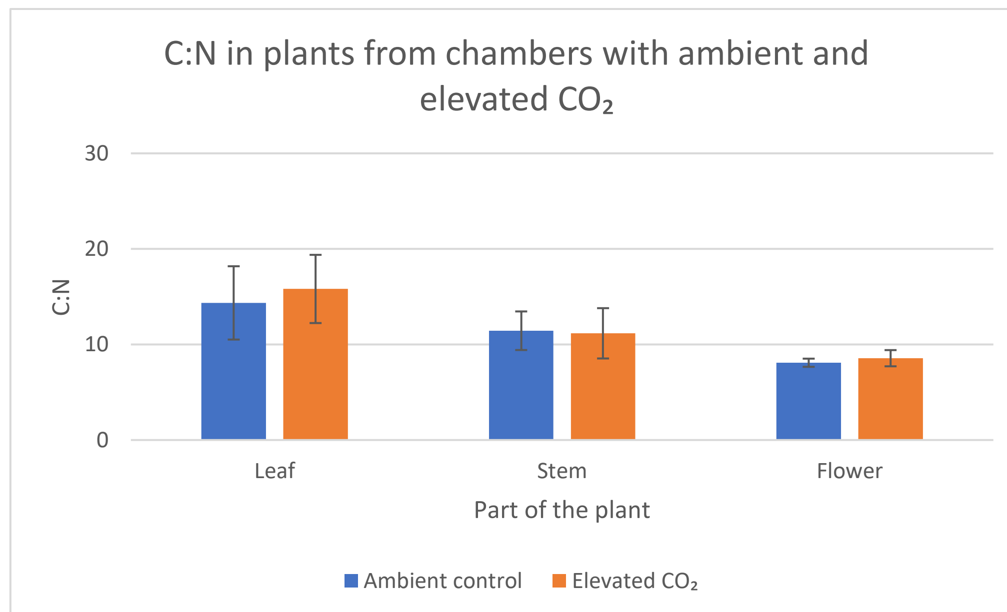



Figure 22: The C:N ratio means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber 1, elevated $CO_2$ is chamber 2.

*EXPERIMENT 2*

The C:N ratio was very similar in the leaves and stems in both chambers. The ratio was significantly lower in the flowers, especially in chamber 2 with a decline of 75% from ~40 in the leaves and stems to 11 (Figure 23). There was an increase in the C:N ratio in chamber 2 in all plant tissues. The increase was significant in the leaves and the stem (Table 14). The average increase was 79.7% and 66.5%, respectively. There was also a slight increase in the flower.

Table 14: The C:N ratio mean, SD, and estimated p-value from samples of plant tissue from *Arabidopsis thaliana* after one growth season. P-value from t-test between chambers. All significant p-values are bolded.

| **C:N** | **Leaf** | **Stem** | **Flower** |
|---|---|---|---|
| **Average Ambient** | 22.880 | 23.979 | 10.528 |
| **Average Elevated** | 41.120 | 39.930 | 11.720 |
| **SD Ambient** | 6.576 | 4.566 | 0.776 |
| **SD Elevated** | 8.777 | 11.505 | 0.857 |
| ***p*** | **9.158E-09** | **0.021** | 0.065 |

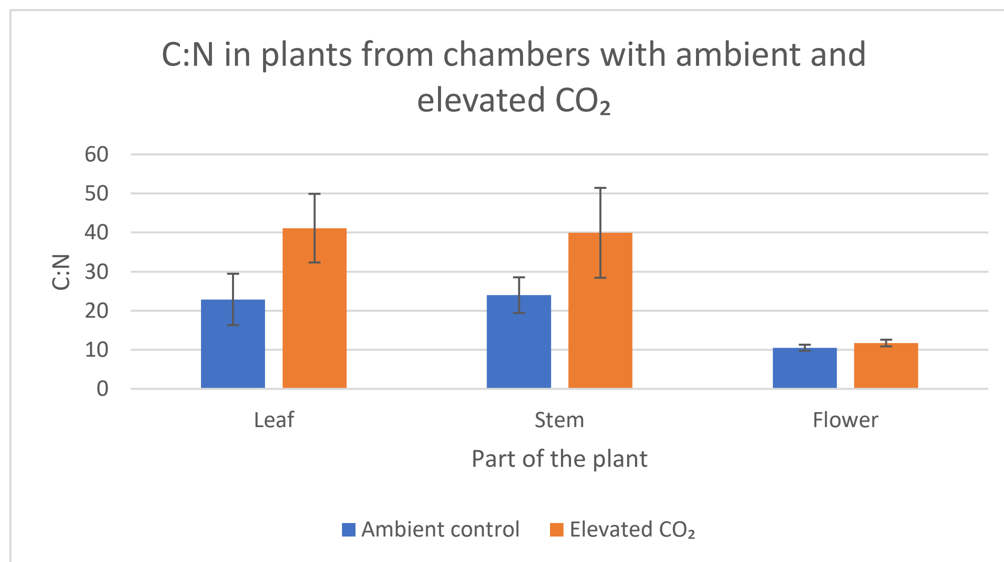



Figure 23: The C:N ratio means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber 1, elevated $CO_2$ is chamber 2.

## N:P RATIOS

### *EXPERIMENT 1*

The N:P ratio was quite similar in all tissue (Figure 24). There was no significant change in the N:P ratio between chambers, but a decrease in N:P in all plant tissues. Variation was generally high in all tissues, except for the stem (Table 15).

Table 15: The N:P ratio mean, SD, and estimated p-value from samples of plant tissue from *Arabidopsis thaliana* after one growth season. P-value from t-test between chambers.

| **N:P** | **Leaf** | **Stem** | **Flower** |
|---|---|---|---|
| **Average Ambient** | 4.465 | 4.787 | 4.814 |
| **Average Elevated** | 3.788 | 4.489 | 4.115 |
| **SD Ambient** | 1.355 | 0.253 | 0.990 |
| **SD Elevated** | 1.336 | 0.945 | 0.983 |
| ***p*** | 0.120 | 0.565 | 0.326 |

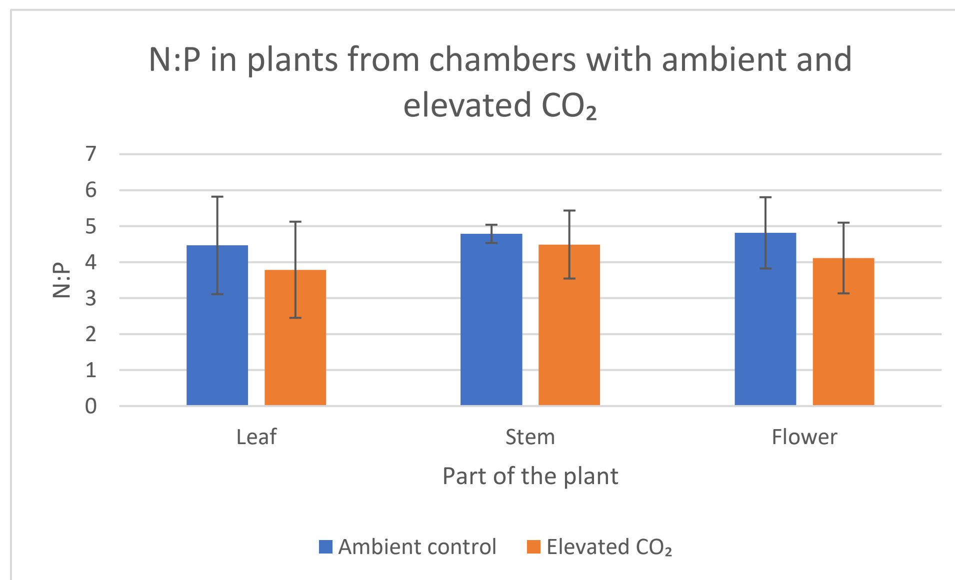



Figure 24: The N:P ratio means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber 1, elevated $CO_2$ is chamber 2.

### *Experiment 2*

The N:P ratio was quite similar in all tissues in chamber 1. N:P in the flowers was higher than the leaves and stems in chamber 2, with the stems having the lowest ratio. There was no significant difference between tissues due to high variation (Figure 25). N:P decreased from chamber 1 to chamber 2 in the leaf and the stem. None of the changes between chambers were significant (Table 16). The flower had a slight increase in N:P from chamber 1 to chamber 2.

Table 16: The N:P ratio mean, SD, and estimated p-value from samples of plant tissue from *Arabidopsis thaliana* after one growth season. P-value from t-test between chambers.

| N:P | Leaf | Stem | Flower |
|---|---|---|---|
| **Average Ambient** | 5.076 | 4.937 | 5.108 |
| **Average Elevated** | 4.632 | 3.936 | 5.175 |
| **SD Ambient** | 2.114 | 1.579 | 0.759 |
| **SD Elevated** | 2.362 | 1.856 | 0.397 |
| ***p*** | 0.535 | 0.385 | 0.878 |

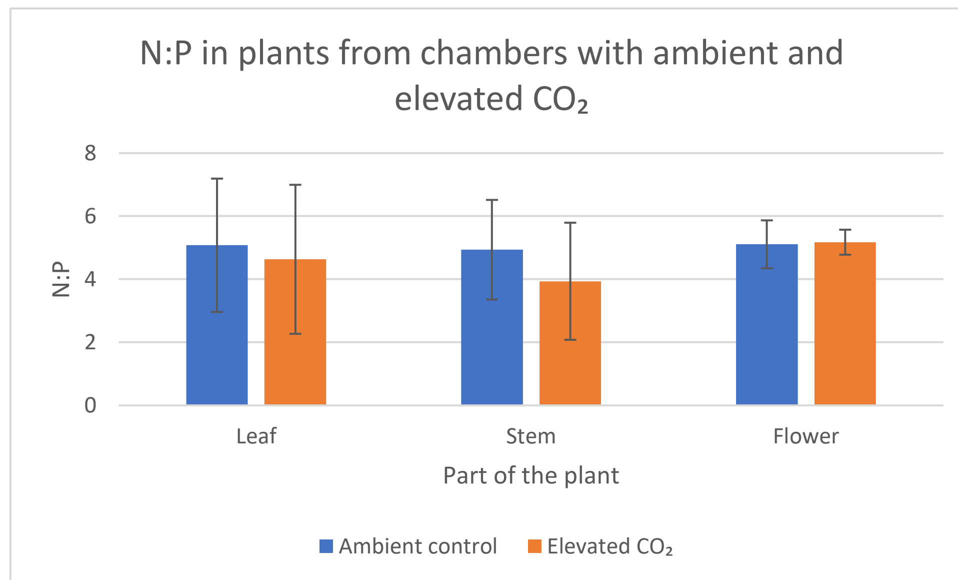



Figure 25: The N:P ratio means ± SD in *Arabidopsis thaliana*, after one growth season. Ambient control (~400 ppm) and elevated (~700 ppm) $CO_2$ concentrations. Ambient control is chamber 1 and elevated $CO_2$ is chamber 2.

Table 17: Average results and p-values from all t-tests between chambers. All significant p-values are bolded.

| | | | Exp1 | | | Exp2 | | |
|---|---|---|---|---|---|---|---|---|
| | | | **Ch1** | **Ch2** | **p-value** | **Ch1** | **Ch2** | **p-value** |
| **Biomass** | | | 0.280 | 0.264 | 0.207 | 0.317 | 0.294 | 0.281 |
| **Chlorophyll** | | | 26.430 | 28.180 | **0.033** | 21.522 | 28.087 | **1.282E-06** |
| **Element concentration** | **%C** | **Leaves** | 36.838 | 36.646 | 0.659 | 43.166 | 42.107 | 0.254 |
| | | **Stem** | 36.155 | 38.278 | 0.078 | 47.533 | 47.602 | 0.952 |
| | | **Flower** | 43.469 | 40.099 | **0.023** | 49.785 | 49.634 | 0.909 |
| | **%N** | **Leaves** | 2.746 | 2.429 | 0.128 | 2.038 | 1.103 | **1.664E-07** |
| | | **Stem** | 3.223 | 3.583 | 0.424 | 2.026 | 1.266 | **0.005** |
| | | **Flower** | 5.386 | 4.803 | **0.017** | 4.750 | 4.242 | **0.040** |
| | **%P** | **Leaves** | 0.639 | 0.681 | 0.411 | 0.437 | 0.269 | **4.268E-05** |
| | | **Stem** | 0.676 | 0.759 | 0.434 | 0.452 | 0.398 | 0.676 |
| | | **Flower** | 1.156 | 1.271 | 0.517 | 0.938 | 0.803 | **0.009** |
| **Ratios** | **C:P** | **Leaves** | 60.638 | 57.619 | 0.543 | 107.489 | 166.965 | **< 0.001** |
| | | **Stem** | 55.492 | 52.825 | 0.760 | 123.106 | 169.102 | 0.450 |
| | | **Flower** | 38.818 | 33.368 | 0.359 | 53.320 | 60.506 | **0.039** |
| | **C:N** | **Leaves** | 14.350 | 15.810 | 0.220 | 22.880 | 41.120 | **9.158E-09** |
| | | **Stem** | 11.438 | 11.166 | 0.859 | 23.979 | 39.930 | **0.021** |
| | | **Flower** | 8.088 | 8.562 | 0.307 | 10.528 | 11.720 | 0.065 |
| | **N:P** | **Leaves** | 4.465 | 3.789 | 0.120 | 5.076 | 4.632 | 0.535 |
| | | **Stem** | 4.787 | 4.489 | 0.565 | 4.937 | 3.936 | 0.385 |
| | | **Flower** | 4.814 | 4.115 | 0.326 | 5.108 | 5.175 | 0.878 |

## Effects of light and $CO_2$ on element concentrations and ratios

Light had a significant effect on all concentrations and ratios except for N:P.
$CO_2$ had a significant effect on %N, %P, C:P and C:N.
Light*$CO_2$ has a significant effect on %N, %P, C:P and C:N.

%C was only affected by light, with a significant difference between experiments. The effect of elevated $CO_2$ on %N and %P differed between the two experiments, and both chambers in experiment 2 were significantly different in %P from experiment 1.

C:P and C:N were significantly higher in experiment 2, with a significant difference between chambers.

Table 18: Significance levels of the explaining factors found in Generalized linear model analyses, starting with full-factorial models. DW of the shoot was added as a covariate. Estimated marginal means comparisons were done and treatment groups with the same letters in the four last columns are not significantly different. ns: not significant, na: not applicable.

| | | **Light** | **$CO_2$** | **DW** | **Light*$CO_2$** | **Light*DW** | **$CO_2$*DW** | **Light*$CO_2$*DW** | **Marginal means comparisons** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | **Exp1** | | **Exp2** | |
| | | | | | | | | | **Ch1** | **Ch2** | **Ch1** | **Ch2** |
| **Biomass** | | 0.007 | ns | na | ns | na | na | na | a | a | a | a |
| **Chl A µg/cm²** | | 0.000 | 0.000 | 0.004 | 0.000 | ns | ns | ns | b | b | a | b |
| **Content** | **C** | 0.817 | 0.034 | 0.000 | ns | 0.032 | 0.017 | ns | a | a | b | b |
| | **N** | 0.000 | 0.000 | 0.000 | 0.011 | ns | ns | ns | c | bc | b | a |
| | **P** | 0.470 | 0.024 | 0.000 | 0.000 | 0.023 | ns | ns | c | c | b | a |
| **Conc** | **%C** | 0.000 | 0.052 | 0.029 | ns | ns | 0.031 | ns | a | a | b | b |
| | **%N** | 0.000 | 0.000 | ns | 0.008 | ns | ns | ns | c | bc | b | a |
| | **%P** | 0.000 | 0.029 | ns | 0.000 | ns | ns | ns | c | c | b | a |
| **Ratio** | **C:P** | 0.000 | 0.000 | ns | 0.000 | ns | ns | ns | a | a | b | c |
| | **C:N** | 0.000 | 0.000 | 0.881 | 0.000 | ns | 0.009 | ns | a | a | b | c |
| | **N:P** | ns | ns | 0.019 | ns | ns | ns | ns | a | a | a | a |

## Discussion

The experimental set up with the two chambers and the different $CO_2$-levels worked very well. Also the second experiment with elevated light (still with two different $CO_2$-concentrations) demonstrated a light effect *per se*, yet the direct comparison between the two experiments must be done with some caution, since they were run at different times, and the temperature was somewhat higher in the second experiments. There were a few outbreaks of moss, but rather marginal and unlikely to have affected the plants.

$CO_2$ remained very stable around the desired levels of ambient (ca 400 ppm) and elevated (ca 700 ppm). Also temperature and humidity remained stable between chambers. However, as mentioned, experiment 2 was run from the end of May to the end of June, while experiment 1 was run from the end of March to mid-May. This resulted in somewhat higher temperature and humidity in experiment 2, with a larger variation.

The plants grew well in all experiments, but clearly faster in experiment 2, likely reflecting the somewhat higher temperature. The plants in experiment 1 needed 47 days to get to flowering and still only a few of them had flowered, while in experiment 2, the plants were harvested after 36 days and they were larger with more flowers (Figure 26 and 27). In experiment 1, a couple of the plants had purple leaves in chamber 2. In experiment 2, several plants had purple leaves in chamber 1, and almost all plants had purple leaves in chamber 2. This indicates that the plants have produced anthocyanin, likely as a stress-response to elevated $CO_2$ (Tallis et al., 2010) and increased light (Das et al., 2011). This could also be a direct response to reduced N or P levels (Close & Beadle, 2003).

### Biomass

Contrary to expectations, elevated $CO_2$ did not cause increased plant biomass. In fact a small, yet insignificant decrease was recorded in both experiments. The increase in chlorophyll indicates an acceleration of the photosynthesis, which again should lead to an increased biomass. A reduction points to another limiting factor, when light and $CO_2$ both were at optimal levels. Lack of nutrients are unlikely, since standard soils with the same levels of nutrients were used for both levels of $CO_2$. The plants were watered regularly when needed, but the decrease could be a result of reduced transpiration and turgor pressure due to closed stomata, which could be a response to elevated $CO_2$ even with high (>50%) relative humidity (RH).

Phosphorus deficiency has been shown to result in a down-regulation of the photosynthesis rate (Brooks et al., 1988), and nutrient deficiency has been found to reduce growth in elevated $CO_2$ (Poorter, 1998). Nevertheless, the main result in experiments with $C_3$ plants and elevated $CO_2$ is increased biomass (Poorter & Navas, 2003). With elevated light, the reduction in average biomass with elevated $CO_2$ was somewhat higher, but both chambers had a (significantly) higher biomass than with normal light. This indicates that increased light stimulated growth to a certain

extent, although also the temperatures were somewhat higher in this experiment. *Arabidopsis* has been found to maintain a relatively constant size and growth over temperatures ranging from 16°C to 30°C, however (Hua et al., 2001), hence while elevated temperature have promoted growth rates, it would not be expected to affect final biomass.

A somewhat higher variation was found in experiment 2. Plants can acclimate to the elevated $CO_2$ to conserve nutrients by reducing the photosynthetic rate, but the carbon gain should still be greater than with ambient $CO_2$ (Leakey et al., 2009). Elevated $CO_2$ has been shown to stimulate root biomass, often more than shoot biomass. This could change the R/S ratio, but there are varying results between studies (Madegowda & Hatfield, 2013). The roots were not weighed in this experiment because they were too thin, but the amount of growth in the small pots would be limited and should not have contributed to the somewhat counterintuitive effect of elevated $CO_2$.

### CHLOROPHYLL

The chlorophyll concentrations increased with elevated $CO_2$. With increased light, the chlorophyll levels were reduced in the chamber with ambient $CO_2$, but the plants with elevated $CO_2$ were not affected.

Elevated $CO_2$ has been shown to accelerate the photosynthesis by increasing the carbon available for Rubisco in the chloroplasts (Leakey et al., 2009). This increased Rubisco efficiency will lead to an increased demand for energy, which can be achieved with an increased amount of chlorophyll in the leaves. With increased light, less chlorophyll is needed to achieve the same photosynthesis rate (Hessen et al., 2002). When the plants reach light saturation, $CO_2$ fixation becomes the limiting factor for photosynthesis in the ambient chamber (Formighieri, 2015). The plants with elevated $CO_2$ and light had the same chlorophyll concentration as the plants without elevated light, this could indicate another limiting factor after reaching both light- and $CO_2$ saturation.  Increased temperature in experiment 2 could affect the rate of photosynthesis and hence increase the demand for chlorophyll, but comparing the results between the experiments indicate that this is not the case. The increase in chlorophyll matches the expectations with elevated $CO_2$ levels, but it did not, however, increase the biomass.

Figure 26: Plants from experiment 1 ready for harvesting after 47 days

Figure 27: Plants from experiment 2 ready for harvesting after 36 days

## Element content

Element content was measured in different plant tissues to test the hypotheses that elevated $CO_2$ and light would alter the elemental composition of the plant in a tissue-specific manner. It was expected to find an increase in carbon and reduced levels of nitrogen and phosphorus, something that would result in an increase in C:N and C:P, and potentially also a decrease in N:P, given that N-concentrations (proteins) were more affected than P (nucleic acids and phospholipids). These skewed elemental ratios have been shown to affect both small and large herbivores (Agrell et al., 2000; Craine et al., 2017).

### Carbon content

The carbon (C) content increased linearly with the DW and constituted a stable fraction of the dry weight of the shoot in both chambers. This was an expected result, as bigger plants have the ability and capacity to take up more C, and because C stands for a large fraction of the total weight. In experiment 1, the weight range was almost identical in the two chambers, which means that the plants did not take up more C with elevated $CO_2$. This coincides with the results of the biomass. The main result of elevated $CO_2$ in $C_3$ plants is an increase in photosynthesis rate,

resulting in an increased uptake of C (Drake et al., 1997). Growth at elevated $CO_2$ results in higher concentrations of non-structural carbohydrates (Farrar et al., 2000). Results from Free-Air $CO_2$ Enrichment (FACE) studies show an increase in carbohydrate synthesis by 19-46% in $C_3$ plants exposed to elevated $CO_2$ (Leakey et al., 2009). When $CO_2$ increases, this will also increase the velocity of carboxylation, which will reduce loss of $CO_2$ from photorespiration (Long et al., 2004). I therefore expected to find a higher uptake and accumulation of C. The C range was slightly higher with elevated light, where the largest plants were bigger and had a higher content than in experiment 1. The findings in both experiments coincides with the results from the GLM, that C increases with the DW, and has the highest increase with elevated light. In experiment 2, the plants were harvested after 36 days, while the harvesting in experiment 1 was done after 47 days. The plants in experiment 2 grew a lot faster and had been flowering longer when they were harvested. This means that a direct comparison between the two experiments should be done with some caution.

### Nitrogen content

The nitrogen (N) content also had a linear increase with the dry weight of the shoot, but the results showed more irregularities in and between the chambers. In experiment 1, total N was quite similar between the chambers, except for a small decrease in chamber 2. In experiment 2, the N weight with elevated $CO_2$ was lower than with ambient control, with a 49% average decrease. This strengthens the hypothesis that increased light further reinforces the effects of elevated $CO_2$ on N content. One possible explanation for the reduction in N could be photosynthetic acclimation. When more $CO_2$ becomes available for assimilation, the rate of photosynthesis is increasing and less Rubisco is needed to keep up the efficiency. This acclimation should not be enough to halt the increased C uptake (Leakey et al., 2009). A survey of eight chamber studies found an average reduction in leaf N, total protein, and Rubisco amount of 17%, 14%, and 15%, respectively (Drake et al., 1997). However, a summary of FACE studies found a 20% decrease in Rubisco, but only a 4% decrease in N per unit leaf area (Long et al., 2004). This coincides with another FACE experiment review, which suggests that the decrease in Rubisco is specific, and not a part of the general decrease in leaf protein. They assumed that Rubisco account for 25% of leaf N, the 20% reduction they found in Rubisco could then account for the 5% decrease they found in leaf N (Ainsworth & Long, 2005). Either way, none of these results could explain the 49% reduction in N content found in this experiment.

Converting N content to pollen content (conversion factor 6.25, (Keller et al., 2005)) showed an average reduction in pollen weight with elevated $CO_2$ of 16.5% in experiment 1 and 48.9% in experiment 2. This coincides with a 33% decline in pollen content that has been found with elevated $CO_2$ (Ziska et al., 2016), even though the decline clearly is larger with increased light.

Both increased light and temperature can increase the efficiency of the photosynthesis, which again could result in an even higher uptake of C and less demand for N (Hessen et al., 2002). N content decline could also result from decreased transpiration. With elevated $CO_2$, plants

reduce stomata conduction to improve water use (Drake et al., 1997). This could again stimulate growth, but reduce the mass flow of nutrients from the soil (Loladze, 2002). An increase in RH could further strengthen the closing of stomata and result in a decrease in N accumulation (McDonald et al., 2002). If the biomass increase as a result of increased carbohydrate production, this would equally dilute the concentration of the other elements (Loladze, 2002). Studies have shown that the root architecture and uptake capacity can be altered with elevated $CO_2$ (Taub & Wang, 2008), but since this study was done in small pots, it is unlikely that this would have a profound effect on the results. There has been found a reduction of protein content in $C_3$ plants of 6-23% with elevated $CO_2$. This coincides with the reduction in N found in other studies (Medek, 2017). A reduction of protein in $C_3$ grasses of 6.3-7.8% with elevated $CO_2$ was found, and the difference between reduction in N and other elements concluded that dilution could not be the only explanation (Myers et al., 2014).

### Phosphorus content

The phosphorus (P) content showed similar trends as the N content, with a much higher variation than the C contents. N and P are tightly linked via protein synthesis where P is needed for ribosomes and N for amino acids. While P in DNA should be fairly constant, the number of ribosomes could cause major changes in P. Something to note is that in experiment 1, the average P content was slightly higher (not significantly) with elevated $CO_2$, but in experiment 2, average P content was 43% lower with elevated $CO_2$. The small changes in N and P in experiment 1 could coincide with the small change in C. The slight increase with elevated $CO_2$ in experiment 1 could be a sign of growth, but this does not coincide with the biomass results. The reduction with increased light could be due to carbohydrate or biomass dilution. Another explanation could be a higher demand for P to maintain growth, caused by the increase in light (Hessen et al., 2002). The decrease in P between experiments was larger than the decrease in N, this was not expected as there is no down-regulation of P with a more efficient photosynthesis. On the contrary, increased photosynthesis rate should increase the demand for P for phosphorylation in the photosynthesis apparatus (Gifford et al., 2000). Further on, the reduced transpiration would not affect the P uptake in the roots in the same way as the N uptake, as the roots take up P mainly by diffusion, not by mass flow (Taub & Wang, 2008). The decreased transpiration could actually lead to a higher P uptake as a result of increased soil moisture (Loladze, 2002). There are fewer papers on the effects of elevated $CO_2$ on P, and the results are varied, from large reductions to no significant change (Gifford et al., 2000). Again, one would expect that higher levels of carbohydrates should increase C:P ratios as well as P per DW. Conroy et al found an average decrease in P of 46% in *Eucalyptus grandis* when grown in elevated $CO_2$ at different rates of P fertilizer (Conroy et al., 1992).

## TISSUE SPECIFIC CONCENTRATION

Generally, the elevated $CO_2$ did not seem to have a distinct effect on any of the elements in experiment 1, except for slight signs of changes in the different tissues. When increased light was added in experiment 2, there was a clear increase in C and decreases in N and P in all tissues, which was even more evident with elevated $CO_2$.

### CARBON CONCENTRATION

The average C concentration in experiment 1 was significantly higher in the flowers than in the other tissues in both chambers, with a decrease with elevated $CO_2$. In experiment 2, the C concentration was higher in the stem than in the leaves, and in the flowers than in other tissues. The C concentration seemed to be almost unaffected with elevated $CO_2$ in experiment 2, but there was a large increase in all tissues from experiment 1. The increase in C concentration with increased light would again suggest an increase in photosynthesis products, as there was an increase of about 10% in C and biomass with elevated light both with ambient and elevated $CO_2$. The lack of increase in C with elevated $CO_2$ in experiment 1 indicates that more available C for assimilation is not enough to make a noteworthy increase in photosynthesis. The increase in C with elevated light, despite the decrease in N and P would indicate that these elements are not limiting factors for photosynthesis. The more probable reasons for increased C and photosynthesis would be elevated light, even though temperature and humidity could also have some positive effect on the efficiency of the plant, especially when $CO_2$ availability is not a limiting factor (Drake et al., 1997). It is difficult to separate the effect of elevated light from the effect of increased temperature and humidity, but the chlorophyll results indicate that elevated light have some effect on photosynthesis. A meta-analysis of FACE experiments found a C concentration increase of 6%, together with declines in other elements with elevated $CO_2$ (Loladze, 2014).

### NITROGEN CONCENTRATION

The average N concentration in experiment 1 was clearly higher in the flowers than in other tissues and the leaves had the lowest concentrations in both chambers. N concentration decreased in the flowers with elevated $CO_2$. In experiment 2, N decreased in all tissues with elevated $CO_2$, it was also slightly lower than in experiment 1.

As the decrease in N was more profound with increased light, this strengthens the hypothesis that increased light adds to the effects of elevated $CO_2$. Dilution of N from a higher C accumulation could be one of the reasons of the decrease between experiments, but because of the little change in C and biomass between chambers in experiment 2, it would not explain the clear decrease in N concentration. The meta-analysis of the concentrations of N in aboveground tissues done by Loladze revealed a 14% average decline in concentrations with elevated $CO_2$ (Loladze, 2014).

PHOSPHORUS CONCENTRATION

The average P concentration was almost twice as high in the flowers than in the other tissues with ambient $CO_2$ in experiment 1. The variation was high in all tissues in all treatments. With increased light in experiment 2, the concentration was more than twice as high in the flowers than in the other tissues in both chambers. There was a decline in all tissues with increased light, and a decline from ambient to elevated $CO_2$ of 38% in the leaves and 14% in the flowers in experiment 2. The meta-analysis of FACE experiments done by Loladze found an average decrease in P concentration of 9% with elevated $CO_2$ (Loladze, 2014).

The flowers had the highest concentration of elements in all treatments, yet with some differences between the two light levels. This was expected, as they are all important nutrients for development of the reproductive tissues (Dordas, 2009). %C and %N decreased with elevated $CO_2$ in experiment 1 (standard light), and %P and %N decreased with elevated $CO_2$ in experiment 2 (high light intensity). The decline of 15% in N with elevated $CO_2$ (in experiment 1) coincides with other studies done on seeds at different levels of $CO_2$ (500-800 ppm), where a 15% decline was found on average among nonlegume $C_3$ plants (Jablonski et al., 2002). The decrease in N concentration in the flowers with elevated $CO_2$ (in experiment 2) was lower than in experiment 1, 10.7%. Seen together with the substantial decrease in the other tissues, this could be a sign of reallocation of N from leaves and stem to the reproductive tissues.

There was an evident increase in %C in the stem from experiment 1 to experiment 2, while the specific content of N and P clearly decreased. With elevated light, N concentration decreased 37.5% in the high $CO_2$ treatment. An analysis of 75 papers on changes in N concentration with elevated $CO_2$ showed an average decrease in above-ground N of 14%, with a decrease in stem N of 9% (Cotrufo et al., 1998). This coincides with the results in this study; a decrease of 9% (yet insignificant) in the stem in experiment 1, but not with the striking decrease of 37.5% in experiment 2. This demonstrates that light indeed have an additive effect of elemental concentrations and elemental ratios, and notably in the stem.

Leaf %C increased from experiment 1 to experiment 2, while %N and %P decreased. There was also a decrease in N and P with elevated $CO_2$ in experiment 2. Compared to the 16% decrease in leaf %N that Cotrufo et al found with elevated $CO_2$, the reduction here was 11.6% in experiment 1 and remarkable 46% in experiment 2. This increase is a lot higher than what has been found in other experiments when only increased $CO_2$ has been used. The decrease in N with elevated $CO_2$ and light was highest in the leaf, this was expected as the photosynthesis mainly takes place here (Gifford et al., 2000).

## Element ratios

The changes in element contents caused by necessity also changes in element ratios in the different tissues. The increase in C and decrease in N and P both impacted the C:N and C:P ratios.

### C:P

In experiment 1, the C:P ratio was higher in the leaves than in the flowers and did not change much between chambers. The stem had large variation in C:P with ambient $CO_2$ because of low P in one single plant. C:P in the leaves increased between experiments and with elevated $CO_2$ in experiment 2. The flowers had the lowest ratio, but it increased with elevated $CO_2$ in experiment 2. The data on effects of elevated $CO_2$ on C:P ratio are variable, but the trend seems to be an increase (Gifford et al., 2000; Hessen et al., 2002).

### C:N

The C:N ratio was also quite similar between chambers in experiment 1, in all tissues. This was expected, from the clear correlation between C and N uptake, unlike the P uptake, which is more correlated with environmental variables (Moe et al., 2019). There was a difference between tissues, with the highest ratio in the leaves and lowest in the flowers. With elevated light, the ratio increased largely in all tissues. C:N increased in leaves and stems in experiment 2 with elevated $CO_2$. An increase in C:N has been shown to correlate with increased atmospheric $CO_2$ and a decline in pollen protein (Ziska et al., 2016). Further, a 13% increase in DOM:CP (digestible organic matter:crude protein concentration) in cattle diet was fitting well to the increase found in C:N (Craine et al., 2017).

### N:P

The N:P ratio was higher in the stem than in the leaves with ambient $CO_2$. There was not found any other significant differences between tissues or treatments. My hypothesis was an overall decrease in N:P, however, because of the large reduction in P in the flowers and leaves, which makes up most of the dry weight of the shoot, the N:P ratio did not change significantly.

The reduction in N and P concentration and increase in C concentration found in the meta-analysis of Loladze translates into a ~7% decrease in N:P, 16% increase in C:P and 25% increase in C:N (Loladze, 2014).

In my experiments, the leaves had an increase in C:P of 76% between ambient $CO_2$ chambers with ambient and elevated light, and 68% between ambient and elevated $CO_2$ in experiment 2.

C:N in the leaves showed a decrease of 63% between ambient $CO_2$ chambers with ambient and elevated light, and 79% between ambient and elevated $CO_2$ experiment 2.

## CONCLUSION

One of the main conclusions drawn from these experiments is that elevated $CO_2$ does not necessarily increase C content and overall biomass. Further, the effects of elevated $CO_2$ combined with increased light on plant stoichiometry are clear, and in some elements, profound. The results showed that the interaction of $CO_2$ and light affects all plant tissues, with strongest impacts in the leaves where the photosynthesis takes place, but that the element ratios in stems and flowers are also affected. The increased efficiency causes a reduction in both P and N, where the effect is most distinct in N. The responses of the different elements result in substantial shifts in ratios, and both C:P and C:N are greatly increased. The reduction of N (and hence proteins) and P will reduce the quality of forage for plant eaters, which states that elevated $CO_2$ is not necessarily positive neither for plants nor for their consumers.

This emphasizes the need for further research done on the combination of environmental factors, as the results show that the effects from each factor are additive, promoting the impacts from $CO_2$. It should be stressed here that the elevated levels of $CO_2$ (700 ppm) clearly is a *business as usual* scenario and likely in the upper end (hopefully) of future atmospheric $CO_2$ concentrations (IPCC, 2014b). Still the $CO_2$ levels continue to rise, and affect plants both directly via the $CO_2$ uptake and indirectly through changes in temperature and precipitation. These drivers interact in complex ways, notably via stomata responses. As N and P (as well as other elements) generally is scarce in natural environments, it is crucial that future studies focus on changes in plant stoichiometry. The change in element uptake is predicted to provide a strong feedback on the trophic transfers of the ecosystems, as plants are a key global resource, vital to almost all environmental processes.

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# APPENDIX

Table 1: Experiment 1. Wet weight and dry weight of each shoot, and their respective element concentrations. The sample number indicates where several samples has been taken from the same shoot to analyse different tissue. Part of plant: blad = leaf, stilk = stem, blomst = flower.

| **Weighing** | | | | **Analysis** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Chamber** | **Plant no** | **Wet weight** | **Dry weight** | **Chamber** | **Sample no** | **Part of plant** | **N %** | **C %** | **% P** |
| 1 | 1 | 2.0531 | 0.2323 | 1 | 1 | blad | 2.709 | 37.768 | 0.652 |
| 1 | 2 | 2.031 | 0.2160 | 1 | 2 | blad | 3.290 | 35.585 | 0.716 |
| 1 | 3 | 2.1951 | 0.2328 | 1 | 3 | blad | 3.284 | 35.941 | 0.696 |
| 1 | 4 | 2.3066 | 0.2859 | 1 | 3 | stilk | 3.921 | 34.415 | 0.823 |
| 1 | 5 | 2.4431 | 0.2683 | 1 | 4 | blad | 2.200 | 37.617 | 0.584 |
| 1 | 6 | 2.3519 | 0.2580 | 1 | 5 | blad | 3.495 | 37.254 | 0.513 |
| 1 | 7 | 2.5257 | 0.2935 | 1 | 5 | stilk | 3.077 | 36.882 | 0.605 |
| 1 | 8 | 2.6727 | 0.3005 | 1 | 5 | blomst | 5.802 | 43.761 | 1.204 |
| 1 | 9 | 2.8964 | 0.3281 | 1 | 6 | blad | 2.997 | 34.801 | 0.583 |
| 1 | 10 | 2.4836 | 0.2862 | 1 | 7 | blad | 2.071 | 36.244 | 0.796 |
| 1 | 11 | 2.6191 | 0.3211 | 1 | 8 | blad | 2.576 | 37.310 | 0.562 |
| 1 | 12 | 2.1486 | 0.2365 | 1 | 8 | stilk | 3.168 | 33.819 | 0.164 |
| 1 | 13 | 1.9083 | 0.2372 | 1 | 8 | blomst | 5.568 | 42.929 | 0.989 |
| 1 | 14 | 2.3921 | 0.2589 | 1 | 9 | blad | 2.320 | 36.610 | 1.022 |
| 1 | 15 | 3.1809 | 0.3594 | 1 | 9 | stilk | 2.645 | 37.887 | 0.592 |
| 1 | 16 | 2.3921 | 0.3080 | 1 | 9 | blomst | 5.145 | 43.185 | 0.864 |
| 1 | 17 | 2.2443 | 0.2900 | 1 | 10 | blad | 1.753 | 36.409 | 0.571 |
| 1 | 18 | 2.8742 | 0.3343 | 1 | 11 | blad | 1.833 | 37.116 | 0.773 |
| 1 | 19 | 2.32 | 0.2726 | 1 | 12 | blad | 4.315 | 36.503 | 0.857 |
| 1 | 20 | 2.4246 | 0.2887 | 1 | 13 | blad | 1.613 | 34.422 | 0.531 |
| 2 | 1 | 1.9837 | 0.2676 | 1 | 14 | blad | 3.014 | 35.451 | 0.695 |
| 2 | 2 | 1.5423 | 0.1983 | 1 | 14 | stilk | 3.306 | 37.773 | 0.685 |
| 2 | 3 | 2.3987 | 0.3169 | 1 | 14 | blomst | 5.201 | 43.339 | 1.317 |
| 2 | 4 | 2.2044 | 0.2696 | 1 | 15 | blad | 3.949 | 38.831 | 0.541 |
| 2 | 5 | 1.7073 | 0.2033 | 1 | 15 | blomst | 5.215 | 44.130 | 1.403 |
| 2 | 6 | 2.2351 | 0.2605 | 1 | 16 | blad | 2.303 | 36.916 | 0.388 |
| 2 | 7 | 2.2066 | 0.2657 | 1 | 17 | blad | 2.097 | 38.184 | 0.439 |
| 2 | 8 | 2.2322 | 0.3384 | 1 | 18 | blad | 2.982 | 37.548 | 0.556 |
| 2 | 9 | 2.1282 | 0.3067 | 1 | 19 | blad | 2.937 | 37.180 | 0.628 |
| 2 | 10 | 2.2113 | 0.2777 | 1 | 20 | blad | 3.188 | 39.077 | 0.671 |
| 2 | 11 | 2.3525 | 0.2891 | 2 | 1 | blad | 1.895 | 36.574 | 0.744 |
| 2 | 12 | 2.2331 | 0.2885 | 2 | 2 | blad | 1.892 | 36.230 | 0.615 |

| | | | |
|---|---|---|---|
| 2 | 13 | 2.2164 | 0.2740 |
| 2 | 14 | 1.7375 | 0.1717 |
| 2 | 15 | 2.6688 | 0.2896 |
| 2 | 16 | 2.4057 | 0.3084 |
| 2 | 17 | 2.2699 | 0.2585 |
| 2 | 18 | 1.7733 | 0.2184 |
| 2 | 19 | 1.9892 | 0.2477 |
| 2 | 20 | 1.7763 | 0.227 |

| | | | | | |
|---|---|---|---|---|---|
| 2 | 2 | stilk | 2.812 | 36.255 | 0.781 |
| 2 | 2 | blomst | 4.567 | 43.125 | 0.946 |
| 2 | 3 | blad | 1.739 | 37.607 | 0.876 |
| 2 | 4 | blad | 3.683 | 38.139 | 0.470 |
| 2 | 5 | blad | 2.211 | 36.618 | 0.815 |
| 2 | 6 | blad | 3.116 | 38.851 | 0.576 |
| 2 | 7 | blad | 2.343 | 38.509 | 0.630 |
| 2 | 8 | blad | 2.299 | 38.340 | 0.805 |
| 2 | 8 | stilk | 2.668 | 39.156 | 0.543 |
| 2 | 8 | blomst | 4.639 | 40.584 | 1.678 |
| 2 | 9 | blad | 1.546 | 36.446 | 0.350 |
| 2 | 10 | blad | 2.133 | 36.751 | 0.597 |
| 2 | 11 | blad | 2.310 | 35.038 | 0.539 |
| 2 | 12 | blad | 2.019 | 38.646 | 0.612 |
| 2 | 13 | blad | 2.399 | 37.561 | 0.523 |
| 2 | 14 | blad | 2.591 | 35.068 | 0.660 |
| 2 | 14 | stilk | 4.516 | 37.469 | 1.818 |
| 2 | 14 | blomst | 5.163 | 38.241 | 1.288 |
| 2 | 15 | blad | 2.726 | 34.935 | 0.792 |
| 2 | 16 | blad | 3.091 | 33.483 | 1.063 |
| 2 | 17 | blad | 2.623 | 34.665 | 0.489 |
| 2 | 17 | stilk | 4.280 | 39.157 | 0.762 |
| 2 | 17 | blomst | 3.558 | 36.664 | 1.446 |
| 2 | 18 | blad | 2.301 | 37.005 | 0.866 |
| 2 | 19 | blad | 3.337 | 36.877 | 0.759 |
| 2 | 20 | blad | 2.320 | 35.577 | 0.838 |
| 2 | 20 | stilk | 3.638 | 39.351 | 0.951 |
| 2 | 20 | blomst | 4.841 | 41.881 | 0.996 |

Table 2: Experiment 2. Wet weight and dry weight of each shoot, and their respective element concentrations. The sample number indicates where several samples has been taken from the same shoot to analyse different tissue. Part of plant: blad = leaf, stilk = stem, blomst = flower. DW of plant no 1 was not included in the analysis.

| **Weighing** | | | | **Analysis** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Chamber** | **Plant no** | **Wet weight** | **Dry weight** | **Chamber** | **Sample no** | **Part of plant** | **% N** | **% C** | **% P** |
| 1 | 1 | 2.64353 | -0.11207 | 1 | 1 | blad | 1.353 | 41.869 | 0.296 |
| 1 | 2 | 2.1735 | 0.2602 | 1 | 1 | stilk | 2.190 | 45.657 | 0.514 |
| 1 | 3 | 2.3601 | 0.2982 | 1 | 1 | blomst | 4.296 | 49.570 | 0.995 |
| 1 | 4 | 1.7699 | 0.2088 | 1 | 2 | blad | 2.851 | 43.504 | 0.462 |
| 1 | 5 | 2.3747 | 0.3216 | 1 | 3 | blad | 2.668 | 43.167 | 0.470 |
| 1 | 6 | 2.389 | 0.3302 | 1 | 4 | blad | 2.493 | 42.719 | 0.383 |
| 1 | 7 | 2.3504 | 0.3185 | 1 | 5 | blad | 1.300 | 43.614 | 0.286 |
| 1 | 8 | 2.4893 | 0.282 | 1 | 6 | blad | 2.113 | 44.024 | 0.664 |
| 1 | 9 | 2.7703 | 0.3938 | 1 | 7 | blad | 2.284 | 42.653 | 0.452 |
| 1 | 10 | 2.8103 | 0.4131 | 1 | 7 | stilk | 2.264 | 47.820 | 0.618 |
| 1 | 11 | 2.3712 | 0.3175 | 1 | 7 | blomst | 5.187 | 49.708 | 0.839 |
| 1 | 12 | 2.4401 | 0.2912 | 1 | 8 | blad | 2.232 | 43.108 | 0.444 |
| 1 | 13 | 2.3321 | 0.3376 | 1 | 9 | blad | 1.552 | 43.101 | 0.384 |
| 1 | 14 | 1.8356 | 0.288 | 1 | 10 | blad | 2.117 | 46.056 | 0.319 |
| 1 | 15 | 1.8666 | 0.2349 | 1 | 10 | stilk | 2.120 | 47.713 | 0.363 |
| 1 | 16 | 3.6938 | 0.4181 | 1 | 10 | blomst | 4.967 | 49.920 | 0.891 |
| 1 | 17 | 2.5298 | 0.3092 | 1 | 11 | blad | 2.289 | 43.522 | 0.269 |
| 1 | 18 | 3.0173 | 0.4355 | 1 | 12 | blad | 2.702 | 43.519 | 0.315 |
| 1 | 19 | 2.7175 | 0.3549 | 1 | 13 | blad | 1.528 | 43.691 | 0.474 |
| 1 | 20 | 1.8062 | 0.2176 | 1 | 13 | stilk | 2.033 | 47.757 | 0.556 |
| 2 | 1 | 1.035 | 0.184 | 1 | 13 | blomst | 4.779 | 49.861 | 0.986 |
| 2 | 2 | 1.4195 | 0.2594 | 1 | 14 | blad | 1.241 | 43.261 | 0.432 |
| 2 | 3 | 1.5511 | 0.256 | 1 | 15 | blad | 1.495 | 39.078 | 0.756 |
| 2 | 4 | 2.8045 | 0.3833 | 1 | 16 | blad | 2.775 | 46.228 | 0.290 |
| 2 | 5 | 1.8482 | 0.2969 | 1 | 17 | blad | 1.618 | 41.051 | 0.606 |
| 2 | 6 | 1.4392 | 0.2442 | 1 | 18 | blad | 1.408 | 44.389 | 0.407 |
| 2 | 7 | 1.6412 | 0.2335 | 1 | 19 | blad | 1.854 | 44.334 | 0.537 |
| 2 | 8 | 2.8398 | 0.4091 | 1 | 19 | stilk | 1.526 | 48.717 | 0.210 |
| 2 | 9 | 2.1542 | 0.4162 | 1 | 19 | blomst | 4.519 | 49.866 | 0.979 |
| 2 | 10 | 1.107 | 0.2114 | 1 | 20 | blad | 2.889 | 40.432 | 0.498 |
| 2 | 11 | 1.8465 | 0.307 | 2 | 1 | blad | 1.101 | 43.782 | 0.199 |
| 2 | 12 | 1.9588 | 0.2897 | 2 | 2 | blad | 0.796 | 43.211 | 0.211 |
| 2 | 13 | 2.1693 | 0.3478 | 2 | 2 | stilk | 1.177 | 49.291 | 0.396 |
| 2 | 14 | 1.952 | 0.3476 | 2 | 2 | blomst | 4.030 | 51.068 | 0.872 |

| | | | |
|---|---|---|---|
| 2 | 15 | 1.4786 | 0.2841 |
| 2 | 16 | 2.4796 | 0.4019 |
| 2 | 17 | 1.6421 | 0.2686 |
| 2 | 18 | 1.5062 | 0.2506 |
| 2 | 19 | 1.4489 | 0.2351 |
| 2 | 20 | 1.4839 | 0.2536 |

| | | | | | |
|---|---|---|---|---|---|
| 2 | 3 | blad | 1.145 | 44.623 | 0.180 |
| 2 | 4 | blad | 1.586 | 45.260 | 0.182 |
| 2 | 5 | blad | 1.194 | 44.895 | 0.347 |
| 2 | 5 | stilk | 1.062 | 49.076 | 0.156 |
| 2 | 5 | blomst | 4.532 | 51.083 | 0.822 |
| 2 | 6 | blad | 0.908 | 43.692 | 0.193 |
| 2 | 7 | blad | 1.944 | 43.213 | 0.410 |
| 2 | 8 | blad | 1.194 | 44.004 | 0.240 |
| 2 | 9 | blad | 1.242 | 43.199 | 0.503 |
| 2 | 10 | blad | 0.759 | 43.316 | 0.332 |
| 2 | 11 | blad | 1.438 | 44.677 | 0.198 |
| 2 | 11 | stilk | 1.499 | 45.492 | 0.632 |
| 2 | 11 | blomst | 4.204 | 45.283 | 0.814 |
| 2 | 12 | blad | 0.888 | 43.215 | 0.291 |
| 2 | 13 | blad | 0.878 | 32.994 | 0.174 |
| 2 | 13 | stilk | 1.691 | 44.973 | 0.617 |
| 2 | 13 | blomst | 6.437 | 68.415 | 0.727 |
| 2 | 14 | blad | 1.117 | 43.075 | 0.311 |
| 2 | 15 | blad | 0.704 | 34.985 | 0.258 |
| 2 | 16 | blad | 1.495 | 55.979 | 0.129 |
| 2 | 17 | blad | 0.725 | 34.026 | 0.232 |
| 2 | 18 | blad | 0.979 | 43.384 | 0.356 |
| 2 | 19 | blad | 0.924 | 42.363 | 0.240 |
| 2 | 19 | stilk | 0.900 | 49.177 | 0.188 |
| 2 | 19 | blomst | 4.201 | 51.102 | 0.778 |
| 2 | 20 | blad | 1.042 | 42.122 | 0.393 |

Table 3: Chlorophyll concentration in µg/cm² from both experiments.

| **Chlorophyll concentration** | | |
|---|---|---|
| | **EXP1** | **EXP2** |
| **Sample ID** | **µg/cm2** | **µg/cm2** |
| 1 | 25.11368 | 19.29979 |
| 2 | 21.23579 | 20.36084 |
| 3 | 22.19123 | 19.42484 |
| 4 | 22.17789 | 23.85853 |
| 5 | 28.12561 | 21.73642 |
| 6 | 24.92281 | 22.71789 |
| 7 | 26.30737 | 23.58189 |
| 8 | 27.51018 | 20.43663 |
| 9 | 28.60912 | 23.44547 |
| 10 | 27.30456 | 21.78189 |
| 11 | 31.14035 | 22.71789 |
| 12 | 26.44632 | 21.90695 |
| 13 | 28.09614 | 21.51663 |
| 14 | 25.10211 | 18.27284 |
| 15 | 24.90596 | 22.76337 |
| 16 | 28.91825 | 24.34737 |
| 17 | 27.43825 | 23.64253 |
| 18 | 27.92772 | 22.53221 |
| 19 | 31.0200 | 18.48126 |
| 20 | 24.11298 | 17.62105 |
| 21 | 24.69789 | 25.83284 |
| 22 | 26.85263 | 26.92989 |
| 23 | 26.67965 | 25.41789 |
| 24 | 28.89719 | 38.32674 |
| 25 | 25.58421 | 26.25347 |
| 26 | 27.07263 | 26.37853 |
| 27 | 26.97018 | 36.30884 |
| 28 | 30.95509 | 29.12968 |
| 29 | 29.3614 | 23.616 |
| 30 | 28.15053 | 24.62779 |
| 31 | 24.86491 | 30.10168 |
| 32 | 31.54105 | 31.80695 |
| 33 | 31.2793 | 29.61284 |
| 34 | 27.19018 | 31.95474 |
| 35 | 30.83684 | 18.756 |
| 36 | 25.52667 | 33.68274 |
| 37 | 29.3393 | 23.08168 |
| 38 | 30.92281 | 25.37242 |
| 39 | 27.13333 | 25.63389 |
| 40 | 29.74982 | 28.908 |