

PRE-INDUSTRIAL TO 2000 PPM; SOYBEAN RESPONSE TO INCREASING CO₂

BY

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THESIS

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Abstract

Many studies addressing the effects of rising carbon dioxide concentrations ($[\text{CO}_2]$) on agricultural crops have demonstrated the critical role that this gas has on plant physiology. It is now essential to assess the responses of our major crop systems to even higher $[\text{CO}_2]$ as these photosynthetic and physiological responses may determine our food security in the next century. The majority of previous studies have focused on scenarios published in the Intergovernmental Panel on Climate Change (IPCC) reports. None of these scenarios have accurately predicted the rise in recent years of $[\text{CO}_2]$; in fact, measured $[\text{CO}_2]$ has exceeded the worst-case IPCC emissions scenario (A1F1). In this study, I tested soybean (*Glycine max* L. cv 93B15) responses to eight different $[\text{CO}_2]$ levels in growth chambers at the University of Illinois Plant Sciences Laboratory. Five individual plants were grown for five weeks in each of eight chambers with $[\text{CO}_2]$ ranging from pre-industrial (250ppm) to a level much higher than any predicted for the next century (2000ppm). The objective of this experiment is to assess the physiological and photosynthetic responses to $[\text{CO}_2]$ exceeding levels predicted by current models. Measurements included plant developmental stages, photosynthesis rates and underlying biochemistry, respiration, as well as plant growth and yields. I predict that soybean biomass accumulation and photosynthesis will increase linearly with increases in $[\text{CO}_2]$ due to the decrease in photorespiration; however, above a certain threshold, the benefits of continued increases in $[\text{CO}_2]$ will diminish. The data show that physiological development was delayed as $[\text{CO}_2]$ increased. Plant height and total leaf area increased with higher $[\text{CO}_2]$. Photosynthesis increased with increasing $[\text{CO}_2]$ up until the 1000ppm treatment, after which it plateaued. Stomatal

conductance showed a decreasing trend with increasing $[\text{CO}_2]$. These results indicate soybean productivity will increase as $[\text{CO}_2]$ continues to rise, but as the concentrations exceed the “worst-case” scenarios, physiology, growth, and yields will begin to plateau.

I dedicate this to Chris and Gary Drag, my parents. Without their support and patience I would not be where I am today.

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CHAPTER 1

INTRODUCTION

The atmospheric CO₂ concentration has been measured at the Mauna Loa Observatory since 1958 near the Pacific Ocean, where Northern Hemisphere air is well mixed, and has been showing steady increases ever since (Jones 2013). In May 2013, the observatory recorded a mean daily atmospheric carbon dioxide concentration ([CO₂]) of 400 ppm for the first time. This rise in [CO₂] has become a major global concern, and scientific evidence supports that anthropogenic activities have had the greatest impact on the ever-increasing concentrations of [CO₂] (Canadell et al. 2007). In the last decade, the annual rate of increase was recorded at 2.0 parts per million (ppm) per year which was 0.3ppm greater than the thirty year mean (Hartmann et al. 2013). Through photosynthesis, plants take up CO₂ from the atmosphere for growth. Thus, significant changes in [CO₂] are likely to lead to significant implications for plant and ecosystem productivity.

Growth and productivity of plants that exclusively utilize the C₃ photosynthetic pathway, including many of the most widely grown crops such as soybean, is shown to increase with rising [CO₂] (Bowes 2003). This is due principally to a decrease in photorespiration as CO₂ outcompetes O₂ for the active site in Rubisco (Bowes 1991). With long-term exposure to increased CO₂, some species acclimate or become less responsive to these increases (Kramer 1981; Ainsworth et al. 2002; Bernacchi et al. 2005). Other studies have concluded that some plant species do not show this acclimation response to prolonged exposure (Arp and Drake 1991). Photosynthetic acclimation has been found to be quite variable between species and has

been described as an elevated-[CO₂]-induced down-regulation of photosynthesis (Bernacchi et al., 2005). Arp and Drake (1991) speculated that annual species and young seedlings tend to show photosynthetic acclimation due to sink limitations. Studies testing the effects of extreme levels of CO₂ on plants have reported leaf necrosis and damage to other tissues with drastic enrichment of CO₂ (Peet et al. 1986). This is thought to be caused by a greater amount of starch accumulation in the tissue (Sasek et al. 1985; Madsen 1975; Peet et al. 1986). Increased starch and carbohydrate accumulation in the leaf has also been thought to trigger photosynthetic acclimation, when excess photosynthate has limited sinks to fill (Makino and Mae 1999).

The trend toward higher [CO₂] does not appear to be approaching a plateau anytime in the near future. In fact, the worst-case scenarios (IPCC A1F1 prediction) of future global carbon emissions predicted by the IPCC are being exceeded by the current actual emissions (Brysse et al. 2013). Before the Industrial Revolution [CO₂] was around 260ppm (Raynaud and Barnola 1985). The current atmospheric [CO₂] concentration is unprecedented in the last 650,000 years, which could even extend to the millions of years (Canadell et al. 2007). The majority of elevated [CO₂] experiments, particularly chamber studies and those using Free Air Concentration Enrichment (FACE) technology have focused on scenarios published in the Intergovernmental Panel on Climate Change (IPCC) reports assuming a business as usual approach or the IPCC A1 emission scenarios (Brysse et al. 2013). It is absolutely essential to test the effects of a wider range of [CO₂] on the major food crops. However, this can only be tested in controlled environments, as a fully replicated open-air field trial would not be economically feasible.

Soybean (*Glycine max*) is a field crop grown around the globe that ranks first in worldwide oilseed production (Singh and Hymowitz 1999), leading to a vast number of studies addressing the effects of increased CO₂ on soybean physiology and development. These experiments have been deployed in greenhouses, growth chambers, FACE-sites, and open-top field chambers. A meta-analysis covering the majority of the studies on soybean (Ainsworth et al. 2002) found that the overall effect of increased CO₂ caused significant increases in light saturated leaf photosynthetic rates, total canopy assimilation, growth rates, biomass, yield, branch number, leaf number, and stem heights. The studies in the analysis that included more extreme [CO₂] showed further stimulation of these parameters. Reductions were found in stomatal conductance and specific leaf area (SLA) (Ainsworth et al. 2002). These effects are consistent with the responses of many other C₃ and indeterminate species (Arp and Drake 1991; Peet et al. 1985). Soybean has been reported to show a significant response to increases in CO₂ concentration (Rogers and Dahlman 1993; Kramer 1981) and is widely studied as a model C₃ legume species (Ferguson and Gresshoff 2009; Ainsworth et al. 2002).

The focus of this thesis is to test the response of soybean growth and photosynthesis to a range of [CO₂] spanning pre-industrial levels up to a concentration much higher than any prediction for the next century. I hypothesize that soybean biomass accumulation and photosynthesis will increase linearly with increases in [CO₂] due to the decrease in photorespiration; however, based on Rubisco kinetics, I also predict that above a certain threshold, the benefits of continued increases in [CO₂] will diminish. In this study, I aim to capture the response of soybean photosynthesis and physiology to this range of [CO₂].

CHAPTER 2

MATERIALS AND METHODS

2.1 Site Description

The experiment was conducted in the Plant Science Laboratory at the University of Illinois. Soybean plants were grown, starting from germination, for five weeks in custom built growth chambers that control $[\text{CO}_2]$ and temperature. Each of the eight chambers covers 1.25 m^2 and measure 1.2 m tall. CO_2 was injected into each chamber independently through solenoid valves (Model ETO-3M-12VDC, Clippard Instrument Laboratory, Inc., Cincinnati, OH USA). In the chambers controlling $[\text{CO}_2]$ at 1500 ppm and below, an SBA-4 CO_2 analyzer (PP-systems, Inc., Amesbury, MA USA) with an upper limit of 2000 ppm were used. In the 1750 and 2000 ppm chambers, SBA-5 analyzers (PP Systems, Inc.) calibrated for 5000 ppm were deployed because the SBA-4 analyzers were unstable near their upper limit. The gas analyzers are accurate to $<1\%$ of the span concentration over the calibrated range. The analyzers were calibrated both before and two weeks into the experiment using a span gas. The $[\text{CO}_2]$ in the lowest two treatments (250 ppm and ambient) were achieved using custom built CO_2 scrubbers filled with granulated self-indicating Sodasorb (W.R. Grace and Company, Versailles, OH USA). It was necessary to lower ambient and pre-industrial $[\text{CO}_2]$ to attain current atmospheric $[\text{CO}_2]$ due to the high ambient $[\text{CO}_2]$ in the greenhouse.

Each chamber was assigned a different CO_2 concentration that was rotated weekly to reduce chamber bias. The CO_2 concentrations used in the experiment were 250, 395(ambient),

750, 1000, 1250, 1500, 1750, and 2000 ppm. Temperature was controlled at 23 °C during the day and 17 °C at night using custom heating and cooling units built into the chambers to stabilize the set-points. The chambers frames were covered in 0.2 mm clear Dura-Lar film (Grafix, Inc.) that allowed natural sunlight in the chamber from the glasshouse. Natural light was supplemented using 1000W metal halide lamps mounted 1.4m above the chambers. The photoperiod was set for 14 hours starting at 6 am and ending at 8 pm. Photosynthetically active radiation (PAR) was measured with Apogee SQ-110 quantum sensors at the top of the canopy within each chamber starting in the 2nd week and then throughout the experiment.

Five labeled 14.5 L pots were used in each chamber, each containing one plant (totaling 40 plants). Each pot was filled with ~0.02 m³sunshine LT1 soil mix and supplemented with 0.06 L of 5-9-12 Osmocote Plus extended release fertilizer 10 days after emergence. The plants were watered every other day throughout the duration of the experiment. Three soybean seeds, Cultivar 93B15 (Pioneer Seeds, USA), were sown in each pot and thinned to one plant following emergence. The plants did not receive any pesticide applications, inoculations, or additional fertilizer and did not show any signs or symptoms of diseases, pests or N deficiency. The plants were systematically rotated clockwise as they were moved to different chambers to minimize border effects.

2.2 Measurements

Plant height and development data were collected three times a week from emergence until harvest. Developmental stage was determined based on the stages defined in Ritchie et al. (1993). The development of all plants within each chamber was recorded and averaged.

Photosynthetic measurements were taken using a portable gas exchange system (LI-6400; LI-COR, Inc., Lincoln, NE USA) calibrated based on the manufacturers specifications. Gas exchange photosynthetic-CO₂ response, diurnal response, and midday point measurements were taken twice during the experiment. The first set of measurements was conducted when plants were in early vegetative development (V1-V2) and the second during late vegetative/early reproductive (V6-R2) development. Along with the second sampling set, mitochondrial respiration was measured during the night starting an hour after dusk. Custom built leaf cuvettes were installed on the LI-6400 which measured respiration on entire trifoliate leaves. Each trifoliate was removed from the plant after measuring in order to determine the leaf area using a leaf area meter (LI-3000), which was used to correct gas exchange rate per unit area. Midday gas exchange measurements were collected at 1 pm (all times are Central Daylight Savings Time) on three plants in each chamber. The temperature was controlled at 23 °C for all measurements and the gas exchange system's leaf chamber fluorometer was controlled to maintain the same [CO₂] as each treatment. The diurnal measurements were collected from two plants in each treatment every other hour beginning at 9 am and finishing at 6 pm. CO₂ concentrations in the leaf chamber were set to match the chamber values and light was set to the ambient PAR measured in the room. The daily integral of carbon assimilation (A') was calculated for each concentration for comparison using the total daily values. Photosynthetic-CO₂ response (A/C_i) measurements were collected on the youngest most fully expanded leaf using an auto-program feature of the portable gas exchange system. This program sampled photosynthetic parameters at the rate determined by time required for the measurement system to stabilize to the 13 different [CO₂] in the following order: 400, 300, 200, 100, 50, 400, 400, 700, 1000, 1300, 1900, 2100 ppm. The resulting curve

was used to determine the limiting factors affecting assimilation. The data were then analyzed according to the method described in Long & Bernacchi (2003) and adjusted for temperature based on Bernacchi et al. (2001 & 2003) using the PS-FIT software package (<http://www.life.illinois.edu/bernacchi/links.html>). This software uses the leaf model of photosynthesis (Farquhar et al., 1980) to predict the maximum rates of electron transport (J_{max}) and maximum velocity of carboxylation by Rubisco ($V_{c,max}$) from the measured responses. Two curves were measured in each chamber on both measurement dates. The data were then entered into the model for analysis and interpretation.

After the 5th week of the experiment, the plants were removed from the chambers, leaves were harvested from each plant, and leaf area was determined using a leaf area meter (LI-3000, Licor, Inc.). Two leaf punches from the youngest most fully expanded leaf, measuring 1.9cm in diameter, were taken from three plants in each treatment to determine specific leaf area (SLA, leaf area per unit mass). The plants were then destructively harvested separating leaves, stems, and root biomass. Tissue from each plant was sampled and analyzed separately so the statistical analyses could reflect the variance between plants within each treatment. Leaf number, total leaf area, basal diameter, stem heights, number of pods, and number of nodes were all measured and recorded specific to each plant. The biomass was then dried and weighed to determine the dry weights of leaves, stems, and root tissue and summed to get a total dry weight of each plant. The averages of all of these parameters were then determined using the summations of the five plants in each chamber.

2.3 Statistical Analysis

Regression analysis was used for all parameters in the study using the Analysis feature associated with the Sigmaplot 12.5 software package (Systat Software, Inc., San Jose, CA USA). The shape of the responses of each parameter to $[\text{CO}_2]$ was used to determine the fit and type of regression used. Height, development, and all final harvest parameters yielded a linear relationship so a simple linear regression was used on the averages of the five individual plants per chamber. The trend encountered with photosynthetic assimilation and stomatal conductance was more complex. The midday and diurnal assimilation data was best described with a single rectangular hyperbola, 2-parameter equation. Stomatal conductance data from the midday measurements were fit using a polynomial, inverse second order equation. The A/C_i curves were input into the PS-Fit tool to calculate the best fit of the data and return J_{max} and $V_{c,max}$ estimates for each curve. The J_{max} and $V_{c,max}$ data showed a linear response and were then analyzed using simple linear regression.

CHAPTER 3

RESULTS

3.1 Fumigation Control

All treatments were within ± 50 ppm of the set-point when averaged over the entire experiment with the exception of the 750 ppm treatment which averaged about 150 ppm higher than the set-point (Figure 1).

3.2 Development and Plant Heights

Plant development was delayed as CO₂ concentrations increased (Figure 2). The delay caused by elevated [CO₂] became evident as the plants entered the third vegetative stage (V3), about 25 days following emergence. This difference in development among the treatments was statistically different starting on the 27th day ($p < 0.001$). The height of the plants increased with [CO₂] (Figure 3). The difference was statistically significant among treatments on every measurement day following the 16th day after emergence ($p < 0.001$ on all days except the 20th day of the experiment in which $p < 0.01$). By the end of the experiment the difference in height from the lowest treatment to the highest was about 15 cm (Figure 3).

3.3 Midday Gas Exchange Measurements

Both measurement days showed that photosynthesis increased as the CO₂ concentration increased. However, this increase began to level off at [CO₂] above 1000 ppm (Figures 4 a,c). The first measurement date on the 16th day following emergence yielded a significance value of

$p < 0.01$ when comparing $[\text{CO}_2]$ and photosynthesis (Figures 4a). The difference between treatments with respect to stomatal conductance was not significant on this day (Figures 4 b). On the second measurement day, the 30th day following emergence, both photosynthesis and stomatal conductance were found to show significant correlations with $[\text{CO}_2]$ ($p < 0.001$, Figure 4 c,d). Again, the photosynthesis values gave an increasing trend with the treatment that leveled off near 1000 ppm (figure 4 a). Stomatal conductance values decreased with increasing $[\text{CO}_2]$ (Figure 4 d).

3.4 Diurnal Measurements

The two diurnal measurement dates showed a similar trend. On the first day (18th day after emergence) the daily integral of photosynthesis showed a positive correlation with $[\text{CO}_2]$, similar to the midday results with a $p < 0.01$ (Figure 5 a). On the 2nd measurement day (32nd day) the trend toward increasing integrated photosynthesis with rising $[\text{CO}_2]$ was even more pronounced with a $p < 0.001$ (Figure 5 b).

3.5 A/C_i Measurements

The A/C_i dataset for both days was analyzed using PS-Fit. The maximum rates of Rubisco carboxylation ($V_{c,max}$) and the electron transport (J_{max}) were not statistically significant on the 19th day after emergence, showing no differences between treatments (Figures 6a, c). On the 35th day after emergence there was a decreasing linear trend for both $V_{c,max}$ and J_{max} with increasing $[\text{CO}_2]$ with p -values of 0.0008 and 0.014 respectively (Figures 6 b,d).

A minor decrease in mitochondrial respiration on the following day (36th day after emergence) with increases in [CO₂] was observed when the data were fit using a linear regression with a *p*-value of 0.04 (Figure 7).

3.6 Destructive Harvest

All final harvest parameters collected showed an increasing linear trend with increasing [CO₂] except for SLA. When averaged and combined the total biomass showed a significant increasing trend with increasing [CO₂] (*p* = 0.004, Figure 8). An increasing trend was also found for basal diameter, leaf number, branch number, node number, leaf area, and stem height as well (Figure 9). Specific leaf area (SLA) did not show any significant difference or trend among treatments.

CHAPTER 4

DISCUSSION

Fumigation within the chambers was adequately maintained for the duration of the experiment (Figure 1). The concentrations within the chambers were maintained for the majority of the experiment other than the times that the chambers were opened to take measurements or rotate the plants.

There were significant differences in plant height and development among treatments. This supports my hypothesis that plant height increased linearly with increasing [CO₂]. By the end of the experiment the highest [CO₂] treatments were, on average, more than 10 cm taller than the lowest treatments. Significant differences in plant heights when comparing ambient [CO₂] and 550 ppm over two years using FACE technology have been observed previously using the same soybean cultivar (Morgan et al. 2005). The differences were noticeable later in development but the results are comparable to what we had found. There were no differences in development until the third week of the experiment when the lowest treatments entered V5 and the high treatments were entering R1. On average, the lowest treatments entered reproductive development several days before the higher treatments. This delay is similar to previous reports for soybean (Castro et al., 2009) using the same soybean cultivar within a FACE experiment. They found that the early reproductive stages were the most affected by CO₂ enrichment possibly due to the formation of extra nodes on the plants under increased CO₂. Differences were also found later in reproductive development (Castro et al., 2009).

Overall, the observed differences in photosynthesis with increases in CO₂ support the hypothesis that carbon assimilation will increase with rising [CO₂], and plateau above a certain threshold. From the midday and diurnal measurements, it is evident that photosynthesis increases with rising [CO₂], although this response is not linear. It has been well documented that C3 species show increased photosynthetic assimilation and with increases in [CO₂]. My findings are consistent with a meta-analysis encompassing the majority of publications prior to 2002 that focused on soybeans response to elevated [CO₂] (Ainsworth et al. 2002). The photosynthetic data followed a hyperbolic trend, which seemed to increase steadily until 1000 ppm where it began to plateau. Stomatal conductance on the first midday sampling showed no significant difference among treatments. The differences in assimilation rates were also less apparent on this measurement day than all of the others. This may have been a result of limiting light on this day with photosynthetically active radiation measuring $\sim 400 \mu\text{mol m}^{-2} \text{s}^{-1}$. Overall, the early vegetative gas exchange measurements (including midday, diurnal, and A/C_i) showed similar assimilation and stomatal conductance rates to the same sampling protocol that was completed during late vegetative/ early reproductive stages. Increases in carbon assimilation and decreases in stomatal conductance were found to remain linear until [CO₂] reaches 1000 ppm.

The first sampling in early vegetative development did not show a significant difference in $V_{c,max}$ or J_{max} across treatments. One possible explanation for this is that the plants were rapidly growing at these stages and there was no sink limitation for the photosynthate. The second measurement set in late vegetative/early reproductive stages revealed a linear down regulation of $V_{c,max}$ and J_{max} as [CO₂] increased, evidence of photosynthetic acclimation. There was plenty of carbon for the plant to take up in the higher treatments, but a lack of a strong sink

for the carbon may have occurred later in development. These findings may be caused by the use of chambers in this study. It has been found that pot size, chamber conditions, and nitrogen availability have a strong effect on photosynthetic responses, specifically acclimation (Bernacchi et al. 2005; Ainsworth et al. 2002; Sage 1994). Arp (1991) reported that growing plants in containers can have a strong influence on photosynthetic acclimation when pots are relatively small, and a significant correlation can emerge between the volume of the pot used and photosynthetic capacity. After removing the roots during the destructive harvest, it was clear that the roots were not pot-bound and considering the large containers used in this experiment it seems unlikely that this was responsible for the acclimation that was found. Nitrogen limitations can also impose acclimation (Arp 1991; Sage 1994), however the extended release fertilizer used was sufficient for the longevity of the experiment and no signs of nutrient deficiencies were apparent. Temperature can also cause photosynthetic acclimation, but was evenly controlled in every chamber.

The results from the destructive harvest supported the hypothesis that biomass accumulation will increase with increasing $[\text{CO}_2]$. The final root, stem, and leaf weights revealed a linear trend that increased with rising $[\text{CO}_2]$. With a diminished source limitation, the increases in photosynthesis allow for increases in biomass accumulation and the development of the plant. However, it has been shown that the extent to which assimilation rates impact biomass accumulation and yield were not equally stimulated as one would expect (Ainsworth et al. 2002). This is thought to be a result of the sink limitation that is apparent late in soybean development. Again, all harvest parameters measured revealed linear increases with increases in $[\text{CO}_2]$ except

for SLA. This was also a parameter that was found to decrease with increasing $[\text{CO}_2]$ in the meta-analysis by Ainsworth (2002).

For future investigations using the similar treatments, it would be interesting to test if the nodulation effects on photosynthesis would drive similar responses. With the nodules acting as carbon sinks, this might provide evidence whether photosynthetic acclimation is driven by a lack of a sink for photosynthate. Testing starch accumulation in leaves as well as leaf nitrogen content would also be useful in explaining the photosynthetic results. The similarities found between the data presented and field experiments testing similar treatments show that using controlled environment chambers is a dependable way to test the predicted future atmospheric conditions on our most important field crops. These results, thus, could be used to further understand, at a larger scale, how soybean will perform under future CO_2 concentrations.

REFERENCES

- Ainsworth E.A., Davey P.A., Bernacchi C.J., Dermody O.C., Heaton E.A., Moore D.J., Morgan P.B., Naidu S.L., Yoo Ra H., Zhu X., Curtis P.S., & Long S.P. 2002. A meta-analysis of elevated [CO₂] effects on soybean (*Glycine max*) physiology, growth and yield. *Global Change Biology*. 8: 695-709.
- Arp W.J. 1991 Effects of source-sink relations on photosynthetic acclimation to elevated CO₂. *Plant, Cell, and Environment*. 14: 869-875.
- Arp W.J. & Drake B.G. 1991. Increased photosynthetic capacity of *Scirpus olneyi* after 4 years of exposure to elevated CO₂. *Plant, Cell, and Environment*. 14: 1003-1006.
- Bernacchi C.J., Pimentel C., & Long S.P. 2003. In vivo temperature response functions of parameters required to model RuBP-limited photosynthesis. *Plant, Cell, and Environment*. 26: 1419-1430.
- Bernacchi C.J., Morgan P.B., Ort D.R., & Long S.P. 2005. The growth of soybean under free air [CO₂] enrichment (FACE) stimulates photosynthesis while decreasing in vivo Rubisco capacity. *Planta*. 220: 434-446.
- Bowes G., 1991. Growth at elevated CO₂: photosynthetic responses mediated through Rubisco. *Plant, Cell and Environment*. 14: 795-806.
- Bowes G., 1993. Facing the inevitable: plants and increasing atmospheric CO₂. *Annual Review Plant Physiology. Plant Molecular Biology*. 44:309-332.
- Brysse K., Oreskes N., O'Reilly J., & Oppenheimer M. 2013. Climate change prediction: erring on the side of least drama? *Global Environmental Change*. 23:327-337.
- Canadell J.G., Quéré C.L., Raupach M.R., Field C.B., Buitenhuis E.T., Ciais P., Conway T.J., Gillett N.P., Houghton R.A., & Marland G. 2007. Contributions to accelerating atmospheric CO₂ growth from economic activity, carbon intensity, and efficiency of natural sinks. *Proceedings of the National Academy of Sciences*. 104 (47) 18866-18870.
- Castro J.C., Dohleman F.G., Bernacchi C.J., & Long S.P. 2009. Elevated CO₂ significantly delays reproductive development of soybean under Free-Air Concentration Enrichment (FACE). *Journal of Experimental Botany*. 60: 2945-2951.
- Ferguson B.J. & Gresshoff P.M. 2009. Soybean as a model legume. *Grain Legumes*. 53
- Hartmann D.L., Tank Klein A.M.G., Rusticucci M., Alexander L.V., Brönnimann S., Charabi Y., Dentener F.J., Dlugokencky E.J., Easterling D.R., Kaplan A., Soden B.J., Thorne P.W., Wild M., & Zhai P.M. 2013: Observations: Atmosphere and Surface. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker T.F., Qin

- D., Plattner G.-K., Tignor M., Allen S.K., Boschung J., Nauels A., Xia Y., Bex V., and Midgley P.M. (eds.)). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Kramer P.J. 1981. Carbon dioxide concentration, photosynthesis, and dry matter production. *Bioscience*. 31:29-33.
- Jones, N. 2013. Troubling milestone for CO₂. *Nature Geoscience*. 6, 589.
- Long, S. P. & C. J. Bernacchi. 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany*. 54: 2393-2401.
- Madsen E. 1975. Effect of CO₂ enrichment on growth, development, fruit production and fruit quality of tomato from a physiological viewpoint. *Phytotronics*. III: 318-330
- Makino A. & Mae T. 1999. Photosynthesis and plant growth at elevated levels of CO₂. *Plant Cell Physiology*. 40: 999-1006.
- Morgan P.B., Bollero G.A., Nelson R.L., Dohleman F.G., & Long S.P. 2005. Smaller than predicted increase in aboveground net primary production and yield of field-grown soybean under fully open-air [CO₂] elevation. *Global Change Biology*. 11: 1856-1865.
- Peet M.M., Huber S.C., & Patterson D.T. 1986. Acclimation to high CO₂ in monoecious cucumbers. *Plant Physiology*. 80: 63-67.
- Ritchie S.W., Hanway J.J., Benson G.O., Herman J.C., & Lupkes S.J. 1993. How a soybean plant develops. Special Report No 53. Iowa State University of Science and Technology, Ames, IA.
- Rogers, H.H. & Dahlman, R.C. 1993. Crop Responses to CO₂ enrichment. *Vegetation*. 104/105: 117-131.
- Sage R.F. 1994. Acclimation of photosynthesis to increasing atmospheric CO₂: the gas exchange perspective. *Photosynthesis Research*. 39: 351-368.
- Sasek, T.W., Delucia, E.H., & Strain, B.R. 1985. Reversibility of photosynthetic inhibition in cotton after long-term exposure to elevated CO₂ concentrations. *Plant Physiology*. 78, 619-622.
- Ziska L.H. 1998. The influence of root zone temperature on photosynthetic acclimation to elevated carbon dioxide concentrations. *Annals of Botany*. 81: 717-721.

FIGURES

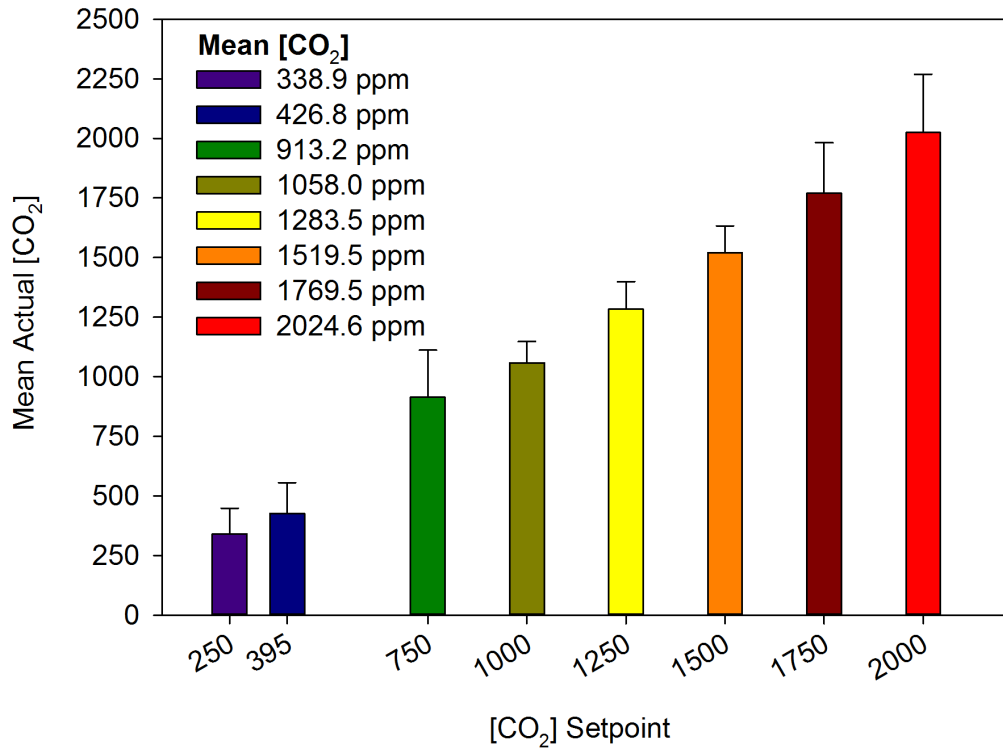


Figure 1. The mean [CO₂] of each chamber over the entire length of the experiment calculated from 60 second data.

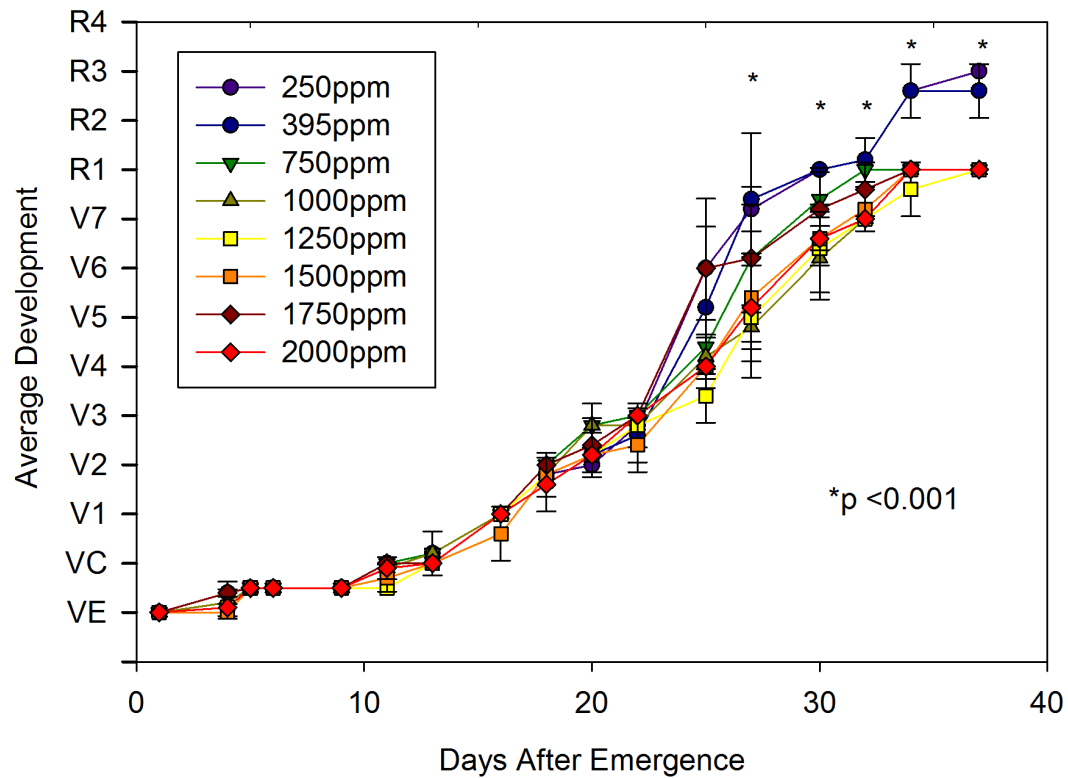


Figure 2. The progression through vegetative and reproductive development for soybean at each [CO₂] treatment. The symbols represent the mean of 5 plants per chamber on each day and the error bars represent one standard error of the mean. Statistics were performed using the averages from each chamber on each day. Statistically significant differences ($p < 0.001$) across treatments within a day are represented with an asterisk above the symbols.

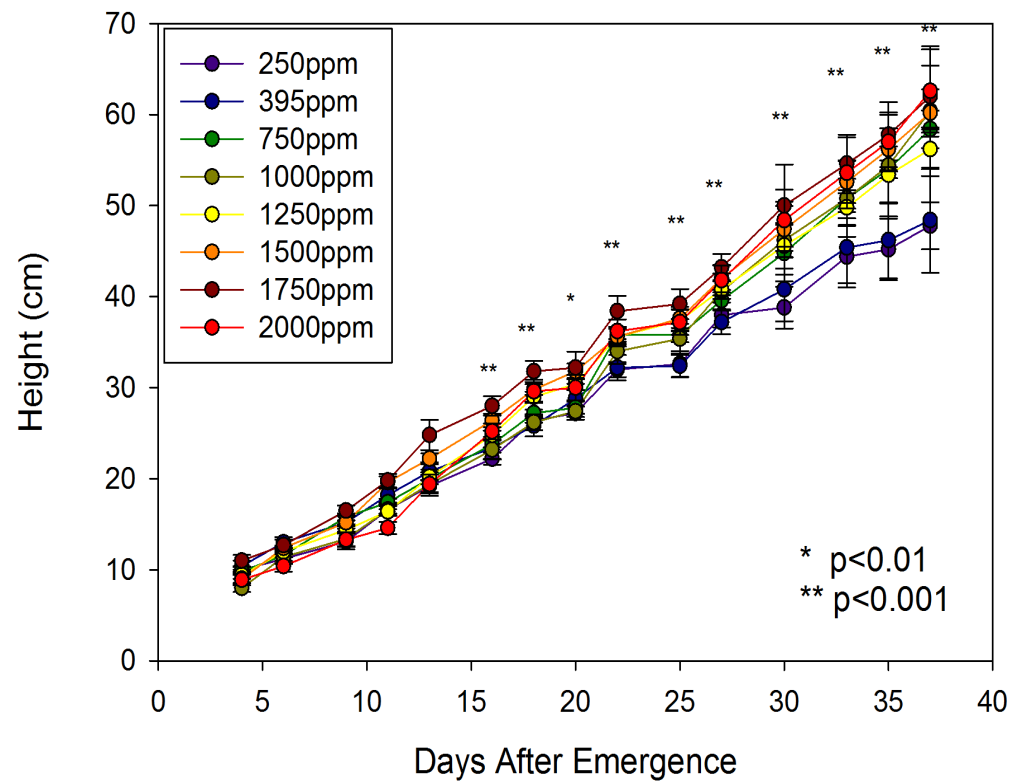


Figure 3. Average plant height over the duration of the experiment. Symbols and error bars are as in Figure 2.

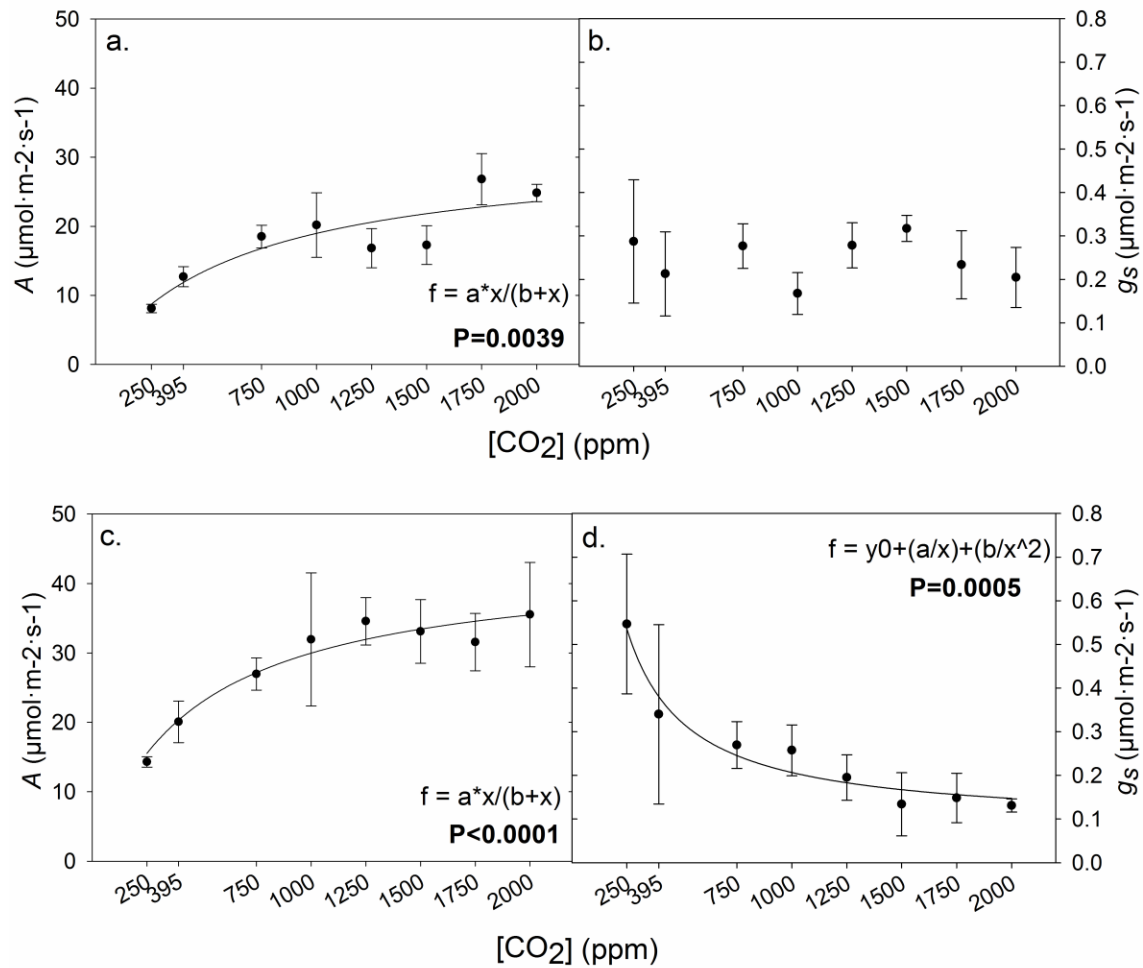


Figure 4. Mean midday photosynthesis (a,c) and stomatal conductance (b,d) on the 16th (a,b) and 30th (c,d) day after emergence. Each symbol is the mean of three plants per treatment and the error bar represents the standard deviation of the mean. Statistically significant responses are indicated by a fit plotted through the data.

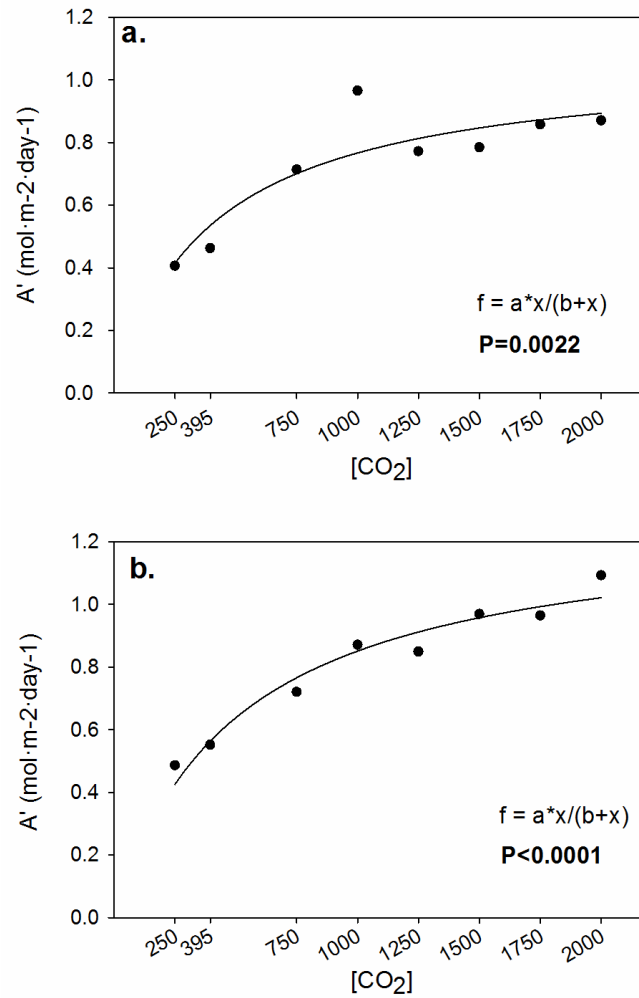


Figure 5. Total daily photosynthesis acquired on the diurnal measurement dates. Graph (a) corresponds to the diurnal on March 20th (18th day after emergence) and (b) is from April 3rd (32nd day after emergence). The graphs are fit using a rectangular hyperbola equation stated in each graph.

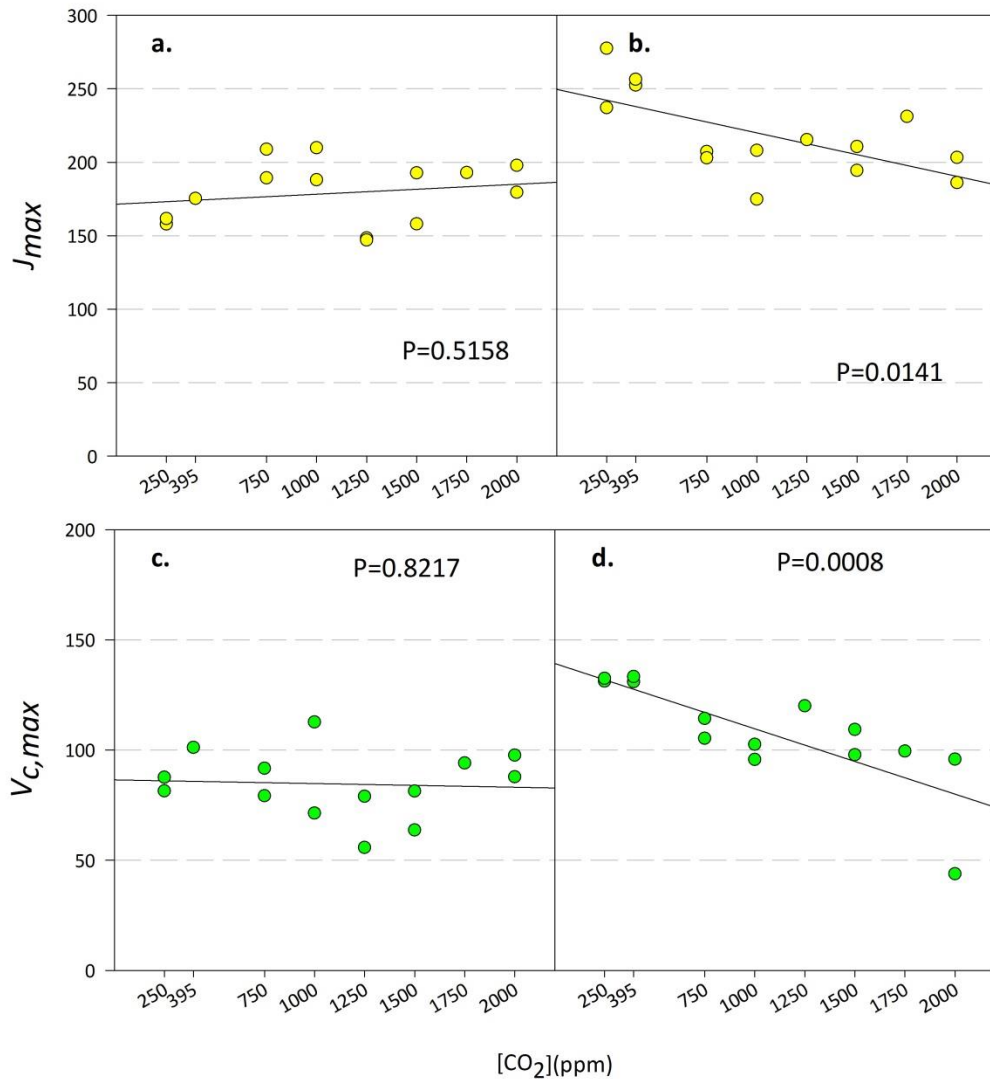


Figure 6. $V_{c,max}$ and J_{max} values calculated from the A/C_i measurements using the PS-Fit model. Graphs (a,c) are from March 21st (19th day after emergence) and (b,d) are from April 5th (34th day after emergence). P- values are given for linear regression fit.

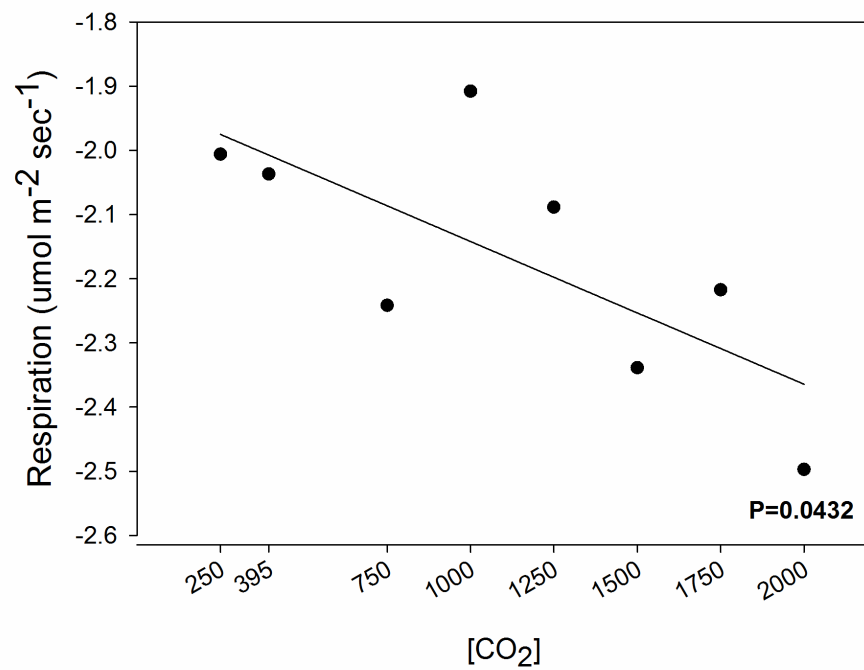


Figure 7. Night Respiration measured on the 35th day after emergence. The p-value is given for the linear regression fit.

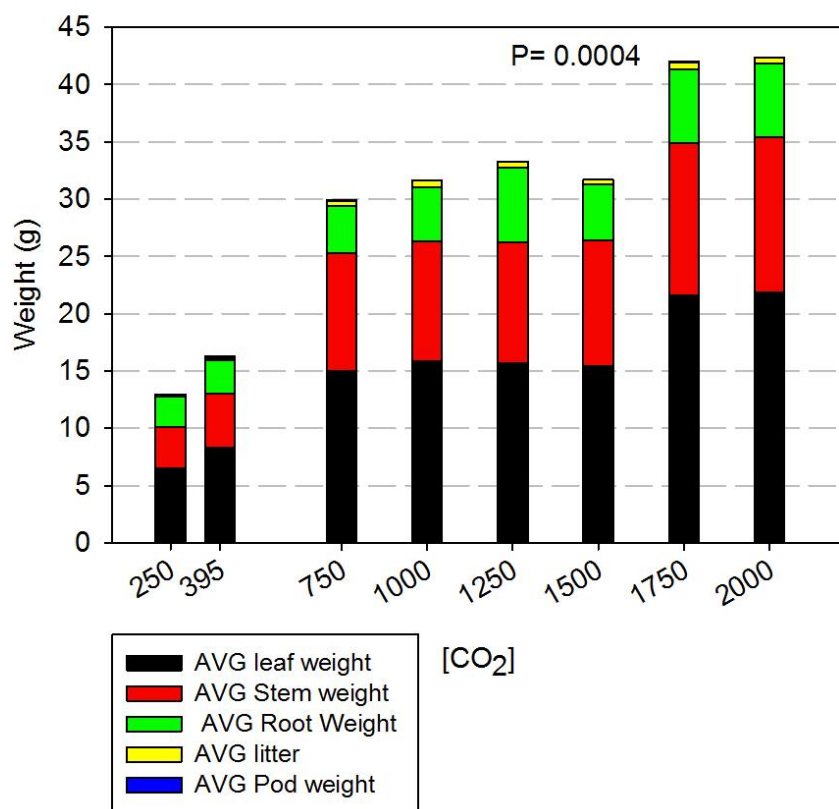


Figure 8. Total dry biomass as a function of growth [CO₂]. The data were fit using a simple linear regression ($p = 0.004$).

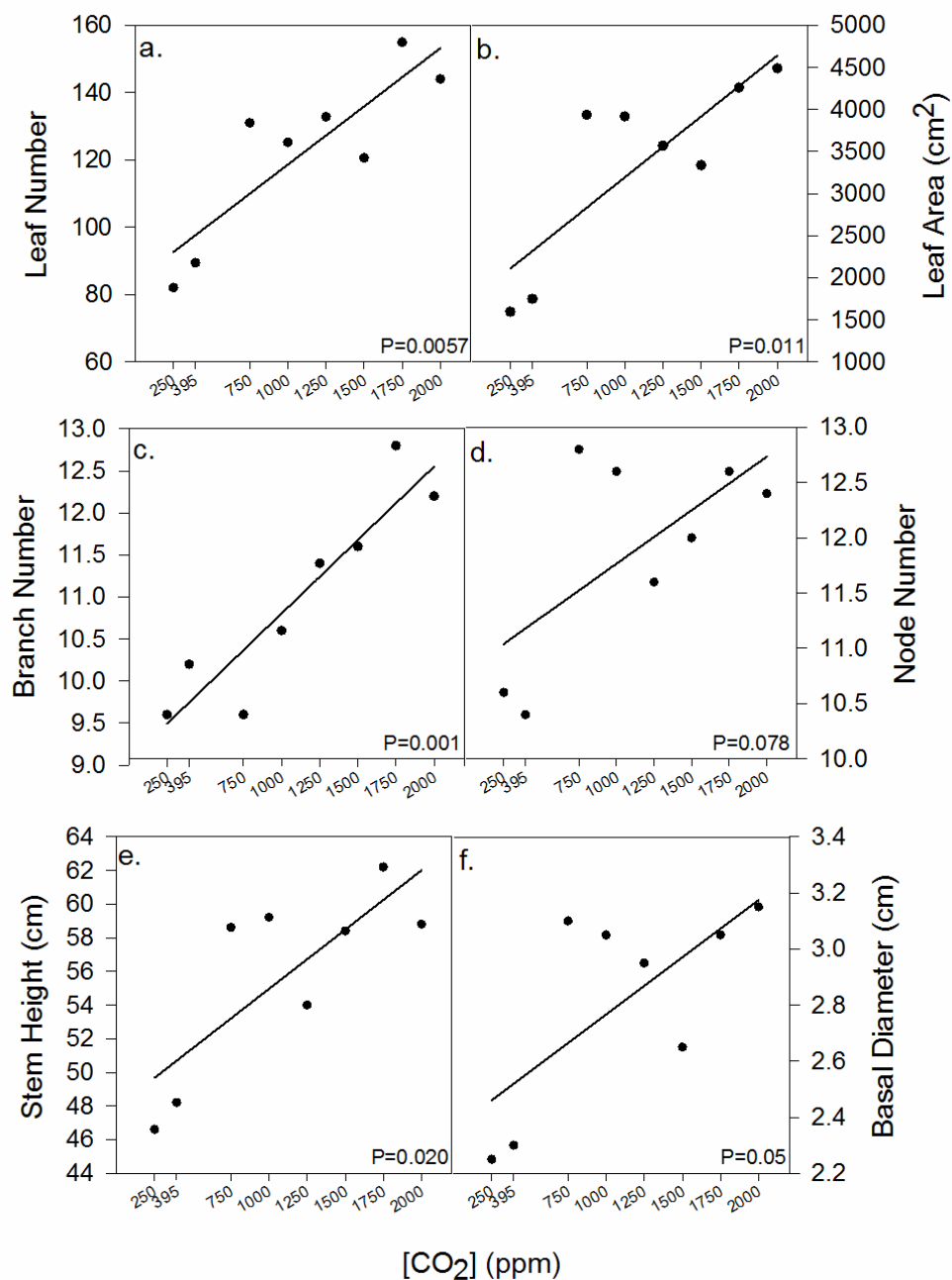


Figure 9. Final harvest parameters including leaf area, leaf number, stem height, branch number, node number, and basal diameter. Each point represents the average from each treatment. The data were analyzed using simple linear regression.