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Does elevated [CO_2] mitigate the impact of climate extremes on northern red oak seedlings: a multifactor approach

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Abstract

There exists little doubt that global climate change takes place and that this change will entail important consequences for plant communities. An increasing number of studies have investigated the effect of elevated [CO_2] and increased temperature on plant physiology, but very few have investigated the effect of increased [CO_2] on drought stress during temperature extremes, which are increasingly likely to occur.

We investigated the interactions of elevated [CO_2], decreased soil water availability and heatwaves of differing severities and their effect on net photosynthesis, water relations and chlorophyll fluorescence of one-year old potted northern red oak (*Quercus rubra* L.) seedlings grown in treatment chambers with high fertility. We constructed eight climate-controlled chambers and assigned each chamber one of eight treatment combinations: ambient (380 ppm) or elevated (700 ppm) [CO_2], ambient (+0°C), increased (+3°C), heatwave (+6°C) or extreme heatwave (+12°C) temperature. The heatwaves were applied for a respective amount of time so that each heat treatment would yield the same heat sum. One half of the plants per chamber were watered to field capacity and the other half at wilting point. Net photosynthesis (A_{net}), stomatal conductance (g_s), transpiration (E), leaf vapour pressure deficit (VPDl) and chlorophyll fluorescence were measured in the morning and the afternoon prior to, during and one week following the heat wave.

Heatwave-induced stress significantly reduced A_{net} in the afternoon in all treatments, but actually increased A_{net} in the morning for low water treatments, and the low water seedlings in the elevated [CO_2] treatments reached higher values than their well-watered peers at this time. Elevated [CO_2] increased A_{net} and the effective quantum yield of photosystem II (Φ_{PSII}), markedly reduced g_s in all treatments, and mitigated heat and drought stress significantly. Decreased water availability significantly lowered A_{net} prior to and after the heatwave, but led to an importantly smaller decrease in A_{net} during the heatwave in the ambient [CO_2] treatment and even a rise in A_{net} during the heatwave in the elevated [CO_2] treatment.

Our findings suggest extreme temperature events and drought inhibit many physiological processes, but their interaction may lead to acclimation responses able to suppress part of this inhibition. Predicted increases in atmospheric [CO_2] will thus not only mitigate aforementioned stresses, they might even eliminate them.

Samenvatting

Er wordt nog maar weinig aan de globale klimaatverandering en zijn ver strekkende gevolgen voor plantengemeenschappen getwijfeld. Een groeiend aantal studies heeft reeds de effecten van verhoogde [CO_2] en temperatuur op plantenfysiologie bestudeerd, doch zeer weinigen hebben het effect van verhoogde [CO_2] op droogtestress tijdens temperatuurextrema, dewelke steeds waarschijnlijker zullen voorkomen, bestudeerd.
We onderzochten de interacties van verhoogde [CO_2], verminderde waterbeschikbaarheid en verschillende hittegolven en hun effect op netto fotosynthese, water relaties en chlorofyl fluorescentie van gepotte éénjarige Amerikaanse eik (*Quercus rubra* L.) zaailingen in serres. We bouwden acht klimaat gecontroleerde serres en kenden elke serre één van acht behandelingen toe: omgevings- (380 ppm) of verhoogde (700 ppm) [CO_2], omgevings- (+0°C), verhoogde (+3°C), hittegolf (+6°C) of extreme hittegolf (+12°C) temperatuur. De hittegolven waren van dergelijke duur dat elke hitte behandeling dezelfde hittesom onderging. Per serre werd één helft van de zaailingen bewaterd tot op veldcapaciteit en de andere helft tot op het verwelkingspunt. Netto fotosynthese (A_{net}), stomatale conductie (g_s), transpiratie (E), het dampdruk tekort (VPDl) en chlorofyl fluorescentie werden 's ochtends en 's namiddags voor, tijdens en na de hittegolf gemeten.
Hittegolf stess reduceerde 's namiddags A_{net} significant in alle behandelingen, maar verhoogde deze 's ochtends voor de lage waterbehandelingen, en de verhoogde [CO_2] laag water haalde dan hogere waarden dan hoog water. Verhoogde [CO_2] verhoogde A_{net} en de effectieve quantum opbrengst van photosysteem II (Φ_{PSII}) en reduceerde g_s belangrijk in alle behandelingen, en verzachtte hitte- en droogtestress significant. Verminderde waterbeschikbaarheid verlaagde A_{net} belangrijk voor en na de hittegolf, maar verhoogde A_{net} tijdens de hittegolf relatief voor omgevings [CO_2] en absoluut voor verhoogde [CO_2].
Onze resultaten suggereren dat extreme temperaturen en droogte vele fysiologische processen inhiberen, maar dat hun interactie tot acclimatieverschijnselen kan leiden die een deel van deze inhibitie onderdrukken. De voorspelde [CO_2] toename zal niet alleen genoemde stress verzachten, maar mogelijk zelfs teniet doen.

List of abbreviations

Abbreviation	Meaning	Unit
A_{dark}	Dark respiration	[μmol CO_2 m^{-2} s^{-1}]
A_{net}	Net photosynthesis	[μmol CO_2 m^{-2} s^{-1}]
C_A	Ambient atmospheric [CO_2] treatment	-
C_E	Elevated atmospheric [CO_2] treatment	-
C_i	Intercellular CO_2 concentration	[μmol mol^-1]
E	Transpiration rate	[mmol H_2O m^{-2} s^{-1}]
FACE	Free Air CO_2 Enrichment	-
F_v/F_m	Maximum quantum yield	[-]
F'_v/F'_m	Energy harvesting efficiency	[-]
g_s	Stomatal conductance to water vapour	[mol H_2O m^{-2} s^{-1}]
H	High water treatment	-
L	Low water treatment	-
PAR	Photosynthetic active radiation	[mol m^{-2}]
Φ_{PSII}	Effective quantum yield (PSII-efficiency)	[-]
Q_p	Photochemical quenching	[-]
T_0	Ambient temperature treatment	-
T_3	Ambient +3 °C temperature treatment	-
T_6	Ambient +6 °C temperature treatment	-
T_{12}	Ambient +12 °C temperature treatment	-
VPDl	Leaf vapour pressure deficit	[kPa]
WUE_I	Instantaneous water use efficiency	[μmol CO_2 $mmol^{-1}$ H_2O]

Contents

Chapter 1

Introduction

Global climate change has proven to be a popular subject in both scientific and non-scientific circles. Researchers view it as a compelling new cogitation topic, political leaders experience it as a substantial baffle to their ends, and the media use it as a means to draw attention. In general it is portrayed as something to be feared and avoided. The aim of this study however is not to discuss the validity of this last statement but to add to the ever growing yet still lacking knowledge on the impact this climate change could produce. It will further examine the effect climate change may have on ecosystems and plants in particular.

The main constituents of global climate change can roughly be described as (i) the rise of atmospheric [CO_2]; (ii) the increase in mean annual temperature and (iii) the shift in global weather patterns and precipitation. Atmospheric [CO_2] has not been this high in 26 million years and is projected to surpass 700 μmol mol^{-1} by the end of the century (Houghton *et al.*, 2001; Pearson and Palmer, 2000). Projected global average surface warming will amount to 2.8°C by the end of the 21st century (Keeling and Whorf, 2005; IPCC, 2007a) and the chance of heatwave occurrence could drastically increase due to a rise in the concentration of several atmospheric greenhouse-gases (Karl and Nicholls, 1997; Meehl and Tebaldi, 2004; Schär *et al.*, 2004). Lastly, increased variance of precipitation is expected everywhere, with wet areas becoming wetter and dry and arid areas becoming more so (Dore, 2005).

In this study, we subjected the northern red oak (*Quercus rubra* L.) to different levels of these three environmental parameters. In summary, we compared seedlings of northern red oak grown in today's atmospheric [CO_2] to those grown in the projected atmospheric [CO_2] of 700 μmol mol^{-1}, seedlings experiencing different temperature regimes to one another, and well watered seedlings to those who were under continuous drought stress. We combined these treatments to reach a total of 16 factor combinations, which allowed us to constitute a delineation of possible future prospectives of ecosystem expansion.

Chapter 2

Literature study

This literature study will systematically highlight the current knowledge on the different components of which our research topic consists. As mentioned in the previous chapter, this research was conducted in the conceptual framework of a future climatic change, the main constituents of interest of which are focused on in later sections. In addition some basic knowledge of the morphology of the examined species will be addressed. Lastly an overview of similar and cocurrent studies will be provided.

2.1 Climate change

There is an increasing consensus that global climate change occurs (Harris *et al.*, 2006; IPCC, 2007a) and that the next century will be characterized by shifts in global weather patterns and climate regimes (Watson and Team, 2001; McCarthy *et al.*, 2001; Munasinghe and Swart., 2005). According to the IPCC A1B model, which assumes very rapid economic growth, rapid introduction of new and more efficient technologies and a balanced use of fossil and non-fossil energy sources (Nakicenovic *et al.*, 2000), leading to an annual rise of atmospheric [CO_2] of 2μmol mol^{-1} yr^{-1} due to anthropogenic emissions, atmospheric [CO_2] will rise to 700 μmol mol^{-1} and the global average surface temperature will increase with 2.8°C by the end of the 21st century (Houghton *et al.*, 2001; Keeling and Whorf, 2005; IPCC, 2007a). Also modelling work by Karl and Nicholls (1997); Meehl and Tebaldi (2004); Schär *et al.* (2004) showed that an increase in the concentration of atmospheric greenhouse-gases not only could lead to an increase in mean temperature, but to an increase in temperature variability, which could drastically increase the chance of heatwave occurrence. Furthermore increased variance of precipitation is expected everywhere (Dore, 2005). It is expected that wet areas become wetter, and dry and arid areas become more so. In general high latitudes (Northern Hemisphere) will experience increased precipitation, China, Australia and the Small Island States in the Pacific will experience reduced precipitation and equatorial regions will undergo the highest increase in variability (Trenberth, 1998; Dore, 2005).

Since the native range of the examined species (Figure 2.1) is situated in the southeast of North America, some specific climatic changes for this region are summarized.

The rise in [CO_2] and other greenhouse gases is expected to cause an increase in the annual temperature in eastern North America of 3.6°C (IPCC, 2007b). In addition, Diffenbaugh and Ashfaq (2010) used a large suite of climate model experiments to find the possibility that intensification of hot extremes could result from relatively small increases in greenhouse gas concentrations, suggesting that current mitigation targets arising from political negotiations may not be sufficient to avoid dangerous climate change. Regional precipitation too is subject to diverging predictions. Wang *et al.* (2010) analyzed 60-yr rainfall data and concluded that interannual variability of summer precipitation in the southeastern United States intensified, leading to stronger summer droughts and pluvials. According to Trenberth (1998) and IPCC (1996), future climatic changes may lead to a slight decrease in the amount of precipitation in this region. However, most models show increases in precipitation over much of North America (Dore, 2005). Also the increase in air temperature predicted by the A1B model could result in a slight increase in precipitation in eastern North America. Schindler (1997) does note that rates of evaporation and perhaps transpiration are also likely to increase with increasing temperatures, which may lead to declines in runoff and river flows in regions in which changes in precipitation do not offset increasing rates of evaporation and transpiration, leading to decreased overall water availability.

2.2 Description of *Quercus rubra*

The species on which measurements were conducted during this experiment was the northern red oak, also known as common red oak, eastern red oak, mountain red oak, gray oak (Burns and

Honkala, 1990) or *Quercus rubra* L. Northern red oak is the only native oak extending northeast to Nova Scotia. It grows from Cape Breton Island, Nova Scotia, Prince Edward Island, New Brunswick, and the Gaspé Peninsula of Quebec, to Ontario, in Canada; from Minnesota South to eastern Nebraska and Oklahoma; east to Arkansas, southern Alabama, Georgia, and North Carolina. Outliers are found in Louisiana and Mississippi (Sander, 1965; Little, 1971).

Northern red oak can be found in regions with varying climates, with mean annual precipitation ranging from about 760 mm in the Northwest to about 2030 mm in the southern Appalachians, mean annual temperature from about 4°C in the northern part of the range to 16°C in the extreme southern part. These different climates experience different frost-free periodes, averaging 100 days in the North and 220 days in the South. Annual snowfall ranges from a trace in southern Alabama to 254 cm or more in the Northern States and Canada (Sander, 1965; Little, 1971). Its native range is depicted in Figure 2.1.

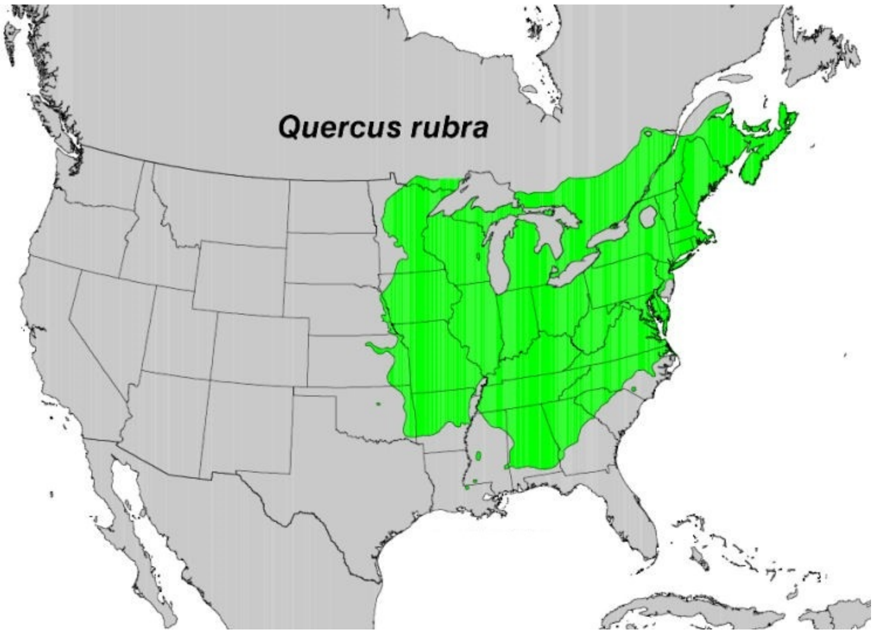


Figure 2.1: Native range of Northern red oak (Little, 1971)

Northern red oak seedlings that are established naturally or by planting at the time an old stand is clearcut, regardless of how large the clearcut area, do not grow fast enough to compete with the vigorous woody sprouts and other vegetation (Beck, 1970), yet sprouts readily. New sprouts grow rapidly and are usually straight and well formed (Sander, 1971, 1972).

As mentioned, seedlings of the Northern red oak shoot grow episodically, slow and generally restricted to one growth flush under undisturbed or lightly disturbed forest stands. It will also average only a few centimeters annually at best (Sander, 1979). Multiple shoot growth flushes may occur in the same growing season when moisture, light, and temperature conditions are favorable enough (Reich *et al.*, 1980). Most of the annual root elongation occurs during the rest periods (Burns and Honkala, 1990).

Of specific interest to this study are findings by Sander (1965) and Seidel (1972), who found that available soil moisture can be a critical factor affecting first year survival of northern red oak seedlings and that, when the taproot is able to penetrate the soil, seedlings survive considerable moisture stress later in the growing season. The most critical factor however, affecting not only first year survival, but also survival and growth in subsequent years appears to be light intensity (McGee, 1968; Sander, 1979). Caspersen and Kobe (2001) ranked *Quercus rubra* drought insensitive and second most likely to survive at low growth rates.

Recent studies on the behaviour of *Quercus rubra* L. during changing environmental conditions show that the effects may be greatest (or most notable) when examining seedlings of northern red oak (Hättenschwiler *et al.*, 1997). Apostol *et al.* (2007) found that changing root zone temperatures significantly affected photosynthesis, stomatal conductance and transpiration. These treatment effects ceased after three weeks, despite persisting reductions in growth. Tardif *et al.* (2006) conducted climate response experiments on northern red oak and white oak (*Quercus alba* L.) and reported that radial growth in *Quercus rubra* was particularly sensitive to climatic conditions in the early growing season (May to July). Lastly, Collins and Carson (2004) reported that, although most studies describing tree species distributions across environmental gradients only focus on the abundance of adults, in the absence of disturbance, site characteristics have the strongest effects on abundance patterns of oaks during the sapling stage.

2.3 Effect of shift in environmental conditions

Our experimental setup, which was briefly described in the introduction and will be thoroughly addressed in Chapter 4, involved subjecting the plants on which measurements were conducted to different levels of three environmental variables likely to undergo a shift in the future climate change. These variables were atmospheric [CO_2], amount of available water and temperature.

The effect of these parameters has been the subject of many studies, but few studies examined the effect of a possible interaction and even fewer to the extent of this study. Note that *Quercus rubra* L. is seldom used in this kind of studies, despite it being highly valued as an ornamental and intensively planted because of its symmetrical shape and brilliant fall foliage, aside from its obvious ecological role as a source of food and shelter for squirrels, deer, turkey, mice, voles and other mammals and birds (Burns and Honkala, 1990). Most studies using trees to study these effects use species such as loblolly pine (*Pinus taeda* L.) and douglas fir (*Pseudotsuga menziesii* L.) (Saxe *et al.*, 1998), favoring needles and evergreens over deciduous species. Niinemets *et al.* (2011) state that photosynthesis and other physiological characteristics respond more strongly to elevated levels of CO_2 in leaves with more robust structures, such as evergreen sclerophylls, due to generally large limitations in internal diffusion in C3 plants. Therefore studies involving other species than northern red oak will also be discussed.

2.3.1 Effect of increased [CO_2]

Most authors agree that the effects of increased atmospheric [CO_2] can be divided into effects in the short and in the long term (Cure and Acock, 1986; Gunderson and Wullschleger, 1994; Curtis, 1996; Stirling *et al.*, 1997; Hättenschwiler *et al.*, 1997; Saxe *et al.*, 1998; Curtis and Wang, 1998; Long *et al.*, 2004; Ainsworth and Rogers, 2007; Maier *et al.*, 2008). Plants with the C3 photosynthetic pathway respond in the short term to increased [CO_2] via increased net photosynthesis and decreased transpiration and stomatal conductance. Even in C4 plants, photosynthesis and production may be indirectly increased through improved water status, since stomatal conductance decreases in roughly inverse proportion to the increase in [CO_2] (Myers *et al.*, 1999). In the longer term this increase in photosynthesis is often offset by downregulation of the photosynthetic capacity (Long *et al.*, 2004). Also note that, though most experimental setups were not designed to integrate drought stress, many authors indirectly gather information about plant water relations at elevated [CO_2]. Specific literature on this subject will be reviewed in section 2.3.4.

Myers *et al.* (1999) determined the photosynthetic capacity of adult loblolly pine during one full growth season. They found that an application of ambient + 200 ppm [CO_2] resulted in a maximum increase in photosynthetic rate of 52%. *Glycine max* exposed to 550 ppm CO_2 showed an increase of 17% for net primary production (Morgan *et al.*, 2005). Reviewing FACE experiments, Long *et al.* (2006) found an increase of 13% and 9% for rice, 10% and 13% for wheat, 25% and 19% for soybeans and 0% and 6% for C4 crops, respectively for biomass and photosynthesis. Curtis and Wang (1998) looked at more than 500 reports of effects of elevated [CO_2] on photosynthesis and observed a mean enhancement of 54%. Past experiments may have been exaggerating photosynthetic enhancement by elevated [CO_2] however. Recent studies by Morgan *et al.* (2005); Long *et al.* (2006) show that many of these experiments were conducted within chambers, and that FACE experiments show significantly different results.

While reviewing the literature on the behaviour of C3 plants in a doubled atmospheric [CO_2], Cure and Acock (1986) found that photosynthesis was initially enhanced by 52%, but this enhancement declined to 29% for long-term growth. Gunderson and Wullschleger (1994) examined the effect of elevated [CO_2] on 39 tree species and found that short-term measurements of the [CO_2]-response revealed on average a 65% increase of photosynthesis, which declined to 44% during the longer term measurements. Hättenschwiler *et al.* (1997) compared *Quercus ilex*, grown since they were seedlings and for thirty years under 650 ppm CO_2 to trees of the same age at ambient [CO_2] control sites and noted a 12% increase in final radial stem width for the elevated [CO_2] site, although this stimulation was mainly due to responses when the trees were young. Elevated [CO_2] did have a relatively greater positive effect at any given tree age on tree ring width in years with a dry spring. Another interesting finding was that high [CO_2] grown *Quercus ilex* trees reached the same stem basal area at the age of 26 years as control trees at 29 years, which they proposed was due to a higher turnover of carbon. Contradicting this last study, modeling by Zalud and Dubrovsky (2002) appeared to make adult plants more responsive, which might be explained by their choice of crop, which was corn, a C4 crop. Other findings, such as the increase in biomass and photosynthesis, were compliant with other studies.

Various explanations for and mechanisms of the response of photosynthetic rate and production as well as their downregulation have been proposed. The direct initial effects are summarized in Figure 2.2. Most authors agree that the main factor of photosynthesis enhancement lies with Rubisco (Ribulose-1,5-bisphosphate carboxylase oxygenase) (Saxe *et al.*, 1998; Long *et al.*, 2004; Ainsworth and Rogers, 2007; Niinemets *et al.*, 2011). This enzyme has two main functions, being a carboxylase and an oxygenase, where the first one is one of the crucial steps in the photosynthetic process. The CO_2 specificity factor for Rubisco is the ratio of the specificity for CO_2 relative to the specificity for O_2. The mean estimate for the CO_2 specificity of Rubisco is ca. 90 (range =

60-128) (Ainsworth and Rogers, 2007). Because the ratio of the [CO_2]:[O_2] at the active site is 6.3:263 ca. 0.024, the relative rate of carboxylation to oxygenation in a C3 leaf at 25°C is 0.024x90 ca. 2.2 (von Caemmerer and Quick, 2000). By the end of the century, the [CO_2] at the active site of Rubisco in C3 plants will have risen from 6.3 to 15 μM (based on Houghton *et al.* (2001)), which will increase the photosynthetic rate in two ways. The first one is that at this concentration Rubisco, which is substrate limited under current conditions (von Caemmerer and Quick, 2000), will increase its carboxylation rate. The second way is that an increase in [CO_2] will inhibit the oxygenation reaction, thus reducing the [CO_2] and associated 2PG flux to photorespiratory pathways (Long *et al.*, 2004; Ainsworth and Rogers, 2007). The last effect is that elevated [CO_2] induces decreased transpiration and improved leaf water status via reduced stomatal conductance, which favor increased leaf area growth and thus production (Saxe *et al.*, 1998; Ainsworth and Rogers, 2007).

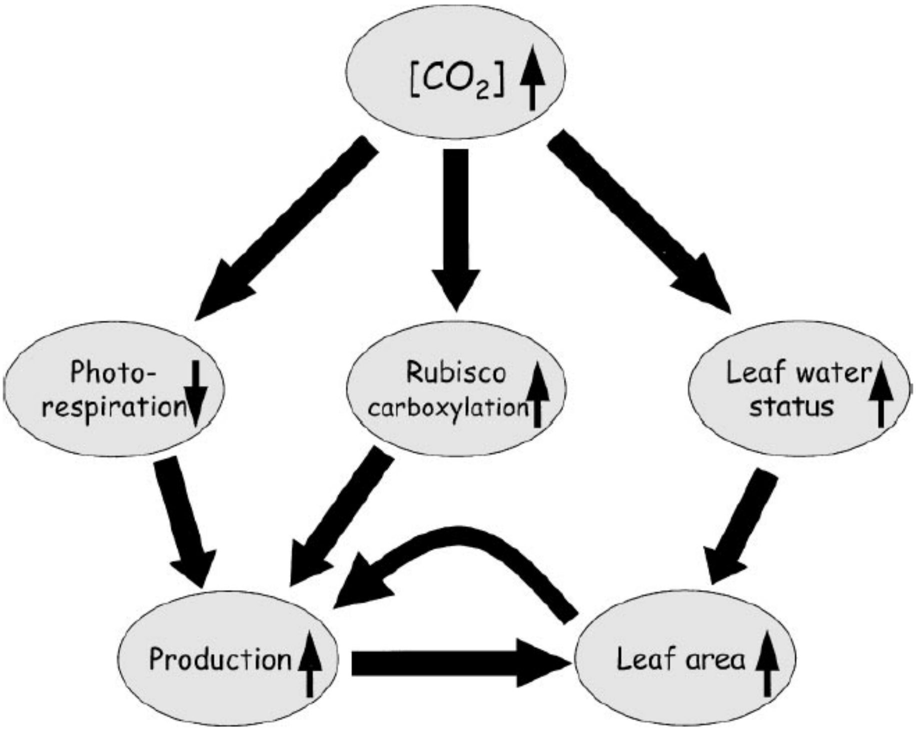


Figure 2.2: Schematic of the direct initial effects of rising [CO_2] on C3 plant production. Taken from Long *et al.* (2004).

It is unclear whether and when photoacclimation or downregulation of photosynthesis under prolonged exposure to elevated [CO_2] occurs. There are studies where no such effect can be observed, while many others show it in a significant manner (Saxe *et al.*, 1998). Regardless of the previous matter, two possible explanations for photosynthetic downregulation in C3 plants have been proposed.

The first explanation is based on the development of a source-sink imbalance. At the cell level and leaf level, the export of C-compounds is rate-limiting, resulting in negative feedback on Rubisco activity and photochemical processes (Saxe *et al.*, 1998). It is suggested that acclimation is caused by an accumulation of soluble sugars, in particular glucose and fructose, with the sucrose content in source leaves reflecting the balance of supply (photosynthesis) and demand (growth, storage, nutrient assimilation), and changes in the sucrose pool being able to communicate whole-plant carbon flux (Long *et al.*, 2004), which are sensed by a hexokinase, if sink strength is insufficient. Increased carbohydrate content is the most pronounced and universal change observed in the leaves of C3 plants grown at elevated [CO_2] (Drake *et al.*, 1997). The sugars reduce the abundance of transcripts of genes involved in photosynthesis and up-regulate the genes for carbohydrate storage and utilization (Gunderson and Wullschleger, 1994; Saxe *et al.*, 1998). The hypothesis would be that excess carbohydrate at elevated [CO_2] feeds back on photosynthetic gene expression and leads to acclimation (Long *et al.*, 2004). Carbohydrate accumulation is often poorly correlated with a loss of photosynthetic capacity however (Moore *et al.*, 1998). Several authors have nonetheless proposed this as a possible reason for their observations of downregulation (Arp, 1991; Myers *et al.*, 1999; Osborne and Beerling, 2003).

The second explanation relies not on the whole-plant based but on the area based photosynthetic rate and lies in a reduction of leaf Rubisco concentration (Saxe *et al.*, 1998). This reduction may be the consequence of a nonselective decrease in leaf nitrogen (N) content (Long *et al.*, 2004) or a redeployment of N within the leaf (Parry *et al.*, 2003; Ainsworth and Rogers, 2007). Several studies have focused on this hypothesis. BassiriRad *et al.* (1997, 2003); BassiriRad (2000) and Zerihun *et al.* (2000) found that a steady uptake of N was indeed of crucial importance in order to maintain an enhanced photosynthetic rate, while Zerihun and BassiriRad (2000, 2001) observed that, regardless of N supply and uptake, *Helianthus annuus* L. showed no acclimation response of any kind. Curtis (1996) and BassiriRad *et al.* (1999, 2001) performed in-field measurements as well as meta-analytical operations and concluded that leaf N was significantly reduced, but only when expressed on a mass basis.

The effects of elevated [CO_2] on plant water relations are various. Without going into details a summary of observed effects will be given. Across a wide range of species (Wullschleger *et al.*, 2002) four main effects could be marked: (i) proliferation of fine roots (*Triticum aestivum* L., Gifford (1979); *Quercus alba* L., Norby *et al.* (1986)); (ii) enhanced water use efficiency (*Medicago sativa* L., Luis *et al.* (1999); *Prunus avium* L., Centritto *et al.* (1999)); (iii) reductions in stomatal conductance (*Acer saccharum* Marshall; *Populus tremuloides* Michx, Holtum and Winter (2010); *Pinus taeda* L., Ellsworth (1999, 2000)); (iv) solute accumulation in the leaves (*Betula populifolia* Marsh, Morse *et al.* (1993); *Pinus taeda*, Tschaplinski *et al.* (1995)). Observed consequences of these changes are roughly (i) higher water availability (Hanson and Weltzin, 2000); (ii) enhanced drought tolerance (Beerling *et al.*, 1996); (iii) reduction of transpiration and increase in plant water potential (Centritto *et al.*, 1999; Medlyn *et al.*, 2001); (iv) osmotic adjustment and dehydration tolerance of leaves (Ellsworth, 1999).

2.3.2 Effect of increased temperature and heatwave induced stress

It was noted in section 2.1 that an annual increase in mean temperature may be attained via different scenarios, and that this increase is increasingly likely to produce itself in the form of seasonal heatwaves. It will be shown in section 4.3 and Figure 4.1 how the occurrence of heatwaves was simulated in this study. Literature on the effect of heatwaves is more recent and will be addressed seperately from the effect of increased temperature, since we expected the effects to be different as well.

The response of the photosynthetic metabolism in C3 plants has been summarized in Figure 2.3. Direct effects occur in the mesophyll and involve changes in the activity of Rubisco (Berry and Björkman, 1980; von Caemmerer and Quick, 2000; Sage and Kubien, 2007; Lloyd and Farquhar, 2008; Yamori and von Caemmerer, 2009). The Rubisco capacity to consume RuBP (ribulose bisphosphate) is generally the predominant limitation on net photosynthesis (A_{net}) at light saturation and CO_2 levels below the current ambient concentration of 380 ppm (Sage and Kubien, 2007). As air temperature rises, A_{net} increases due to increased Michaelis-Menten kinetics (Berry and Björkman, 1980; von Caemmerer and Quick, 2000). At the same time however, an inhibition of the maximum rate of electron transport (J_{max}), associated with an increase in the cyclic flow of electrons around PSI (Lloyd and Farquhar, 2008), causes A_{net} to decrease appreciably before the Rubisco carboxylase has reached its optimum . Also, as temperature increases, the relative rate of carboxylation to oxygenation is reduced and the flux of 2PG (2-phosphoglycolate) into photorespiration increases (Long, 1991). These mechanisms vary across species and are important in order to understand the difference in temperature optima across these species (Sage and Kubien, 2007). It is also possible that very high temperatures cause irreversible destruction of the thylakoids, who are responsible for the regeneration of RuBP, but interestingly enough, the temperature high enough to accomplish this depends upon the temperature at which the leaves have developed (Berry and Björkman, 1980), which could point to a greater adaptational capacity of seedlings.

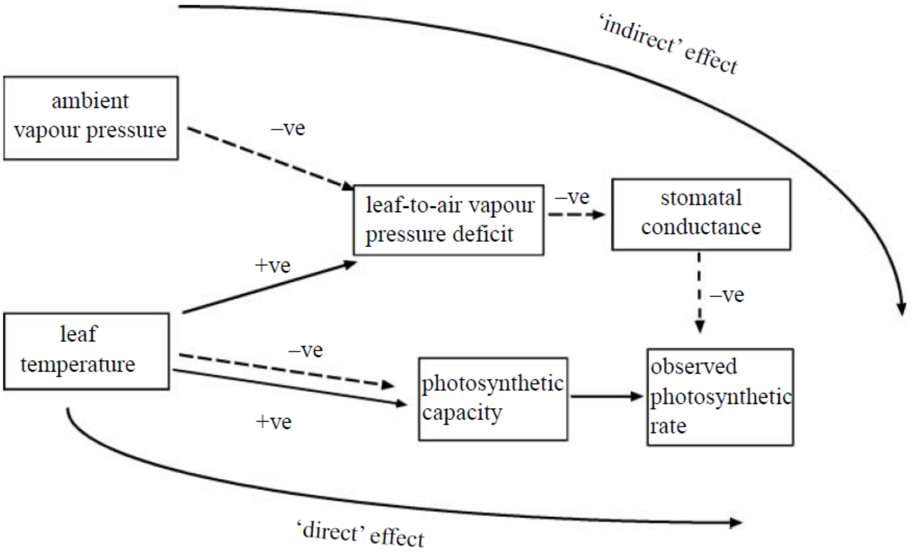


Figure 2.3: Schematic showing direct and indirect effects of temperature on leaf photosynthetic metabolism. In this figure 've' represents the photosynthetic electron chain. From Lloyd and Farquhar (2008).

Indirect effects are situated around the stomatal area and include a decreased solubility of CO_2 (von Caemmerer and Quick, 2000) and the eagerness of the stomata to reduce the rate of water loss through transpiration, caused by an increased evaporative demand, by closing. With this stomatal closure a reduction in CO_2 assimilation rate is associated because CO_2 is being supplied to the chloroplast in a reduced rate (Sage and Kubien, 2007; Lloyd and Farquhar, 2008).

Several studies have focused on the effect of increased temperature on physiological and other parameters. Kouril *et al.* (2004) studied the temperature dependence of chlorophyll fluorescence intensity in barley leaves under weak and actinic light excitation during linear heating from room temperature to 50°C and documented a rise in fluorescence they thought reflected an accumulation of Q_A- (the primary electron acceptor in PSII) even under weak light excitation due to the thermal inhibition of the S_2Q_A- recombination, and and enhanced back electron transfer from Q_B (the secondary electron acceptor in PSII) to Q_A. Loka and Oosterhuis (2010) looked at the short and long term effect of high night temperatures on cotton respiration, ATP levels and carbohydrate content. Both treatment sets showed significant increases of respiration (up to 56% and 39% respectively) and decreases in leaf ATP levels. Carbohydrate content was unchanged during the short term experiments but was reduced significantly (up to 70% for sucrose and 39 % for hexose) during the long term experiments. Dias *et al.* (2011) examined the photosynthetic responses to heat stress in *Triticum aestivum* L. and *Triticum turgidum* subspecies *durum*. *Triticum aestivum* revealed a significant decrease in A_{net} and a significant increase in C_i, whereas these parameters in *Triticum turgidum* shifted in the opposite direction. Contradicting these findings, they also measured that the maximum photochemical efficiency of photosystem II (F_v/F_m) decreased significantly for *Triticum turgidum* and increased significantly for *Triticum aestivum*. Both species showed a decrease in mean stomatal conductance. They hypothesized that, even within such close related species, these differences were due to different protection mechanisms and genomic expressions. Yamori *et al.* (2010, 2011) performed experiments on the effect of light intensities and leaf nitrogen content on the temperature dependence of the CO_2 assimilation rate. They found that the chloroplast CO_2 concentration (C_{trans}), at which the transition from RuBP carboxylation to RuBP regeneration limitation occurred, increased with leaf temperature and was independent of growth light intensity (Yamori *et al.*, 2010), and that the limiting step of photosynthesis at different temperatures depended on leaf N content and was mainly determined by N partitioning between Rubisco and electron transport components (Yamori *et al.*, 2011). Integrating these two findings, one could conclude that the leaf N partitioning changes with temperature as well.

As with elevated [CO_2], it is unclear whether and when acclimation of photosynthesis and other parameters under prolonged exposure to elevated temperature occurs. In this case the underlying mechanisms are less defined and highly species dependent (Berry and Björkman, 1980), which may well explain whether plants are able to acclimate or not, since several authors (Teskey and Will, 1999; Ow *et al.*, 2008a,b; Nedlo *et al.*, 2009) recorded a significant response yet no signs of acclimation to elevated temperature.

It is getting increasingly likely that extreme events such as heat waves will be an unavoidable consequence of climate change (Karl and Nicholls, 1997; Keeling and Whorf, 2005; IPCC, 2007a). To date, however, experiments on the impacts on plants are scarce (Boeck *et al.*, 2010). Literature found on this topic was indeed scantily available and quite recent. It is however very important that more research on this subject is conducted, in order to to identify the types and time-scales of climate extremes and to provide a mechanistic framework for extreme climatic events and a definition of extremity (Smith, 2011a,b). Marchand *et al.* (2006) simulated heatwaves using infrared irradiation sources and observed different results during two consecutive heatwaves (10 d each, with a 5-d recovery period in between) in the arctic species *Pyrola grandiflora*, *Polygonum viviparum* and *Carex bigelowii*. Plant conditions, such as leaf relative growth, leaf chlorophyll content and maximal photochemical efficiency were increased during the first heatwave. During the second heatwave, leaf mortality increased, which they hypothesized was indicating that the heat stress lasted too long and negatively influenced the species resistance to high temperature. Even more interesting was that, when plants were exposed to (low) ambient temperatures again, plant performance deteriorated further, possibly indicating loss of cold resistance. Bragazza (2008) assessed the impact of an extreme heat wave on peat mosses of the genus *Sphagnum* and documented an increased mortality of peat mosses forming high hummocks. Important to know is that the heatwave coincided with a drought spell and the distribution of desiccated peat mosses was restricted to the hummock face receiving the greatest amount of solar irradiation. Jentsch *et al.* (2011) reported similar results, with water availability being the determining factor of survival rate.

In this context, Hüve *et al.* (2011) examined in which measure heat stress of varying duration caused instability of photosynthesis in relation to respiratory burst, cell permeability changes and H_2O_2 formation in *Phaseolus vulgaris*. They reported that A_{net} was already strongly inhibited before the temperature (48°C) at which a rise in minimum dark fluorescence and a burst of respiration, during which H_2O_2 was released, was observed. Further increasing the temperature resulted in enhanced membrane permeability. They also stressed the importance of the heat dose (amount of heat over the entire duration of the heat pulse) received and the implications towards a 'point of no return', which indicated irreversible cellular damage and reduction of the photosynthetic metabolism because of H_2O_2 accumulation.

2.3.3 Effect of drought induced stress

There exists little doubt about the absolute necessity of water to plants. Therefore only recent literature on the influence of drought stress will be provided in this section. General information will be given when relevant in Chapter 6.

Sanchez-Rodriguez (1999) examined the effect of long term drought stress on photosynthetic carbon metabolism in *Casuarina equisetifolia*. They observed that CO_2 assimilation decreased while C_i remained high, and suggested this was due to drought stress induced stomatal closure. Activities and activation states of Rubisco remained unaltered, while the leaf concentration of photosynthetic metabolites, glucose, fructose and sucrose decreased, pointing to a possible redistribution of carbon. This was investigated by Dai *et al.* (2007), who researched the effect of short (3 d) and longer (15 d) term drought stress on the distribution of newly-fixed ^{14}C-photoassimilate in one year old *Malus domestica* Borkh. seedlings. For the first 5 days of drought stress, the enhanced sink strength of fine roots in prolonged plants compensated for the reduced carbon availability due to the decreased total carbon fixation. As a result, fine roots obtained the same or even greater amounts of ^{14}C-photoassimilates than those of the control plants, but the compensatory effect became insufficient as drought stress continued. When the plants were rewatered, 5-day lags occurred for the carbon fixation rate. The amount of photoassimilates obtained by the fine roots in the drought stressed plants did not return to pre-stress levels but remained at higher levels. This greater investment of photoassimilate into the roots during the rewatering period was thought to provide abundant carbon substrates and energy for the restoration of the metabolic activity of the roots. An earlier study by Chiatante *et al.* (1999) also documented increased investment of photoassimilates into the root system, but in another way. *Pinus nigra* fine root meristems were not supported during nor recovered after periods of drought stress. Instead new lateral meristems from the cortical tannin zone above the fine root tip were formed.

Many other studies found a positive correlation between drought stress and a reduction in photosynthetic capacity due to a change in whole tree as well as leaf level water relations (Haupt-Herting and Fock, 2001; Catovsky *et al.*, 2002; Turnbull *et al.*, 2002; da Silva and Arrabac, 2004; Samarah, 2005; Pérez *et al.*, 2007). Others found that the main factor controlling stomatal mechanisms was the availability of water (Catovsky *et al.*, 2002; Ribas-Carbo *et al.*, 2005; Buckley, 2005). Caspersen and Kobe (2001) observed that the relationship between probability of mortality and growth did not vary with soil moisture for *Quercus rubra*, *Tsuga canadensis* and *Pinus strobus* but did so for other tree species. Ellsworth (2000) looked at seasonal CO_2 assimilation in a *Pinus taeda* canopy and concluded that summer drought, rather than high temperature, had negative impact on A_{net} and annual CO_2 uptake.

Other interesting findings about possible protective mechanisms were the synergistic reduction of plants under drought stress and N-fertilization, meaning that over time water deficiency severely lowered leaf water potential and, together with fertilization, almost totally depressed nitrogenase activity in *Leucaena* seedlings (Mrema *et al.*, 1997). Drought stress in *Zea mays* L. induced abscisic acid accumulation but this accumulation was not inhibited by free radicals nor oxidants, but only by reducing agents, which has consequences on the current view on dehydration induced free radicals (Jia and Zhang, 2000). Japanese mountain birch (*Betula ermanii*) leaves showed a higher rate of electron transport at low C_i after long-term drought acclimation (Kitao *et al.*, 2003).

2.3.4 Interactions of environmental conditions

In this section we will review literature on possible interactions between combinations of afore mentioned parameters, since we would suspect that some parameters would mitigate each other, while others may show synergystic interactions (Saxe *et al.*, 1998).

Interaction between [CO_2] and temperature

A considerable amount of studies has been performed on the interaction between elevated [CO_2] and temperature, probably due to the majority of climate change models pointing to a rise of both these parameters (see section 2.1). As for the occurrence of an interaction between elevated [CO_2] and temperature, it should be stressed that opposite observations have been reported across and within (*Acer sachharum*, Williams *et al.* (2003); Norby *et al.* (2000)) species.

It was well described in section 2.3.1 that one of the main effects of elevated [CO_2] is improved plant water relations, whereas the opposite can be said for elevated temperature. This leads to a shift in drought resistance and response strategies (Saxe *et al.*, 1998; Wullschleger *et al.*, 2002; Long *et al.*, 2004). One such response was determined by Cen and Sage (2005) on sweet potato (Figure 2.4). Elevated [CO_2] increased the A:T slope as well as the temperature optimum, while the breadth of this optimum is narrowed. It is also shown clearly how P_i regeneration limitation becomes more pronounced with rising CO_2 levels (Sage and Kubien, 2007).

Guak *et al.* (1998) grew Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings at elevated

$[CO_2]$ (+200 ppm) and temperature (+4°C) for 2.5 years. They reported no interaction effects on cold hardiness of the needles, but elevated CO_2 level did significantly reduce the negative impact that elevated temperature had on bud burst and growth. Lin *et al.* (1999) investigated the possible impact of a CO_2-temperature interaction on soil CO_2 efflux of Douglas fir, but found no significant effects.

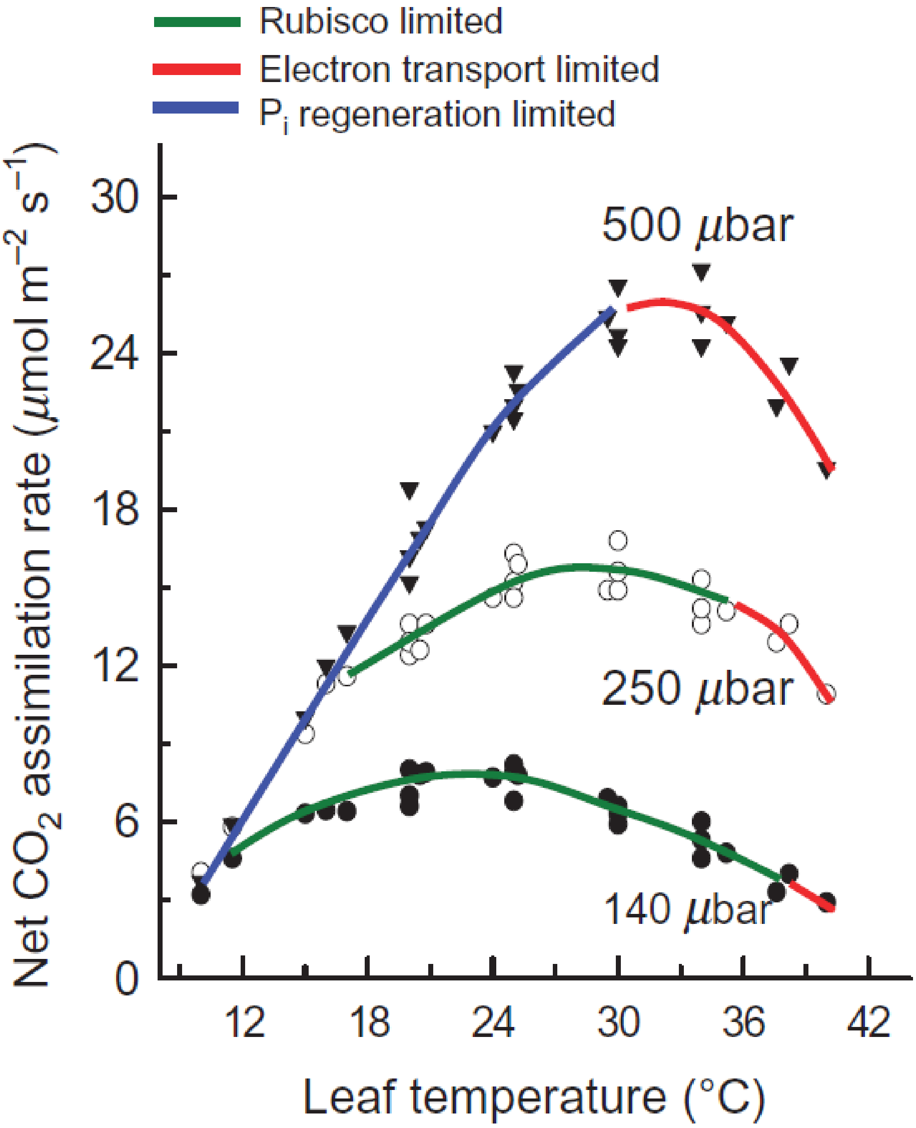


Figure 2.4: A_{net} response to different levels of intercellular $[CO_2]$; P_i= inorganic phosphate. Data from Cen and Sage (2005), full lines were modeled after Farquhar *et al.* (1980).

Tingey *et al.* (2007) reported that elevated temperature stimulated photosynthesis in Douglas fir systems, but that elevated $[CO_2]$ showed no effect in the elevated temperature treatment, which they thought was due to a change in needle area. In an earlier study it was found that long term exposure to elevated temperature and [CO_2] altered nitrogen allocation, but in the opposite manner. Therefore the combination yielded no significant changes to the ambient control plants (Tingey *et al.*, 2003). Lewis *et al.* (2001) found no interaction effects either, suggesting that the effects of elevated [CO_2] on net photosynthetic rates in Douglas fir are largely independent of temperature. In a follow-up study, where they focused on water relations, results showed that elevated [CO_2] did not significantly ameliorate temperature induced water loss and reduction of water use efficiency (Lewis *et al.*, 2002).

Zha *et al.* (2003) measured respiration rates of Scots pine (*Pinus sylvestra*), grown for 4 years in different [CO_2] and temperature treatments. Although he did not find any significant difference in growth respiration, maintenance respiration in the combined elevated treatment differed sizeably (58%) from the other treatments. An interactive effect ($P < 0.05$) was also observed on stomatal density and concentrations of nutrients (Luomala *et al.*, 2005), whereas Kilpeläinen *et al.* (2006) did not find any CO_2-effect across temperature treatments on Scots pine height growth. In a study on secondary compounds in needles it was reported that, though temperature alone did not have any significant effect, it enhanced the effect elevated [CO_2] had. Surprisingly enough, in a cocurrent study, it was shown that the combination of elevated [CO_2] and temperature significantly increased monoterpene emission, while each factor alone decreased it (Räisänen *et al.*, 2008a,b).

Examples of reported interactive effects of elevated temperature and [CO_2] are *Yucca whipplei*, rice (*Oryza sativa*), soybean (*Glycine max*), *Poa annua*, dwarf apple (*Malus domestica*), sugar maple (*Acer saccharum*) and wheat (*Triticum aestivum*) (Vu *et al.*, 1997; Stirling *et al.*, 1997; Huxman *et al.*, 1998; Ro *et al.*, 2001; Norby *et al.*, 2000; Alonso *et al.*, 2008), while no interactive effects were found in *Poa alpina, Bellis perennis, Plantago lanceolata, Helianthemum nummularium*, loblolly pine, sugar maple, *Phytolacca americana, Eucalyptus saligna* or *Eucalyptus sideroxylon* (Stirling *et al.*, 1997; Teskey, 1997; Williams *et al.*, 2000, 2003; He *et al.*, 2005; Ghannoum *et al.*, 2010).

A last interesting finding was that, in grain-sorghum (*Sorghum bicolor* (L.) Moench), elevated [CO_2] exacerbated the negative high temperature effects on yield and harvest, because it would significantly increase tissue temperatures compared to the ambient treatment (Prasad *et al.*, 2006).

Interaction between [CO_2] and drought induced stress

The physiological mechanism behind CO_2-induced increases in plant water relations was amply addressed in section 2.3.1. The importance of the integration of a water availability component in elevated [CO_2] research has been stressed by Saxe *et al.* (1998); Ellsworth (1999); Wullschleger *et al.* (2002) and Holtum and Winter (2010). Following are studies conducted with the mensurable intent of examining elevated [CO_2] and drought stress interactions.

In a 30 year experiment on *Quercus ilex* a significant beneficial effect of elevated [CO_2] on tree water relations under drought stress was indicated (Hättenschwiler *et al.*, 1997). [CO_2] levels of up to 700 ppm delayed drought induced growth inhibition in rice (*Oryza sativa*) and were concluded to provide a reduction of near 10% in water use (Baker *et al.*, 1997). A similar experiment was performed by Widodo *et al.* (2003) and examined diurnal variations and recovery rates. It was found that plants under CO_2 levels of 700 ppm were able to maintain midday leaf photosynthesis where ambient [CO_2] plants were not, and that they recovered to their original rate of photosynthesis more rapidly. Elevated [CO_2] (700 ppm) resulted in a 34% increase in A_{net}, a 31% decrease in g_s in well watered northern red oak seedlings, where drought stressed seedlings demonstrated up to a 69% increase in A_{net}, and only a 23% decrease in gs (Anderson and Tomlinson, 1998). It was this increase that was considered to provide extra carbon resources to drought stressed seedlings, decreasing the sink strength of the plant roots (Tomlinson and Anderson, 1998).

Johnson *et al.* (2002) found a mitigating effect of drought stress by elevated [CO_2] (700 ppm) in willow (*Salix* spp.), but not in poplar (*Populus* spp.). Belote *et al.* (2003) found that, during a wet year, total above-ground net primary productivity (ANPP) in *Lonicera japonica* and *Microstegium vimineum* communities subjected to elevated [CO_2] (565 ppm) did not differ from the ambient control communities. During a dry year, total ANPP was significantly different in both treatments. Experiments on cherry (*Prunus avium*) seedlings resulted in quite different findings. [CO_2] levels of 700 ppm showed no fundamental change in the plant response to drought stress, which may have indicated that increased diffusional limitations due to this drought stress obstructed higher C_i levels in the seedlings (Centritto, 2005). Corn plants treated with elevated [CO_2] (800 ppm) were reported to withstand severe drought stress considerably better than untreated plants and even used up to 49% less water (Chun *et al.*, 2011).

Interaction between temperature and drought induced stress

Few will doubt the devastating effect the simultaneous currence of drought and extreme heat may have on plant physiology. Some literature examples could still provide a reference situation for the damage inflicted without the interference of increased [CO_2] however, which should be helpful to place the next section in perspective.

Although known for remarkable acclimation capacity, the schlerophyll shrub *Heteromeles arbutifolia* was not able to maintain its leaf temperature under the limits of chloroplast thermostability when exposed to both heat (above 40°C) and drought (diurnal average relative humidity below 30%) (Valladares and Pearcy, 1997). When temperature was above 35°C, thermostability of PSII was strongly enhanced in drought-stressed leaves of wheat (*Tritium aestivum*), and no significant differences in fluorescence parameters were recorded between moderately and severely drought-stressed plants, indicating that drought stress modified the PSII chemistry rather in advance by down-regulating photosynthetic electron transport (Lu and Zhang, 1999). It was suggested by Xu and Zhou (2005) that an increase in mean temperature significantly exacerbated the adverse effects of soil drought stress, and that their synergistic interactions might reduce *Leymus chinensis* productivity in the future. In alfalfa plants, elevated temperature and drought reduced plant dry mass and leaf area, especially when both stresses were combined (Aranjuelo *et al.*, 2007). There was no difference in photosynthesis in relation to water availability however, which could mean the reduced growth was caused by a reduction in leaf area.

Bragazza (2008) performed a study that identified a climatic treshold, defined by the ratio of precipitation to temperature. When mean monthly P:T dropped beneath this treshold, which they recorded as 6.5 mm:°C, an irreversible dessication of peat mosses was triggered. After a period of 4 years of there was still no sign of any recovery since the drought, which could be bad news for organisms not able to adapt to abrupt climate anomalies.

Boeck *et al.* (2011) conducted a combined factorial study on experimental plant communities containing three common herbaceous species (*Plantago lanceolata, Rumex acetosella* and *Trifolium repens*). Since they observed that heat waves are usually preceded by a dry period (Boeck *et al.*, 2010), they subjected the plants to drought periods of up to 31 days, but only imposed the heating treatment during the last 10 days of the drought. They found that treatments of just heat or drought did show that the photosynthetic rate was lowered, but recovered after the treatments. The combined effect of a heat wave and a drought however was so dramatic that photosynthetic rates decreased more markedly than in the other treatments (to near zero), and never recovered back to control levels.

[CO_2], temperature and drought combined

Very few studies on this combination could be found, which is surprising taking into account that there is an impressive amount of literature on climate change impact. A possible explanation for this might be that most climate change models focus on [CO_2] and temperature, without the explicit allusion to increased drought. As found by Boeck *et al.* (2010) however, heatwaves are often anticipated by drought spells.

One of the earliest studies was performed by Hamerlynck *et al.* (2000), who subjected 1-year-old Mojave Desert evergreen shrub (*Larrea tridentata*) seedlings for 9 days to air temperatures of up to 53°C, CO_2-levels of 360, 550 and 700 μmol mol^{-1} and two water regimes (well-watered and drought-stressed). In 5 of the 6 treatments, heat stress markedly decreased net photosynthetic rate, stomatal conductance, and the photochemical efficiency of photosystem II. Only the well-watered treatment of 700 ppm [CO_2] maintained A_{net} and g_s similar to pre-stress levels.

Qaderi *et al.* (2006) grew canola (*Brassica napus*) plants under two temperature (22 and 28°C), two [CO_2] (370 and 700 ppm) and two watering (watered to field capacity and wilting point) regimes. They conducted three successive experiments on the plants in which they found that drought-stressed plants grown under higher temperature at ambient [CO_2] had decreased stem height and diameter, leaf number and area, dry matter, leaf area ratio, shoot/root weight ratio, net CO_2 assimilation and chlorophyll fluorescence. Elevated [CO_2] and high water availability significantly reduced the negative effect of temperature on these parameters. Wertin *et al.* (2010) investigated the effect of elevated [CO_2] (700 ppm), elevated temperature (+2°C above ambient) and decreased soil water availability on net photosynthesis and water relations of one-year old loblolly pine (*Pinus taeda*) seedlings across the species' native range. The seedlings showed little temperature effect, but A_{net} was significantly increased by elevated [CO_2], which also mitigated drought induced A_{net} reductions.

In a more recent paper on the effect of extreme drought events in a future climate, grassland communities were subjected to several drought levels (0, 15, 22 and 35 days of drought), two temperature levels (ambient and ambient +3°C) and two CO_2-levels (ambient and 620 ppm), and overall plant productivity was determined throughout the growing season. Their results were surprising, since at the end of the growing season, both in the ad- and absence of drought, community productivity showed no response to the future climate (Naudts *et al.*, 2011). Different drought treatments did have a significant effect on the evolution of the productivity during the season however. Early in the growing season plant productivity was positively affected by future climate in the absence of drought,. but this effect tended to turn negative later in the season, resulting in a disappearance of the overall effect. During drought there was a stronger decrease in A_{net} in future than in current climate due to stronger stomatal closure, which is inconsistent with results in other species. Therefore the benificial effect of increased [CO_2] stagnated. After a period of recovery, plant productivity was not affected by climate at the end of the growing season.

A last study examined the combined effect of elevated [CO_2] (510 ppm, CO_2), night-time warming (ambient +1.4°C, T) and drought (D) on evergreen dwarf shrub (*Calluna vulgaris*) (Albert *et al.*, 2011). They found a synergistically stimulating interaction of T X CO_2 and an antagonistic interaction between D X CO_2 on plant carbon uptake. The response of photosynthesis in the full factorial combination ($TDCO_2$) could be explained by the main effect of experimental treatments (T,D,CO_2) and the two-factor interactions (D X CO2, T X CO_2).

2.4 Current level of knowledge and conducted studies

This brief summary of current available literature allows us to abstract some things:

- Elevated [CO_2] is expected to enhance A_{net} and overall production, although this may be downregulated after prolonged exposure or at later development stages
- An increase in mean temperature will shift plants more towards or more away from their temperature optimum

- The occurrence of heatwaves will sizeably stress plant communities, though the measure of this stress is dependent on the length and severity of the heatwave
- Elevated [CO_2] may mitigate negative effects of drought and heatwaves
- The effects of combined changes in environmental conditions is far more complicated than the mere combination of their effects

As for the current level of conducted studies:

- There have been few studies on interactive effects on trees, and even fewer on broadleaf trees
- There is a rising tendency towards research on climate extremes (heat waves and drought spells)
- Different species show different reactions, pointing towards different response mechanisms

We need more studies of interactive effects, including a wider range of species grown under different climates. (Long *et al.*, 2004). Multifactor experiments remain important, for testing concepts, demonstrating the reality of multiple factor influences, and reminding us that surprises can be expected (Norby and Luo, 2004).

Chapter 3

Objectives

There is little to be found in literature concerning the interaction of temperature, water and CO_2 and its influence on physiological parameters such as photosynthesis, fluorescence and stomatal conductance. The main objective of this master thesis will be to determine the effect of water availability during a heat wave on water relations and photosynthesis of *Quercus rubra* L. in ambient and elevated [CO_2]. Our specific working hypotheses were:

Hypothesis 1: Seedlings in the low water treatment will have reduced photosynthesis and transpiration compared to those in the high water treatment.

- Photosynthesis and transpiration will be lower in low water seedlings compared to high water seedlings both prior to and during a heat wave, regardless of the time of the day.
- The decrease in photosynthesis and transpiration from the morning to the afternoon will be greatest in low water seedlings compared to high water seedlings.
- Heat waves will reduce photosynthesis and transpiration to a greater extent in the low water treatment than in the high water treatment.

Hypothesis 2: Elevated [CO_2] will mitigate heat stress induced reductions in photosynthesis in both low and high water treatments, though the effect will be greatest in low water seedlings.

- Seedlings grown in the elevated [CO_2] treatment will have a higher rate of photosynthesis prior to the heat wave event, regardless of the water treatment.

- Seedlings grown in the elevated [CO_2] treatment will have photosynthesis reduced to a smaller extent than seedlings in the ambient [CO_2] treatment, regardless of the water treatment.

- Photosynthesis will be greatest in the high water and elevated [CO_2] treatment and lowest in the low water and ambient [CO_2] treatment prior to heat waves.

- Seedlings from the high water and elevated [CO_2] treatment will have the smallest reduction in photosynthesis with heat wave induced stress, while seedlings grown in the low water and ambient [CO_2] treatment will have the greatest reduction in photosynthesis.

Chapter 4

Materials and methods

4.1 Study site

The study site was located at Whitehall Forest of the University of Georgia in Athens (33°57’N, 83°19’W, altitude 230 m). Eight treatment chambers, half-cylinder in shape and measuring 3.62 m length by 3.62 m width by 2.31 m height were constructed at the site. The treatment chambers were constructed according to the method described in Boyette and Bilderback (1996) with lumber bases and PVC pipe frames supporting 6 mil clear polyethylene film (GT Performance Film Green-Tek Inc. Edgerton, WI, USA). The chambers were placed in an open field, spaced 3.7 m apart to minimize shading and oriented facing south to maximize daily sun exposure. Each of the eight chambers had the same dimensions, the chambers were oriented in the same direction and all equipment within the chambers was located at the same spot to minimize any potential chamber effect.

4.2 Plant material

Seedlings of northern red oak (*Quercus rubra* L.) were planted December 1, 2009 in 0.5 L pots in potting medium. The red oak seeds were obtained from a wild collection in Tennessee (Louisiana Forest Seed Company, Lecompte, LA USA). The northern red oak provenance is from the south region of the species range (Figure 2.1).

The average stem height and diameter of the seedlings before the start of the experiment (June 28, 2010) were 31.2 cm and 7.5 mm. The seedlings were watered 4 times a day until saturation. In April, May, June and July each pot was fertilized with 30 g of 15-9-12 extended release fertilizer (Osmocote plus #903286, Scotts-Sierra Horticultural Products, Marysville, OH, USA). In May, 0.04 mL Imidacloprid was applied topically to the soil in each pot to control pests (Bayer Advanced 12 months tree and shrub insect control; Bayer; Monheim am Rhein, Germany). A topical fungicide was sprayed on the seedlings in June.

4.3 Experimental design and setup

The design and setup of this experiment were based on Wertin *et al.* (2010). Each chamber was assigned to one of sixteen treatment combinations. These combinations were produced by three factors, namely atmospheric [CO_2], chamber temperature and watering scheme. The [CO_2] treatment discerned ambient [CO_2] (C_A) and elevated [CO_2] (C_E). Ambient and elevated atmospheric [CO_2] treatments were 380 μmol CO_2 mol^{-1} and 700 μmol CO_2 mol^{-1}, respectively. The temperature treatment discerned respectively 0°C (T_0), 3°C (T_3), 6°C (T_6) and 12°C (T_{12}) above the ambient temperature measured outside the chambers. The water treatment involved watering to field capacity (H) and watering to a volumetric water content just above the wilting point (L). These treatment combinations are summarized in Table 4.1.

Table 4.1: Treatment combinations

				Temperature (°C)			
				+0	**+3**	**+6**	**+12**
CO_2	**Ambient**	**H_2O**	**High**	C_AT_0H	C_AT_3H	C_AT_6H	$C_AT_{12}H$
			Low	C_AT_0L	C_AT_3L	C_AT_6L	$C_AT_{12}L$
	Elevated	**H_2O**	**High**	C_ET_0H	C_ET_3H	C_ET_6H	$C_ET_{12}H$
			Low	C_ET_0L	C_ET_3L	C_ET_6L	$C_ET_{12}L$

This setup allows investigation of the occurrence of a mitigating effect due to higher CO_2-concentrations. Treatments were initiated May 2, 2010. In this study we obtained data from before, during and after the heat wave applied from 20 to 26 August.

Target air temperatures in the chambers were the ambient temperature +0°C (T_0), +3°C (T_3), +6°C (T_6) and +12°C (T_{12}). In order to simulate heat waves we subjected the + 6°C and +12°C treatment respectively each two weeks and each four weeks to a heat wave for the duration of one week. The +0°C treatment was used as a reference treatment to represent the current situation, whereas the +3°C treatment was used as a control to determine the effect of an increased heat sum on plant physiology. The temperature treatment scheme is summarized in Figure 4.1.

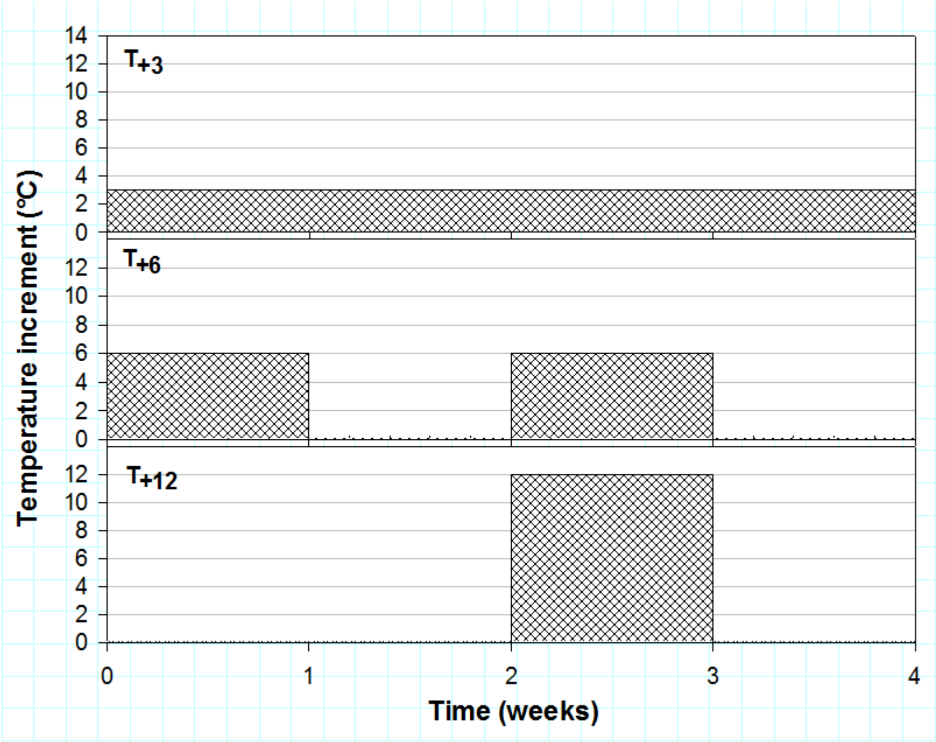


Figure 4.1: Heat treatments

To maintain [CO_2] at the desired levels, a nondispersive infrared CO_2-sensor (Model GMT222, Vaisala Inc., Woburn, MA, USA) continuously measured [CO_2] in each chamber and directly controlled a solenoid valve which released CO_2 into the chambers as necessary from a cylinder of industrial grade compressed 100% CO_2 (Airgas National Welders, Toccoa, GA, USA). An oscillating fan was placed within each chamber to disperse the CO_2 evenly throughout the chamber.

Chamber temperature was measured by type T thermocouples every 3 min and averaged and recorded every 15 min using a datalogger (Campbell 23X, Logan UT, USA), which continuously compared the air temperature inside each chamber to the outside temperature measured with matched thermocouples 1.45 m south of the chambers. Each thermocouple was housed in a ventilated radiation shield (Model SRS100, AmbientWeather, Chandler, AZ, USA) mounted on a pole 1 m above ground level. The dataloggers controlled an air conditioner (Model FAM186R2A, Frigidaire, Augusta, GA, USA) to maintain the treatment air temperatures. Additionally, in the elevated temperature chambers a time switch controlled heaters (Model 3VU33A, Dayton Electric, Niles, IL, USA) to increase air temperature at night and on cloudy days.

Volumetric water content in the pots was measured by ECH2O EC5 soil moisture probes (Decagon Devicesand) and recorded every 10 min using the 23X datalogger. Probes were inserted in 4 plants per water treatment per chamber. For the high water treatment, probes served as a control for the watering scheme, which provided the treatment with 4 watering events of 6 minutes, during which 189.3 mL was applied via an automated irrigation system and drip emitters (Supertif - PLASTRO, Kibbutz Gvat D. N. Ha'Amakim, Israel). For the low water treatment, when the soil moisture content dropped below a treshold value, the datalogger initiated a signal that activated a solenoid and all the low water treatment oaks for that chamber received 5.25 mL water over 10 seconds.

Treatments were initiated May 2 2010. Chamber air vapour pressure deficit was neither measured nor controlled.

Photosynthetic active radiation (PAR) was measured outdoors and inside one chamber with quantum radiation sensors (Model LI-190SZ, LiCor Biosciences, Lincoln, NE, USA). PAR sensors were mounted on top of the leveled radiation shields. All PAR sensors were connected to the datalogger (Campbell 23X, Logan UT, USA) and recorded at the same frequency as temperature.

Thirty seedlings of northern red oak were placed in each chamber. Northern red oak seedlings were placed together with *Pinus taeda* seedlings (from a separate experiment, Ameye *et al.* (2011)), with each species separate to a side of the chamber and randomly assigned to one of six blocks per side; three blocks were assigned to a high water treatment and the three others to a low water treatment. Thus the *Pinus taeda* seedlings were placed on one side of the chamber and the northern red oaks on the other side. Pots were evenly spaced within the chamber. Each month treatment combinations, blocks, and pots within blocks, were randomly rotated between the houses to minimize any potential chamber effect. An overview of the setup of these pots is given in Figure 4.2.

4.4 Measurements

4.4.1 Measurement protocol

To determine the effect of heatwaves on gas exchange, measurements were made prior to, during, and after one representative heat wave. Measurements were conducted two times per day (09.00 and 15.00 hours) on four seedlings from each of the sixteen treatment combinations (temperature, [CO_2], and water). The measurements took place on ten days during the growing season, of which three days before the start of the heat treatment (August 12, 18 and 19), four days during the heat treatment (August 20, 23, 24 and 26) and three days after the heat treatment (August 27, 29 and 31). To maximize the amount of PAR as well as heat stress experienced by the seedlings, measurements were performed on sunny or mostly sunny days. All measurements were conducted on representative foliage from the most recent fully developed flush, which was the only flush that had grown that season for most seedlings.

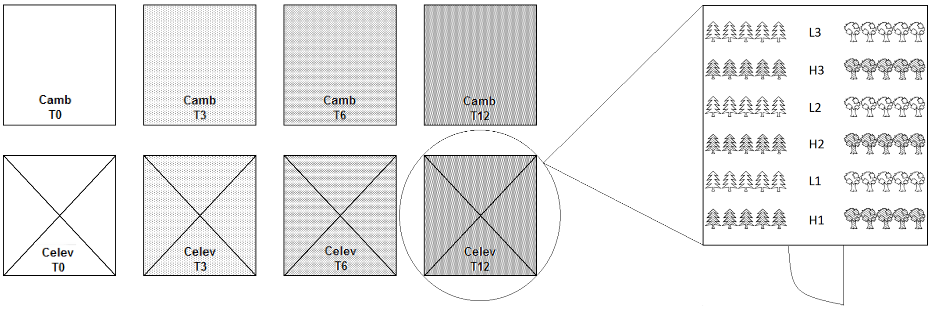


Figure 4.2: Experimental setup

4.4.2 Open flow gas exchange system

Physiological parameters were measured using a portable photosynthesis system (Model LI-6400, LiCor Biosciences, Lincoln, NE, USA) fitted with a fluorescence head (6400-40 Leaf Chamber fluorometer, LiCor Biosciences, Lincoln, NE, USA). Net photosynthesis (A_{net}), stomatal conductance (g_s), vapour pressure deficit (VPDl) internal [CO_2] (C_i) and transpiration were measured on the selected seedlings throughout the experiment. The chamber temperature of the fluorescence head was set to match the actual temperature measured in the treatment chambers at the start of the measurement. The light source of the fluorescence head was set at 1500 μmol photons $m^{-2}s^{-1}$,

[CO_2] was set to either 380 or 700 μmol CO_2 mol^{-1} for C_A and C_E respectively (LI-COR Biosciences, 2008).

4.4.3 Fluorescence parameters

Fluorescence parameters were also measured using the portable photosynthesis system (Model LI-6400, LiCor Biosciences, Lincoln, NE, USA) fitted with a fluorescence head (6400-40 Leaf Chamber fluorometer, LiCor Biosciences, Lincoln, NE, USA). When performing measurements it was made sure that the cross-section of the cuvette was entirely covered by the measured leaf. F'_v/F'_m Φ_{PSII} and Q_p were measured on the same seedlings as the physiological parameters. These parameters were used to determine whether the seedlings had sustained any damage and whether a change in photosynthetic metabolism had taken place.

4.4.4 Other calculations

Instantaneous water use efficiency (WUE_I, μmol CO_2 mmol^{-1} H_2O) was calculated for each seedling at each measurement time by dividing the rate of A_{net} by the rate of transpiration to obtain a value which was an indication for the amount of carbon fixated per amount of water used.

Leaf vapour pressure deficit (VPDl, kPa) was also calculated by the LI-COR system, but we found that the system used an equation dating from 1981 (LI-COR Biosciences, 2008) , which is why we recalculated it as described in Goldstein *et al.* (1998), which confers more updated values to the constants in the empirical equation:

VPDl$=0.6108 \cdot exp\left(\frac{17.27 \cdot T_l}{T_l+237.3}\right) \cdot \left(1-\frac{RH}{100}\right)$,

where T_l is the leaf temperature (°C) and RH (%) is the relative humidity of the chamber air surrounding the leaf.

4.5 Data analysis

4.5.1 Statistical approach

An average from the three measurement days before the heat wave was taken to serve as a baseline. Values from before the heat wave of physiological parameters such as A_{net} were compared to the first and last day of the heat wave and to the first and the last measurement after the heat treatment. This facilitates checking for accumulative damage during the heat wave and whether there was any recovery after the heat wave.

There were a total of 16 treatment combinations in this study: 2 [CO_2] treatments x 4 temperature treatments x 2 water treatments. Measured and calculated parameters were analysed using a three-way repeated measures analysis of variance (RMANOVA) with treatment period (three levels) and time of day (two levels) as fixed factors on a physiological parameter (i.e. A_{net}, g_s, WUE_I, VPDl, E, Φ_{PSII}, F'_v/F'_m and Q_p). One-way ANOVAs, accompanied by Holm-Sidak's multiple comparison test, were used to determine differences among treatments in each parameter

4.5.2 Software

Statistical tests were performed using proc mixed (mixed model) analyses with contrast statements and multiple-means comparisons to test the effect of treatment in SAS 9.2 (SAS Institute Inc., Cary, NC, USA) and R (R.Development.Core.Team, 2009).

Visualizations and regressions were performed using Sigmaplot 11.0 (Systat Software Inc., Chicago, IL, USA).

Chapter 5

Results

Net photosynthesis (A_{net}) is considered as one of the most important parameters when looking at plant health and performance (Pinto, 1980; Longstreth and Nobel, 1980; Saxe *et al.*, 1998; Long *et al.*, 2004). Therefore the focus of this chapter will be mainly on photosynthesis. In Chapter 6, the results of the A_{net} analysis will be used as a perspective from which to analyse other measurements and calculations unless mentioned otherwise.

5.1 Environmental conditions

Ambient temperature, outside PAR, chamber temperature and chamber [CO_2] were continuously measured and recorded as stated in section 4.3 and are presented in Figure 5.1 and Table 5.1 for August 2011 and May through September 2011, respectively.

Table 5.1: Mean (SE) air temperature (°C) and atmospheric [CO_2] (μmol mol^{-1}) in the eight treatment chambers in Athens, Georgia, USA during the treatment period (May 2 through September 10)

Treatment	$\mathbf{C}_A\mathbf{T}_0$	$\mathbf{C}_A\mathbf{T}_3$	$\mathbf{C}_A\mathbf{T}_6$	$\mathbf{C}_A\mathbf{T}_{12}$
Temperature	27.5 (0.8)	30.4 (0.7)	30.6 (1.2)	30.5 (2.5)
[CO_2]	403.4 (8.1)	412.9 (8.9)	411.1 (9.9)	399.9 (3.3)
Treatment	$\mathbf{C}_E\mathbf{T}_0$	$\mathbf{C}_E\mathbf{T}_3$	$\mathbf{C}_E\mathbf{T}_6$	$\mathbf{C}_E\mathbf{T}_{12}$
Temperature	27.4 (0.8)	30.3 (0.7)	30.7 (1.8)	30.5 (2.0)
[CO_2]	714.9 (6.4)	706.7 (5.6)	708.2 (5.3)	719.9 (7.5)

Treatments ran from May 2 to September 10. Air temperatures during this period were maintained per chamber according to their treatment. Mean daily atmospheric [CO_2] was maintained near 380 mmol mol^{-1} in the ambient [CO_2] chambers and near 700 mmol mol^{-1} in the elevated [CO_2] chambers (Table 5.1). Mean incoming PAR was 63.4 mol m^{-2} day^{-1}. Mean ambient temperature was 26.9°C.

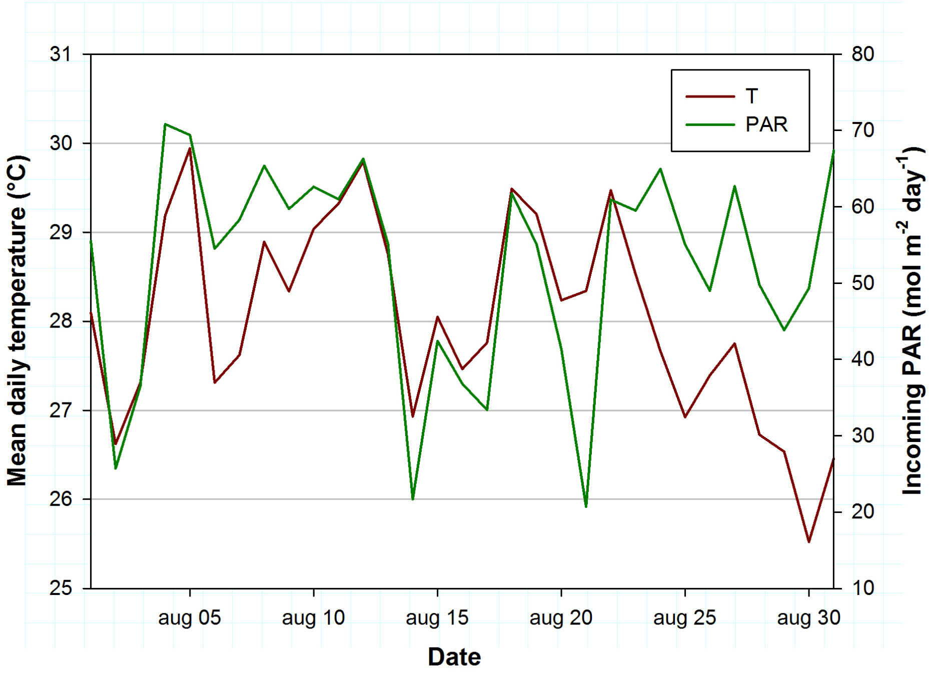


Figure 5.1: Mean daily ambient temperature and incoming PAR for August 2011

5.2 Physiological parameters

5.2.1 Net photosynthesis

Due to the surmised complexity of the interactions between the different factors ([CO_2], temperature, water availablity, treatment period and time of day), a graphical as well as statistical analysis was chosen based on its aptitude to process such interactions. The statistical approach was discussed in section 4.5.1 and will be addressed later. For the graphical approach a full factorial breakout of the data was carried through, which allows clear visualisation of the effect of different factor combinations. Only the most interesting combinations are shown. Important to consider is that each data point is the arithmetic mean of 12 measurements on four plants (as explained in section 4.4.1), and thus represents the value of one treatment during one measurement session.

We tried to find the response of A_{net} to changes in ambient [CO_2], temperature and water availability. Figures 5.2 and 5.4 show the value of all data points as a function of leaf temperature, Figure 5.3 shows mean values of treatment populations and Figure 5.5 shows A_{net} as a function of measurement period for all treatment combinations.

In Figure 5.2 a distinction was made between ambient and elevated [CO_2] treatments. It should be clear that the latter not only reaches higher values than the former, but also shows a greater spread.

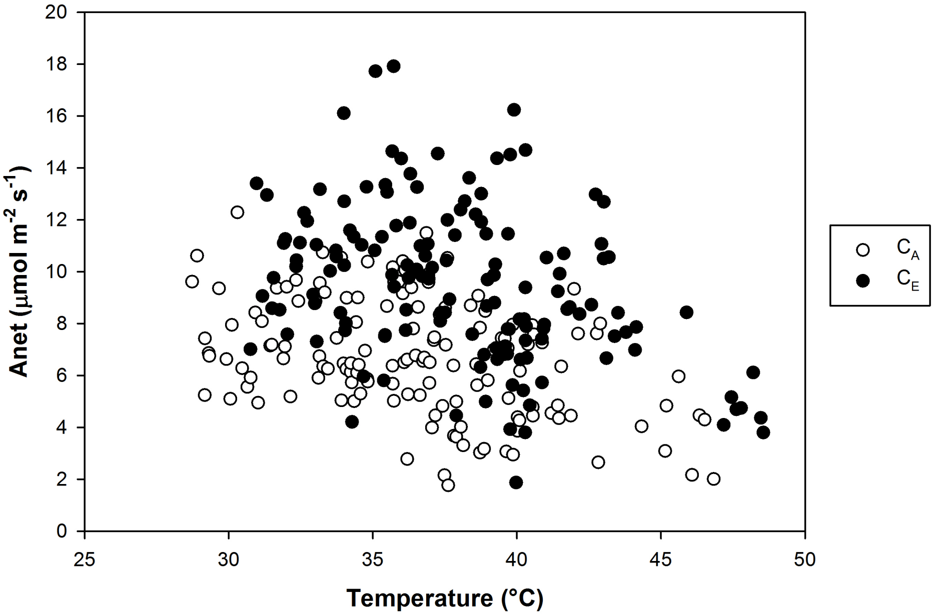


Figure 5.2: Net photosynthesis (A_{net}) as a function of temperature. Elevated [CO_2] shows generally higher values but also a greater spread.

In order to determine the cause for this greater spread in the elevated [CO_2] population, and to see whether or not a correlation could be found, the data was further categorized into its temperature treatments. A sparse negative correlation could be seen per [CO_2] x temperature treatment, although of more interest was the slight decline that could be seen across the treatments. We therefore took the arithmetic mean for each population and displayed them with their respective errors in Figure 5.3.

The result proved rather rewarding, since this figure allowed for several deductions to be made. It is for instance clear that a large part of the variance of the elevated [CO_2] treatment is due to the T_0 treatment. Also, as a measure of control, the temperature variance should be greatest in T_{12}, since this treatment experienced the hottest as well as the coolest temperatures, and smallest in T_0

and T_3, since these treatments stayed at the same relative temperature throughout all measurement periods. Further observations will be addressed as well as discussed in the next chapter.

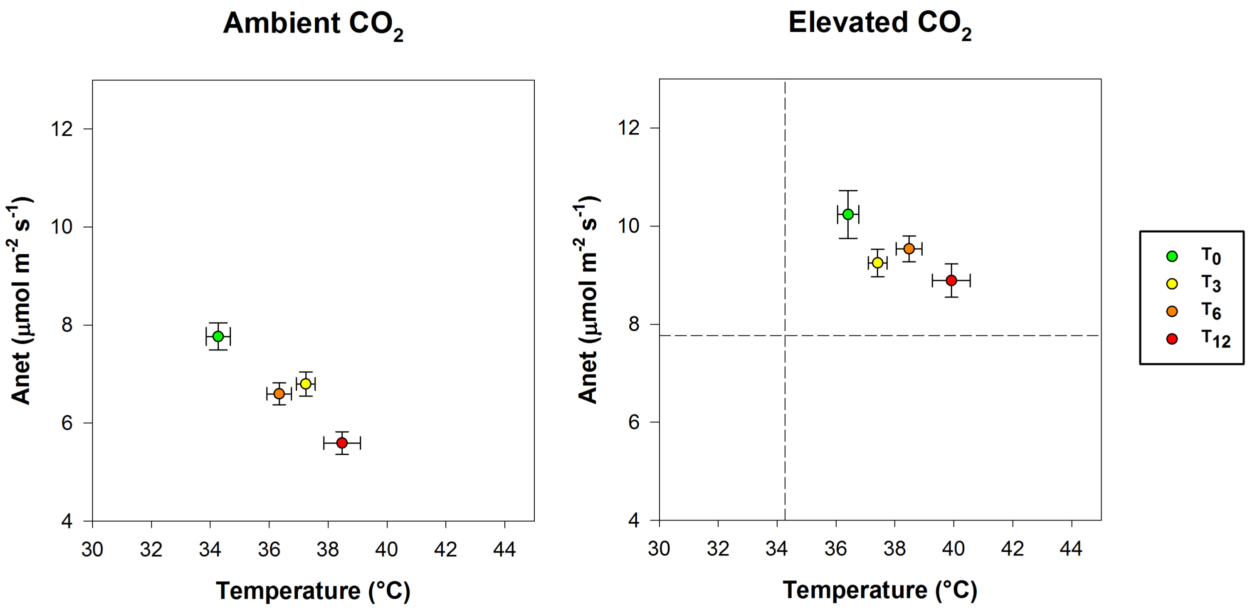


Figure 5.3: Net photosynthesis (A_{net}) as a function of temperature. The dashed line represents the location of T_0 in ambient [CO_2]. Each point represents the mean (± SE) of all data points in that treatment.

To examine the effect of water availability on the response of A_{net} to rising temperature, the next figure involved breaking the data down into water treatments. Since we expected that different temperature treatments would yield different plant water relations, we reduced our scope to water x temperature treatments. We also hypothesized that different water regimes would show different diurnal patterns, so a last further breakdown of the data occurred to include the time of day in which the data was gathered. The result is displayed in Figure 5.4.

The final figure for this section gives A_{net} throughout the experiment. Since graphical as well as statistical analysis indicated a significant difference between A_{net} in the morning and in the afternoon, we chose to present the data with the same discrepancy (Figure 5.5). The measurements taken before the heatwave event were integrated in an arithmetic mean value named pre-Treatment. Four other days were chosen from the heatwave and the recovery period. These days were selected on a treatment related relevance base. Note that, though these days are displayed in a successive series, there was a period of 21 days between the first and the last day of measurements. Values were scaled to the value of H x T_3 on the first day in the same [CO_2] treatment, which allows comparing values between water treatments but NOT between [CO_2] treatments.

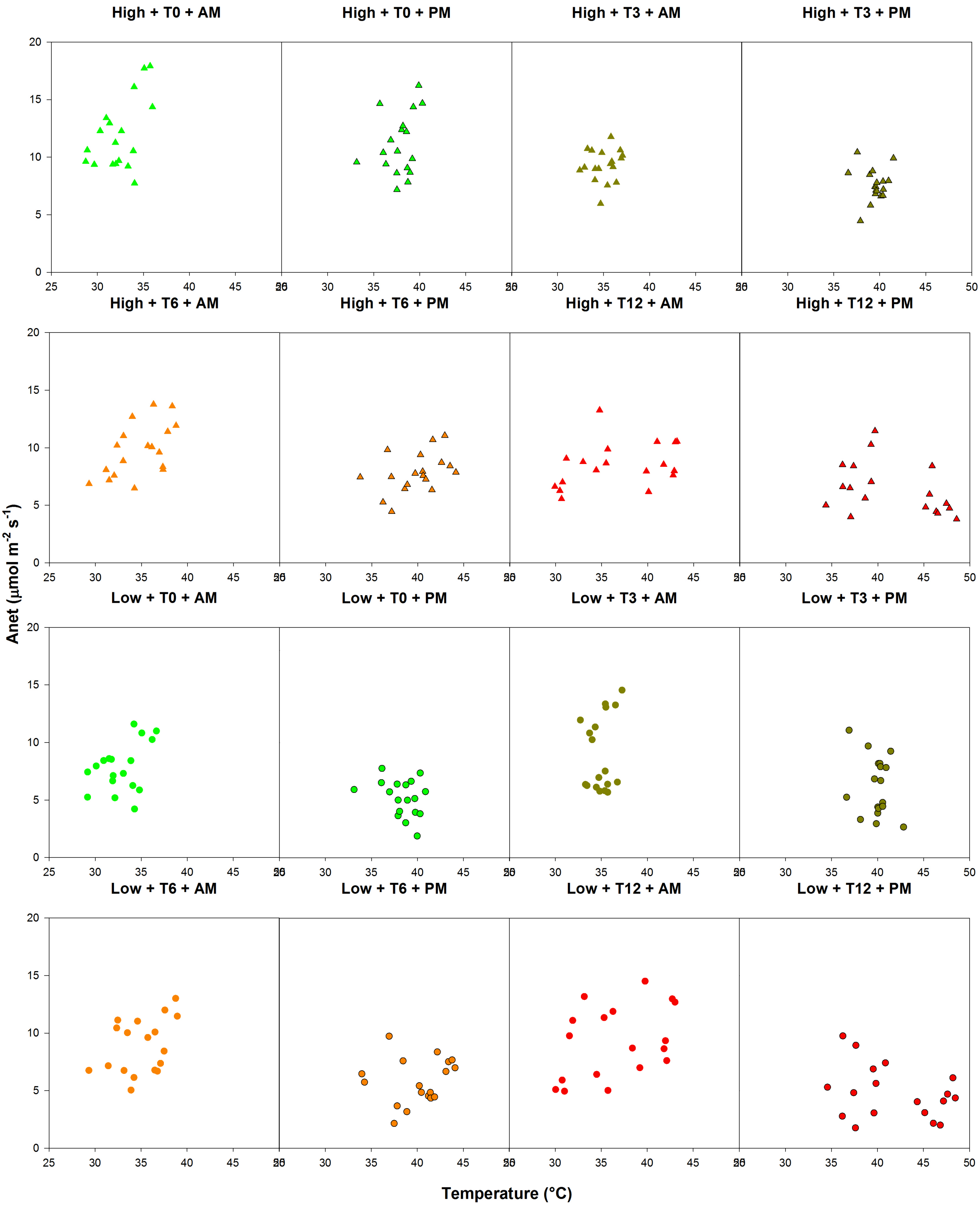


Figure 5.4: Net photosynthesis (A_{net}) as a function of leaf temperature. The data is categorized into its temperature treatments to show the diurnal pattern of different water regimes.

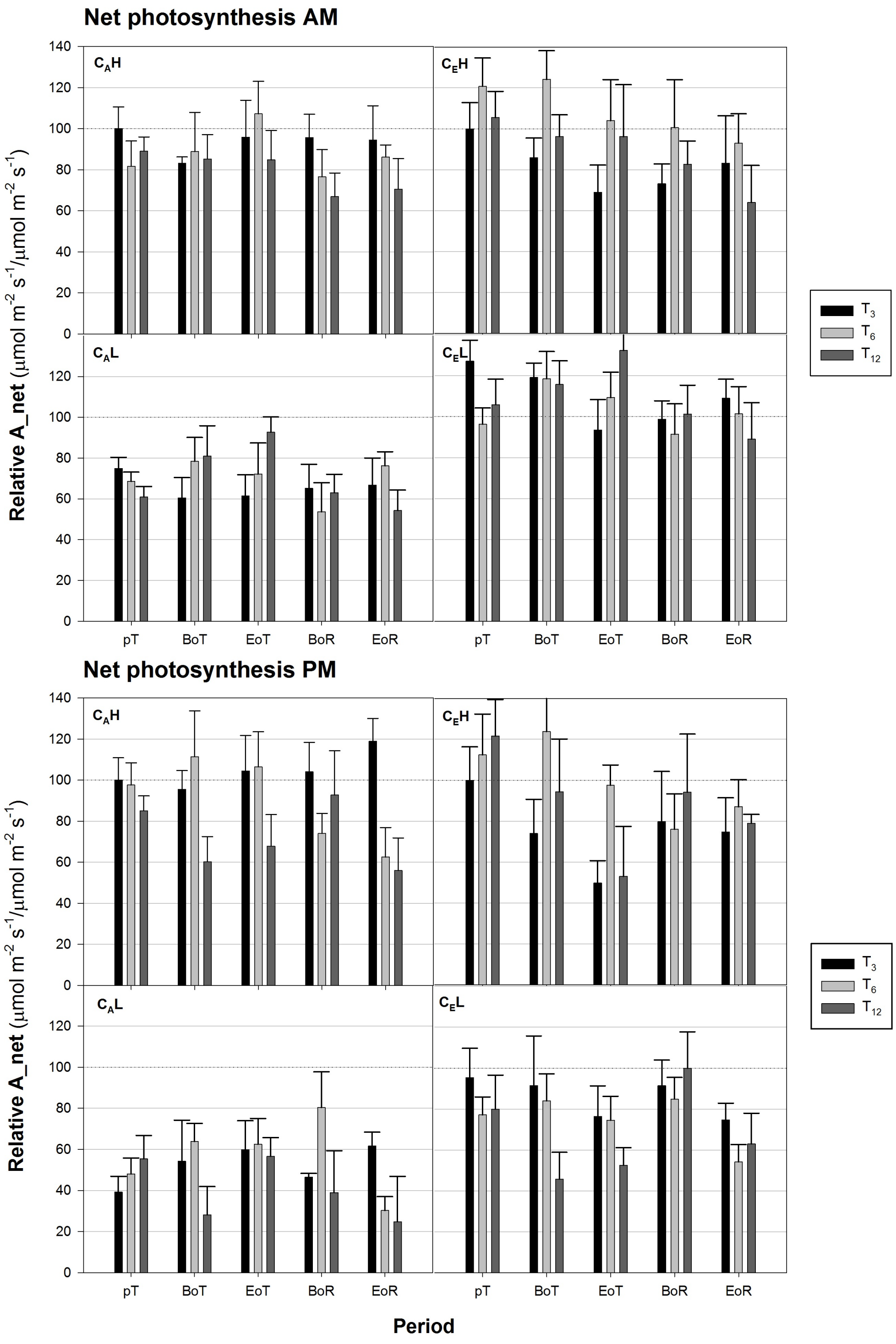


Figure 5.5: Net photosynthesis (A_{net}) (± SD) throughout the experiment. Values per time of day and per [CO_2] treatment were scaled to T_3 on the first day. C_A= Ambient [CO_2]; C_E= Elevated [CO_2]; H= High water; L= Low water; pT= pre-(Heatwave)Treatment; BoT= Beginning of Treatment ; EoT= End of Treatment; BoR= Beginning of Recovery; EoR= End of Recovery.

5.2.2 Intercellular [CO_2] and stomatal conductance to water vapour

G_s may be a very useful parameter in the framework of our research, since it not only serves as an indicator for plant response to elevated [CO_2], it also reacts strongly to drought conditions (Cochard *et al.*, 1996; Lu *et al.*, 1996; Peak and Mott, 2010). Therefore we looked at the relationship between A_{net} and g_s in Figure 5.6. Stomatal closure is also directly linked to C_i (Morison, 1987; Mott, 1988; Hetherington and Atkinson, 1990; Mott, 1990; Chen *et al.*, 1999; Yamori *et al.*, 2011), which is why g_s was plotted as a function of C_i in Figure 5.7. Finally, Figure 5.8 shows g_s, in absolute values, throughout the experiment.

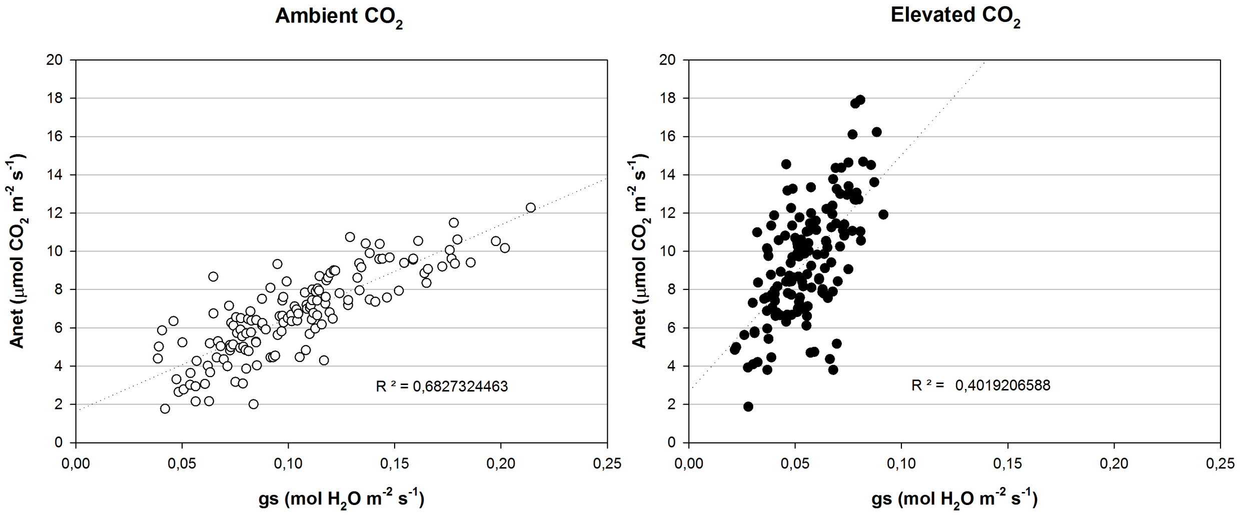


Figure 5.6: Net photosynthesis (A_{net}) as a function of stomatal conductance (g_s). Both treatments show a linear relationship but with a different slope.

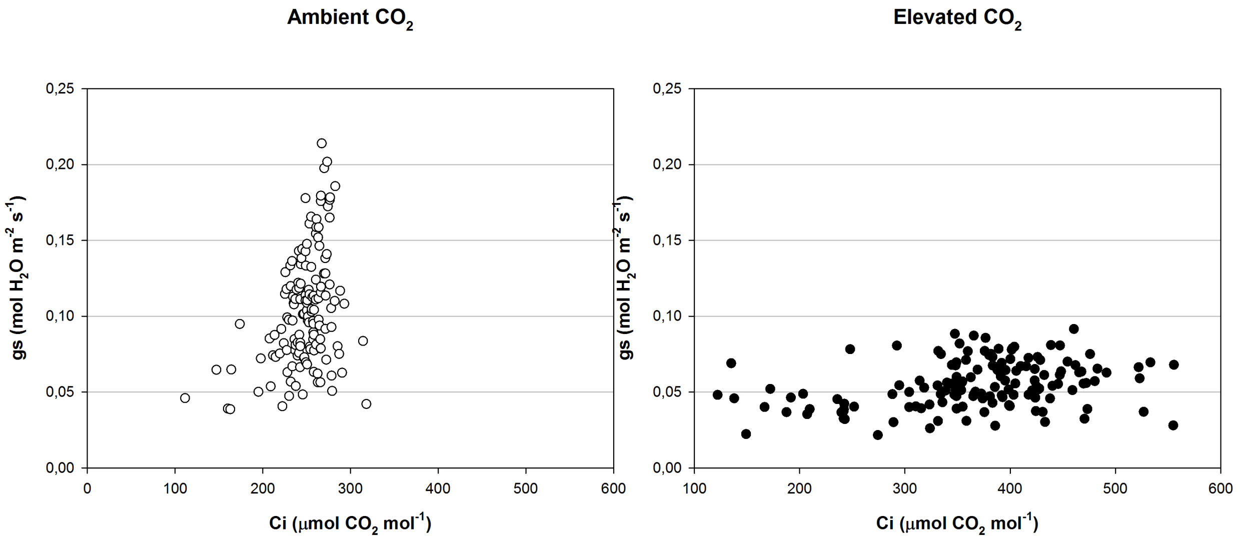


Figure 5.7: Stomatal conductance (g_s) as a function of intercellular [CO_2] (C_i). Both populations react in an opposite fashion.

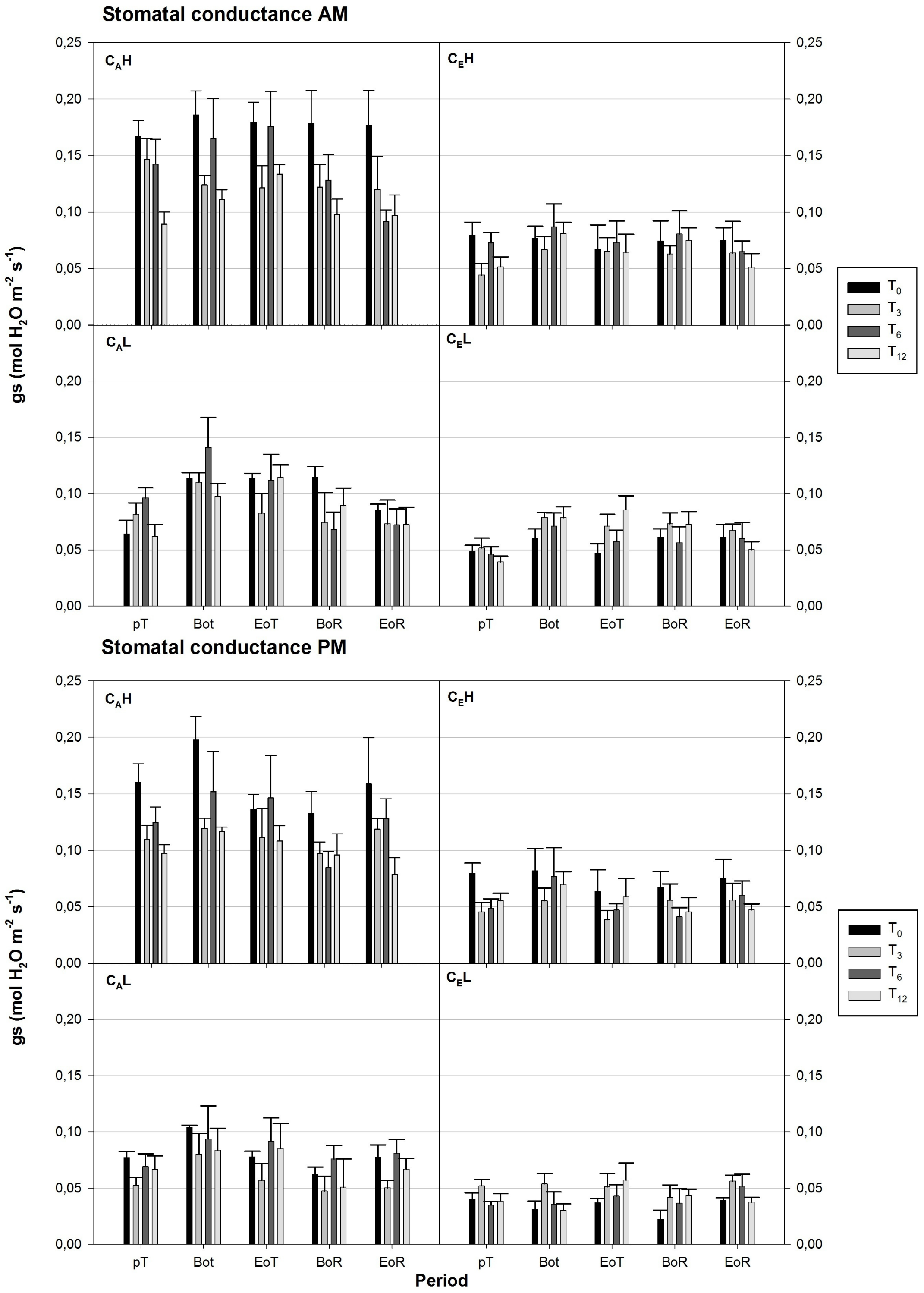


Figure 5.8: Stomatal conductance (g_s) throughout the experiment. pT= pre-(Heatwave)Treatment; BoT= Beginning of Treatment ; EoT= End of Treatment; BoR= Beginning of Recovery; EoR= End of Recovery.

5.2.3 Leaf vapour pressure deficit

According to Cochard *et al.* (1996); Lu *et al.* (1996); Goldstein *et al.* (1998); Saxe *et al.* (1998) and da Silva and Arrabac (2004) stomatal conductance is linked to leaf level water and vapour pressure deficit. According to our data however, a decoupling of g_s and VPDl seems to have occurred. According to Peak and Mott (2010), temperature alone should have little to no effect on g_s. In specific cases of constant VPD, g_s is expected to rise slightly. Our data on the contrary suggest that an extreme temperature event may have caused a permanent change in the regulation of the stomata, causing a reduction in its conductance, although it is not sure whether this change should be attributed to acclimation or to damage. Figure 5.9 shows the response of g_s to different values of VPDl for different temperature treatments.

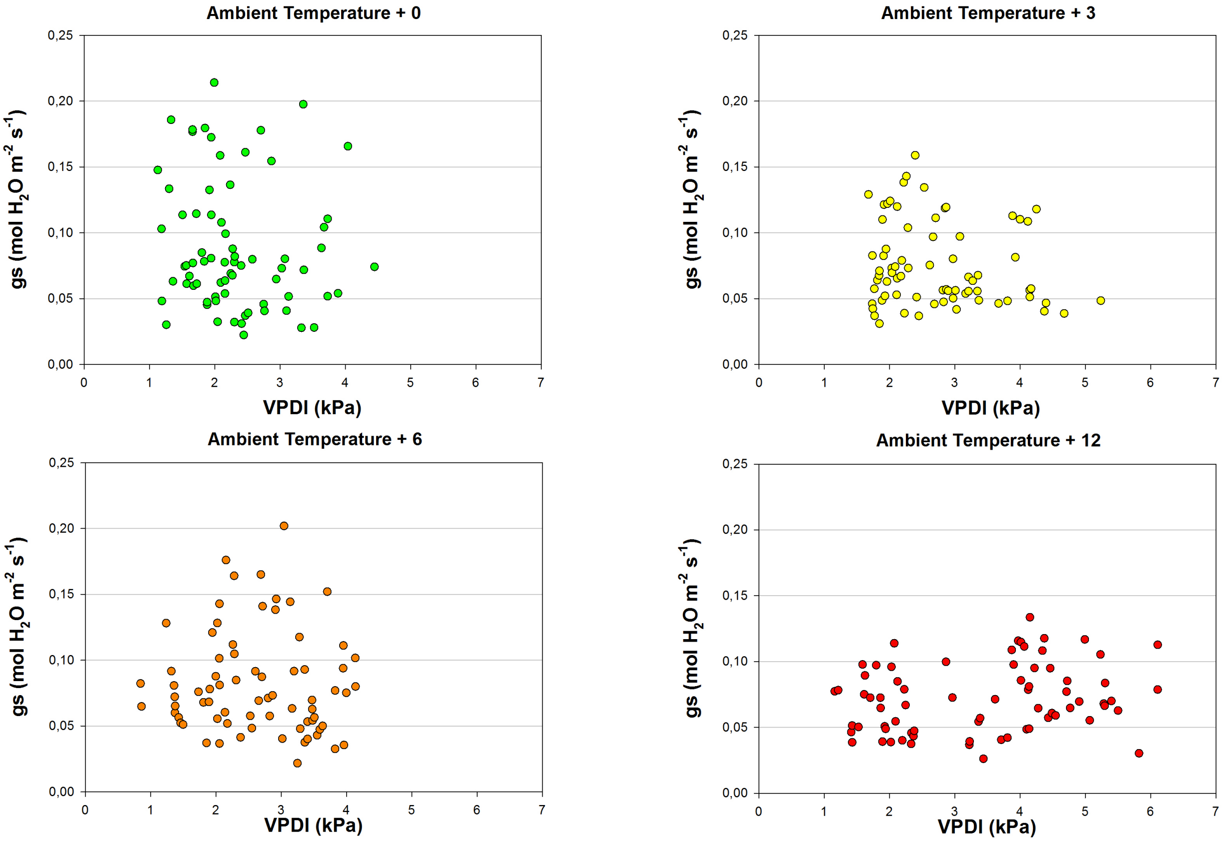


Figure 5.9: Stomatal conductance (g_s) as a function of leaf vapour pressure deficit (VPDl). Note how T_{12} never surpasses g_s= 0.14 along the entire VPDl range and across all water and [CO_2] treatments.

5.3 Fluorescence parameters

5.3.1 A brief introduction to fluorescence

In order to understand the following section in a sufficient manner some basic terminology is reviewed. Figure 5.10 shows the sequence of a typical fluorescence trace.

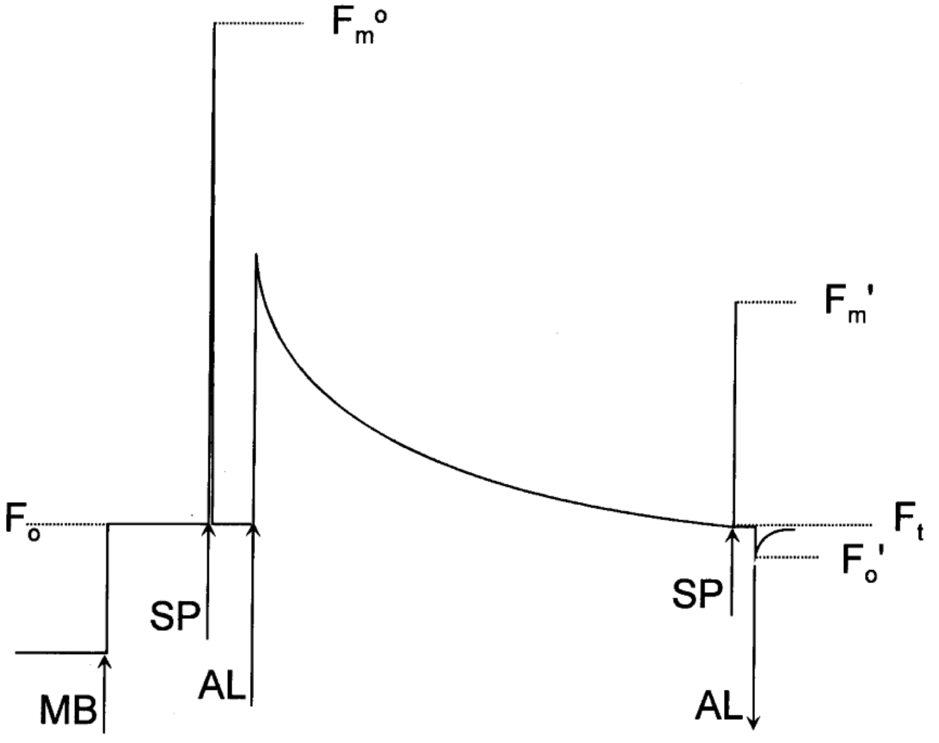


Figure 5.10: Sequence of a typical fluorescence trace. From Maxwell and Johnson (2000).

The figure should be interpreted from left to right. First a measuring light is switched on (MB ↑) and the zero fluorescence is measured (F_0). Application of a saturating flash of light (SP ↑) allows measurement of the maximum fluorescence level F_m^0. An actinic light, which drives photosynthesis, is then applied (AL ↑) and, after a period of time, another saturating light flash (SP ↑) allows the maximum fluorescence in the light (F'_m) to be measured. The level of fluorescence immediately before the saturating flash is termed F_t. Turning off the actinic light (AL ↓), typically in the presence of far-red light, allows the zero level fluorescence 'in the light' (F'_0) to be measured. (Tutorial after Maxwell and Johnson (2000))

5.3.2 Energy harvesting efficiency and effective quantum yield

There are three important fluorescence parameters often used in literature (Maxwell and Johnson, 2000; LI-COR Biosciences, 2008). F_v/F_m (= $(F^0_m$-$F_0)$ /F_m) is called the maximum quantum yield of PSII and is calculated with values measuremed on dark acclimated leaves. This parameter will not be used in the following section. We will however look at two measures for the effective (light acclimated) quantum yield. These two are both popular in literature and have shared the same name in different reports. For further disambiguation, F'_v/F'_m (= $(F'_m$-$F'_0)$ /F'_m) will be defined as the energy harvesting efficiency (by oxidized PSII reaction centers), whereas Φ_{PSII} (= $(F'_m$-$F_t)$ /F'_m)will be defined as the effective quantum yield. Since both are equally valued as a measure for photosynthetic efficiency, both will be given as an explaining variable for A_{net}. Figure 5.11 shows the relationship between A_{net} and F'_v/F'_m.

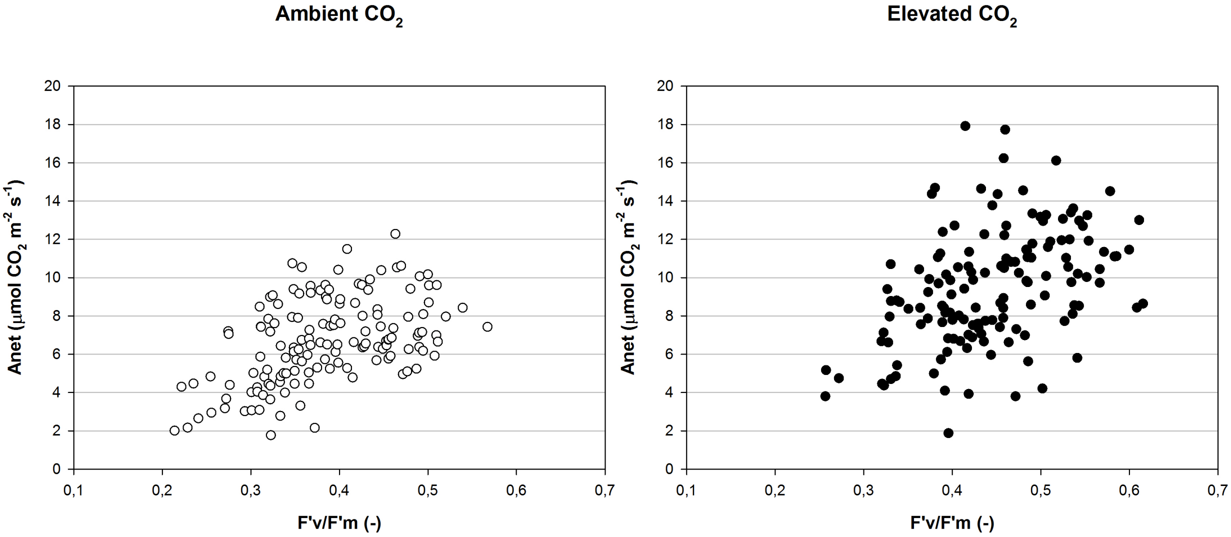


Figure 5.11: Net photosynthesis (A_{net}) as a function of energy harvesting efficiency (F'_v/F'_m). There is a slight positive correlation visible.

A clearer distinction in response to different levels of $[CO_2]$ can be seen in Figure 5.12, where A_{net} is plotted as a function of Φ_{PSII}.

Because of the different behaviour of both populations in correspondence with A_{net}, it was deemed interesting enough to plot these two against each other. The resulting relationship is displayed in Figure 5.13.

Note that, although they show little resemblance in their respective relations across $[CO_2]$ treatments, both measures nevertheless give information about the efficiency of the photosynthetic metabolism, which could provide rather interesting insights about [CO_2] induced photosynthetic alterations. The underlying reason for their discrepantic behaviour will be discussed in depth in the next chapter.

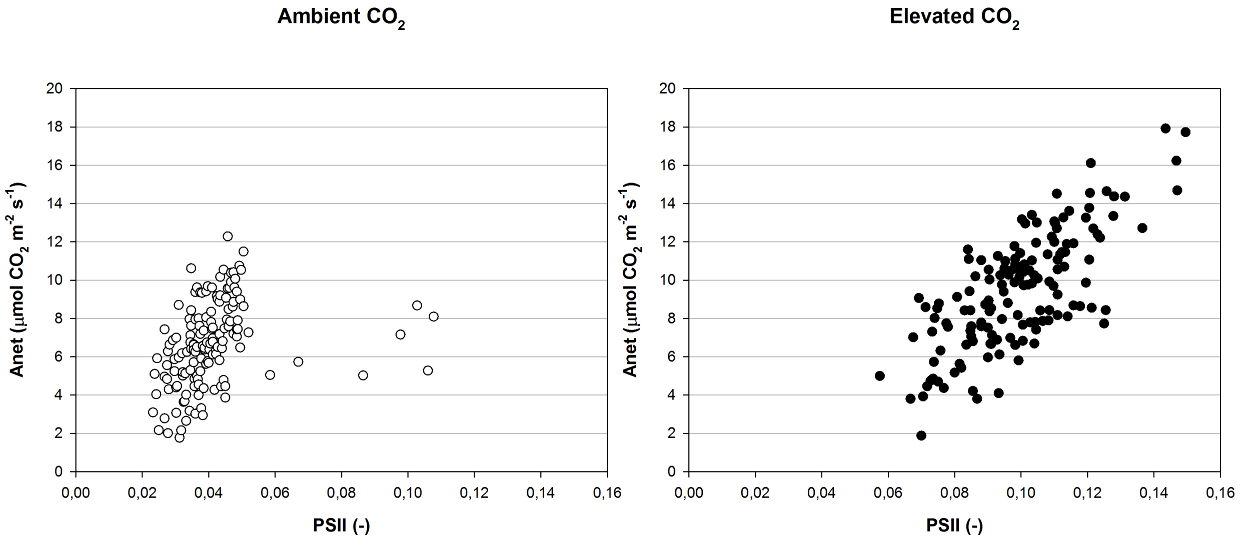


Figure 5.12: Net photosynthesis (A_{net}) as a function of effective quantum yield (Φ_{PSII}). There is a clear difference in the response of both [CO_2] levels.

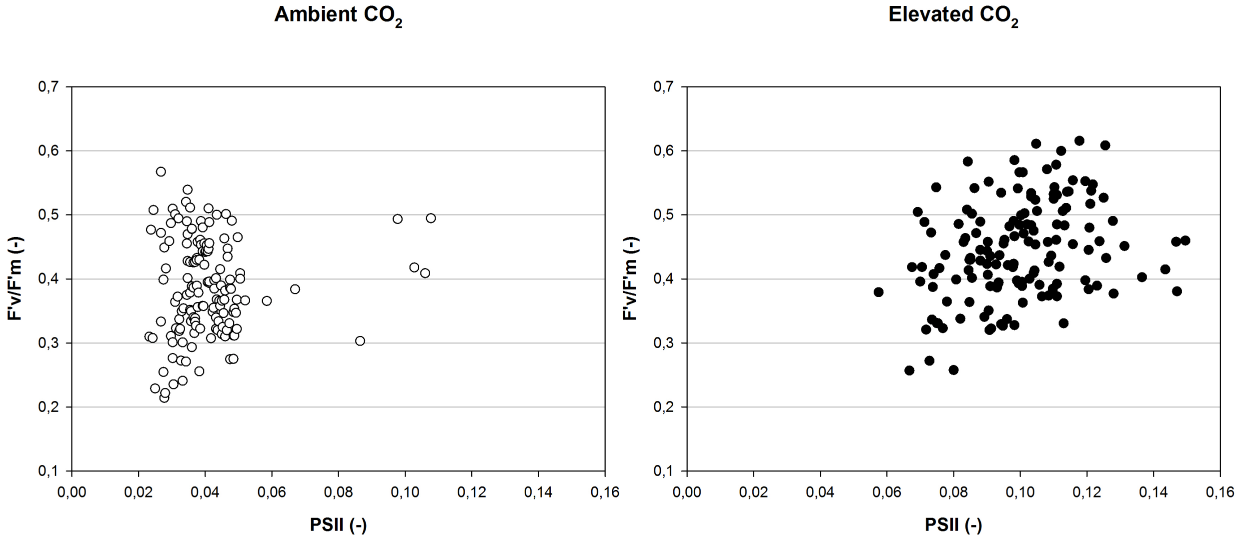


Figure 5.13: Energy harvesting efficiency (F'$_v$/F'$_m$) plotted against corresponding effective quantum yield (Φ_{PSII}) values. These two are both used often in literature, though our data indicates strong discrepancies between the behaviour of different [CO_2] levels.

Since the correlation between A_{net} and Φ_{PSII} seemed more distinct than between A_{net} and F'$_v$/F'$_m$, we decided to present the absolute values of the former throughout the experiment in Figure 5.14.

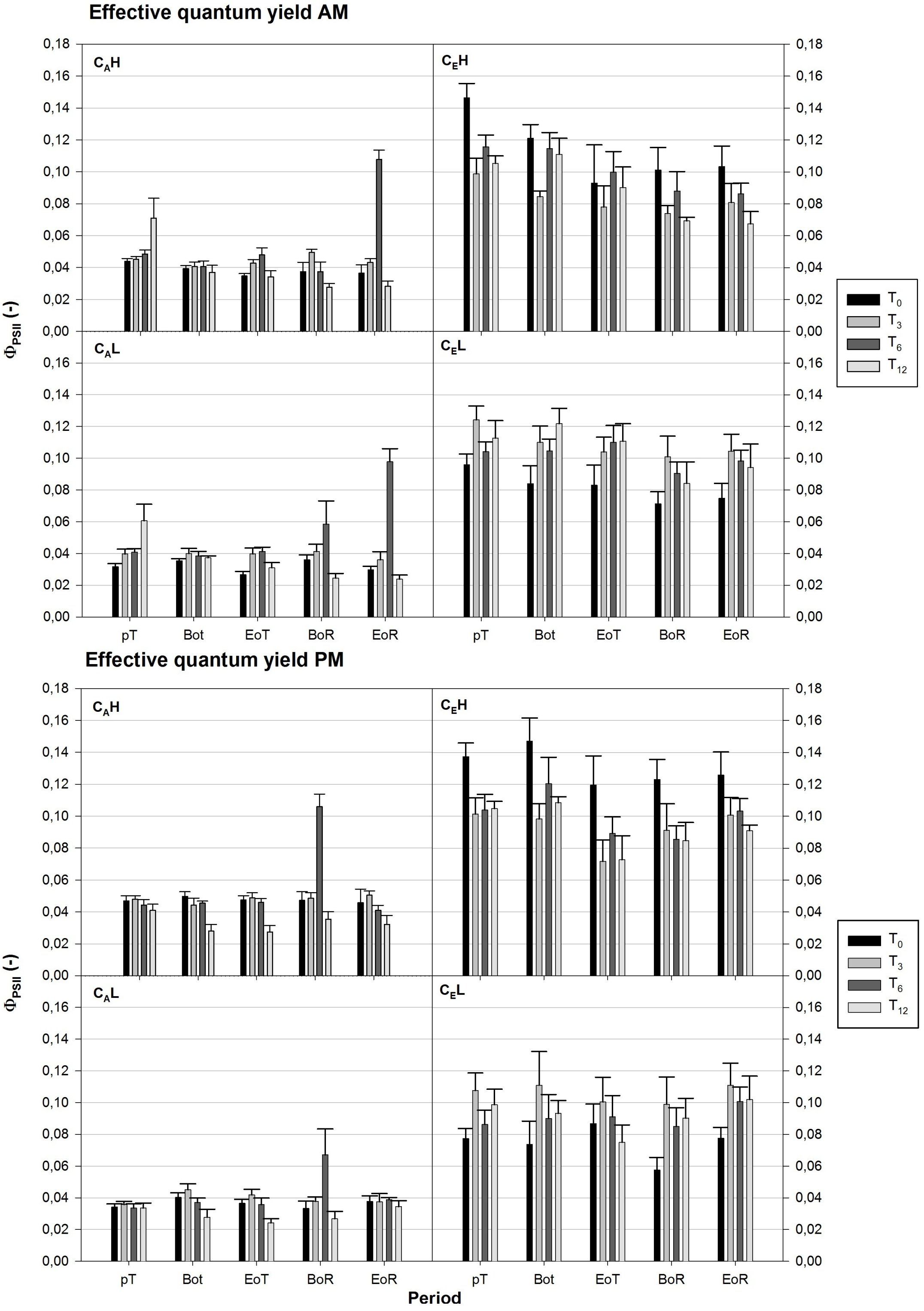
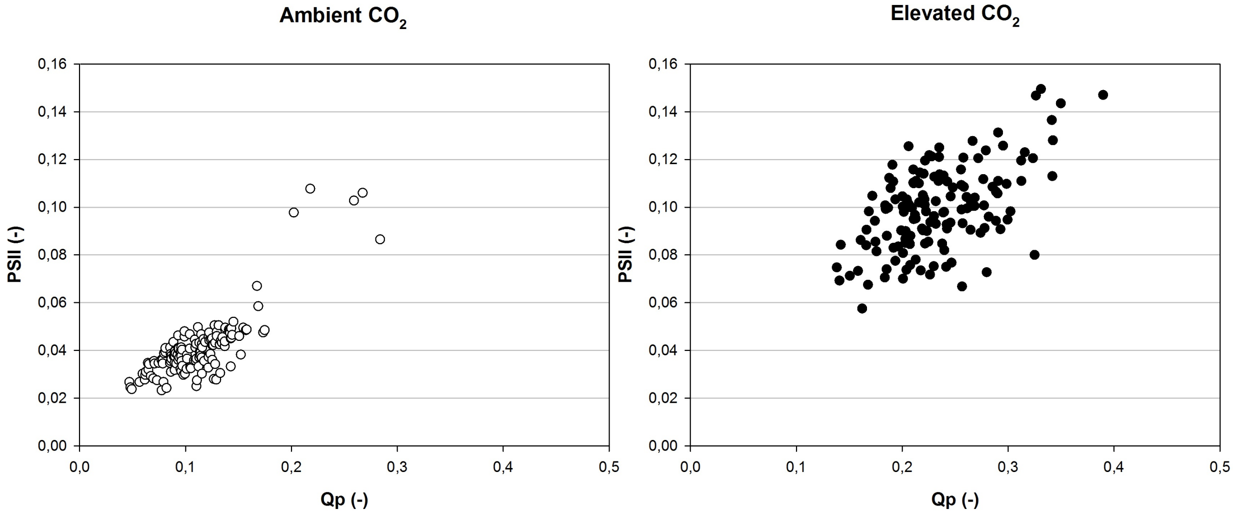


Figure 5.14: Effective quantum yield (Φ_{PSII}) throughout the experiment. pT= pre-(Heatwave)Treatment; BoT= Beginning of Treatment ; EoT= End of Treatment; BoR= Beginning of Recovery; EoR= End of Recovery.

5.3.3 Photochemical quenching

Photochemical quenching (Q_p= (F'_m-F_t) / (F'_m-F'_0)) is the increase in the rate at which electrons are transported away from PSII (right after the progressive closure of PSII centers, or right after AL ↑ in Figure 5.10), mainly due to the light-induced activation of enzymes involved in carbon metabolism and the opening of stomata (Maxwell and Johnson, 2000).

Figure 5.15 shows the relationship between Φ_{PSII} and Q_p. This particular comparison was chosen because it seemed logical to examine to which extent an increased quantum yield corresponded with an increase in Q_p. The outcome of this analysis was intriguingly compatible with some previous results and will be elucidated in the next chapter.


Ambient CO_2

Elevated CO_2

Figure 5.15: Relationship between effective quantum yield (Φ_{PSII}) and photochemical quenching (Q_p). The strong correlation suggests intermediate [CO_2] levels would yield intermediate values.

Data regarding non-photochemical quenching, which involves an increase in heat dissipation efficiency, were unfortunately enough of insufficient expediency to use in this context. The magnitude of this parameter may and will be deducted from other data however, and may thus be used in an indicative fashion.

5.4 Water relations

5.4.1 Transpiration

As amply discussed in sections 2.3.1 and 2.3.3, one of the main factors controlling net photosynthetic rate is stomatal regulation. Although it is generally seen as the most important, some authors have suggested that there are other factors than g_s influencing the rate of transpiration (E) (Beerling *et al.*, 1996; Centritto *et al.*, 1999; Ellsworth, 1999; Medlyn *et al.*, 2001; Peak and Mott, 2010). Therefore Figure 5.16 shows the rate of transpiration as a function of g_s for the different temperature treatments.

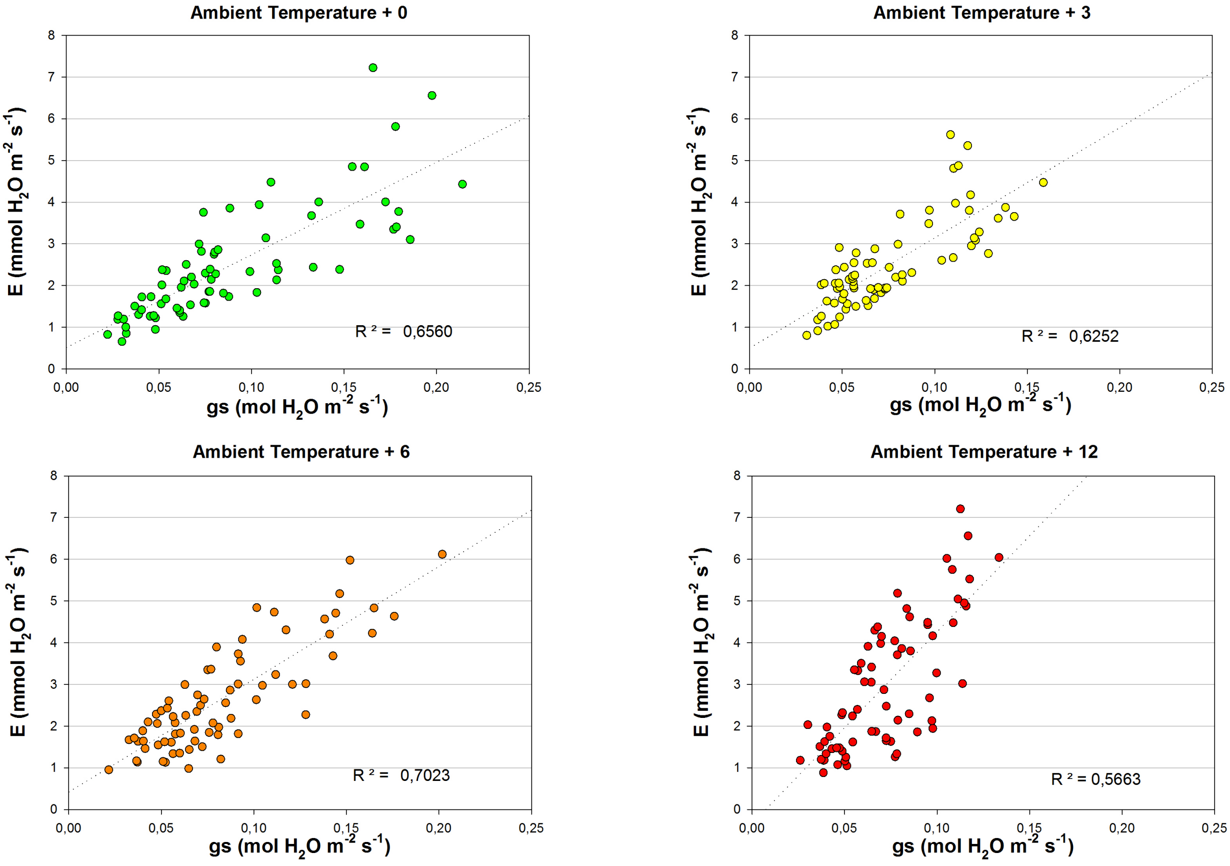


Figure 5.16: Transpiration rate (E) as a function of stomatal conductance (g_s). There is a strong positive correlation within temperature treatments.

Figure 5.17 shows the absolute values of E for all treatments throughout the experiment.

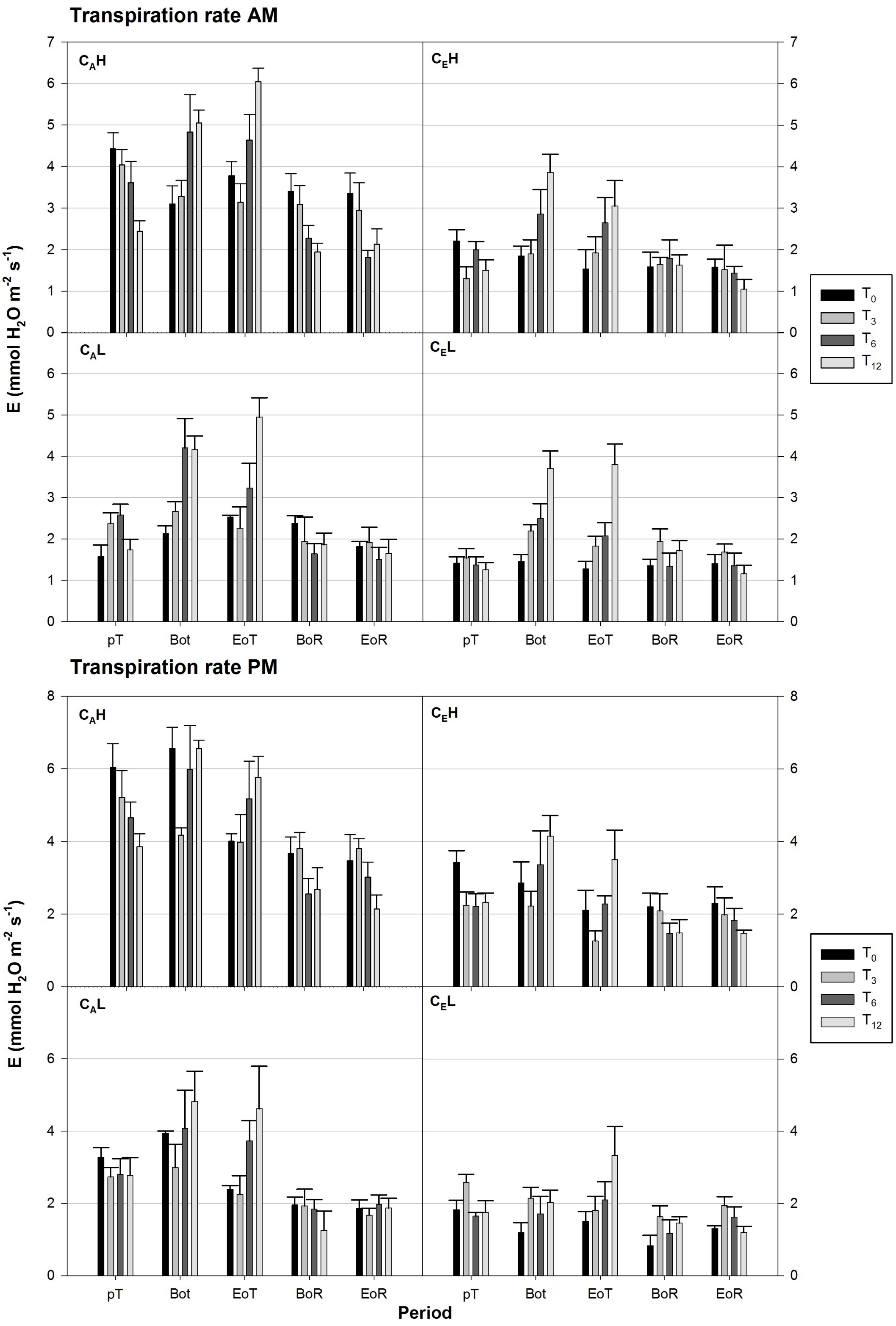


Figure 5.17: Transpiration rate (E) throughout the experiment. pT= pre-(Heatwave)Treatment; BoT= Beginning of Treatment ; EoT= End of Treatment; BoR= Beginning of Recovery; EoR= End of Recovery.

5.4.2 Instantaneous water use efficiency

There are two methods frequently used to calculate instantaneous or intrinsic water use efficiency (WUE$_I$). One is dividing A$_{net}$ by g$_s$, whereas the other method divides by transpiration. The latter was chosen in this particular study, since it was conducted in the specific framework of plant water relations. It was plotted as a function of VPDl in Figure 5.18 because of the close link to water relations both parameters share.

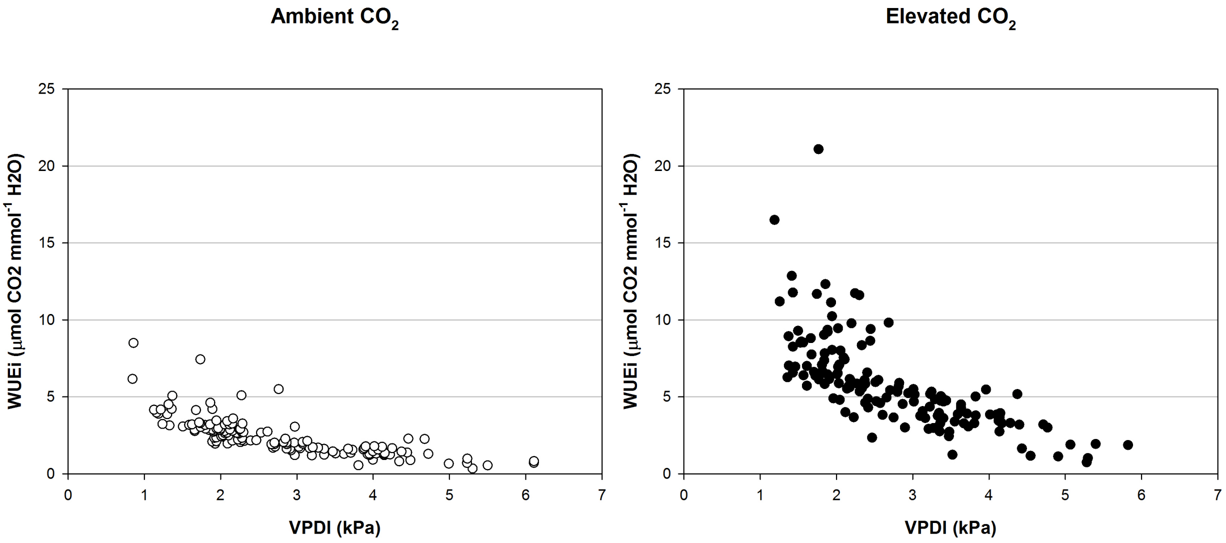


Figure 5.18: Instantaneous water use efficiency (WUE$_I$) as a function of leaf vapour pressure deficit (VPDl). There is an inverse correlation visible.

5.5 Statistical analysis of the treatments

In this section we will provide an RM ANOVA for significance of treatment effects on various parameters, as well as a summary of the values for A$_{net}$, g$_s$, E, Φ_{PSII} and Q$_p$ comparing the high and low water treatment.

The RM ANOVA was carried through with time of day (AM/PM) and period (Before heatwave, During heatwave, Recovery) as repeated factors. The results are summarized in Table 5.2. The absolute effect of the different treatments can be approached by looking at the mean value of each variable in each treatment combination. These values can be found in Table 5.3. Note that these values represent the mean value for ALL measurements made in this treatment, which means morning and afternoon values as well as pre-heatwave, during heatwave and recovery values were taken into account.

Table 5.2: Anova for all parameters, by treatment: [CO_2] (CO_2), temperature (T), water availability (W) and time of day (time). Unshown combinations yielded no significance in any variable. Significance: 0 ”***” 0.001 ”**” 0.01 ”*” 0.05 ”.” 0.1 ” ” 1

	A_{net}	g_s	C_i	VPDl	E	WUE_I	Φ_{PSII}	F'_v/F'_m	Q_p
CO_2	***	***	***		***	***	***	***	***
T	*	**		***	*	***			
W	***	***	*	***	***		*	*	***
time	***	***	***	***	***	***		***	***
CO_2 x T		.							
CO_2 x W		***			***			*	
CO_2 x time			*	*		***			
T x W	***	**					***		.
T x time				***					
W x time	**			*	**		*		
CO_2 x T x W	*					*	***		

Table 5.3: Mean value of A_{net} (μmol CO_2 m^{-2} s^{-1}), g_s (mol H_2O m^{-2} s^{-1}), E (mmol H_2O m^{-2} s^{-1}), Φ_{PSII} (-) and Q_p (-) for all treatments. A significant difference ($P < 0.05$) between water treatments is depicted by an asterisk * behind the low water value.

Treatment combination			**Parameter**				
CO_2	**T**	**W**	**A_{net}**	**g_s**	**E**	**Φ_{PSII}**	**Q_p**
C_A	T_0	H	9.78	0.16	4.16	0.04	0.11
		L	5.86*	0.08*	2.28*	0.03	0.09
	T_3	H	8.40	0.12	9.93	0.05	0.14
		L	5.19*	0.07*	2.33*	0.04	0.11
	T_6	H	7.55	0.13	3.92	0.05	0.13
		L	5.65	0.09*	2.79*	0.04	0.12
	T_{12}	H	6.24	0.10	4.04	0.04	0.10
		L	4.95	0.08	3.07*	0.03	0.09
C_E	T_0	H	13.31	0.07	2.15	0.12	0.29
		L	7.17*	0.04*	1.33*	0.08*	0.19*
	T_3	H	8.49	0.05	1.77	0.09	0.25
		L	9.99	0.06	1.91	0.11*	0.24
	T_6	H	10.05	0.06	2.17	0.10	0.24
		L	9.02	0.05	1.69	0.09	0.21
	T_{12}	H	8.67	0.06	2.47	0.09	0.23
		L	9.11	0.05	2.26	0.09	0.22

5.6 Outcome of hypotheses

In this section our specific working hypotheses will be tested by presenting the data in the form of a table which allows high efficiency in deduction and gauging. The interpretation of these tables will be given in Chapter 7.

5.6.1 High water versus low water

We hypothesized that A_{net} and E would be lower in the low compared to the high water seedlings regardless of the time of day or the measurement period (Table 5.6), that the decrease in A_{net} and E from morning to afternoon would be greatest in the low compared to the high water seedlings (Table 5.4) and that heat waves would reduce A_{net} and E to a greater extent in the low than in the high water treatment (Table 5.5).

Table 5.4: Comparing mean net photosynthesis (A_{net}, μmol CO_2 m^{-2} s^{-1}) and transpiration rate (E, mmol H_2O m^{-2} s^{-1}) across time of day for both water treatments. Significance ($P < 0.05$) is depicted by an asterisk *.

Water treatment	AM	PM	ΔA_{net}	AM	PM	ΔE
High water	9.87	8.23	1.64*	2.67	3.48	-0.81*
Low water	8.73	5.50	3.23*	2.06	2.35	-0.29*

Table 5.5: Comparing mean net photosynthesis (A_{net}, μmol CO_2 m^{-2} s^{-1}) and transpiration rate (E, mmol H_2O m^{-2} s^{-1}) across the heatwave throughout days for both water treatments. D= During heatwave. Significance ($P < 0.05$) is depicted by an asterisk *.

Period x Water	T_0	T_{12}	ΔA_{net}	T_0	T_{12}	Δtrans
D1 x H	12.68	7.82	4.86*	3.59	4.90	-1.31*
D1 x L	7.59	6.60	0.99	2.18	3.68	-1.50*
D2 x H	12.59	6.97	5.61*	3.69	5.13	-1.44*
D2 x L	7.76	7.44	0.32	1.97	4.50	-2.53*
D3 x H	10.54	7.02	3.52*	2.86	4.59	-1.73*
D3 x L	6.54	7.99	-1.45	1.93	4.17	-2.25*
D4 x H	9.49	6.09	3.40*	2.37	4.29	-1.91*
D4 x L	5.17	5.98	-0.81	1.52	3.52	-1.99*

Table 5.6: Comparing mean net photosynthesis (A_{net}, μmol CO_2 m^{-2} s^{-1}) and transpiration rate (E, mmol H_2O m^{-2} s^{-1}) across water treatments throughout days prior to and during the heatwave. B= Before heatwave; D= During heatwave. Significance ($P < 0.05$) is depicted by an asterisk *.

Period x time	High water	Low water	ΔA_{net}	High water	Low water	Δtrans
B1 x AM	11.59	8.96	2.63*	2.70	1.68	1.02*
B1 x PM	9.58	5.79	3.78*	4.13	2.73	1.39*
B2 x AM	10.63	9.02	1.62*	2.69	1.78	0.90*
B2 x PM	9.05	4.89	4.16*	3.36	2.11	1.25*
D1 x AM	10.41	9.71	0.70	3.34	2.88	0.46*
D1 x PM	8.79	5.29	3.51*	4.48	2.86	1.62*
D2 x AM	10.98	10.38	0.60	3.7	2.93	0.78*
D2 x PM	7.92	5.45	2.47*	4.39	3.39	1.00*
D3 x AM	9.80	9.23	0.57	3.34	2.74	0.59*
D3 x PM	7.26	5.16	2.09*	3.51	2.72	0.79*
D4 x AM	8.2	6.78	1.43*	2.63	2.07	0.56*
D4 x PM	7.56	5.47	2.09*	3.53	2.48	1.06*

5.6.2 Mitigation by elevated [CO_2]

We hypothesized that C_E would have higher A_{net} values prior to the heat wave event regardless of water treatment (Table 5.8), C_E would have A_{net} reduced to a smaller extent than C_A regardless of water treatment (Table 5.7), A_{net} would be greatest in C_EH and lowest in C_AL prior to heat waves (Table 5.9) and that C_EH would have the smallest reduction in A_{net} with heat wave induced stress while C_AL would have the greatest reduction in A_{net} (Table 5.10).

Table 5.7: Comparing mean net photosynthesis (A_{net}, μmol CO_2 m^{-2} s^{-1}) for both [CO_2] treatments during the heatwave. D= During heatwave. Significance ($P < 0.05$) is depicted by an asterisk *.

Period x CO2	T_0	T_{12}	ΔA_{net}
D1 x A	8.25	5.48	2.77*
D1 x E	12.03	8.94	3.08*
D2 x A	9.29	6.50	2.79*
D2 x E	11.05	7.91	3.14*
D3 x A	8.74	6.38	2.36*
D3 x E	8.34	8.62	-0.29
D4 x A	6.92	4.95	1.97
D4 x E	7.74	7.12	0.63

Table 5.8: Comparing mean net photosynthesis (A_{net}, μmol CO_2 m^{-2} s^{-1}) across [CO_2] treatments. B= Before heatwave; D= During heatwave. Significance ($P < 0.05$) is depicted by an asterisk *.

Measurement period	**Ambient [CO_2]**	**Elevated [CO_2]**	Δ**A**$_{net}$
B1	6.55	11.42	-4.87*
B2	6.64	10.16	-3.51*
D1	6.70	10.39	-3.69*
D2	7.76	9.60	-1.84*
D3	7.24	8.48	-1.24*
D4	6.37	7.65	-1.28*

Table 5.9: Comparing mean net photosynthesis (A_{net}, μmol CO_2 m^{-2} s^{-1}) for all [CO_2] x water treatments prior to the heatwave. B= Before heatwave.

CO2 x Water	**B1**	**B2**
E x H	12.97	11.72
E x L	9.86	8.59
A x H	8.19	7.97
A x L	4.89	5.31

Table 5.10: Comparing mean net photosynthesis (A_{net}, μmol CO_2 m^{-2} s^{-1}) for all [CO_2] x water treatments during the heatwave. D= During heatwave. Significance ($P < 0.05$) is depicted by an asterisk *.

Period x CO2 x water	**T**$_0$	**T**$_{12}$	Δ**A**$_{net,rel}$
D1 x E x H	15.39	9.49	38.33%*
D1 x E x L	8.66	8.39	3.09%
D1 x A x H	9.97	6.15	38.32%*
D1 x A x L	6.52	4.81	26.23%
D2 x E x H	13.28	7.15	46.16%*
D2 x E x L	8.81	8.67	1.58%
D2 x A x H	11.89	6.79	42.86%*
D2 x A x L	6.71	6.21	7.41%
D3 x E x H	10.56	7.64	27.64%
D3 x E x L	6.11	9.60	-57.21%*
D3 x A x H	10.51	6.39	39.12%*
D3 x A x L	6.97	6.37	8.59%
D4 x E x H	10.23	6.86	32.91%*
D4 x E x L	5.26	7.38	-40.12%
D4 x A x H	8.76	5.32	39.25%*
D4 x A x L	5.07	4.58	9.70%

Chapter 6

Discussion

6.1 Experimental concerns

In Chapter 2 it was emphasized that short term effects of [CO_2] fertilization may greatly differ from long term effects. Therefore care should be taken when interpreting the results of this study, since the period during which our treatments were run (± 5 months) could be classified as a short term.

Of equal importance is that our research was conducted in greenhouses or closed top chambers. Although they allow for greater control of environmental conditions, Norby *et al.* (1997) points at the importance of mimicking unmanaged habitats in which other environmental resources such as nitrogen or water are generically limiting. In a reviewing meta-analysis, Curtis (1996) found that FACE and open-top chamber experiments resulted in greater responses of A_{net} of 41 species (one of which was *Quercus rubra*) compared to closed top chamber experiments. Curtis and Wang (1998); Morgan *et al.* (2005) and Long *et al.* (2006) compared models using FACE data to those using chamber data and saw great differences in the results. Intuitively one would expect experiments run in the open to resemble reality to a greater degree.

Other remarks are that our measurement period may have been in the late growing season, where *Quercus rubra* was found to be less sensitive to climatic conditions (Sander, 1979; Tardif *et al.*, 2006). We furthermore used seedlings as test subjects, where findings by Hättenschwiler *et al.* (1997); Collins and Carson (2004) and Niinemets (2010) suggested that the response to elevated

[CO_2] is strongly dependent on the life stage of the trees. A last remark is that the seedlings were grown in pots, where the roots had ± 8 L to grow, which may have had consequences for the response of g_s (Arp, 1991; Curtis, 1996).

6.2 Physiological parameters

6.2.1 Net photosynthesis

The data displayed in Figure 5.2 were compliant with most literature. Elevated [CO_2] generally yielded higher values for A_{net} than ambient [CO_2], but showed a greater variance in its population.

Part of this variance is explained in Figure 5.3, where $C_E T_0$ has by far the largest standard error. Upon closer inspection of this treatment in Figure 5.4 it becomes clear why. Elevated [CO_2] not only gave rise to the highest A_{net} values in T_0H, but in T_0L to the lowest A_{net} values as well. This could mean that the interaction between elevated [CO_2] and heat stress is not just a one-way mitigation but rather one that operates in both directions, where heat stress-induced acclimation responses make the elevated [CO_2] seedlings more resistant to drought. The observed increase in variance of leaf temperature in higher heat treatments is a logical consequence of the heatwave event, which produced a broadening of the temperature data range.

More information may be extracted from Figure 5.3 than a mere explanation for increased variance however. Only erroneously could one overlook the negative correlation between A_{net} and leaf temperature manifested in both [CO_2] treatments. In this context Hüve *et al.* (2011) conducted experiments on the herbaceous bean plant (*Phaseolus vulgaris*) with varying heat pulse lengths and intensities that underscored the threshold-type loss of photosynthetic functioning, and indicated that the degree of photosynthetic deterioration and cellular damage depended on accumulated heat-dose. Our results seemingly contradict this however, which may again indicate species dependency of the heat response.

Of particular interest is that $C_E T_{12}$, the arithmetic mean of data taken during measurement conditions that included severe heatwave and drought stress, is still situated higher than $C_A T_0$, a situation where no relative heat stress occurred anywhere in the measurement period. Also note how, for the same difference in mean leaf temperature (± 4°C), elevated [CO_2] showed a smaller decrease in A_{net}, though this decrease was not significant. Another interesting observation is that T_3 and T_6 are located very close to each other in both [CO_2] treatments, and that they seem to have switched 'places' going from ambient to elevated [CO_2]. A salient trait of this switch is that not only A_{net} but also leaf temperature shifts to an 'opposing' value. This may indicate that these data were measured very close to a temperature dependent breaking point, possibly where A_{net} shifts from Rubisco carboxylase limited to PSI and photorespiration limited.

Our last but hypothetically not least observation is that C_ET_0, C_ET_3, C_ET_6 and C_ET_{12} seem to have higher leaf temperature values than their respective ambient [CO_2] peers, though this may not be equally prominent for T_3 and T_6 due to aforementioned mechanisms. The reason for this is highly subjected to the author's conjecture and will be thoroughly addressed in section 6.6.

The final graph shows A_{net} throughout the experiment and is displayed in Figure 5.5. Values were scaled to T_3H per [CO_2] treatment per time of day because this allowed for easy comparison between water and heat treatments, respectively. Two important observations can be made in this figure. Firstly, we can see that the heatwave event has opposite effects in the morning across water treatments. In the morning the heatwave caused T_{12} to rise relatively in the low water treatment, whereas it remains at more or less the same level in the high water treatment. The second observation is nested within the first and portends that the rise of A_{net} in the low water treatment in the morning during the heatwave is of such magnitude that it effectively surpasses A_{net} values of the high water treatment. Again this indicates the possible existence of a two-way mitigation by elevated [CO_2] and heat induced acclimation responses.

6.2.2 Intercellular [CO_2] and stomatal conductance to water vapour

Figure 5.6 shows A_{net} as a function of g_s, an assumption that is supported by most authors and may primarily be explained, in C_3 plants, by an increased resistance to CO_2 diffusion in the mesophyll (Tenhunen *et al.*, 1984; Santrucek and Sage, 1996; Saxe *et al.*, 1998; Buckley *et al.*, 2003; Sage and Kubien, 2007). It is clear that a rise in g_s is associated with a rise in A_{net} in both [CO_2] treatments, but that the slope of the response is much higher for elevated than ambient [CO_2]. The underlying cause was copiously addressed in section 2.3.1 and may be summarized by an increased activity of Rubisco carboxylase for various reasons.

As stomatal closure is directly linked to intercellular [CO_2] (Morison, 1987; Mott, 1988; Hetherington and Atkinson, 1990; Mott, 1990; Chen *et al.*, 1999; Yamori *et al.*, 2011), g_s was plotted as a function of C_i in Figure 5.7. The result is somewhat surprising, since our comparison yielded a near to complete decoupling of g_s and C_i. Maybe even more intriguing is that this decoupling has manifested itself in opposite modes for the different [CO_2] treatments.

Generally g_s declines as C_i increases (Mott, 1988), but this behaviour can not be found in our results. One possible explanation may be that the calculation of C_i by the LI-COR system is not exact in all situations but only provides a rough estimate. If we were to assume this however, other correlations that were determined to be compliant with literature would be coincidential, and such an assumption would lead us no further. Let us assume then that our values are correct. The lack of response in the ambient [CO_2] treatment may just be due to the small interval across which C_i varies, which corresponds to a review of Long *et al.* (2004), where the remarkable constancy of C_i that is often observed, is explained by an impressive response rate of the stomatal opening.

A more interesting situation presents itself in the elevated [CO_2] treatment. We see little to no effect of a change in C_i across a rather large interval on g_s. One possible explanation may be that, as Niinemets *et al.* (2011) hypothesized, an increase in [CO_2] led to a more robust leaf structure, allowing for less sensitivity of g_s to environmental parameters. A second explanation might be given by a review by Ainsworth and Rogers (2007). Stomatal closure requires the guard cell membrane potential to be depolarized. Studies have shown that elevated [CO_2] increases the activity of outward rectifying K^+ channels, decreases the activity of inward rectifying K^+ channels, enhances anion channel activities, stimulates Cl^- release from guard cells and increases guard cell [Ca^{2+}]. These changes effectively depolarize the membrane potential of guard cells and cause stomatal closure, and this process is enhanced by elevated [CO_2]. It is possible that acclimation responses caused the depolarisation balance to shift and included a buffer mechanism similar to hysteresis.

Lastly Figure 5.8 shows absolute g_s throughout the experiment. There is a significant reduction in g_s in elevated compared to ambient [CO_2] and in low compared to high water seedlings. During the heatwave, T_{12} reached higher values than prior to or following the heatwave. Statistical analysis supported that there was a strong interactive effect of water and temperature treatment.

6.2.3 Leaf vapour pressure deficit

The relationship between g_s and VPDl was given in Figure 5.9, because we observed a response that was incoherent with literature. According to Sage and Kubien (2007) stomata can open with rising temperature when VPD is low. This observation was nuanced by Peak and Mott (2010), proposing a model where a rise of g_s due to increased temperature is only possible at a constant difference in the mole fractions of water in the air inside and outside the leaf. Our results however show an acclimation of g_s exclusive to T_{12}, where it never surpasses the value of 0.14. This might in any case be a contributing factor to the heat stress induced drought resistance mentioned in section 6.2.1.

6.3 Fluorescence parameters

6.3.1 Energy harvesting efficiency and effective quantum yield

A_{net} was plotted as a function of F'_v/F'_m and Φ_{PSII} in Figure 5.11 and Figure 5.12, respectively. A comparison of their corresponding values was presented in Figure 5.13. It is obvious that, although both measures are popular and often used for the same purpose in literature (Maxwell and Johnson, 2000; LI-COR Biosciences, 2008), our data suggest that they differ significantly in their response to elevated [CO_2].

Mathematically this difference can easily be explained. F'_v/F'_m compares the entire light acclimated fluorescence range to the fluorescence maximum, whereas Φ_{PSII} only compares the fluorescence range upwards from the the steady-state level to the fluorescence maximum (see Figure 5.10). The implications that this difference has on the other hand are of more importance and complexity.

What the above means in functional terms is that elevated [CO_2] brought little change to the light acclimated fluorescence minimum, which in simplified terms is a measure for the maximum amount of oxidized PSII centers in the light. Elevated [CO_2] did however cause significant changes in the light acclimated steady-state fluorescence, which is a measure for the actual amount of oxidized PSII centers in the light.

When considering our findings from the above perspective, one might conclude that elevated [CO_2] only slightly increased the relative amount of available PSII centers but significantly increased the relative amount of active PSII centers. Consequences of this phenomenon are further addressed in section 6.6.

Since Φ_{PSII} showed a greater response to different levels of [CO_2], its absolute values throughout the experiment were given in Figure 5.14. Elevated [CO_2] significantly increased Φ_{PSII} in all treatments.

6.3.2 Photochemical quenching

Figure 5.15 shows the relationship between Φ_{PSII} and Q_p, since we were interested in the extent to which an increased quantum yield corresponded with an increase in Q_p. Analysis shows that an increase of Φ_{PSII} is accompanied by an even greater increase in Q_p. The implications of this relationship will again be thoroughly discussed in section 6.6.

6.4 Water relations

6.4.1 Transpiration

Figure 5.16 shows the transpiration rate as a function of g_s across the different heat treatments. Comparing the slopes of the different graphs reveals that higher heat treatments yield equally high rates of transpiration for lower values of g_s. This corresponds to suggestions by Beerling *et al.* (1996); Centritto *et al.* (1999); Ellsworth (1999); Medlyn *et al.* (2001) and Peak and Mott (2010), who proposed other regulatory systems for transpiration. In this case it is suggested that the greater rate of transpiration is mainly due to the larger VPDl, which in its turn is a consequence of the extreme temperature applied during the heatwave. This finding corresponds to the lack of correlation between g_s and VPDl in Figure 5.9.

Figure 5.17 shows the absolute values of E throughout the experiment. The heatwave event is easily spotted by looking at the peaks in transpiration corresponding to T_6 and T_{12}. It is faintly visible how the low water treatment is less governed by diurnal patterns than the high water treatment.

6.4.2 Instantaneous water use efficiency

WUE_I was plotted as a function of VPDl in Figure 5.18. It is self explanatory that elevated [CO_2] would yield higher values, since it not only increases A_{net}, it also decreases E compared to ambient [CO_2]. Of more interest is the shape of the relationship. Extreme values for one variable yield oppositely extreme values for the other variable. In between lies an interval where the relation is quasi-linear. This interval is broadened in the elevated [CO_2] treatment, which might be explained by elevated [CO_2] induced production of fine roots able to maintain greater balance in the plant water relations by increasing soil water availability (Wullschleger *et al.*, 2002).

6.5 Statistical analysis of the treatments

In this section, a selection from the ample amount of information extractable from Tables 5.2 and 5.3 will be given.

Table 5.2 provides information about the significance of treatment combinations. Most prominent is the [CO_2] treatment, which was found to be highly significant for each parameter, with the dismal exception of VPDl, which might suggest no significant interaction between [CO_2] mediated leaf temperature and relative humidity. Another interesting observation is that A_{net} is significantly affected by each individual parameter, but not by their first level combinations, which might indicate no mitigating effect of elevated [CO_2]. Their second level combination however was found significant, which again could mean that there is a multi-directional mitigation operation.

The most impressive data in Table 5.3 would be the A_{net} values of C_ET_{12}, where the low water treatment actually approximate C_AT_0H, indicating that the occurrence of a heatwave was nearly completely counteracted by synergistic mitigation by elevated [CO_2] and drought acclimated responses.

6.6 Suggestions for a conceptual model that envelops the light mediated energy balance

The following section will attempt to provide answers to questions which, to the best of the author's knowledge, have remained unanswered or even unasked. A systematic approach will be used, where each new element will be explained upon introduction into the conceptual model.

As was previously addressed, Figure 5.3 shows that the elevated [CO_2] treatment seems to yield higher leaf temperatures. Two explanations are suggested: (i) partial closure of stomata and increased leaf resistance to water vapour loss result in increased tissue temperature and (ii) stress induced decreases in the photorespiratory chain lead to increased energy dumping via heat dissipation.

The first one may just be the main cause for this observation. Our results, in compliance with literature, show that elevated [CO_2] significantly reduced g_s and E, thus inhibiting one of the main systems for plant temperature regulation. Oddly enough only one study could be found where specific attention was directed to this increase in tissue temperature. Prasad *et al.* (2006) found that, instead of mitigation, elevated [CO_2] led to exacerbation of heat stress. A reduced rate of leaf vapour loss was suggested to be the cause.

The second mechanism may not be of equal quantitative importance as the first one, although the author believes that several previously unmarked phenomena may be integrated. Therefore, if not an explanation for increased leaf temperature, it might just prove to be a comprehensive model for these phenomena.

Figure 6.1 gives a schematic overview of sinks for energy obtained by light harvesting. As previously mentioned, this energy may either be dissipated as heat or used in photochemistry. A measure for these mechanisms was given by Q_N and Q_p respectively, where Q_N is non-photochemical quenching.

$\hookrightarrow$ We observed that elevated [CO_2] only slightly increased the amount of available PSII centers, but significantly increased the amount of active PSII centers. This increase was caused by a decrease of the light acclimated steady-state fluorescence. Interestingly enough this last parameter may be used to deduct some information about Q_p.

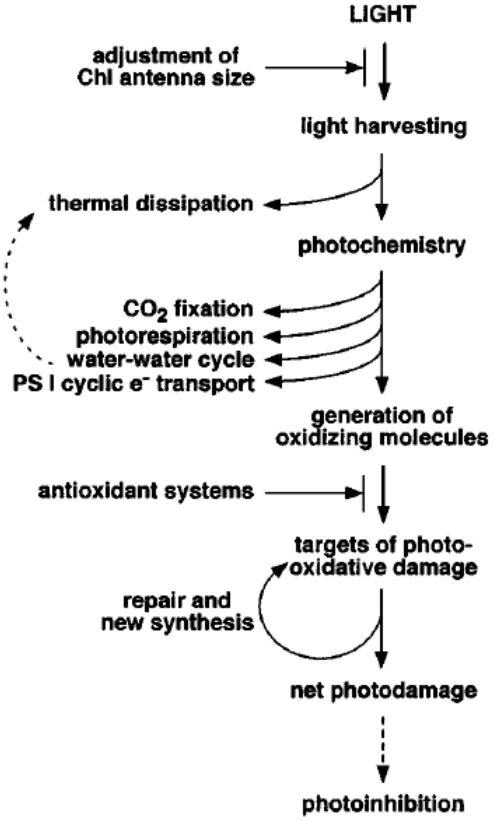


Figure 6.1: Schematic diagram of photoprotective processes occurring within chloroplasts (Niyogi, 1999).

$\hookrightarrow$ Remember how Φ_{PSII} compared the fluorescence range upwards from the the steady-state level to the fluorescence maximum (see Figure 5.10)? Q_p compares the same value, though not to the light acclimated fluorescence maximum but to the entire light acclimated fluorescence range. In functional terms Q_p determines the ratio of Φ_{PSII} to F'_v/F'_m, which may be interpreted as the relative activity of the photosynthetic metabolism.

Figure 5.15 shows an enhanced response of Φ_{PSII} to increasing Q_p due to elevated [CO_2], which might indicate that not only the amount of active PSII centers have strongly increased, there might actually be an increase in available PSII centers. The last and most excessive statement would be that elevated [CO_2] reduces the gap between dark and light acclimated fluorescence.

$\hookrightarrow$ Before we lose ourselves in speculation, let us put the above into context. According to Lloyd and Farquhar (2008) the maximum electron transport rate, responsible for Rubisco regeneration, is inhibited by cyclic flow of electrons around PSI, which is part of the photochemistry pathway for electrons. This flow increases as PSII is in need of more protection. It was suggested by Wingler *et al.* (2000) that an increase in PSII activity would entail such an increased need for protection.

$\hookrightarrow$ The last component of this model makes the following suggestion: due to a relatively reduced rate of photochemical quenching, other energy sinks must be utilized, one of which is leaf level heat dissipation. This increase in leaf level heat dissipation would create a positive feedback on the cyclic flow of electrons around PSI, which would further inhibit photochemical electron transport rate, until saturation of the cyclic flow occurs (Streb *et al.*, 2005). It would have been very useful to have the actual Q_N data in this context, but as mentioned in section 5.3.3 these data were unavailable.

Although seemingly complex, some surmised connections were made between this model and literature. Many authors recorded a decreased leaf N concentration on a mass base but not on an area base (BassiriRad, 2000; Zerihun and BassiriRad, 2001; Maier *et al.*, 2008). Research by Wingler *et al.* (2000) shows that photorespiratory carbon metabolism is intimately linked to nitrogen metabolism in the leaf. Due to a decreased electron transport rate, Rubisco regeneration is thus effectively inhibited, leading to lower leaf Rubisco concentrations.

Another connection was found in a review by Ainsworth and Rogers (2007), who found compelling evidence that the Calvin cycle and photosynthetic electron transport operated in guard cell chloroplasts at similar rates to those in mesophyll cells. There was one hypothesis suggesting that photosynthetically derived ATP was shuttled from guard cell chloroplasts to the cytosol, where it would drive proton pumping and cation uptake at the plasmalemma. This was supported by findings that guard cell [ATP] decreased with higher [CO_2], which would mean that Q_p or Φ_{PSII} would have be high enough to support Calvin cycle activity in the guard cells. The great news is that our findings prove (or at least indicate) that these parameters are much higher in elevated [CO_2]. This would be another explanation for the insensitivity of g_s observed in Figure 5.7.

6.7 Implications on climate change

This study mimicked our future climatic situation in several ways, but all of them entailed an increase in atmospheric [CO_2] and a mean rise in annual temperature. Few would disagree that the most important questions in this context are ”Will the plants survive?”, followed by ”Will they do better or worse?” and ”How much better or worse?”. Our results suggest that at the very least *Quercus rubra* will survive, and that it will do a lot better than today, especially under stress conditions.

We found that heat and drought stress had a significantly negative impact on photosynthesis in today's [CO_2], whereas future [CO_2], that should accompany aforementioned stresses, strongly mitigated these stresses, and in some cases even made these parameters benefit from them. The possible reasons for this are various and complex, but our and previous studies suggest that a substantial alteration in plant water relations is responsible.

The future may look uncertain and direful to some, but our forest ecosystems may very well adapt and find a new balance nevertheless, as they always have.

Chapter 7

Conclusions

7.1 General inferences

We started out with very specific working hypotheses, and have succeeded in conducting our experiment aptly enough to be able to validate these statements.

Hypothesis 1: ”*Seedlings in the low water treatment will have reduced photosynthesis and transpiration compared to those in the high water treatment.*”

- Surprisingly we found that low water seedlings from the elevated [CO_2] treatment achieved higher photosynthetic rates during the heatwave in the morning than their well watered counterparts, and that this phenomenon grew further in the heatwave, though this was entirely different in the afternoon. This raises the question as to how A_{net} evolves throughout a day, which asks for studies with continuous measurement methodologies. The same could be said for transpiration. This could mean a stomatal response to heat stress is responsible.
- Taking the previous observations into extra account, low water seedlings showed significantly greater decrease in A_{net} from the morning to the afternoon than the well watered seedlings. The opposite was true for transpiration, however, which complies with our previous conclusion, since a lowered stomatal conductance reduces transpiration.
- While it was hypothesized that the low water treatment would undergo greater heat stress than the high water treatment, this was very much untrue. The reduction in A_{net} was far

greater in the well watered seedlings. Again this points to a possible stomatal mediated acclimation response.

- Transpiration skyrocketed during the extreme heatwave (T_{12}) compared to the control treatment, although this should not be interpreted as a loss of stomatal control, rather than as an increased air VPD due to the extreme ambient temperature.

Hypothesis 2: ”*Elevated [CO$_2$] will mitigate heat stress induced reductions in photosynthesis in both low and high water treatments, though the effect will be greatest in low water seedlings.*”

- Elevated [CO_2] seedlings had significantly higher rates of photosynthesis than ambient [CO_2] seedlings, both prior to and during the heat wave incident.
- Elevated [CO_2] seedlings had experienced a significantly smaller reduction in A_{net} than ambient [CO_2] seedlings during the heatwave, and this difference increased further in the heatwave.
- The seedlings from the high water and elevated [CO_2] treatment had the highest photosynthetic rates prior to the heatwave, while seedlings from the low water and ambient [CO_2] treatment had the lowest rates.
- While the above observations might suggest that elevated [CO_2] would do better than ambient [CO_2] or the high water treatment would do better than the low water treatment our findings indicate otherwise. Water was the most determining factor and, as stated in the previous hypothesis, low water seedlings had their A_{net} reduced to a lesser extent than high water seedlings, even in such a way that the ambient [CO_2] by low water treatment suffered less heat induced stress than the elevated [CO_2] by high water treatment. Again this suggests a possible stomatal mediated acclimation response by the low water seedlings.

7.2 Future directions

As indicated in the above section, we believe that direct or indirect continuous measurements of A_{net} could prove to be valuable, since it would allow for recording the diurnal photosynthetic pattern on the one hand and for expediently determining the mean photosynthetic rate for one day on the other hand. Another suggestion is to insert a prolonged measurement period in future experiments, by which we mean successive heatwaves and recovery periods. This study yielded some inconclusive results that could have been put in the right context if previous and future data had been available.

Also, as was adressed in section 6.1, the behaviour of plants in closed top chambers is often different or more explicit than in open top chambers or in FACE experiments. Therefore it might be interesting to repeat this experiment in a similar manner outside of the greenhouses.

Another suggestion is the implementation of leaf composition measurements. Many authors have already suggested that a lack of leaf N might be causing a downregulation of CO_2-stimulated photosynthesis (BassiriRad *et al.*, 1997; BassiriRad, 2000; Zerihun *et al.*, 2000; Zerihun and BassiriRad, 2001; Maier *et al.*, 2008). Our temperature treatments as well showed several physiological discrepancies on the leaf level, and knowing the composition of the stomatal and mesophyllic tissue might shed some light on these murky mysteries.

The interactions between the environmental conditions that are likely to change in the near future remain poorly understood still, but our research may just have added another piece to an ever growing puzzle.

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