

The effects of CO₂ fertilization and phosphorous availability on the photosynthesis of plants

A controlled growth chamber experiment on Holcus lanatus and Solanum dulcamara

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Preface

This thesis was written as part of the Msc. program Sustainable Development – Global Change and Ecosystems at Utrecht University. Use has been made of the facilities of Utrecht University and growth chambers of the biology department. I was supervised by dr. Hugo de Boer who assisted me during the whole process of the thesis. I want to thank him for his advice, feedback and providing me with the plant seeds and materials making it able to perform a good experiment. I also want to thank Shuqiong Wang for helping me with the calculation of nutrient concentration and creation of the nutrient solutions and dr. Jerry van Dijk as second reader for his feedback.

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Abstract

Rising CO₂ levels can have a positive effect on the photosynthesis of plants through CO₂ fertilization. At the same time changes are occurring in nutrient cycles through the use of fertilizers. The effects of CO₂ fertilization together with phosphorus availability on photosynthesis have been tested *Holcus lanatus* *Solanum dulcamara*. Controlled growth chambers with CO₂ levels of 200, 400 and 800 ppm have been used where plants were either treated with nutrient solutions of 1:1 or 45:1 N:P ratio. Through the means of photosynthesis measurements and *A/Ci* curves, different photosynthetic variables have been determined and analyzed. P treatment had an effect on light saturated photosynthesis (A_{sat}) in the sense that high P availability led to higher A_{sat} values for both species. Increased CO₂ concentration did not lead to increased A_{sat} for *S. dulcamara*. For *H. lanatus* higher CO₂ level did cause higher A_{sat} values with high P availability, but this was not observed under low P conditions. This resulted in an interaction effect between CO₂ level and P availability, indicating that low P availability limits increase of A_{sat} under elevated CO₂ concentration. Photosynthetic capacity showed for both P treatments a decrease with increasing CO₂ levels caused by downregulation of maximal Rubisco-limited rate of photosynthesis (V_{cmax}) and maximal electron transport-limited rate of photosynthesis (J_{max}) with increasing CO₂ level. V_{cmax} decreased to a larger extent than J_{max} resulting in a decrease in $V_{cmax} \cdot J_{max}$. The changes in V_{cmax} , J_{max} and $V_{cmax} \cdot J_{max}$ were more profound in the transition from low to ambient CO₂ level than from ambient to high CO₂ level. Stomatal conductance (g_s) declined with increasing CO₂ level, but only showed an effect of P availability for *H. lanatus*, where high P treatment caused a higher g_s . Positive correlations were found within the CO₂ treatments when plotting V_{cmax} , J_{max} and g_s against A_{sat} . As photosynthetic capacity declined with increasing CO₂ but A_{sat} did not decline it can be concluded that photosynthesis becomes more efficient by CO₂ fertilization. Even without increase in A_{sat} , plants can still benefit from elevated CO₂ by for example increased water and nitrogen use efficiency. The larger transitions of variables from low to ambient CO₂ suggest CO₂ fertilization will have smaller effect in the future than it had until now. The results of the P treatments show nutrients cannot be excluded in looking at the effect on photosynthesis under elevated CO₂.

Abbreviations:

A : assimilation rate of CO_2

C_i : intercellular CO_2 concentration

C_a : atmospheric CO_2 concentration

A/C_i curve: photosynthetic response to intercellular CO_2

A_{sat} : light saturated photosynthesis at CO_2 concentration of growing conditions

A_{max} : light saturated photosynthesis at saturated CO_2 concentration

A_{400} : light saturated photosynthesis at CO_2 conditions of 400 ppm

g_s : stomatal conductance

V_{cmax} : capacity of Rubisco maximum rate of carboxylation

J_{max} : RuBP regeneration expressed as the maximum rate of electron transport

C_{it} : intercellular CO_2 value of the transition of V_{cmax} limitation to J_{max} limitation

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1. Introduction

The concentration of CO₂ in the atmosphere has risen since the industrial revolution due to the burning of fossil resources and the clearing of forests (Blanco et al., 2014). Although this brings many negative impacts due to the consequential climate change, it could have a positive effect on the growth of terrestrial vegetation by stimulating photosynthesis (Ainsworth & Rogers, 2007), which is termed CO₂ fertilization. This fertilization effect can, for example, be seen in the extension of forests in some parts of the world. A study by Zhu et al. (2016) looked at satellite images and models to investigate four key drivers of Leaf Area Index (LAI) changes during 1982–2009. Between 25 and 50% of the global vegetated area showed an increase of growing season integrated LAI, while only 4% showed decrease. The greening trend was for 70% explained by CO₂ fertilization. As the CO₂ concentration in the atmosphere will continue to increase, it is important to look at how plants will react to elevated CO₂ concentrations.

Rising CO₂ has a variety of effects on plants. Under elevated CO₂ concentrations the rate of photosynthetic carbon fixation by leaves increases. Ainsworth & Rogers (2007) found an increase of 40% in leaf photosynthetic rates from a variety of plants under a CO₂ increase from 475 to 600ppm. Plants also respond to atmospheric CO₂ concentrations by changing their stomatal conductance (g_s). Typically, g_s is decreased under high CO₂ levels plants. As a result, plants can decrease water loss through the stomata (Ainsworth & Rogers, 2007). This is expected to decrease the water use of the entire plant. At larger scales, reductions in plant water use may have consequences for the hydrological cycle of entire ecosystems, with soil moisture levels and runoff both increasing under elevated CO₂ conditions (Leakey et al, 2009). Elevated CO₂ generally causes plants to grow faster and gives an increase in dry matter. This can also increase the yield of harvestable crops (Taub, 2010). Furthermore, the chemical composition of plant tissue changes under elevated CO₂ concentration. Due to the increase in photosynthesis, sugars and starches increase where leaf nitrogen decreases. This can also cause that the protein levels in plants decrease, affecting species in higher trophic levels as the food quality goes down. This can also be seen in protein levels of crops for human use (Taub et al, 2008).

Despite the clear effects of elevated CO₂ on photosynthesis and plant growth, the key question that remains is to what extent these responses are constrained by nutrient limitation in the future (Lukac et al., 2010; De Graaff et al., 2006; Goll et al., 2010; Lewis et al., 2010). Nitrogen (N) and Phosphorus (P) are considered the most important nutrient for plants. N is vital because it is a major component of chlorophyll, the compound by which plants use sunlight for photosynthesis. It is also a major component of amino acids, the building blocks of proteins making many biochemical reactions possible. N is used for energy-transfer compounds, such as ATP. Finally, it is a significant component of nucleic acids such as DNA (Mosaic, 2016). Plants require P for plant growth and development. Limited inorganic phosphate (P_i) supply results in numerous perturbations in plant growth and development and strongly affects plant yields (Rychter & Roa, 2005). Plants specifically require P for capturing and converting the energy of the sun into useful plant compound. P is also a vital component of DNA and RNA. The structures of both DNA and RNA are linked together by phosphorus bonds. P is also a vital component of ATP which is formed during photosynthesis and has P in its structure (Jin et al., 2015; Pandey et al., 2015). Critically, previous research on the effects of elevated CO₂ on photosynthesis in combination with nutrients has focused on N and to a lesser extent on P (Lewis et al., 2010).

The C, N and P cycles are among the most anthropogenically altered biogeochemical cycles on Earth (Falkowski et al., 2000). N and P availability can have an effect on how plants will react to elevated CO₂ levels. While the inputs of C and N through human-induced inputs keep increasing, are the

inputs of P much smaller. This is leading to a change in C:P and N:P that has never been seen before. These changes are affecting organism and ecosystem functioning and structure, the carbon cycle, climate and agriculture. For land ecosystems, coupled climate-carbon models generally do not include N and P limitation, suggesting vegetation growth and carbon storage are likely overestimated (Peñuelas et al., 2013).

Currently 180-190 Tg of phosphate rock is mined globally every year. The P demand is rising as the world population is continuing to grow, requiring more crop production and an increasing trend in a more P intensive meat/dairy based diet is taking place (Desmidt et al., 2015). Phosphate rock is a finite resource that is being depleted and cannot be manufactured, which can give problems when demand increases. Increasing scarcity can limit crop growth and food security as P is an essential nutrient for plants (Cordell & White, 2011). The stoichiometry of N:P can give inside about N or P limitation in terrestrial ecosystems. Large N inputs increase the productivity of plants and can lead to over-enrichment, causing N:P in plants to increase and can even result in P limitation. Increasing use of P fertilizer can have the opposite effect (Huang et al., 2015). The analysis of Huang et al. (2015) suggests that N generally decreases in plants under CO₂ enrichment. P levels are however much more variable, although a decrease is also often observed. N:P ratio in plants decreases under elevated CO₂ due to larger decreases in N relative to P. The decline in N:P under elevated CO₂ can be related to increased photosynthetic N use efficiency, increased need for ATP which demands more P than N and increased requirement of P-rich ribosomal RNA needed for the growth. Furthermore, how the ratio of N:P responds to rising CO₂ depends on the inputs of N and P in the ecosystem. N or P supplies can offset any downregulation of photosynthesis in response to CO₂ enrichment. Furthermore, the input of N may alter the P content in the plant, whereas changes in P input is not known to alter N content. Analysis by Sardans et al. (2012) reported the same about decreases in N and P in plants. Also the ratio between C:N and in most cases C:P decreased under elevated CO₂. They also state a possible cascading effects on the whole ecosystem through lower C:N ratios in litter and effects on herbivores through decreased food quality. Both Huang et al. (2015) and Sardans et al. (2012) point out that differences can be seen in responses of C:N:P to elevated CO₂ between species and climate zones. P may become the limiting resource as N:P increases through the use of fertilizers, although in some cropland ecosystems the solubility of N may reduce this (Sardans et al., 2012).

2. Theory

CO₂ is needed by plants to perform photosynthesis (A). In this process, Ribulose-1,5-bisphosphate-carboxylase/oxygenase (Rubisco) is responsible for CO₂ fixation. At the active site of Rubisco, ribulose-1,5-bisphosphate (RuBP) reacts with CO₂ to create two 3-phosphoglyceric acid (3PGA) molecules. Besides this carboxylase, Rubisco also reacts with O₂ to create a 2-phosphoglycollate (2PG) and another 3PGA molecule. The 3PGA enters the Calvin cycle, but the 2PG is used in the photorespiratory pathway. This results in 75% recovery of carbon and the release of half of a molecule of CO₂ for every molecule of 2PG metabolized (figure 1). At 25 °C around 23% of the carbon fixed by photosynthesis is lost due to photorespiration and as biochemical energy is used without producing sugar, photorespiration is seen as a wasteful process (Ainsworth & Rogers, 2007; Bloom, 2009). The carboxylation and oxygenation reactions are competing with each other and dependent on the CO₂: O₂ ratio at the active site. A low concentration of CO₂ in the active site will offset the affinity of Rubisco for CO₂ as a low concentration of O₂ will offset its affinity for O₂ (Ainsworth & Rogers, 2007). As a result, changes in atmospheric CO₂ concentrations strongly influence the amount of carbon taken up through photosynthesis.

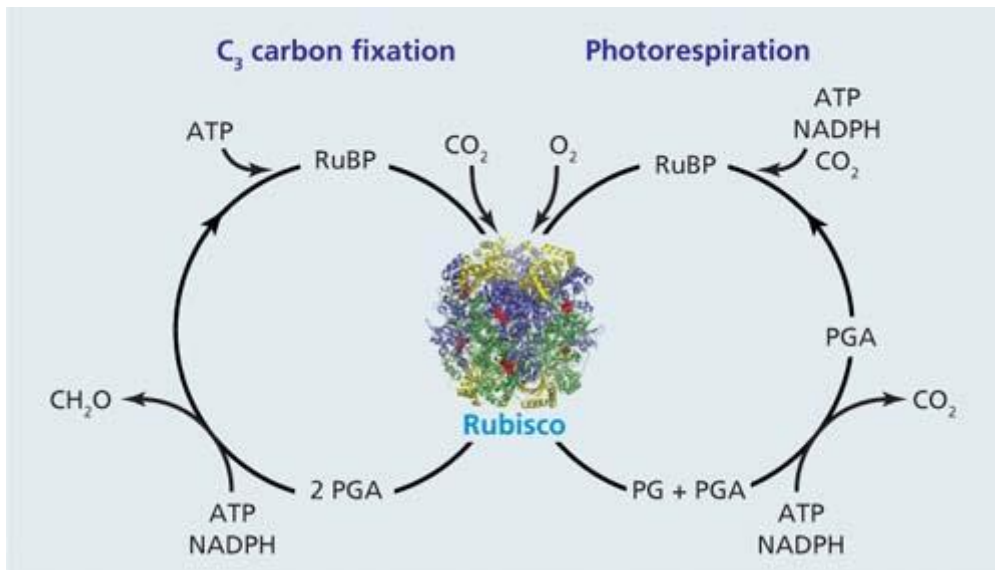


Figure 1: Competing processes of carboxylation and oxygenation through Rubisco with the Calvin cycle on the left and the photorespiratory pathway on the right (Bloom, 2009).

The direct increase in photosynthesis due to elevated CO₂ results from two properties of Rubisco. The first one is that the K_m of the enzyme for CO₂ is close to the current atmospheric concentration, making that the rate of carboxylation will increase with increasing CO₂. The second one is that CO₂ competitively inhibits the oxygenation reaction and this way reducing photorespiration. This effect is particularly important because it increases the efficiency of net carbon CO₂ uptake by decreasing photorespiratory CO₂ loss and diverting ATP and NADPH away from photorespiratory metabolism to photosynthetic assimilation. Thus, because the efficiency of net photosynthesis increases, rate increases regardless of whether other factors limit gross photosynthetic rate (Long et al., 2004).

Photosynthesis has two important components, which both could be limiting photosynthesis. These are the capacity of Rubisco expressed as the maximum rate of carboxylation (V_{cmax}) and RuBP regeneration expressed as the maximum rate of electron transport (J_{max}) (Ainsworth & Long, 2004). Due to P deficiency photosynthesis is commonly reduced as a result of a disturbed concentration of P_i in the cytosol. A_{max} was reduced by 83% on the short term when P_i was sequestered (Turnbull et al., 2007). For the longer term, P limitation can result in decreases in V_{cmax} , J_{max} and triose phosphate utilization (TPU). TPU is associated with the P_i metabolism and is in a way part of RuBP regeneration limitation. So it can be seen as part of J_{max} , but is by some treated separately from it (Yang et al., 2015). It is in some studies suggested that P limitation also leads to reduced stomatal conductance and concentrations of CO₂ in sub-stomatal cavities. So photosynthesis is affected by P through the pathway of the atmosphere to the chloroplasts (Warren, 2011). A study by Tissue & Lewis (2010) on the photosynthetic responses of P supply under glacial through future atmospheric CO₂ levels on cottonwood, showed P supply increasingly limited A_{sat} as CO₂ limitation went down under increasing CO₂ levels. Where P supply had a little limited effect from glacial to modern CO₂ levels, the impact from modern to future levels had a large effect. They suggest that this is mainly due to the reductions in V_{cmax} and g_s . When photosynthesis is limited by the capacity of Rubisco, the activities of electron transport and P_i regeneration are downregulated so that the rate of RuBP regeneration matches the rate of RuBP consumption by Rubisco. Similarly, when A is limited by electron transport or P_i regeneration, the activity of Rubisco is downregulated to balance the limitation in the rate of RuBP regeneration (Rychter & Roa, 2005). Photosynthesis is at current CO₂ level Rubisco limited for most plants, as CO₂ increases more carbon fixation will take place. For regeneration of RuBP, more

ATP is needed. The control of photosynthesis shifts from being limited by Rubisco to limitation by the capacity of regeneration of RuBP (Ainsworth & Rogers, 2007). Both V_{cmax} and J_{max} decrease under elevated CO_2 levels. The reductions in V_{cmax} are however higher than the reductions in J_{max} . This can be coupled to the idea that increasing CO_2 levels cause a shift from Rubisco limitation to a more RuBP regeneration limitation (Ainsworth & Rogers, 2007). Inadequate supply of P can result in a decrease in RuBP regeneration and as RuBP regeneration becomes more limited at higher CO_2 levels it may hereby also limit the increase in photosynthesis under elevated CO_2 levels (Rychter & Roa, 2005). This corresponds to the results of Tissue and Lewis (2010) where P supply becomes increasingly limited at higher CO_2 levels.

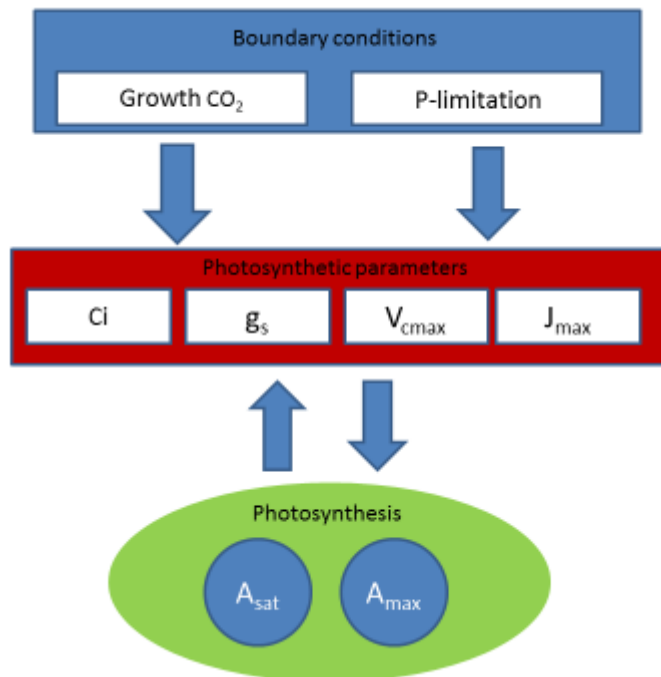


Figure 2: conceptual model of the main effects of CO_2 increase and P availability on photosynthesis, V_{cmax} and J_{max} . Increased atmospheric CO_2 concentration leads to increased C_i , which is causing increased carboxylation and decreased oxygenation by Rubisco. This results in an increase in photosynthesis. This allows V_{cmax} and J_{max} to be downregulated. Besides this, g_s can be reduced without decreasing CO_2 diffusion. Low P availability is causing a decrease in both V_{cmax} and J_{max} resulting in a decrease in carbon fixation and lower photosynthesis compared to high P conditions. Low P may also lower g_s , resulting in a decrease in photosynthesis. CO_2 increase is also causing a shift from limitation by V_{cmax} to limitation by J_{max} expressed by a decrease in $V_{cmax}:J_{max}$ and a higher C_i .

2.1 Research questions and hypothesis:

2.1.2 Research questions

This MSc. thesis research aims to quantify how photosynthesis, via adaptation of photosynthetic parameters adapts to different CO_2 levels and how this adaptation is affected by P limitation. To achieve this aim, answer will be given to the question: *How is photosynthesis and photosynthetic parameters of plants affected by combined changes in CO_2 growth conditions and P-limitation?* This thesis can be subdivided into three parts, each giving answer to the following subquestions: 1. *How are the variables A_{sat} , A_{400} , A_{max} and g_s affected by combined changes in CO_2 growth level and P-limitation?*; 2. *How do the photosynthetic parameters V_{cmax} , J_{max} and $V_{cmax}:J_{max}$ adapt to combined changes in CO_2 and P treatments?*; 3. *How is photosynthesis affected by V_{cmax} , J_{max} and g_s under different CO_2 and P treatments?*

2.1.3 Hypotheses

It is expected that A_{sat} will increase with increasing CO₂ level as a result of the CO₂ fertilization effect. The decrease in photorespiration will make the photosynthetic process more efficient. Elevated CO₂ conditions allow the plant to leave their stomata more closed without decreasing CO₂ diffusion from the atmosphere. The increased efficiency in A under elevated CO₂ levels allows the plant to downregulate V_{cmax} and J_{max} . CO₂ elevation leads to a shift of Rubisco limitation (V_{cmax}) to limitation by RuBP regeneration (J_{max}). This is expected to lead to a decrease in the $V_{cmax}:J_{max}$ ratio as V_{cmax} can be downregulated to a larger extent than J_{max} . This will also cause the Cit to increase with increasing CO₂ level. Ci will be regulated to fit the Ca causing no change in Ci/Ca between the treatments. When A is normalized by A_{400} or A_{max} the effects of V_{cmax} and J_{max} should come forward. This would in contrary to A_{sat} result in a decrease of A_{400} and A_{max} . P treatment is expected to cause a decrease of V_{cmax} and J_{max} and this way reduce A_{sat} . g_s will not be affected by P treatment. A_{400} and A_{max} will be lower with low P conditions with larger differences between the P treatments with higher CO₂ level. It is thought that P deficiency will limit J_{max} more than V_{cmax} . From this it is expected that as a shift takes place from V_{cmax} limitation to a J_{max} limitation with elevated CO₂, P deficiency will increasingly limit A_{sat} with increasing CO₂ concentrations. This interaction effect between CO₂ and P availability will result in higher increases in A_{sat} from low to ambient CO₂ level than from ambient to high CO₂ level and will be more profound under low P conditions.

This all leads to three hypotheses that this research expects to find:

1. A_{sat} is expected to increase with increasing CO₂ level. We expect larger increase from low to ambient than from ambient to high CO₂ level. A_{sat} is higher for high P and smaller increases with increasing CO₂ level with low P. A_{400} and A_{max} decrease with increasing CO₂ level. P availability will make A_{400} and A_{max} to be lower at low P conditions. This is caused by reductions in V_{cmax} and J_{max} . As these reductions are mainly during low to ambient CO₂ conditions, A_{400} and A_{max} are expected to also show a large reduction here. g_s will decrease with increasing CO₂ level, but P availability will not have an effect on g_s .
2. We expect that V_{cmax} and J_{max} will both decrease with elevated CO₂. The decrease V_{cmax} is expected to be larger than J_{max} resulting in reduction of $V_{cmax}:J_{max}$. Cit is expected to go up and Ci/Ca will be adjusted to stay around the same level. P limitation will result in a lower V_{cmax} and J_{max} .
3. Higher values of V_{cmax} and J_{max} will result in higher values of A_{sat} looking at the CO₂ treatments separately. g_s will have no relationship with A_{sat} as P treatment is not expected to have an effect on g_s .

3. Methods

3.1 Plant material

In this experiment, two plant species have been treated with different levels of CO₂ and P. The chosen species are *Solanum dulcamara* (bittersweet) which is a semi-woody C₃ herbaceous perennial vine and *Holcus lanatus* (Yorkshire fog) which is a perennial C₃ grass.

Seeds were sown in Primasta® potting soil in their respective CO₂ growth chambers. All plants of one species started growing in one bin all together in the potting soil. After 6 weeks the plants had grown enough and 24 individuals of each species were selected and repotted to individual pots with the size of approximately 1 liter filled with crystal sand. The sand has an average diameter of 2,5mm and had

been heated and filtered to prevent any containment of seeds or microbes. The absence of nutrients in the sand will prevent influence of nutrients already present in the soil.

3.2 Growing conditions

Three growth chambers were used to create conditions with three different atmospheric CO₂ levels. The plants were this way exposed to CO₂ levels of around 200 ppm corresponding to the level of the last interglacial, ambient CO₂ concentration of around 400 ppm and a concentration of 800 to represent a possible future concentration. The temperature is kept constant at 21/17 °C during day/night and the daylight time is from 08:00 to 18:00. The plants were checked upon every other day and given sufficient water so no water stress occurred.

3.3 Nutrient treatments

The plants growing in the sand were treated with a nutrient solution with a N:P ratio of either 1:1 or 45:1. Only P was varied, N was kept high and K and other micronutrients were not limited and stayed the same throughout the experiment. This led to 12 plants per species per P treatment per CO₂ level. The plants will get sufficient water fed from the bottom of the pots.

Plants were treated weekly with nutrient solution created in the lab. To let the plants adjust to the nutrient solution, the amount of nutrients the plants got was build up. In the first 3 weeks the plants got 15 ml of solution. The next 2 weeks this was increased to 50 ml solution. The following 3 weeks the solution was twice as concentrated and again 50 ml per week will be given to the plants. The final 3 weeks the solution was three times as concentrated as the first solution and again 50 ml was given weekly. The amount of nutrients and the composition of the solutions can be found in tables 1 and 2.

Table 1: chemicals used to create the 6 different nutrient solutions given in the concentration of mass per liter for high and low P and their composition for week 1-5, 6-8 and 9-11. With the top 4 chemicals used for the macronutrients N, P and K and below that the chemicals used for the several micronutrients.

Chemical	High P g/L			Low P g/L		
	Week 1-5	Week 6-8	Week 9-11	Week 1-5	Week 6-8	Week 9-11
KNO ₃	0,29	0,57	0,86	0,29	0,57	0,86
Ca(NO ₃) ₂	0,51	1,02	1,52	0,51	1,02	1,52
KH ₂ PO ₄	0,44	0,88	1,32	0,01	0,02	0,03
KCl	1,44	0,60	0,34	1,48	1,26	1,04
Micronutrients (mg/L)						
CaCl ₂ . 2H ₂ O	378,49	378,49	378,49	378,49	378,49	378,49
MgSO ₄ . 7H ₂ O	237,51	237,51	237,51	237,51	237,51	237,51
FeSO ₄ .7H ₂ O	40,40	40,40	40,40	40,40	40,40	40,40
EDTA-2Na.2H ₂ O	54,09	54,09	54,09	54,09	54,09	54,09
CuSO ₄ .5H ₂ O	0,16	0,16	0,16	0,16	0,16	0,16
H ₃ BO ₃	4,44	4,44	4,44	4,44	4,44	4,44
MnSO ₄ .H ₂ O	1,49	1,49	1,49	1,49	1,49	1,49
Na ₂ MoO ₄ .H ₂ O	0,20	0,20	0,20	0,20	0,20	0,20
ZnSO ₄ .7H ₂ O	0,88	0,88	0,88	0,88	0,88	0,88

Table 2: concentrations of the macro- and micronutrients derived from the chemicals of table 1 given to the plants for the different weeks and the total input of the nutrient after the full experiment

Nutrient	High P g/L (1:1)			Total (g)	Low P g/L (45:1)			Total (g)
	Week 1-5	Week 6-8	Week 9-11		Week 1-5	Week 6-8	Week 9-11	
N	0,10	0,20	0,30	0,60	0,10	0,20	0,30	0,60
P	0,10	0,20	0,30	0,60	0,002	0,004	0,007	0,013
K	0,99	0,79	0,89	2,67	0,89	0,89	0,89	2,67
Micronutrients in mg/L								
Ca	378,49	378,49	378,49	1135,47	378,49	378,49	378,49	1135,47
Mg	237,51	237,51	237,51	712,53	237,51	237,51	237,51	712,53
Fe	94,48	94,48	94,48	283,44	94,48	94,48	94,48	283,44
Cu	0,16	0,16	0,16	0,48	0,16	0,16	0,16	0,48
B	4,44	4,44	4,44	13,32	4,44	4,44	4,44	13,32
Mn	1,49	1,49	1,49	4,47	1,49	1,49	1,49	4,47
Mo	0,20	0,20	0,20	0,60	0,20	0,20	0,20	0,60
Zn	0,88	0,88	0,88	2,64	0,88	0,88	0,88	2,64

3.4 Measurements

After 5 months since sowing the seeds and 11 weeks of nutrient treatment the plants had grown enough for measurement. With the use of Licor LI-6400XT several aspects of the plants were measured. The block of the device was set at 21°C, the light value was 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to measure at saturated light, the reference CO₂ was 200/400/800 ppm depending on the CO₂ treatment and the relative humidity was kept around 70%. The full area of the 6 cm² cuvette was covered with healthy leaves if possible. To accomplish this for *H. lanatus* multiple leaves were put next to each other without overlap. For *S. dulcamara* some leaves did not cover the full area. Those leaves were photographed and the area was determined using ImageJ. Subsequently the calculated areas were adjusted in the results to be representative. First the A_{sat} , g_s and C_i/C_a were measured in four runs at reference CO₂ of the growing conditions. After this, an A/C_i curves was established of every plant with the C_a values 50, 100, 200, 300, 400, 600, 800, 1000, 1200, 1600, 2000. The data collected with the Licor is provided in excel files. With the data of the A/C_i curves, V_{cmax} , J_{max} and C_{it} can be determined using the program R and from this the $V_{\text{cmax}}:J_{\text{max}}$ ratio was calculated. With use of the package 'plantecophys' the A/C_i curves can be analyzed in R and the results can be collected (Duursma, 2015). A_{400} was not measured separately but filtered out of the A/C_i curves by taking the modeled value at reference CO₂ of 400. The same was done to determine A_{max} by taking the modeled value of the highest measured reference CO₂ (2000 ppm) assuming CO₂ saturation was reached at this CO₂ level. An example of an A/C_i curve is shown in figure 3.

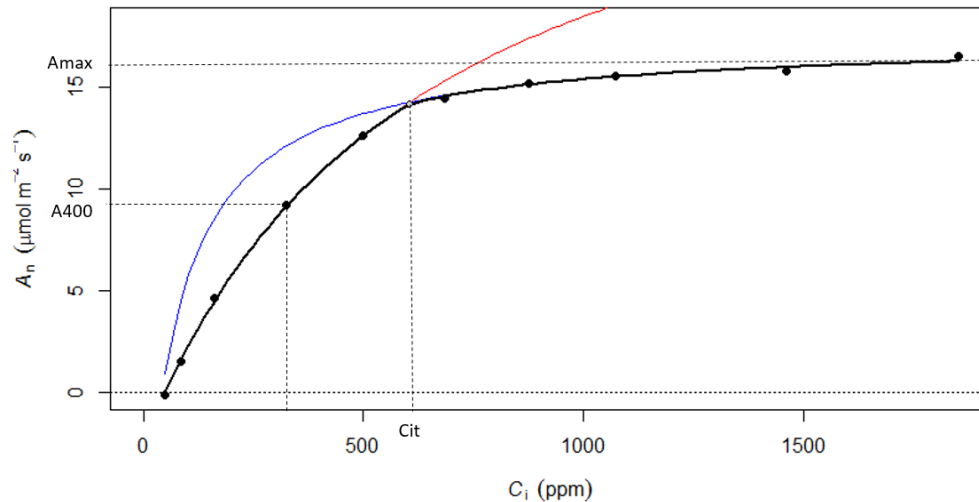


Figure 3: example A/C_i curve (*H. lanatus* green7a). Red line shows the curve of V_{cmax} limitation, blue line shows curve of J_{max} limitation. Cit is the point where both curves intersect. Dotted lines show how A_{400} and A_{max} are determined

A/C_i curves of 38 *S. dulcamara* plants (7 individuals for high and low P treatment under high CO_2 and 6 individuals per other treatment) and 42 *H. lanatus* plants (7 for each treatment) have been done. Individuals that had grown well with healthy leaves were selected for measurement. All measurements of *H. lanatus* were done before starting measuring *S. dulcamara*. The order of measuring within the species was mixed. So first a plant with the low CO_2 /low P treatment was measured, then a plant with low CO_2 /high P continuing with ambient CO_2 /low P etc. On average 6 measurements could be performed per day, every day starting with plants of a different CO_2 chamber to prevent treatments were measured at different moments of the day. All measurements were done before 15:00.

3.5 Statistical analyses

When the data had been collected, statistical analysis could be performed. The independent variables are P (high or low) and CO_2 level (200, 400, 800ppm). The dependent variables are A_{sat} , A_{400} , A_{max} , g_s , V_{cmax} , J_{max} , $V_{cmax}:J_{max}$, Cit and C_i/C_a . Statistics were performed in IBM SPSS Statistics 24.0 and boxplots and scatter plots were also created using this program. Two-way ANOVA's were used to find effects of P and CO_2 treatments on each of the dependent variables and if an interaction effect between P and CO_2 treatment was present. Post-hoc Tukey's HSD tests of the ANOVA's provided mean differences between the low and high P treatments and stepwise mean differences of low to ambient and ambient to high CO_2 level. Furthermore, linear regression of A_{sat} and A_{400} between V_{cmax} , J_{max} , and g_s was performed to acquire the adjusted R^2 . To test if the slopes of the scatter plots were significantly different, the null hypothesis that slope1=slope2 was taken. The statistics for this were performed in excel. For all statistical tests the results were considered significant if $P \leq 0.05$. Test results with $P \leq 0.10$ are marked as potentially significant. These results are not considered significant but can give indications an effect may be present when for example larger test groups are used.

4. Results

In appendix I the results of all the variables can be found of each individual plant. In table 4 the results of the two-way ANOVA's for every variable can be found per species with whether a significant effect was present or not. The different CO₂ treatments are compared to each other as nominal variables. It is however important to keep in mind that the upscaling of low to ambient and from ambient to high CO₂ level is not linear, but a doubling per transition (200, 400 and 800ppm). If the change of the CO₂ treatments per ppm would be taken, this increases the changes from low to ambient relative to the changes from ambient to high CO₂. In table 3 the results of the three-way ANOVA's are presented where the data of both species is included. The table shows there is a lot of significant difference of the variables between the species as well as the interaction effect of CO₂ and P with species. This indicates that the species react different to the treatments. The species are therefore analyzed separate from each other. The results below will show the analyzed data of both species. First photosynthetic leaf characteristics consisting of the variables A_{sat} , A_{400} , A_{max} and g_s will be treated, followed by results of the photosynthetic parameters V_{cmax} , J_{max} , $V_{cmax}:J_{max}$ including the variables Cit and Ci/Ca . The results are supported by outcomes of the ANOVA's in table 4, boxplots of every treatment and the difference in mean of neighboring treatments with corresponding Tukey's HSD significance. In the last part of the results relationships between variables are analyzed.

Table 3: Summary of the tree-way ANOVA's whether a significant CO₂ effect, P effect or interaction effect was present for all the variables

Variable	CO ₂	P	Species	CO ₂ *P	CO ₂ *Species	P*Species	CO ₂ *P*Species
A_{sat}	ns	***	***	***	*	***	*
A_{400}	***	***	***	ns	***	**	ns
A_{max}	***	***	***	ns	***	**	ns
g_s	***	+	**	**	***	*	ns
V_{cmax}	***	***	***	ns	***	ns	+
J_{max}	***	***	***	ns	***	**	ns
$V_{cmax}:J_{max}$	***	ns	**	ns	ns	ns	ns
Cit	***	ns	+	ns	ns	ns	ns
Ci/Ca	**	+	ns	*	ns	ns	ns

ns = not significant + = $P \leq 0,10$ * = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

Table 4: Summary of the two-way ANOVA's of both species whether a significant CO₂ effect, P effect or interaction effect was present for all the variables

	CO ₂	P	CO ₂ *P
S. dulcamara			
<i>A_{sat}</i>	ns	*	ns
<i>A_{max}</i>	***	**	ns
<i>A₄₀₀</i>	***	*	ns
<i>V_{cmax}</i>	***	*	ns
<i>J_{max}</i>	***	**	ns
<i>V_{cmax}:J_{max}</i>	***	ns	ns
<i>g_s</i>	***	ns	ns
<i>C_{it}</i>	***	ns	ns
<i>C_i/C_a</i>	ns	ns	*
H. lanatus			
<i>A_{sat}</i>	**	***	***
<i>A_{max}</i>	***	***	+
<i>A₄₀₀</i>	***	***	*
<i>V_{cmax}</i>	***	***	ns
<i>J_{max}</i>	***	***	+
<i>V_{cmax}:J_{max}</i>	***	ns	ns
<i>g_s</i>	***	**	**
<i>C_{it}</i>	***	+	ns
<i>C_i/C_a</i>	*	ns	ns

ns = not significant + = $P \leq 0,10$ * = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

4.1 Photosynthetic leaf characteristics (*A_{sat}*, *A₄₀₀*, *A_{max}*, *g_s*)

4.1.1 Photosynthesis at growing conditions (*A_{sat}*)

The results show different responses of *A_{sat}* to the combined CO₂ and P treatments in *S. dulcamara* and *H. lanatus* (Fig. 4). For both *H. lanatus* and *S. dulcamara* a significant effect of P treatment on *A_{sat}* was found. The CO₂ treatment did not influence the *A_{sat}* of *S. dulcamara*. As a consequence, no significant CO₂ treatment and no interaction effect between CO₂ and P treatment were found. The results of *H. lanatus* are somewhat different from those of *S. dulcamara*. Both CO₂ and P treatment showed a significant effect on *A_{sat}*. *A_{sat}* increased under high P conditions with increasing CO₂ level while under low P conditions *A_{sat}* seemed to decrease from low to ambient CO₂ and tends to increase again from ambient to high CO₂ with on overall not much change. This adaptation leads to a significant interaction effect between P treatment and CO₂ treatment. The effect of P treatment and the different behavior to CO₂ treatment can be clearly seen in figure 4. At low CO₂ level no significant difference between the P treatments is visible, while at ambient and high CO₂ level a significant difference is present for *H. lanatus*. More response is seen from low to ambient CO₂ under high P conditions as the increase of low to ambient CO₂ level is significant while the increase from ambient to high CO₂ is not.

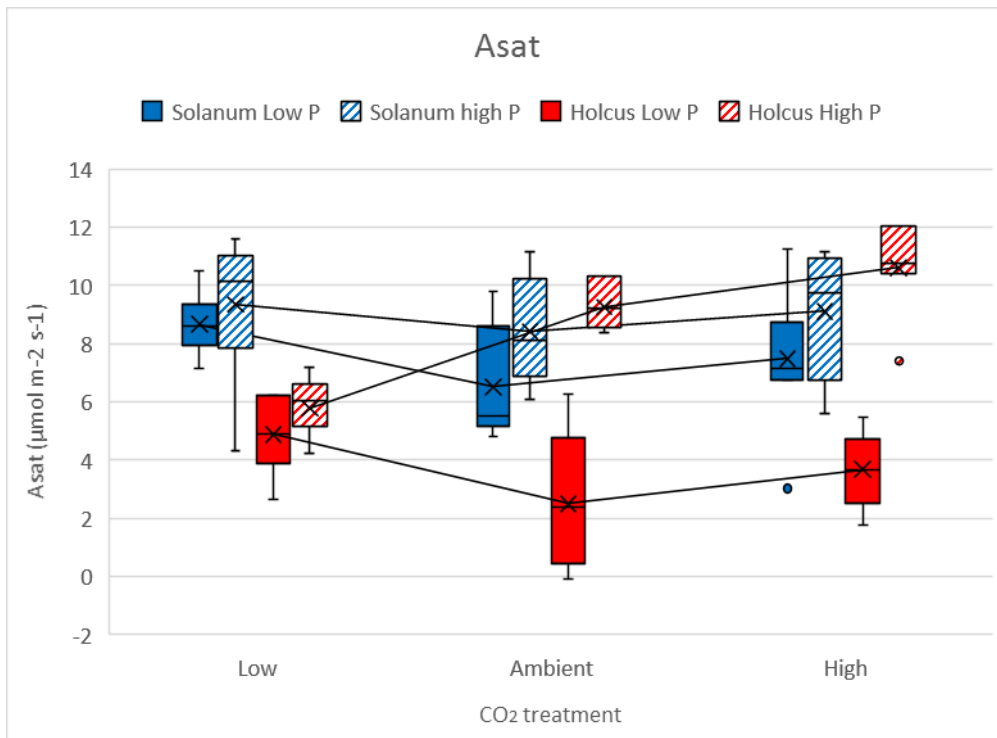


Figure 4: boxplots representing A_{sat} of the different CO_2 and P treatments for both species including mean line between CO_2 transitions.

Table 5 a&b: Mean differences of A_{sat} between neighboring treatments in $\mu mol m^{-2} s^{-1}$ with significance of Tukey's HSD test. Compared treatments represented as: HP-High P; LP-Low P; LC-low CO_2 ; AC-ambient CO_2 ; HC-high CO_2 . Table a showing differences between P treatments, table b showing differences between CO_2 treatments

Table a		CO ₂ treatment		
Species	P treatment	Low	Ambient	High
<i>S. dulcamara</i>	HP-LP	-0,68	-1,92	-1,63
<i>H. lanatus</i>	HP-LP	-0,91	-6,77***	-6,94***

Table b		P treatment	
Species	CO ₂ treatment	Low	High
<i>S. dulcamara</i>	LC-AC	2,16	0,92
<i>S. dulcamara</i>	AC-HC	-0,97	-0,69
<i>H. lanatus</i>	LC-AC	2,38*	-3,48**
<i>H. lanatus</i>	AC-HC	-1,18	-1,35

* = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

4.1.2 Photosynthetic capacity (A_{max} and A_{400})

The results show clear differences in terms of the photosynthetic capacity between CO_2 and P treatments. Figure 5 shows the A/C_i curves plotted out of all measured points for the different CO_2 treatment without distinguishing the P treatments. This shows how A increases with increasing CO_2 concentration. From this the A_{max} can be determined, which shows a lower A_{max} when plants are grown under high CO_2 concentration. This can also be seen in figure 6 where P treatment is included. The results of A_{max} and A_{400} are, in contrary to A_{sat} , normalized values of the photosynthetic capacity by a CO_2 reference value of 400 ppm (A_{400}) or where A is not affected anymore by increasing CO_2 (A_{max}). This eliminates the effect of potential increased A by CO_2 fertilization and will show effects on A of V_{cmax} , J_{max} , $V_{cmax}:J_{max}$ and g_s . The results of A_{max} and A_{400} show an almost identical behavior (figure 6). Both CO_2 and P treatment have a significant effect on A . A negative effect with increasing CO_2 level can be seen and A values are lower with low P availability. Both P treatments of *S. dulcamara* show a larger decline from low to ambient CO_2 level than the transition to high CO_2 treatment. The results of *H. lanatus* only showed this behavior for the low P treatment. The high P treatment showed around the same decrease from low to ambient as from ambient to high CO_2 level. The same behavior over the CO_2 levels for both P treatments of *S. dulcamara* caused no interaction effect. The steep decline from low to ambient and much less decrease from ambient to high with the high P treatment which was not seen at low P treatment with *H. lanatus* did lead to an interaction affect between P and CO_2 . The effect of CO_2 level on A_{max} and A_{400} is different than the effect on A_{sat} . Instead of values that stayed around the same level or showed an increase, now decline in A was observed.

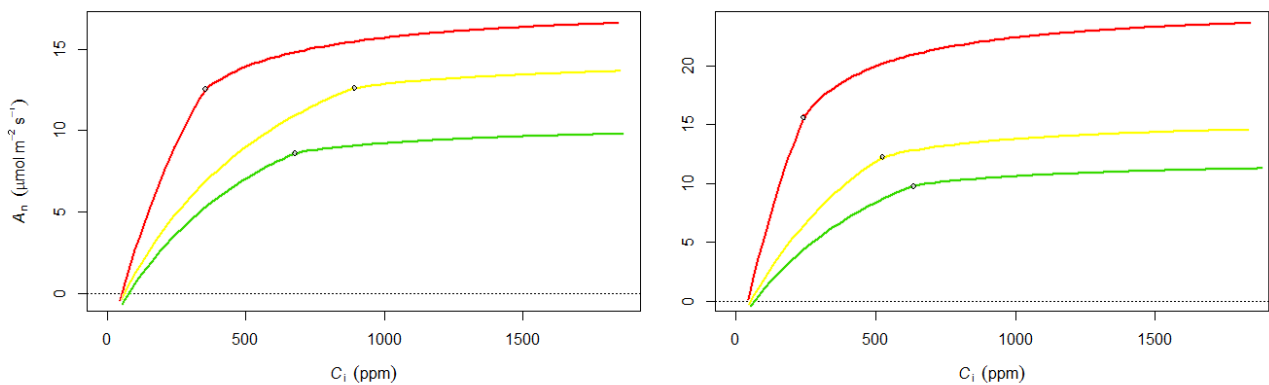


Figure 5: A/C_i curves of *H. lanatus* (left) and *S. dulcamara* (right) of the low (red), ambient (yellow) and high (green) treatments plotted by combining all measured points per CO_2 treatment together.

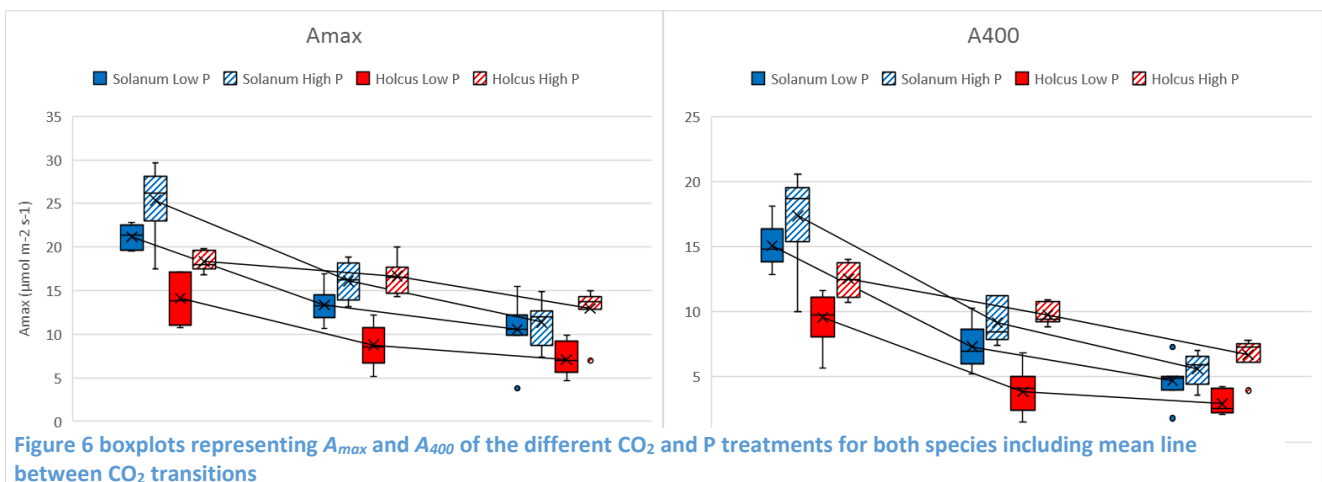


Figure 6 boxplots representing A_{max} and A_{400} of the different CO_2 and P treatments for both species including mean line between CO_2 transitions

Table 6 a&b: Mean differences of A_{400} and A_{max} between neighboring treatments in $\mu\text{mol m}^{-2} \text{s}^{-1}$ with significance of Tukey's HSD test. Compared treatments represented as: HP-High P; LP-Low P; LC-low CO_2 ; AC-ambient CO_2 ; HC-high CO_2 . Table a showing differences between P treatments, table b showing differences between CO_2 treatments

Table a			CO ₂ treatment		
Variable	Species	P treatments	Low	Ambient	High
A_{400}	<i>S. dulcamara</i>	HP-LP	-2,43	-1,59	-0,79
	<i>H. lanatus</i>	HP-LP	-2,90**	-5,73**	-3,70***
A_{max}	<i>S. dulcamara</i>	HP-LP	-4,04	-2,83	-1,01
	<i>H. lanatus</i>	HP-LP	-4,30**	-8,06***	-6,18***

Table b			P treatment	
Variable	Species	CO ₂ treatments	Low	High
A_{400}	<i>S. dulcamara</i>	LC-AC	8,16***	9,01***
	<i>S. dulcamara</i>	AC-HC	2,59	3,39
	<i>H. lanatus</i>	LC-AC	5,68***	2,85**
	<i>H. lanatus</i>	AC-HC	1,02	3,06**
A_{max}	<i>S. dulcamara</i>	LC-AC	8,50***	9,71***
	<i>S. dulcamara</i>	AC-HC	2,76	4,58
	<i>H. lanatus</i>	LC-AC	5,53***	1,77
	<i>H. lanatus</i>	AC-HC	1,90	3,79*

* = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

4.1.3 Stomatal conductance (g_s)

The results show a clear response of g_s to CO_2 for both *S. dulcamara* and *H. lanatus* (Fig 7). For both species g_s is reduced when CO_2 concentration increases. For *S. dulcamara* this reduction is larger from low to ambient CO_2 conditions than from ambient to high CO_2 conditions. Where CO_2 treatment gave a significant effect on g_s and P treatment showed no significant effect. Low and high P were quite similar, resulting that an interaction effect was not found. The results of *H. lanatus* indicate a lower g_s under low P conditions and a significant effect of P was found. With low P availability a sharp decrease from low to ambient CO_2 level was observed which stayed low from ambient to high CO_2 level. High P availability resulted in a more stable decrease of g_s which was with exception of low CO_2 higher than the g_s of low P. This all resulted in a significant effects of P and CO_2 treatment including an interaction effect.

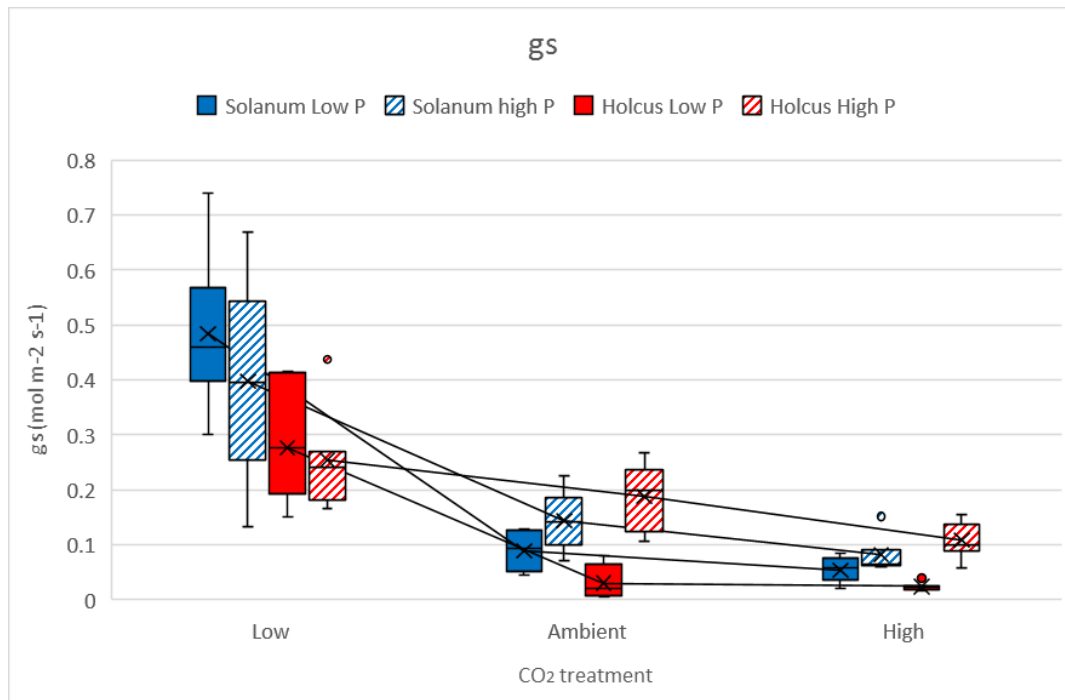


Figure 7: boxplots representing g_s of the different CO_2 and P treatments for both species including mean line between CO_2 transitions.

Table 7: Mean differences of g_s between neighboring treatments in $\mu\text{mol m}^{-2} \text{s}^{-1}$ with significance of Tukey's HSD test. Compared treatments represented as: HP-High P; LP-Low P; LC-low CO_2 ; AC-ambient CO_2 ; HC-high CO_2 . Table a showing differences between P treatments, table b showing differences between CO_2 treatments

Table a		CO_2 treatment		
Species	P treatment	Low	Ambient	High
<i>S. dulcamara</i>	HP-LP	0,086	-0,055	-0,029
<i>H. lanatus</i>	HP-LP	0,022	-0,16***	-0,085

Table b		P treatment	
Species	CO_2 treatment	Low	High
<i>S. dulcamara</i>	LC-AC	0,39***	0,25**
<i>S. dulcamara</i>	AC-HC	0,036	0,061
<i>H. lanatus</i>	LC-AC	0,25***	0,066
<i>H. lanatus</i>	AC-HC	0,0063	0,079

* = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

4.2 Photosynthetic parameters (V_{cmax} , J_{max} , $V_{cmax}:J_{max}$, C_{it} , C_i/C_a)

4.2.1 V_{cmax}

Both P treatment and CO_2 treatment had a significant effect on the V_{cmax} of *S. dulcamara*. A decreasing trend can be seen with increasing CO_2 level, and a lower V_{cmax} at low P conditions compared to high P conditions. The gap between high and low P looks larger with low CO_2 than the ones under ambient or high CO_2 . However, no significant mean differences between the P treatments were found and this did not lead to an interaction effect between CO_2 and P treatment. Between low and ambient CO_2 , a large reduction in V_{cmax} is noticed, where from ambient to high CO_2

the decrease is much smaller. This is supported by significant mean differences from low to ambient CO₂ where the differences between ambient to high were not significant. P and CO₂ treatment also showed a significant effect on V_{cmax} of *H. lanatus*. Under both low and high P availability a decrease is found with increasing CO₂, where low P causes a lower V_{cmax} compared to high P conditions. Their behavior is quite similar resulting in the absence of an interaction effect. Under low CO₂ conditions no significant difference in the mean between high and low P was found, this do was the case for ambient and high CO₂. A larger reduction in V_{cmax} can be seen in the transition of low to ambient CO₂ and only here significant difference was found.

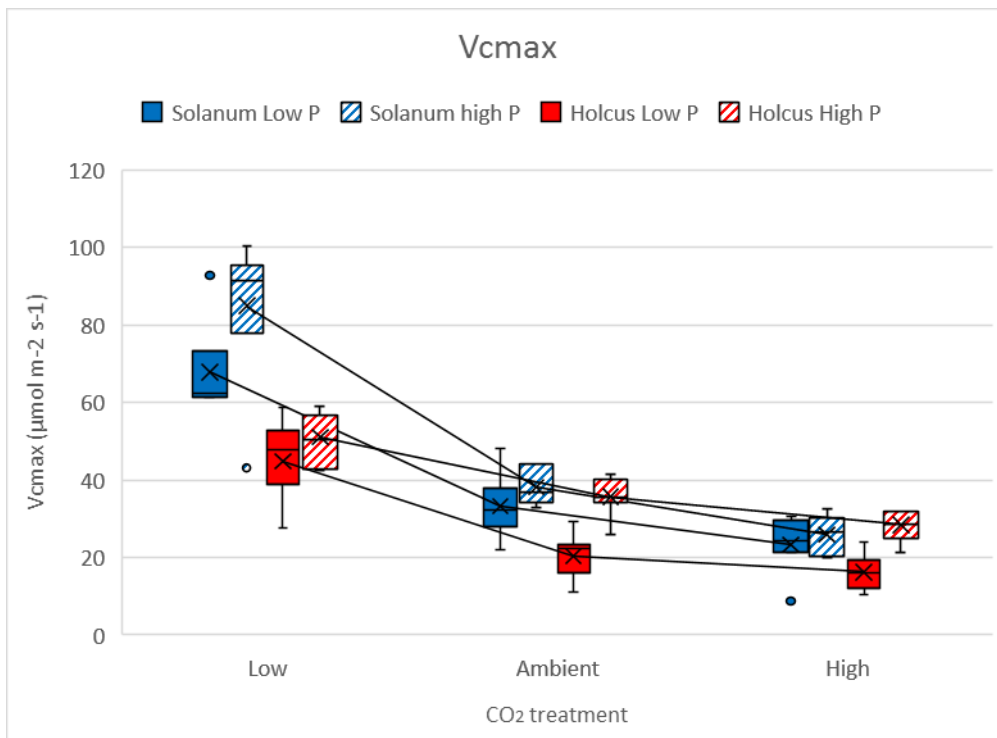


Figure 8: boxplots representing V_{cmax} of the different CO₂ and P treatments for both species including mean line between CO₂ transitions.

Table 8: Mean differences of V_{cmax} between neighboring treatments in $\mu\text{mol m}^{-2} \text{s}^{-1}$ with significance of Tukey's HSD test. Compared treatments represented as: HP-High P; LP-Low P; LC-low CO₂; AC-ambient CO₂; HC-high CO₂. Table a showing differences between P treatments, table b showing differences between CO₂ treatments

Table a		CO ₂ treatment		
Species	P treatment	Low	Ambient	High
<i>S. dulcamara</i>	HP-LP	-17,13	-5,05	-2,64
<i>H. lanatus</i>	HP-LP	-6,25	-15,32**	-12,29*

Table b		P treatment	
Species	CO ₂ treatment	Low	High
<i>S. dulcamara</i>	LC-AC	34,65***	46,73***
<i>S. dulcamara</i>	AC-HC	9,86	12,26
<i>H. lanatus</i>	LC-AC	24,39***	15,33**
<i>H. lanatus</i>	AC-HC	4,18	7,22

* = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

4.2.2 J_{max}

The effects on J_{max} show a similar behavior as V_{cmax} . A decrease with an increasing CO_2 level and a lower J_{max} under low P conditions occurs. P treatment as well as CO_2 treatment show a significant effect on J_{max} , but no interaction effect was observed. For *S. dulcamara* only the mean differences between low and ambient CO_2 are significant, as was also seen with V_{cmax} . For *H. lanatus*, differences between the P treatments are larger than with *S. dulcamara* as can be seen by significant differences between high and low P for every CO_2 level. The decrease for low P conditions is steeper than for high P conditions, but this did not result in a significant interaction effect, although with $P \leq 0,10$ can be considered potentially significant. Under all CO_2 levels significant difference between P treatment was found. Where low P only showed a significant difference from low to ambient CO_2 , high P just gave a significant difference from ambient to high CO_2 . This suggests that for low P availability most decrease in J_{max} occurred during low to ambient CO_2 , while at high P availability this was most during the transition to high CO_2 level.

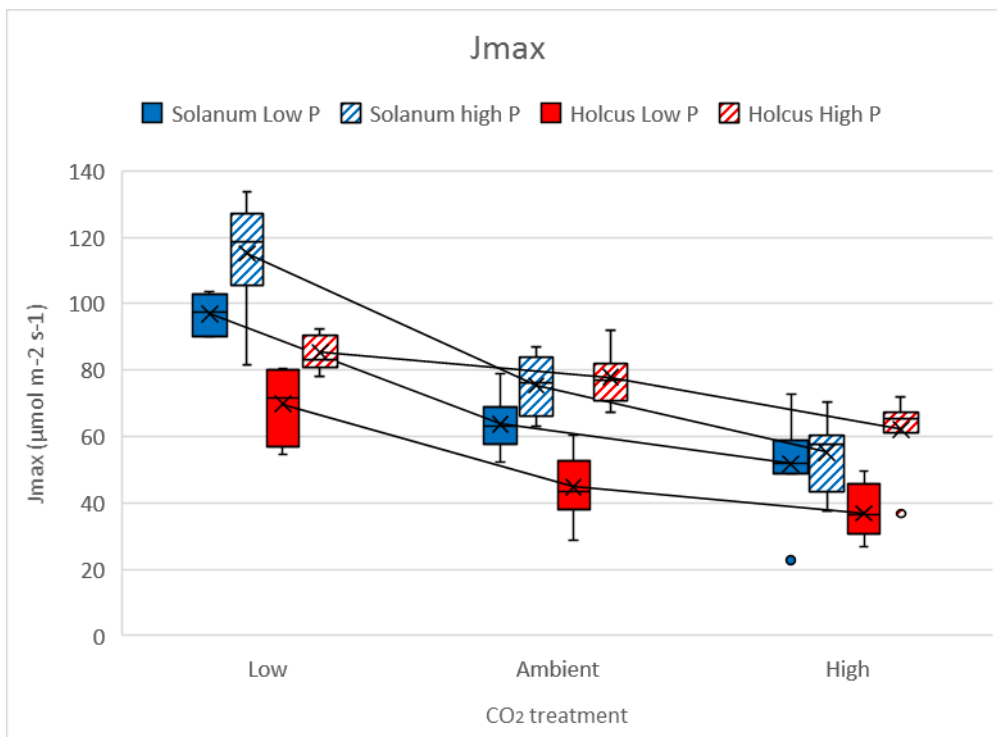


Figure 9: boxplots representing J_{max} of the different CO_2 and P treatments for both species including mean line between CO_2 transitions.

Table 9: Mean differences of J_{max} between neighboring treatments in $\mu\text{mol m}^{-2} \text{s}^{-1}$ with significance of Tukey's HSD test. Compared treatments represented as: HP-High P; LP-Low P; LC-low CO_2 ; AC-ambient CO_2 ; HC-high CO_2 . Table a showing differences between P treatments, table b showing differences between CO_2 treatments

Table a		CO ₂ treatment		
Species	P treatment	Low	Ambient	High
<i>S. dulcamara</i>	HP-LP	-18,31	-11,74	-3,54
<i>H. lanatus</i>	HP-LP	-15,59*	-33,14***	-25,28***

Table b		P treatment	
Species	CO ₂ treatments	Low	High
<i>S. dulcamara</i>	LC-AC	33,18***	39,74***
<i>S. dulcamara</i>	AC-HC	12,03	20,23
<i>H. lanatus</i>	LC-AC	24,97***	7,42
<i>H. lanatus</i>	AC-HC	7,90	15,76*

* = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

4.2.3 $V_{cmax}:J_{max}$

The behavior of $V_{cmax}:J_{max}$ was similar for both species. Only a significant effect of CO₂ level was found on the $V_{cmax}:J_{max}$ ratio as values did not much differ between the P treatments. From low to ambient CO₂ level for both P treatments a decrease was observed. As V_{cmax} and J_{max} both decrease with increasing CO₂ levels, a larger decrease in V_{cmax} than J_{max} can explain this result. Within the P treatments and between ambient and high CO₂ level no significant difference between the groups was observed. The decrease occurs primarily from low to ambient CO₂, supported by significant mean differences. This can be related to the large drop in V_{cmax} between low and ambient CO₂ seen in the results of V_{cmax} above. As V_{cmax} limitation is shifting to J_{max} limitation with high CO₂ level, downregulation of V_{cmax} and J_{max} between ambient to high CO₂ will shift more towards J_{max} explaining the lower decrease in $V_{cmax}:J_{max}$ from ambient to high. The decrease in $V_{cmax}:J_{max}$ still indicates a larger downregulation in V_{cmax} than J_{max} . It must be noted that the differences between ambient to high are not significant, but a different behavior between low to ambient CO₂ and ambient to high CO₂ is clear.

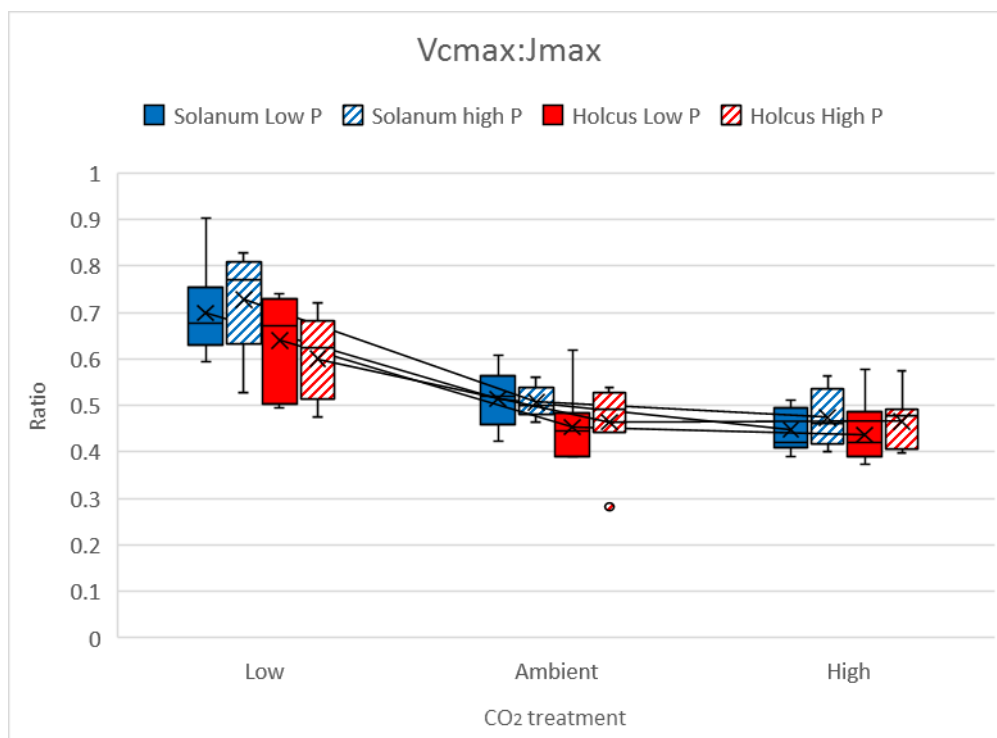


Figure 10: boxplots representing $V_{cmax}:J_{max}$ of the different CO₂ and P treatments for both species including mean line between CO₂ transitions.

Table 10: Mean differences of $V_{cmax}:J_{max}$ between neighboring treatments in $\mu\text{mol m}^{-2} \text{s}^{-1}$ with significance of Tukey's HSD test. Compared treatments represented as: HP-High P; LP-Low P; LC-low CO_2 ; AC-ambient CO_2 ; HC-high CO_2 . Table a showing differences between P treatments, table b showing differences between CO_2 treatments

Table a		CO ₂ treatment		
Species	P treatment	Low	Ambient	High
<i>S. dulcamara</i>	HP-LP	-0,0292	0,0070	-0,0265
<i>H. lanatus</i>	HP-LP	0,0393	-0,0118	-0,0290

Table b		P treatment	
Species	CO ₂ treatment	Low	High
<i>S. dulcamara</i>	LC-AC	0,185**	0,221***
<i>S. dulcamara</i>	AC-HC	0,0663	0,0328
<i>H. lanatus</i>	LC-AC	0,187**	0,136*
<i>H. lanatus</i>	AC-HC	0,0161	-0,00104

* = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

4.2.4 Cit

The transition point where limitation by V_{cmax} shifts to J_{max} goes up with higher CO_2 level for both species. No significant effect of P treatment on Cit was found, however for *H. lanatus* a potential significance was present where the Cit is lower under high P conditions with the ambient and high CO_2 treatment. The increase with increasing CO_2 is much more gradual than the other parameters. Although high P treatment of *S. dulcamara* and low P treatment of *H. lanatus* still show significantly more increase from low to ambient CO_2 .

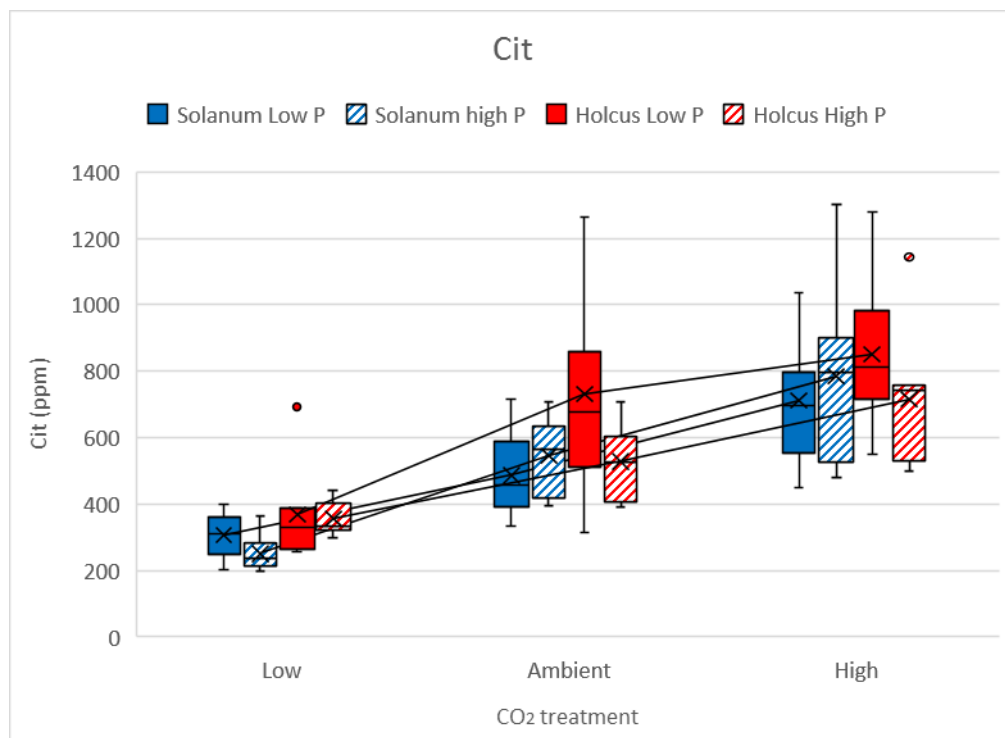


Figure 11: boxplots representing Cit of the different CO_2 and P treatments for both species including mean line between CO_2 transitions.

Table 11 a&b: Mean differences of C_i/C_a between neighboring treatments in $\mu\text{mol m}^{-2} \text{s}^{-1}$ with significance of Tukey's HSD test. Compared treatments represented as: HP-High P; LP-Low P; LC-low CO_2 ; AC-ambient CO_2 ; HC-high CO_2 . Table a showing differences between P treatments, table b showing differences between CO_2 treatments

Table a		CO_2 treatment		
Species	P treatment	Low	Ambient	High
<i>S. dulcamara</i>	HP-LP	56,40	-58,81	-72,19
<i>H. lanatus</i>	HP-LP	12,91	202,32	134,67

Table b		P treatment	
Species	CO_2 treatment	Low	High
<i>S. dulcamara</i>	LC-AC	-180,47	-295,68*
<i>S. dulcamara</i>	AC-HC	-225,59	-238,98
<i>H. lanatus</i>	LC-AC	-362,08*	-172,67
<i>H. lanatus</i>	AC-HC	-119,29	-186,94

* = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

4.2.5 C_i/C_a

Not much change occurs between the treatments when looking at the boxplots of C_i/C_a (figure 12). However, the ANOVA test showed there was a significant effect of CO_2 with *H. lanatus*. This effect mostly found place from low to ambient CO_2 where a decrease in C_i/C_a can be seen. From ambient to high CO_2 almost no change can be observed for both species. For *S. dulcamara* no change over the whole transition of low to high CO_2 can be seen under high P conditions where under low P conditions, C_i/C_a decreases a little resulting in an interaction effect between CO_2 and P level. Overall no drastic changes in C_i/C_a were found where values stayed around 0,7. Table 12 shows no significant difference between the treatments. This indicates that the results of the ANOVA must have been caused by small changes.

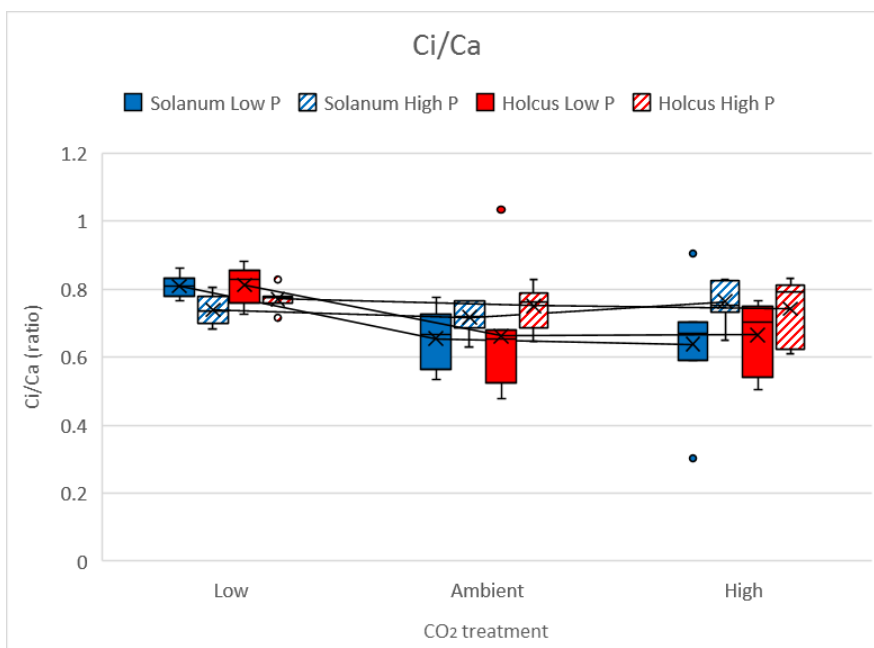


Figure 12: boxplots representing C_i/C_a of the different CO_2 and P treatments for both species including mean line between CO_2 transitions.

Table 12: Mean differences of Ci/Ca between neighboring treatments in $\mu\text{mol m}^{-2} \text{s}^{-1}$ with significance of Tukey's HSD test. Compared treatments represented as: HP-High P; LP-Low P; LC-low CO₂; AC-ambient CO₂; HC-high CO₂. Table a showing differences between P treatments, table b showing differences between CO₂ treatments

Table a		CO ₂ treatment		
Species	P treatment	Low	Ambient	High
<i>S. dulcamara</i>	HP-LP	0,0687	-0,0631	-0,125
<i>H. lanatus</i>	HP-LP	0,0409	-0,0893	-0,0783

Table b		P treatment	
Species	CO ₂ treatment	Low	High
<i>S. dulcamara</i>	LC-AC	0,154	0,0224
<i>S. dulcamara</i>	AC-HC	0,0163	-0,0460
<i>H. lanatus</i>	LC-AC	0,150	0,0198
<i>H. lanatus</i>	AC-HC	-0,00293	0,00810

* = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

4.3 Relationships between variables

The results of A_{sat} and A_{400} have been tested for relationships with the variables V_{cmax} , J_{max} and g_s with CO₂ treatments compared separately. As the results of the behavior of the different variables showed different behavior for the two species will the tests be performed per species and will the results be addressed separately. The results of the linear regression test with the slope of the scatter plots can be found in tables 13 and 14. The scatter plots with statistical difference between slopes can be found in appendix II.

4.3.1 *Holcus lanatus*

The scatter plots divided by the CO₂ treatments of V_{cmax} and J_{max} look very similar. Positive relations were established under all CO₂ conditions. The results of linear regression (table 13) show if the parameters show different behavior with A_{sat} and A_{400} influenced by CO₂ concentration. The slopes of the scatter plots of A_{sat} increase with increasing CO₂ level for V_{cmax} , J_{max} and g_s . The slope of the low CO₂ treatment is more different from ambient and high treatment, where ambient and high are much more similar. This is supported as the low CO₂ slopes of V_{cmax} and J_{max} were tested significantly different from the slopes of the ambient and high CO₂ treatments, but the slopes of ambient and high did not significantly differ from each other. For g_s all CO₂ treatments showed significant difference from each other. These results indicate that under higher CO₂ conditions V_{cmax} , J_{max} or g_s have a more positive relationship with A_{sat} compared to lower CO₂ conditions. So the same increase in V_{cmax} , J_{max} or g_s results in a larger increase in A_{sat} under higher CO₂ conditions. The other way around, this could indicate that downregulation of V_{cmax} or J_{max} will have less effect on A_{sat} under low CO₂ conditions. This may be linked to the results found that larger downregulation takes place from low to ambient CO₂ level than from ambient to high CO₂ level, as the effect of downregulation on A_{sat} increases with increasing CO₂ level. Under low CO₂ conditions a significant relation was found between V_{cmax} and A_{sat} , where for J_{max} and g_s a relation was absent. From this it can be said that the magnitude of V_{cmax} is the main driver for A_{sat} under low CO₂ conditions. This makes sense as V_{cmax} is limiting A at low CO₂ levels. For ambient and high CO₂ conditions, V_{cmax} , J_{max} and g_s all have a significant positive relation with high explanation rates. This causes that all three variables are influencing A_{sat} . Comparing the results with A_{400} it shows that the slopes are much more similar for the different CO₂ treatments. The change of A_{400} is again better explained under ambient and high CO₂ conditions.

Table 13: Adjusted R² values including the slope by which A_{sat} or A₄₀₀ separated by CO₂ treatment is determined by the variable. Significant correlations indicated as bold

		A_{sat} low CO₂	A_{sat} ambient CO₂	A_{sat} high CO₂	A₄₀₀ low CO₂	A₄₀₀ ambient CO₂	A₄₀₀ high CO₂
<i>V_{cmax}</i>	Adj. R ²	0,784***	0,757***	0,816***	0,757***	0,804***	0,915***
	slope	0,12	0,36	0,46	0,22	0,32	0,28
<i>J_{max}</i>	Adj. R ²	0,160	0,888***	0,827***	0,591***	0,927***	0,940***
	slope	0,05	0,19	0,22	0,16	0,17	0,13
<i>g_s</i>	Adj. R ²	0,195	0,833***	0,733***	-0,032	0,780***	0,546**
	slope	3,61	38,42	67,26	5,23	34,54	32,12

* = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

4.3.2 Solanum dulcamara

The results of linear regression of *S. dulcamara* (table 14) show the same kind of behavior over the CO₂ levels as *H. lanatus* for *V_{cmax}* and *J_{max}*. The slopes of the scatter plots also increase with increasing CO₂ level. However, the difference between the treatments seems less profound. The difference in slopes of low CO₂ with the other treatments was still significant with *V_{cmax}*, but was not significant anymore for *J_{max}*, although with a *P* value of 0,083 between low and ambient CO₂ treatment can be marked as potentially significant. *g_s* still showed significant difference between low and ambient CO₂ level. The slope of high CO₂ level seems to be less steep than the one of ambient CO₂, but is still quite similar resulting in no significant difference with neither ambient and low CO₂ treatment. *H. lanatus* did not show significance with *J_{max}* and *g_s* at low CO₂ conditions. This do was the case for *S. dulcamara* which may show that *A_{sat}* is already driven by *J_{max}* and *g_s* at low CO₂ levels. Under high CO₂ conditions a relationship with *g_s* is no longer found. This indicates that under low and ambient CO₂ conditions *V_{cmax}*, *J_{max}* and *g_s* are related to *A_{sat}* where under high CO₂ conditions the influence of *g_s* has fallen away. Values found of linear regression of the variables with *A₄₀₀* showed generally higher explanatory rates. Apart from *g_s* at low CO₂ treatment, are the correlations of *A₄₀₀* with the variables consistent with those found with *A_{sat}*.

Table 14: Adjusted R² values including the slope by which A_{sat} or A₄₀₀ separated by CO₂ treatment is determined by the variable. Significant correlations indicated as bold

		A_{sat} low CO₂	A_{sat} ambient CO₂	A_{sat} high CO₂	A₄₀₀ low CO₂	A₄₀₀ ambient CO₂	A₄₀₀ high CO₂
<i>V_{cmax}</i>	Adj. R ²	0,636**	0,692***	0,658***	0,821***	0,889***	0,781***
	slope	0,09	0,25	0,32	0,15	0,25	0,21
<i>J_{max}</i>	Adj. R ²	0,650***	0,723***	0,768***	0,851***	0,864***	0,776***
	slope	0,10	0,17	0,16	0,18	0,17	0,10
<i>g_s</i>	Adj. R ²	0,410*	0,782***	0,095	0,140	0,530**	0,014
	slope	7,96	35,95	31,04	8,51	27,47	14,11

* = $P \leq 0,05$ ** = $P \leq 0,01$ *** = $P \leq 0,001$

5. Discussion & conclusion

This research investigated how the response of photosynthesis to different atmospheric CO₂ growth levels is modulated by P limitation. In order to achieve this, we studied how the variables A_{sat} , A_{400} , A_{max} and g_s were affected by combined changes in CO₂ growth level and P availability, how the photosynthetic parameters V_{cmax} , J_{max} , $V_{cmax}:J_{max}$, C_i and C_i/C_a adapt to combined changes in CO₂ and P treatments and how photosynthesis is affected by V_{cmax} , J_{max} and g_s under different CO₂ and P treatments.

5.1 Responses of photosynthetic leaf characteristics

The part of the first hypothesis stating that increased CO₂ growth levels will increase A_{sat} and that low P availability would limit this increase, can be partly accepted. Only high P conditions caused increase of A_{sat} with increasing CO₂ level and this increase was larger from low to ambient CO₂. Low P did not show an increase with increasing CO₂, but this did cause an interaction effect which may point to limitation of A_{sat} by P limitation. *S. dulcamara* also showed how P limitation caused a lower A_{sat} . However as for both of the P treatments A_{sat} neither increased or decreased with increasing CO₂ level the hypothesis did not apply to *S. dulcamara* and no interaction effect was found. The results of A_{sat} for *H. lanatus* correspond with those of Tissue & Lewis (2010) and Cambell & Sage (2006). The effects of photosynthesis and P in these were here researched on *Populus deltoides* and *Lupinus albus* L. In both cases no increase in A_{sat} with elevated CO₂ concentration was found when the plants were grown under low P conditions, but like the results of *H. lanatus*, this was the case with plants grown with high P availability, supporting the idea of an interaction effect between CO₂ and P level. This was also seen in the study of Singh et al. (2013) on *Gossypium hirsutum*. Under low P conditions a small decrease in A_{sat} from ambient to elevated CO₂ was found, but led to an increase of A_{sat} under medium and high P conditions. Like *H. lanatus* this resulted in a strong interaction effect between CO₂ and P availability.

There are some points that lower the precision of output data during measuring. An important aspect that should be taken into consideration is that the Licor was set to a certain CO₂ level by the reference CO₂ instead of sample CO₂. This caused that the C_a in the curvet was 5 to 20 ppm lower than planned. To test the impact of this, values have been compensated by assuming linear increase of A between the reference values of 200-400 for low CO₂, 400-600 for ambient CO₂ and 800-1000 for high CO₂. From this the increase of A_{sat} per ppm can be calculated and the A_{sat} at a C_a of 200, 400 and 800 can be estimated. This resulted in underestimated values of A_{sat} of 0,01 to 0,5 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This barely affected the results of the boxplots or the slopes of the linear regression and original values have been used. Also, the CO₂ levels in the growing chambers have not been precisely 200, 400 and 800 resulting in difference with the C_a 's in the Licor. Furthermore, as a single measurement is very time consuming plants are measured during different times of the day. This may make that a plant is more or less active at the moment of measuring. Finally, every plant was only measured once. So differences between leafs are not taken into account. This effect is reduced for *H. lanatus* as multiple leafs were used per measurement. For *S. dulcamara* leaf quality was diverse per plant. Altogether are the results of A_{sat} slightly altered by differences in C_a 's between the Licor and growth chambers. However, the effects of CO₂ and P on A_{sat} still come forward and are considered reliable for analyzing. As the other variables are derived from the A/C_i curves, those results have not been altered by different C_a 's and are therefore reliable.

In further support of the first hypothesis, a reduction in A_{max} and A_{400} was found with increasing CO₂ level and lower values with the low P treatments. With exception of the high P treatment of *H. lanatus*, also a larger reduction from low to ambient CO₂ was seen. The effects of CO₂ and P treatment on A_{max} in this research showed decrease with increasing CO₂ level and increase with

increasing P availability. This was also the case with other experiments with both CO₂ and P variation (Tissue et al., 2010; Cambell and Sage, 2006) or just P variation (Warren, 2011; Chang et al., 2000). Comparison of gas exchange between ambient and elevated CO₂ levels on *S. tuberosum* L. by Fleisher et al. (2012) resulted in significant lower photosynthetic capacity rate with low P availability at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This difference was also seen in the results of *H. lanatus* and *S. dulcamara*, although to a lesser extent with *S. dulcamara*. No difference in photosynthetic rate caused by CO₂ elevation was found by Fleisher et al. (2012). In our study a small decrease of A_{max} and A_{400} was observed from ambient to high CO₂ level. This decrease was however not found significant by Tukey's HSD test. The decrease in A_{max} and A_{400} can be explained by the downregulation of V_{cmax} and J_{max} with elevated CO₂. The plant requires lower photosynthetic capacity to perform the same amount of photosynthesis as the process gets more efficient by decreased photorespiration (Ainsworth & Rogers, 2007).

In both *H. lanatus* and *S. dulcamara* the g_s decreased from low to ambient and from ambient to elevated CO₂. The response of g_s was not the same for *H. lanatus* and *S. dulcamara*. *S. dulcamara* did support the hypothesis that only CO₂ would have a negative effect on g_s , as only an effect of CO₂ on g_s was found. Although not much reduction was seen from ambient to high CO₂, which could have been caused by the already large reduction from low to ambient CO₂, making it unable to decrease much more from ambient to high CO₂. For *H. lanatus*, low P conditions also caused a large reduction in g_s from low to ambient CO₂ which stayed low through high CO₂. High P availability seems to allow the plant to leave their stomata more open under higher CO₂ conditions. Where at low CO₂ level the, g_s was comparable between the P treatments. There was a steady decrease observed where the g_s is significantly higher with the high P treatments than the low P treatments. That g_s is decreased by elevated CO₂ is seen in many studies (Ainsworth & Rogers, 2007; Long et al., 2004; Reddy et al., 2010). Effects of P on g_s have also been observed where increased P input caused increased g_s (Tissue & Lewis, 2010; Fleisher et al., 2012). Mott (1988) deduced that stomatal conductance corresponds to C_i and not C_a . g_s can be reduced at elevated CO₂ as a lower g_s is necessary to maintain C_i/C_a . Decrease in g_s only lowers A to a small extent compared to when no acclimation of g_s would take place, but could greatly decrease transpiration (Long et al, 2004).

5.2 Responses of photosynthetic parameters

The second hypothesis proposed that increasing CO₂ would cause V_{cmax} , J_{max} and $V_{cmax}:J_{max}$ to decrease, C_i to go up and that C_i/C_a would be adjusted to stay around the same level. The effect of P limitation would lower V_{cmax} and J_{max} . This can be accepted for both species. A clear decrease in both V_{cmax} and J_{max} was observed with increasing CO₂ concentration. Comparable to the responses of A and g_s , this reduction was largest from low to ambient CO₂ level. Downregulation of V_{cmax} and J_{max} is often reported with increasing CO₂ concentration and decreases were found with P deficiency (Danyagri & Dang, 2014; Tissue et al., 2010; Cambell & Sage, 2006; Zhang & Dang, 2005; Ainsworth & Rogers, 2007). Also the expected larger decrease of V_{cmax} resulting in a lowering of the $V_{cmax}:J_{max}$ was found in the research. This can be explained by the relatively larger downregulation of V_{cmax} to J_{max} from low to ambient CO₂ as a consequence of the shift from V_{cmax} limitation towards J_{max} limitation with increasing CO₂ level. A stronger decrease in V_{cmax} than J_{max} with increasing CO₂ concentration resulting in a lowering of $V_{cmax}:J_{max}$ was also found in more studies (Leakey et al., 2009; Long et al., 2004; Ainsworth et al. 2005; Bernachi et al., 2004). Furthermore, V_{cmax} and J_{max} were reduced in the low P treatment but this did not result in an interaction effect, which suggests that CO₂ and P only cumulatively affect V_{cmax} and J_{max} . The CO₂-induced changes in V_{cmax} and J_{max} resulted in a reduction of the ratio $V_{cmax}:J_{max}$ under higher CO₂. Interestingly, despite the P-induced changes in both V_{cmax} and J_{max} , the ratio $V_{cmax}:J_{max}$ was not influenced by P availability. Walker et al. (2014) states that most terrestrial biosphere models simulate J_{max} as a linear function of V_{cmax} . Our results of $V_{cmax}:J_{max}$ as well as other studies reporting changes in $V_{cmax}:J_{max}$ suggest that this assumption is incorrect as we

observed consistent changes in this ratio between different CO₂ treatments (Leakey et al., 2009; Long et al., 2004; Ainsworth et al. 2005; Bernachi et al., 2004). Walker et al. (2014) note this caveat and mention that high investment in J_{max} relative to V_{cmax} when carboxylation is limiting photosynthesis would lead to electron transport not used in photosynthesis requiring dissipation of that energy to avoid photoinhibition. Conversely, high investment in J_{max} relative to V_{cmax} would maximize photosynthetic rates when light is limiting photosynthesis.

5.3 Regulation of A by photosynthetic parameters

The results of the linear regression tests with V_{cmax} and J_{max} corresponded with the third hypothesis that V_{cmax} and J_{max} would have a positive relation with A_{sat} and A_{400} . The relation of V_{cmax} and J_{max} within the CO₂ treatments making no division in P treatment always resulted in a positive relation. Those relations did differ over the CO₂ levels where the slopes of the regression lines get steeper with increasing CO₂. This makes that the effect of V_{cmax} and J_{max} gets larger under increasing CO₂ conditions. In contrary to the expectations that g_s would not have a relation, did g_s show the same behavior as V_{cmax} and J_{max} . V_{cmax} and J_{max} seem to have a strong positive correlation with A_{400} . Besides V_{cmax} and J_{max} , g_s also seemed to play a role. Tissue & Lewis (2010) also looked at the relationships between A_{sat} with V_{cmax} , J_{max} and g_s for different CO₂ treatments. Like the results in this experiment, Tissue & Lewis (2010) also found a stronger response with increasing CO₂ level with once again a more profound difference from low to ambient CO₂. Tissue & Lewis (2010) conclude that A_{sat} was mostly driven by V_{cmax} in combination with g_s . They also found a strong relation with J_{max} like in this study, but considered it no explanation for the observed changes in A_{sat} . The findings of Lin et al. (2009) may explain the observed effects of P-limitation as their findings suggested that P deficiency limits RuBP regeneration and this way the photosynthetic rate. As a certain balance between V_{cmax} and J_{max} is strived for by the plant, there is correlation between the two. This results that if one of the two parameters changes, this will influence the other (Walker et al., 2014). However, as shown in this experiment, this adaptation does not need to be linear as the $V_{cmax}:J_{max}$ also changes. This way, both V_{cmax} and J_{max} can have high correlations with A, while only one may be limiting and determining A.

5.4 Conclusion

The results of this research suggest that P deficiency limits A_{sat} independent of CO₂ growth level. The results of *S. dulcamara* did not show increased A_{sat} with higher CO₂ levels for both P treatments causing no interaction effect of CO₂ and P level. *H. lanatus* did only show increased A_{sat} with high P suggesting that under low P conditions, P limits the increase in A_{sat} caused by elevated CO₂ levels. The larger increase in A_{sat} from low to ambient compared to ambient to high CO₂ also suggest that the limitation by P increases at high CO₂ concentration. The decrease in A_{max} and A_{400} is caused by decrease in V_{cmax} , J_{max} and g_s . $V_{cmax}:J_{max}$ seemed to be of lesser influence, so not the ratio but the values of V_{cmax} and J_{max} seemed to be more important. From the results of A_{max} and A_{400} it can be concluded that the photosynthetic capacity of the plants went down with elevated CO₂ levels when the same CO₂ input is given. The very strong positive relations of the linear regressions of A_{400} with V_{cmax} and J_{max} explain this, as well as the positive relationship with g_s . The results that A_{sat} increased (*H. lanatus*) or did not decrease (*S. dulcamara*) show that photosynthesis of plants gets more efficient. The photosynthetic parameters are acclimated by elevated CO₂ as can be seen in downregulation of V_{cmax} , J_{max} and g_s . This makes that even though A_{sat} may not increase substantially under elevated CO₂, the plants do possibly benefit from increased water and nutrient use efficiency. The declined g_s can reduce transpiration and decreased evapotranspiration with elevated CO₂ is observed in literature (Leakey et al., 2009, Long et al., 2004). Rubisco accounts for around 25% of leaf N and investment in Rubisco can be very high (Sage et al., 1987). Reductions in Rubisco due to acclimatization to elevated CO₂ could hereby increase nitrogen use efficiency (Leakey et al., 2009,

Ainsworth & Long, 2004). The same applies to *H. lanatus*, but apparently *H. lanatus* also tends to prefer to increase A_{sat} . This increase in photosynthetic efficiency is backed by the fact that V_{cmax} , J_{max} and g_s all had a strong positive correlation with photosynthesis within their CO₂ treatment, but were all downregulated with increasing CO₂ level without decreasing A_{sat} .

This experiment suggests that photosynthetic capacity of plants cannot be extrapolated linearly to elevated CO₂ levels as, in general, the magnitude of the responses from low to ambient growth CO₂ exceeded the magnitude of the responses from ambient to high CO₂. The effects of a negative feedback on global warming by increased photosynthetic capacity and CO₂ uptake may this way currently be overestimated. Furthermore, P limitation does have an influence related to the photosynthesis. Photosynthesis also does not necessarily increase if the CO₂ concentration increases. This should be taken into consideration when predicting how plants in future atmospheric CO₂ conditions will react.

5.5 Recommendations for future research

As mentioned in the discussion above there have been some limitations to this research which could be improved in future research. Larger groups per treatment would make the statistics more powerful and may create a better outcome by excluding the highest and lowest score per treatment. Now only 6-7 individuals per treatment were taken. As could be seen in the boxplots there was quite a large spread in the results and as the groups are small this has a large effect on the mean values of the treatments. Even though measuring is very time consuming, if more individuals per treatment could be included this would give a better confidence in the results. Even though more individuals could have been used, the results still showed clear outcomes and were enough to achieve the aim of this thesis.

In further research the scope could be widened by including more aspects of the plant in the analysis of how the response to CO₂ level and P availability. For example, the N and P leaf concentration could be determined as well as Rubisco concentration. Also the nutritious value of the plant could be examined which could be important for ecosystem relations and food quality. Furthermore, properties on growth of the plant like LAI, aboveground and root biomass, but also effects on flowering, fruit and seed production/quality of plants. As photosynthesis is measured per m², the total photosynthesis of a plant may increase when biomass or LAI increases even though the photosynthesis per m² stays the same. The experiment now only looked at two P conditions. To look at a clearer trend of the effects of P on photosynthesis a more stepwise increase in P with more than 2 treatments could be applied. Besides this, N was now kept constant high. So besides P, could N also be varied.

Of course do these plants not represent the behavior of all plants, as already could be seen in the differences between *H. lanatus* and *S. dulcamara*. *H. lanatus* and *S. dulcamara* are both species with a C₃ respiratory pathway. It would be interesting to include C₄ species in research. Wand et al. (1999) tested responses of C₃ and C₄ grasses to elevated CO₂ concentration. It was long thought that C₄ plants would not benefit from elevated CO₂ concentrations. However, this may not be as certain as thought before. The results of Wand et al. (1999) do show a lower response for C₄ species, although the photosynthetic response was quite similar to C₃ species. Ainsworth & Long (2004) came to different conclusions regarding the response of C₃ and C₄ plants. A_{sat} was stimulated for both C₃ and C₄, but the magnitude of response was three times higher for C₃ than for C₄. The CO₂ concentrating mechanism in C₄ leaves makes it that photosynthetic stimulation is not necessarily expected. They also state that C₄ species vary in CO₂ saturation level in the leaves. Causing some species to be saturated at current CO₂ levels while others species may not yet be saturated. In a study by Ghannoum et al. (2008) A of C₄ grasses showed a steeper response to P_i in the leaves than C₃ grasses

and also saturated at a lower Pi level. It is suggested that P deficiency interferes with the CO₂ concentrating mechanism of C₄ plants as Pi supply to cycle enzymes is limiting. However, the study still showed a larger P requirement for C₃ photosynthesis than C₄ photosynthesis mainly due to photorespiration. It is concluded that C₄ is more P efficient but also more sensitive to P deficiency. Performing these kind of experiment on plants like trees, grasses, shrubs, but also plants from different climate zones will give a better view of how plants in general will react to increasing CO₂ concentration and nutrient availability. As it may not be possible to look at such a variety of species. Focus could be put towards economically important species. So crops that are most used in agriculture for food, feed, biofuels etc.

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Appendix I: Results individual plants

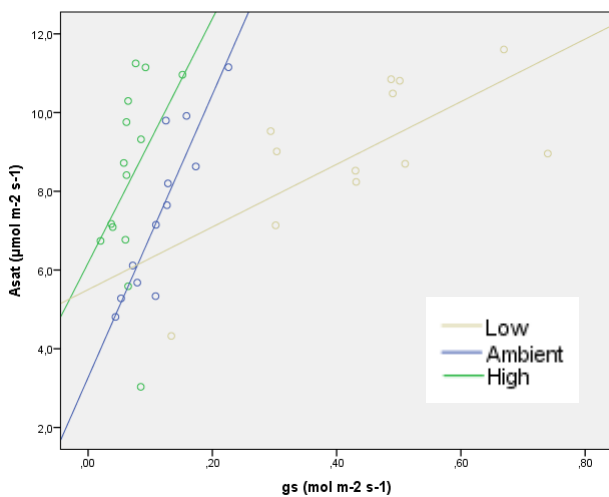
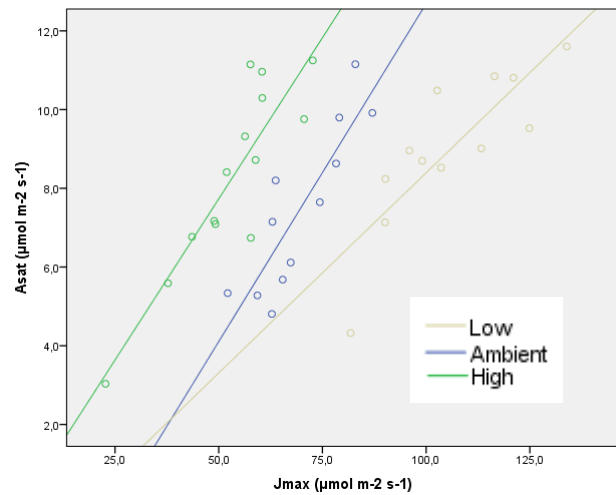
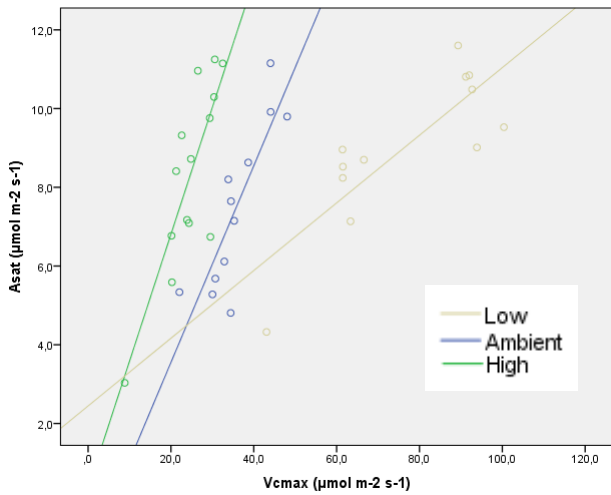
Species	Treatment(CO2_P)	Individual	A _{tot}	A _{max}	A ₄₀₀	g _s	V _{cmax}	J _{max}	V _{cmax} :J _{max}	Cit	Ci/Ca
HolcusLanatus	LowC_lowP	green1l	3,90	16,53	9,60	0,276	39,10	79,12	0,494	387,28	0,857
HolcusLanatus	LowC_lowP	green5l	2,65	11,07	5,35	0,150	27,56	54,71	0,504	694,01	0,829
HolcusLanatus	LowC_lowP	green9l	4,55	10,78	7,95	0,414	38,87	56,82	0,684	331,37	0,881
HolcusLanatus	LowC_lowP	grey10l	4,89	13,82	9,84	0,193	48,60	65,75	0,739	255,21	0,760
HolcusLanatus	LowC_lowP	grey2l	6,25	17,16	11,77	0,288	52,70	80,10	0,658	346,88	0,786
HolcusLanatus	LowC_lowP	grey4l	5,72	17,09	11,09	0,199	58,76	80,48	0,730	305,31	0,727
HolcusLanatus	LowC_lowP	grey6l	6,22	12,56	11,32	0,415	47,91	71,43	0,671	265,94	0,844
HolcusLanatus	LowC_highP	green12l	6,61	19,67	14,02	0,270	59,09	90,58	0,652	326,16	0,762
HolcusLanatus	LowC_highP	green4l	6,06	17,96	13,58	0,242	56,76	83,08	0,683	323,68	0,761
HolcusLanatus	LowC_highP	green6l	4,25	18,76	11,16	0,182	42,42	89,00	0,477	401,96	0,780
HolcusLanatus	LowC_highP	green8l	6,10	17,48	12,50	0,263	50,55	80,92	0,625	333,55	0,775
HolcusLanatus	LowC_highP	grey1l	5,15	19,87	11,89	0,167	49,31	92,49	0,533	369,94	0,716
HolcusLanatus	LowC_highP	grey3l	5,17	17,84	10,63	0,220	42,89	83,22	0,515	440,01	0,775
HolcusLanatus	LowC_highP	grey5l	7,19	16,83	13,45	0,438	56,29	78,22	0,720	300,37	0,830
HolcusLanatus	AmbientC_lowP	green10a	0,44	5,17	1,59	0,005	11,14	28,64	0,389	1264,27	0,593
HolcusLanatus	AmbientC_lowP	green12a	6,27	12,24	6,62	0,080	29,32	60,45	0,485	510,16	0,652
HolcusLanatus	AmbientC_lowP	green2a	4,75	10,76	5,23	0,064	23,45	52,81	0,444	666,68	0,674
HolcusLanatus	AmbientC_lowP	green4a	1,08	7,75	2,37	0,015	16,17	40,18	0,402	827,43	0,680
HolcusLanatus	AmbientC_lowP	grey12a	2,70	6,75	4,19	0,022	23,46	37,98	0,618	314,61	0,478
HolcusLanatus	AmbientC_lowP	grey2a	-0,10	8,55	2,99	0,007	16,83	43,27	0,389	858,51	1,033
HolcusLanatus	AmbientC_lowP	grey6a	2,39	10,17	4,18	0,021	22,35	50,28	0,445	678,93	0,523
HolcusLanatus	AmbientC_highP	green11a	8,54	14,73	9,05	0,124	37,35	70,85	0,527	483,71	0,685
HolcusLanatus	AmbientC_highP	green3a	8,83	17,01	8,65	0,200	35,09	79,24	0,443	706,25	0,789
HolcusLanatus	AmbientC_highP	green7a	9,21	16,54	9,18	0,179	34,09	76,91	0,443	605,32	0,761
HolcusLanatus	AmbientC_highP	green9a	9,27	14,29	9,45	0,268	35,51	67,36	0,527	406,57	0,828
HolcusLanatus	AmbientC_highP	grey1a	10,34	20,00	10,43	0,204	26,05	92,10	0,283	526,98	0,760
HolcusLanatus	AmbientC_highP	grey3a	8,39	16,49	10,00	0,107	41,58	77,14	0,539	390,18	0,647
HolcusLanatus	AmbientC_highP	grey7a	10,31	17,69	10,53	0,237	40,33	81,98	0,492	585,32	0,790
HolcusLanatus	HighC_lowP	green1h	2,53	4,66	2,02	0,022	10,42	26,79	0,389	812,07	0,750
HolcusLanatus	HighC_lowP	green3h	5,48	9,23	3,99	0,025	19,29	45,89	0,420	717,23	0,542
HolcusLanatus	HighC_lowP	green7h	4,40	9,92	4,04	0,019	24,05	49,40	0,487	550,98	0,504
HolcusLanatus	HighC_lowP	grey10h	4,71	5,89	2,84	0,040	18,24	31,62	0,577	845,76	0,737
HolcusLanatus	HighC_lowP	grey12h	1,75	7,24	2,39	0,017	15,91	37,52	0,424	766,19	0,767
HolcusLanatus	HighC_lowP	grey6h	3,65	7,03	2,50	0,022	13,60	36,49	0,373	1280,83	0,652
HolcusLanatus	HighC_lowP	grey8h	3,23	5,60	2,22	0,024	11,91	30,64	0,389	982,55	0,702
HolcusLanatus	HighC_highP	green10h	10,41	14,30	6,30	0,132	28,64	67,44	0,425	1143,93	0,813
HolcusLanatus	HighC_highP	green2h	10,49	13,80	7,30	0,058	32,03	65,47	0,489	531,36	0,611
HolcusLanatus	HighC_highP	green4h	12,03	15,04	7,66	0,100	28,59	71,88	0,398	759,55	0,623
HolcusLanatus	HighC_highP	green6h	7,43	7,02	4,05	0,093	21,20	36,93	0,574	743,79	0,814
HolcusLanatus	HighC_highP	grey11h	11,16	13,72	7,21	0,089	32,03	65,15	0,492	501,40	0,716
HolcusLanatus	HighC_highP	grey1h	12,06	14,17	7,44	0,138	32,03	67,06	0,478	585,90	0,794
HolcusLanatus	HighC_highP	grey7h	10,75	12,87	5,91	0,154	24,93	61,35	0,406	746,98	0,832

SolanumDulcamara	LowC_lowP	purple12l	8,52	22,80	15,41	0,430	61,52	103,61	0,594	398,51	0,805
SolanumDulcamara	LowC_lowP	purple2l	8,96	21,00	15,46	0,740	61,42	95,95	0,640	267,08	0,863
SolanumDulcamara	LowC_lowP	purple9l	8,24	19,64	15,11	0,431	61,47	90,17	0,682	280,73	0,810
SolanumDulcamara	LowC_lowP	white3l	7,14	19,52	13,03	0,302	63,34	90,10	0,703	341,84	0,765
SolanumDulcamara	LowC_lowP	white6l	10,49	22,50	17,56	0,490	92,71	102,66	0,903	201,04	0,784
SolanumDulcamara	LowC_lowP	white8l	8,70	21,69	16,00	0,510	66,57	99,06	0,672	350,59	0,823
SolanumDulcamara	LowC_highP	purple1l	9,53	27,55	19,31	0,294	100,39	124,88	0,804	225,69	0,682
SolanumDulcamara	LowC_highP	purple4l	9,01	24,89	17,72	0,304	93,87	113,30	0,828	199,17	0,707
SolanumDulcamara	LowC_highP	purple7l	11,60	29,69	21,35	0,669	89,31	133,90	0,667	254,03	0,804
SolanumDulcamara	LowC_highP	white2l	10,85	25,68	18,73	0,488	92,01	116,48	0,790	216,79	0,765
SolanumDulcamara	LowC_highP	white4l	4,32	17,56	10,01	0,134	43,07	81,80	0,527	365,29	0,708
SolanumDulcamara	LowC_highP	white5l	10,81	26,80	20,04	0,502	91,19	121,05	0,753	246,37	0,772
SolanumDulcamara	AmbientC_lowP	purple10a	5,28	12,30	6,21	0,053	30,02	59,31	0,506	483,89	0,573
SolanumDulcamara	AmbientC_lowP	purple11a	9,80	16,93	10,26	0,125	48,05	79,07	0,608	332,25	0,648
SolanumDulcamara	AmbientC_lowP	purple2a	4,81	13,12	7,12	0,044	34,42	62,82	0,548	429,99	0,535
SolanumDulcamara	AmbientC_lowP	purple8a	5,34	10,70	5,11	0,109	22,04	52,15	0,423	716,90	0,777
SolanumDulcamara	AmbientC_lowP	white12a	8,20	13,40	8,13	0,128	33,85	63,71	0,531	411,38	0,711
SolanumDulcamara	AmbientC_lowP	white8a	5,68	13,74	6,76	0,079	30,72	65,42	0,470	548,19	0,682
SolanumDulcamara	AmbientC_highP	purple12a	7,65	15,84	7,70	0,127	34,50	74,38	0,464	602,78	0,726
SolanumDulcamara	AmbientC_highP	purple1a	9,92	18,88	10,36	0,158	44,07	87,03	0,506	531,65	0,713
SolanumDulcamara	AmbientC_highP	white1a	11,15	17,94	11,39	0,226	44,03	82,95	0,531	396,17	0,765
SolanumDulcamara	AmbientC_highP	white3a	8,63	16,75	8,81	0,173	38,66	78,29	0,494	609,59	0,765
SolanumDulcamara	AmbientC_highP	white5a	7,15	13,19	8,09	0,109	35,26	62,95	0,560	426,31	0,706
SolanumDulcamara	AmbientC_highP	white9a	6,11	14,18	6,77	0,072	32,88	67,34	0,488	708,95	0,629
SolanumDulcamara	HighC_lowP	purple12h	8,41	10,61	4,56	0,062	21,26	51,92	0,409	762,46	0,702
SolanumDulcamara	HighC_lowP	purple12h1	8,72	12,22	5,04	0,057	24,82	58,88	0,422	689,30	0,671
SolanumDulcamara	HighC_lowP	purple1h	3,03	3,81	1,67	0,085	8,84	22,72	0,389	1036,99	0,906
SolanumDulcamara	HighC_lowP	purple5h	7,09	9,90	4,36	0,040	24,34	49,19	0,495	798,19	0,617
SolanumDulcamara	HighC_lowP	purple9h	6,74	11,94	4,89	0,020	29,52	57,73	0,511	450,09	0,302
SolanumDulcamara	HighC_lowP	white1h	7,17	9,88	5,02	0,037	23,89	48,90	0,489	554,88	0,590
SolanumDulcamara	HighC_lowP	white5h	11,25	15,45	7,18	0,077	30,59	72,68	0,421	696,95	0,679
SolanumDulcamara	HighC_highP	purple10h	11,15	11,99	6,70	0,092	32,49	57,65	0,564	525,06	0,731
SolanumDulcamara	HighC_highP	purple6h	5,59	7,36	3,28	0,064	20,24	37,77	0,536	1300,74	0,804
SolanumDulcamara	HighC_highP	purple8h	6,77	8,68	4,24	0,060	20,14	43,56	0,462	796,27	0,751
SolanumDulcamara	HighC_highP	white12h	10,29	12,62	6,59	0,064	30,40	60,50	0,502	481,97	0,651
SolanumDulcamara	HighC_highP	white6h	9,76	14,89	5,87	0,062	29,38	70,57	0,416	901,32	0,824
SolanumDulcamara	HighC_highP	white8h	9,32	11,64	5,13	0,085	22,61	56,32	0,401	811,01	0,753
SolanumDulcamara	HighC_highP	white8h1	10,96	12,64	6,41	0,152	26,51	60,44	0,439	677,85	0,829

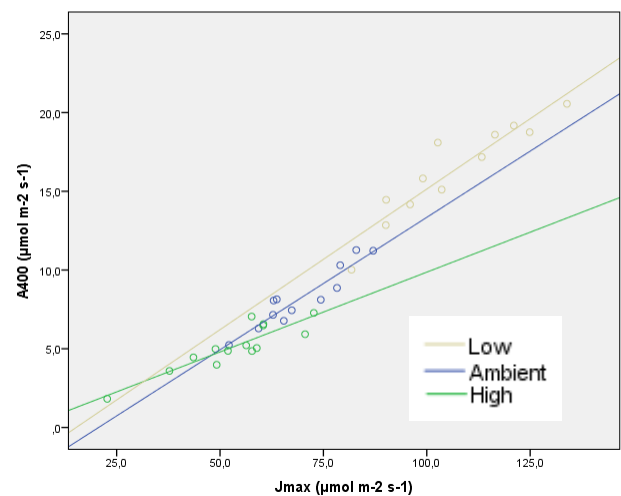
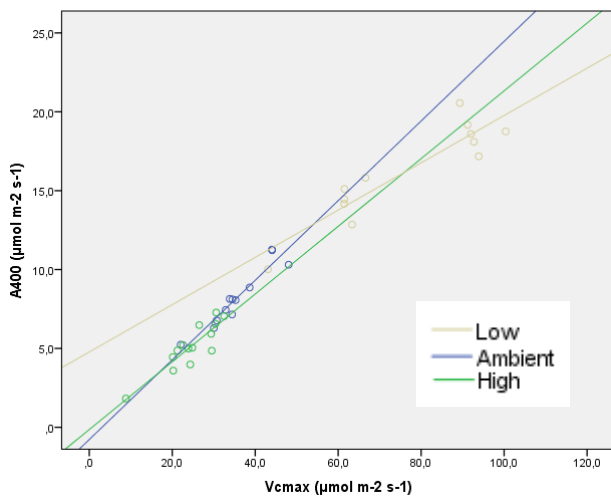
Appendix II: Scatter plots

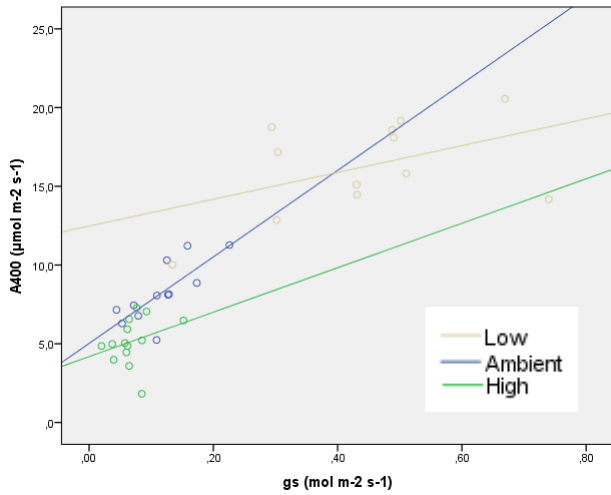
Scatter plots per CO₂ treatment of A_{sat} and A_{400} against V_{cmax} , J_{max} , and g_s including table with significance of statistical difference of slopes for the variables with A_{sat} .

Scatter plots *S. dulcamara*

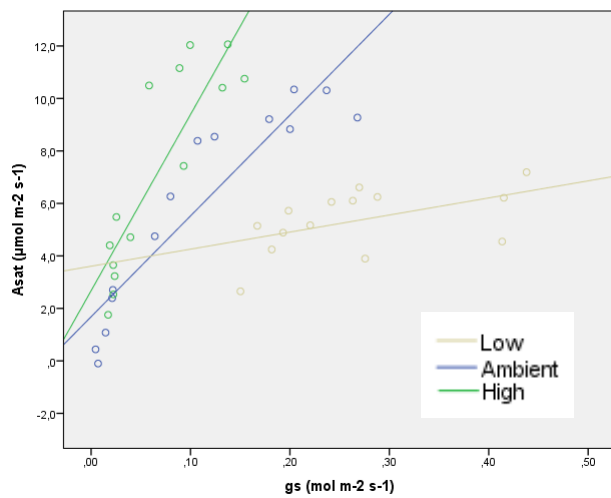
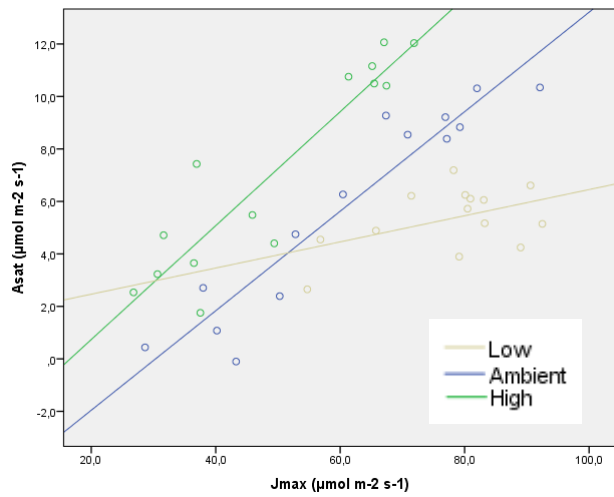
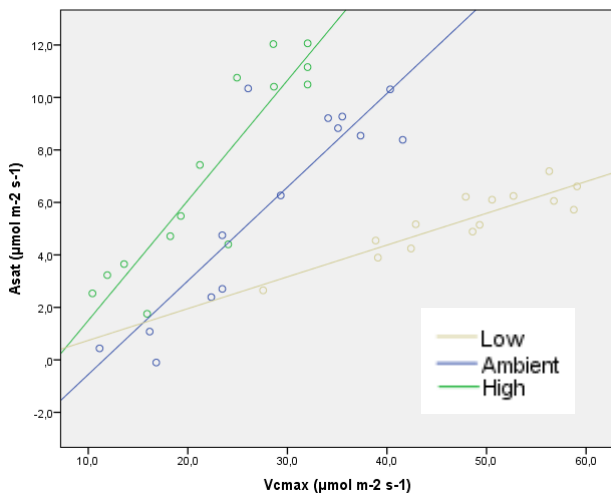


	V_{cmax}	J_{max}	g_s
low-high	0,00168	0,073459	0,269284
low-ambient	0,005646	0,083228	0,000237
ambient-high	0,375018	0,843132	0,816936





Scatter plots *H. lanatus* CO₂ treatment



	V_{cmax}	J_{max}	g_s
Low-high	2,16E-05	0,000507	3,45E-06
Low-ambient	0,000663	0,000735	2,64E-07
ambient-high	0,229525	0,413871	0,025312

