

Productivity and Water Use of Australian Tree Species under Climate Change

By

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STATEMENT OF CANDIDATE

I certify that the work presented in this thesis entitled “Productivity and water use of Australian tree species under climate change” has not been previously submitted for a degree, nor has it been submitted as part of requirements for a degree to any other university of institution other than Macquarie University.

I also certify that this thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

The research presented in this thesis did not require approval from Macquarie University Ethics Committee.

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ABSTRACT

Climate change is likely to impact heavily on Australian forests. Over the next century Australian forests will likely experience an increase in the frequency and severity of drought conditions, and an increase in temperature. Such changing climate conditions may severely disrupt the role of Australian forests in regional and global carbon and water cycles. However, there are considerable gaps in our understanding of the impacts of these changes in climate on forests. In this thesis I examine two key questions for Australian forest responses to climate change. Firstly, it is commonly hypothesized that elevated CO₂ will ameliorate the impact of drought on forest growth and productivity, but there is little evidence to date to support this hypothesis. To address this gap, I examine the impact of elevated CO₂ and variable drought conditions on two *Eucalyptus* species of contrasting drought tolerance. Secondly, we have little understanding of the effect of increasing temperature on leaf level physiology in species from warm climates. To address this gap, I examine the impact of temperature on leaf level physiology of two Australian tropical rainforest species.

It is often hypothesized that elevated CO₂ will impart the greatest relative benefit to forest ecosystems under water limitation, and therefore that elevated CO₂ will reduce the impact of drought. There are two main mechanisms underlying this hypothesis. The first is that lower intercellular CO₂ (C_i) occurring under drought conditions causes a larger enhancement of photosynthesis (A) relative to atmospheric CO₂ concentration (C_a) due to the non-linear response of A to C_i. Further, the higher slope of the A-C_i curve at lower C_a leads to a greater reduction of A, due to drought, under ambient than elevated C_a. Secondly, stomatal conductance is reduced under elevated CO₂, lowering transpiration rate and conserving soil moisture, thus enabling trees under elevated CO₂ to continue to transpire longer into a drought episode. In this thesis, I explore the two mechanisms for CO₂ x drought interactions separately, using a large experiment with two *Eucalyptus* species of contrasting drought tolerance in elevated (700 ppm) and ambient (380 ppm) CO₂ glasshouses.

To explore the first mechanism - lower intercellular CO₂ under drought conditions - seedlings of mesic *Eucalyptus pilularis* and xeric *Eucalyptus populnea* were grown at soil moisture content of either 50% or 100% of field capacity (FC) for 9-11 months. We hypothesized that water-use efficiency (ratio of carbon gain to water loss) would be proportional to growth CO₂ (i.e. in this experiment, elevated CO₂ would cause an increase in WUE of 84%, the ratio of elevated (700 ppm) to ambient CO₂ (380 ppm)). We hypothesised that this increase would be the same for both drought treatments and species, but that lower C_i

in the droughted plants and xeric species would lead to relatively larger CO₂ effects on photosynthesis and biomass growth, and smaller CO₂ effects on transpiration, than in the well-watered plants and the mesic species. These hypotheses were rejected. At the leaf level, instantaneous transpiration efficiency (ratio of photosynthesis to transpiration) responded more than proportionally to growth CO₂ (i.e. greater than 84%). Leaf gas exchange was not affected by growth under long-term moderate drought and did not differ between species, thus leading to a rejection of the low C_i mechanism.

At whole plant scale, the CO₂ effect on whole-plant water use efficiency (WUE) was considerably less than the increase in CO₂. For both species, transpiration rate was similar for plants grown under elevated or ambient CO₂, reflecting an increase in leaf area to compensate for the CO₂-induced reduction in g_s . These results suggest that under elevated CO₂ and long-term moderate drought both mesic *E. pilularis* and xeric *E. populnea* exhibit a capacity to adjust growth processes to match water availability in order to avoid moderate drought stress.

A test of the second proposed mechanism (soil water savings under elevated CO₂), was carried out at the end of the large experiment. This test involved bringing all pots of *E. pilularis* and *E. populnea* back to full field capacity and allowing plants to dry down to predetermined physiological stress levels. There were clear differences among species and antecedent watering treatments in the effect of CO₂ on water stress (identified as a change from the maximum in photosynthesis (A) and stomatal conductance (g_s) observed under well-watered conditions, when pre-dawn leaf water potential (Ψ_{pd}) was close to zero). A delay in water stress in this context represents a longer period of time taken to reach a minimum in A and g_s from well-watered conditions. During the dry down experiment, elevated CO₂ strongly delayed water stress in well-watered *E. populnea* but had no effect on progress of water stress in well-watered *E. pilularis*. Plants of both species grown under low water availability showed some reduction in water stress with elevated CO₂. These responses can be understood from the perspective of individual species ecological strategies: under well-watered conditions *E. pilularis* grows rapidly in response to elevated CO₂, making it vulnerable to future drought, whereas *E. populnea* responds conservatively to elevated CO₂, allowing soil moisture savings when drought occurs.

Lastly, a better understanding of the effect of warming on leaf level physiology for tropical rainforests is needed to assist with better parameterization of global scale models of forest response to climate change. In this experiment I measured the biochemical and stomatal limitations to leaf level photosynthesis in response to temperature on two canopy species at the Australian Canopy Crane Research Station (ACCRS) in Cape Tribulation,

Queensland, Australia. Data were used to perform a sensitivity analysis of a coupled photosynthesis-stomatal model, comparing rainforest parameter values with two parameter sets commonly used for modelling from Leuning (2002) and Kattge and Knorr (2007). The analysis showed that general parameters for C_3 species used in global scale models of forest responses to climate change under predict the optimum temperature of photosynthesis for tropical forest species, even when temperature acclimation is taken into account. The parameter values obtained in this study will prove useful for improving global vegetation models.

CHAPTER 1

1.1 Introduction

Climate change, tied to the continuing rise in atmospheric CO₂, is projected to result in higher temperatures and an alteration to precipitation patterns on a local, regional and global scale during this century (IPCC 2007). The impact of this alteration in climate, on forests, is an issue of ever growing importance due to the integral role that forests play in global and regional carbon and water cycles. Forest ecosystems directly influence local, regional and global climate through exchanges of energy, water and CO₂ (Bonan 2008). Forests additionally serve an essential role as a sink for carbon; however the long-term outlook on forests functioning as carbon sinks is highly ambiguous in light of ongoing climatic change (Sabine *et al.* 2004; Bonan 2008; Anderegg *et al.* 2012). An alteration in the role of forests from the largest terrestrial carbon sink to one of the largest sources of carbon could rapidly accelerate the rate of climate change with hugely detrimental impacts on global environment (Dale *et al.* 2001). Consequently, it is highly imperative that we gain a better understanding the response of forests to environmental factors such as drought and temperature that will most dramatically impact the productivity and water use of forests under future climate (McDowell *et al.* 2011).

An important first step in understanding global patterns of forest response to climate change is identification of regional or even local patterns in forest response to environmental variability (Allen and Breshears 1998). A particularly useful region for looking at water limitation, changing precipitation patterns and temperature increases associated with climate change is Australia, as a result of its standing as the driest inhabited continent and long history of drought events (McAlpine *et al.* 2009). Under future climate, the productivity and water use of Australian forests is expected to change as a result of increasing atmospheric CO₂, rising temperature and a projected increase in the intensity and frequency of drought events (IPCC 2007; Medlyn *et al.* 2011b). Many Australian forest species have adapted to an environment of limited water availability (Merchant *et al.* 2007, 2010; Warren *et al.* 2011a, b), yet the rate at which drought frequency and intensity may increase under future climate may lie outside of many tree species adaptive capacity. Reductions in water supply will likely influence tree species range and diversity through direct influence on patterns of mortality and successional processes. A focus throughout this thesis will be upon *Eucalyptus* species and

Australian rainforest species with two central questions: 1-) how does drought interact with elevated atmospheric CO₂ to impact *Eucalyptus* species? 2-) how does rising temperature impact leaf level physiology of Australian rainforest species?

The framework of this introductory chapter is to provide background information on climate and the impact of particular climate factors on plant growth and physiology. This background information lays the foundation for experiments presented in later chapters. The thrust of the first section of this chapter is on historical and projected patterns in climate change at both a global and a regional scale, with an emphasis on natural patterns and processes within Australia. Following that, information will focus on the role and importance of forests in the global environment, with a particular focus on Australian forests. Additionally, a background will be provided on our current understanding of forest response to elevated CO₂, water availability and the interaction between these two factors. Finally, a brief review will be provided of our current understanding on the response of tropical rainforests to climate change, with an emphasis on the impact of temperature.

1.2 Climate Change

Prior to the Industrial Revolution (circa 1750) the atmospheric CO₂ concentration had been relatively stable at approximately 280 parts per million (ppm) (IPCC 2007). By the year 2005 the atmospheric CO₂ concentration reached 380 ppm, marking the highest level in over 650,000 years (IPCC 2007). And, of even greater concern to overall climate is the recent rate of atmospheric change, with the years 1995 to 2000 exhibiting the largest increase (1.9 ppm yr⁻¹) than at any other time during continuous direct atmospheric estimates (IPCC 2007).

The recent and geologically rapid change in climate is directly attributed to anthropogenic burning of fossil fuels, along with clearing of forested areas (IPCC 2007). Deforestation impacts climate in two ways. Initially the cleared forest is no longer available to take up carbon dioxide, thus creating a loss of a carbon sink. Secondly, the cleared material breaks down and decays over time and becomes a carbon source. In addition, land which is deforested leads to an increase in the albedo or reflectivity of the surface and influences rates of evaporation and evapotranspiration (Bonan 2008). These impacts associated with deforestation contribute to an increase in carbon emissions and thus an increase in the concentration of atmospheric greenhouse gases.

The major concern with the ongoing burning of fossil fuels is the heat trapping ability of greenhouse gases such as carbon dioxide, nitrous oxide and methane. According to the most recent summary on the impacts of climate change (IPCC 2007), the average temperatures at the Earth's surface have risen globally, with important regional variations. Expressed as a global average the temperature at the Earth's surface has risen by 0.74 °C over the hundred year period between 1906 to 2005, while more than half of this warming (0.4 °C) has occurred since 1979 (IPCC 2007).

Climate over the last 100 years within Australia has closely tracked global estimates. Within Australia, average temperatures have increased by 0.9 °C since 1950 (CSIRO 2007). Precipitation has varied regionally with increases in northwestern and central Australia and decreases in the south-east, south-west and central east coast (CSIRO 2007). Conversely, the east coast, state of Victoria and the southwest of Australia have all experienced significant rainfall declines since 1950 (CSIRO 2007). The incidence and intensity of drought increased over the latter half of the 20th century with occurrence of drought much higher in areas seeing lowered precipitation rates (Nicholls 2004; CSIRO 2007). Droughts occurring during the latter half of the century were also exacerbated by an increase in heat waves (Nicholls 2004) and higher surface temperatures (CSIRO 2007).

1.2.1 *Climate Change (Projections)*

Model projections for climate in the year 2099 are for atmospheric CO₂ to reach 600 ppm under a low emissions scenario to a high of 1100 ppm at a high emissions scenario (Sitch *et al.* 2008). This rise in fossil fuel emissions is projected to raise the average global temperature by 1.8 °C to 4.0 °C by the year 2100 (IPCC 2007). Additionally, the increase in global average temperature will lead to alterations of the hydrological cycle and impact ecosystems in a multitude of ways. An increase in temperature will bring about changes in precipitation due to greater rates of evaporation and related increase in water vapor within the atmosphere (Bonan 2008). Due to many interacting environmental factors, an increase in extreme weather events is projected under a future climate. Such extreme weather events include an increase in the number of heat waves, droughts, heavy precipitation events and flooding (IPCC 2007; Hansen *et al.* 2012).

Climate change projections indicate an increased variability of rainfall for many regions of the world in the next 40 years (IPCC 2007; Battisti and Naylor 2009), with the variability greatest in terms of timing versus absolute amounts of precipitation (Trenberth

2005). In regions with mean precipitation increasing, there is a likelihood of more wet days and regions with mean precipitation decreasing are likely to see an increase in the number of extreme dry days (IPCC 2007). Current climate models predict an increase in the severity and frequency of drought for many regions of the world (IPCC 2007). In areas already experiencing drought the projections are for the intensity and frequency of drought events to increase (Trenberth 2005; IPCC 2007) which produces a significant issue for forests close to the lower edge of their precipitation range and or with low adaptive capacity in the face of changing climate (Chmura *et al.* 2011).

Model estimates for temperature increase within Australia are for temperature to rise by 0.8 °C to 1.4 °C by 2030 and by 1.0 °C to 5.0 °C by the year 2070 (IPCC 2007; CSIRO 2007). An increase in the number of hot days is projected along with an overall increase in warm nights (IPCC 2007). Precipitation is projected to change little in the far north and to decrease by 2 to 5% elsewhere (CSIRO 2007). Heavy precipitation events are projected to increase with larger breaks between events (IPCC 2007). Due to increasing temperature, annual potential evapotranspiration is projected to increase over Australia, with the largest increases in the north and east (CSIRO 2007). Lastly, the occurrence and intensity of drought is projected to increase across much of Australia (IPCC 2007), with the largest increase projected for south-western Australia (CSIRO 2007).

1.3 The role of forests

Forests cover approximately 30% (FAO 2006) of the land surface area of the Earth and account for nearly 75% of terrestrial net primary production (Melillo *et al.* 1993; Bonan 2008). Forests provide key interactions with carbon, water and nutrient cycles. An increase in the atmospheric CO₂ concentration, and an associated increase in temperature and changing precipitation cycles, will affect the exchange of energy, carbon, water and nutrients between forests and the environment. The impact of these changes in climate could potentially lead to a massive alteration in forest growth, composition, health and overall ecosystem functioning (Bonan 2008). The adaptive capacity of forest species to climate is generally set by local conditions (Chmura *et al.* 2011). In the face of ongoing climate change, species are often experiencing climate beyond the conditions under which they have existed and adapted (St. Clair and Howe 2007; Aitken *et al.* 2008). Phenotypic plasticity offers some potential in response to environmental variability but it is uncertain if acclimation responses will keep track to the range of climate predicted for many species (Chmura *et al.* 2011). Additionally,

without optimal phenotypic responses, rapid climate change may challenge the capacity of tree species to adapt in place or migrate to suitable areas (Aitken *et al.* 2008).

1.3.1 Forest importance (Australia)

Forests influence and are influenced by climate through physical, chemical and biological processes that affect the cycling of water and carbon between the land surface and atmosphere (Bonan 2008). Australian forests provide a key to understanding forest response to climate as many forest types exist precariously close to climate extremes, inhabiting areas marked by high temperatures, low nutrients, infrequent rainfall and regular occurrence of intense and severe droughts (Medlyn *et al.* 2011).

In Australia, approximately 21% of the land surface is covered in forest, stretching mainly along the eastern seaboard, the Northern Territory and Tasmania (Stork *et al.* 2007). The overall extent of forested land area within Australia sits at approximately 149 million hectares, 147 million of which is native forest and 2 million hectares in plantation forest (Montreal Processes Implementation Group for Australia (MIG) 2008). The two most dominant forest types within Australia are eucalypt (including the genera *Eucalyptus*, *Corymbia* and *Angophora*) and acacia forests, accounting for approximately 86% of native forests (MIG 2008). In addition to making up a majority of the composition of native forests, eucalypt species are also widely used in plantation forests, accounting for approximately 34% of plantation forests within Australia. Beyond eucalypt and acacia forest the remaining composition of forest types includes rainforest, mangroves and wetland forest types (MIG 2008).

1.3.1.1 *Eucalyptus*

Eucalyptus is a very iconic and widespread genus within Australia comprising over 700 species, each with its own particular adaptations and ecological strategy for survival (Williams and Brooker 1997). The *Eucalyptus* genus is almost wholly comprised of species found with Australia (Pryor *et al.* 1995), with additional species native to parts of Indonesia. Due to such a large number of eucalypt species, wholly endemic to the Australian continent, many ecological niches are filled with ecosystems ranging from coastal rainforest to the dry interior and from low lying regions to subalpine sites at tree line (Specht 1972). Eucalypts dominate the forests and woodlands of coastal Australia and vast areas of the arid interior

(Boland *et al.* 1984). As it is such a large genus there are multiple plant traits in operation. However, a few plant traits are commonly shared, such as being broad-leaved evergreens with sclerophyllous leaves. Additionally, the vertical orientation of many eucalypt leaves is also a common shared trait, thought to reduce the high temperatures experienced during summer months for many eucalypt species (Pryor 1976). Eucalypts make up a majority of the forest types within Australia from true forest, to woodland and mallee types (Specht 1970), categories predominately based on height and environment. For example, higher rainfall coastal zones commonly contain a greater proportion of eucalypt forest types whereas within the dry interior is represented by eucalypt mallee forest types (Specht 1970). In higher rainfall zones the response of eucalypt species is commonly much different than species existing within arid zones. For example, mesic grown eucalypt species commonly grow taller and exhibit greater leaf area, with less nutrient limitation than arid grown species. In arid environments eucalypt species typically grow deep roots to reach water unavailable to other plants (Eldridge 1994; Stoneman 1994).

1.4 Elevated CO₂

1.4.1 Effect of elevated CO₂ on non-water limited forests

An increase in atmospheric CO₂ concentration is predicted to stimulate forest productivity directly through increased rates of photosynthesis (Idso *et al.* 1991; Norby *et al.* 1995; Whitehead and Teskey 1995; Roden and Ball 1996a; Norby and Luo 2004; Morgan *et al.* 2004; Nowak *et al.* 2004a; Long *et al.* 2004; Ainsworth and Long 2005; Duursma *et al.* 2011). This direct effect is known as “CO₂ fertilization”, which occurs as plants with the C₃ carbon fixation pathway are not saturated at current levels of atmospheric CO₂ (Ainsworth and Long 2005). Short term increases in photosynthesis with CO₂ enrichment often translate into productivity gains over the long-term. In a comprehensive meta-analysis of over 500 studies looking at the impact of elevated CO₂ on woody tree species, Curtis and Wang (1998) reported that enhanced CO₂ levels led to a significant increase in total biomass and plant net carbon assimilation rates.

1.4.2 Photosynthesis

The widely used photosynthetic model of Farquhar, von Caemmerer and Berry (1980), relates C₃ gas exchange in relation to the underlying limitations to photosynthesis. In the

Farquhar *et al.* (1980) model of C_3 photosynthesis, carbon assimilation rate (A) is driven by intercepted light, CO_2 and temperature. Photosynthetic rate (A) is assumed to be limited by either the carboxylation rate or the electron transport rate, which are represented in the Farquhar *et al.* (1980) model through the model parameters V_{cmax} (maximum Rubisco carboxylation capacity) and J_{max} (maximum RuBP regeneration capacity). However, photosynthesis is also limited by the delivery of CO_2 to the mesophyll, which itself is controlled by two main resistances, namely the stomatal and the mesophyll resistance. These two resistances (stomatal and mesophyll) will be covered in greater detail in the next section. In regards to the carboxylation rate, when grown under increasing atmospheric CO_2 plants typically increase light saturated photosynthesis (Curtis and Wang 1998; Norby *et al.* 1999; Long *et al.* 2004; Ainsworth and Long 2005; Ainsworth and Rogers 2007), resulting from an increase in carboxylation and an inhibition of photorespiration (Long *et al.* 2004). A higher CO_2 concentration increases the leaf intercellular CO_2 concentration (C_i) and the $CO_2:O_2$ ratio at the Rubisco site, which favors carboxylation rather than oxygenation of RuBP; ultimately suppressing photorespiration and enhancing net photosynthesis (Long and Drake 1992; Saxe *et al.* 1998). CO_2 does not saturate rubisco in C_3 species at current levels of atmospheric CO_2 concentration, with saturation occurring nearer ca 500-700 ppm (Sage *et al.* 2002). Evidence from elevated CO_2 experiments suggests that an increase from 380 ppm to 550 ppm leads to an increase in leaf level photosynthesis by 50% (Medlyn *et al.* 1999). As atmospheric CO_2 increases above 550 ppm photosynthesis should be increasingly limited by RuBP regeneration (Rosenthal *et al.* 2011).

1.4.3 Stomatal conductance (g_s)

Along with an increase in photosynthetic rate, plants typically decrease g_s over the short and long-term when grown under elevated atmospheric CO_2 (Norby *et al.* 1999; Medlyn *et al.* 2001; Long *et al.* 2004; Ainsworth and Long 2005; Ainsworth and Rogers 2007). However, an acclimation response of g_s is sometimes observed with long-term growth under CO_2 enrichment (e.g. Santrúcek and Sage 1996; Maherali *et al.* 2002; Domec *et al.* 2009). The effect of a decrease in g_s is a reduction in transpiration, as transpiration unlike photosynthesis is limited only by the stomatal resistance. Numerous climate change experiments display reductions in g_s with growth under elevated CO_2 , with reductions of between 40-50%, although reductions are not universal and some experiments show no change in g_s (Medlyn *et al.* 2001). The exact mechanism causing the decrease in g_s with

increasing CO₂ is uncertain but thought to be a sensing of intercellular CO₂ (C_i) by guard cells rather than CO₂ at the leaf surface (Mott 1988). In the long term, decreases to g_s come about as a result of stomatal density and stomatal index, along with stomatal aperture (Ainsworth and Rogers 2007). According to Drake *et al.* (1997) the leaf intercellular to ambient CO₂ (C_a) ratio (C_i/C_a) remains constant when grown under either ambient or elevated atmospheric CO₂. As a result, plants growing under elevated atmospheric CO₂ conditions consistently experience a higher C_i. This allows species grown under elevated atmospheric CO₂ to maintain higher rates of photosynthesis due to the greater availability of substrate.

The increase in light saturated photosynthesis and decrease in g_s under elevated CO₂ commonly leads to an increase in instantaneous transpiration efficiency (ITE; ratio of photosynthesis to transpiration) at the leaf level (Rogers *et al.* 1983; Eamus 1991; Drake *et al.* 1997; Barton *et al.* 2012). An increase in ITE can come about as a result of an increase in light saturated photosynthesis alone, reductions in g_s alone or a combination of the two occurring in tandem, or with one changing more than the other (Eamus 1991). According to the optimal theory of g_s (defined below), ITE should increase in proportion to C_a (Cowan and Farquhar 1977; Medlyn *et al.* 2011). For example, an increase of C_a from 380 to 700 ppm would mean an increase in ITE of 84%. At the leaf level this amounts to a large reduction in transpiration, but evidence is limited as to whether this reduction leads to water savings at the whole plant level. This is a consequence of potential changes in allocation with CO₂ enrichment, particularly to leaf area, leading to similar or greater overall transpiration losses at the whole plant scale (Eamus 1991; Drake *et al.* 1997; Vivin *et al.* 1996; Wullschlegel *et al.* 2002).

In order to model photosynthesis and transpiration, we need to be able to separate stomatal resistance from the mesophyll limitations to photosynthesis. One means to do this is through measurement and fitting of A-C_i curves. Plotting of such A-C_i curves can provide information on the mesophyll limitations, given through the parameters apparent J_{max} and V_{cmax} (called apparent because they include the effect of mesophyll conductance, as well as the true carboxylation rate and electron transport rate). Conversely, the C_i is determined by stomatal behaviour and can be identified through the use of a recently developed model of stomatal conductance (g_s). Several models of g_s have been developed, some based on optimal theory (e.g. Cowan and Farquhar 1977) and others with a more empirical backing (Ball *et al.* 1987; Leuning 1995). The use of models can often permit a clearer understanding of environmental influence on a given physiological mechanism (Medlyn *et al.* 2011b). Models with direct and simple interpretation of parameters are often quite useful in identifying

controlling mechanisms on forest response under a range of environmental situations. An example is the model of g_s suggested by Medlyn *et al.* (2011) and based on the theory of optimal stomatal behavior by Cowan and Farquhar (1977). According to this theory, stomata balance their roles in carbon gain and water loss by maximising the daily photosynthetic carbon uptake for a given daily water loss. Medlyn *et al.* (2011) combined an empirical model of g_s with the theory of optimal stomatal control, to develop the following

$$g_s = g_0 + 1.6(1 + \frac{g_1}{\sqrt{D}}) \frac{A}{C_a} \quad (1)$$

where D is the leaf to air vapor pressure deficit (D ; kPa), g_1 is a constant that reflects the marginal cost of water to the plant and g_0 is the cuticular conductance (Medlyn *et al.* 2011). The parameter g_1 gives an estimate of the balance between photosynthetic gain to transpiration loss, in essence relaying if water is at a premium or widely available and how these two factors are determined by species and environment. For example, in mesic environments with low vapor pressure deficit (VPD), the marginal cost of water is less than under xeric conditions with high VPD, assuming similar plant functional types and sizes. Additionally, a particular species strategy in taking up and using water may set g_1 . For example, species displaying the anisohydric strategy of g_s are thought to maintain open stomata for longer periods of time throughout the day reacting less to VPD and more to deficiencies due to low soil moisture availability, thus maintaining a higher g_1 overall. Whereas, species displaying an isohydric strategy close stomata earlier during a water stress event thus preventing further transpiration and potentially decreasing g_1 . The utility of g_1 in determining species-specific use of water is in relation to the two parameters making up g_1 . The two parameters include the CO_2 compensation point, Γ^* , and the marginal water cost of carbon, λ , giving g_1 as proportional to the combination of terms $\sqrt{\Gamma^* \lambda}$:

$$g_1 \propto \sqrt{\Gamma^* \lambda}$$

A simplifying assumption that makes g_1 very useful is that the CO_2 compensation point, Γ^* , and its temperature dependence is considered constant for all C_3 vegetation and increases with temperature (Bernacchi *et al.* 2001), suggesting that g_1 should correspondingly increase with temperature. In contrast, the marginal cost of water, λ , is thought to be species specific rather than strictly temperature dependent and therefore may allow for parameter comparison (Medlyn *et al.* 2011). Comparing species from various ecosystems and environments may permit insight into the factors influencing λ and for global change studies provide insight into

whether g_1 changes with growth under elevated CO_2 . Recent findings from Medlyn *et al.* (2011) found a close relationship between growth temperature and g_1 with values highest in warm, tropical angiosperms and lowest in cool, temperate conifers providing evidence of the strong temperature enhancement of the marginal cost of water. Comparing co-existing species may provide greater insight into other factors, beyond temperature controlling the marginal cost of water such as species-specific rates of photosynthesis, g_s or hydraulic architecture (Medlyn *et al.* 2011).

The utility of the Medlyn *et al.* (2011) model of g_s is found in not only comparing between co-existing species, but also in identifying treatment effects on the parameter g_1 , which is expected to remain constant as environmental conditions change. This feature of the parameter g_1 greatly improves direct assessments of plant performance by removing confounding factors from plant species comparisons. This use of the model is analogous to the use of the Farquhar and von Caemmerer (1982) model of photosynthesis to understand differences in species performance or responses to environmental conditions such as CO_2 and temperature, by investigating effects on the model parameters V_{cmax} (maximum Rubisco carboxylation capacity) and J_{max} (maximum RuBP regeneration capacity) (e.g. Medlyn *et al.* 1999; Ainsworth and Rogers 2007; Kattge and Knorr 2009). Another very relevant component in global change scenarios is elevated atmospheric CO_2 , and it is important to identify how the marginal cost of water will change with elevated CO_2 . An example of the utility of the Medlyn *et al.* (2011) model is its simple interpretation of parameters at a range of relevant atmospheric CO_2 concentrations. For example, when the plant canopy is well coupled to the atmosphere such that total transpiration is directly controlled by VPD and g_s , and $g_s \gg g_0$, equation (1) can be transformed to provide:

$$\text{ITE} = \frac{A}{E} = \frac{C_a}{(g_1 \sqrt{D} + D)} \quad (2)$$

Therefore, if g_1 and D are the same between C_a treatments, theory predicts that ITE should remain proportional to C_a , so that any increase in C_a will lead to an increase in ITE and conversely any decrease in C_a will cause a concomitant decline in ITE.

1.4.4 Down-regulation of photosynthesis

In many elevated CO_2 experiments there is often a down regulation of photosynthesis (Gunderson and Wullschlegel 1994; Loehle 1995; Ainsworth *et al.* 2004; McMurtrie *et al.* 2008). This acclimation response is more often observed in CO_2 enrichment experiments of

longer duration, and is associated with an increase in carbohydrate levels, together with reductions in nitrogen, Rubisco protein and Rubisco activity (Rogers *et al.* 1998; Ainsworth *et al.* 2002; Ainsworth and Long 2005). In long-term studies, down regulation is often identified as the culprit for limited gains in productivity under elevated CO₂. Even in situations where elevated CO₂ initially stimulates productivity, over time the stimulation is not maintained and similar gains in productivity occur between species grown under ambient or elevated CO₂. In many studies where down regulation occurs the cause is tied to a sink limitation, brought on by growth in small pots or insufficient nutrients (Curtis and Wang 1998).

1.4.5 Productivity

The impact of higher rates of photosynthesis under elevated CO₂ on non-water and non-nutrient limited forests is generally one of increasing net primary production over the long-term (Idso *et al.* 1991; Norby *et al.* 1995; Whitehead and Teskey 1995; Roden and Ball 1996b; Norby and Luo 2004; Morgan *et al.* 2004; DeLucia *et al.* 2005; Drake *et al.* 2011). Gains in net primary productivity are typically represented through enhancements to aboveground biomass, belowground biomass or a combination of the two. During early life stages, such as the seedling stage for tree species, rates of growth are at a maximum and this is often the same stage when many elevated CO₂ experiments are conducted (Curtis and Wang 1998; Norby *et al.* 1999; Kirschbaum 2011). When scaling up, either by using large trees in experiments or using tree rings and stable isotope ratios as proxies for growth enhancement due to rising CO₂ concentrations over the last century, the evidence still suggests that biomass enhancements occur in the absence of any other major limitation to growth (Körner *et al.* 2005; Norby *et al.* 2005; Leuzinger and Körner 2007). With the introduction of other limitations, or as limitations arise over the lifetime of a tree, results often differ from projections (Oren *et al.* 2001; Norby and Iversen 2006; Iversen and Norby 2008; Uddling *et al.* 2009; Drake *et al.* 2011). Additionally as the length of many experiments is increasing we are starting to get a better understanding of long-term tree and forest response to climate change (Nowak *et al.* 2004a; Ainsworth and Long 2005). For example, in many long term FACE studies allocation patterns are adjusting from greater allocation to aboveground biomass to an increase in below ground partitioning to shorter lived fine roots, root exudates and mycorrhizae (Norby and Luo 2004; DeLucia *et al.* 2005; Drake *et al.* 2011). Any change in allocation could potentially decrease turnover time of carbon and may serve as

a positive carbon cycle feedback and thus accentuate the rate of climate change (Delucia *et al.* 2005). As forests near canopy closure the potential for carbon gain due to leaf area enhancement begins to decline and productivity may level off or even decline (Norby *et al.* 2003).

1.4.6 Biomass allocation

Biomass allocation patterns have been widely studied across many different forest biomes (Grier *et al.* 1981; Gower *et al.* 2001; Giardina *et al.* 2003; Forrester *et al.* 2006; Palmroth *et al.* 2006). A general response, found across biomes at current CO₂ and without any other major limitations, relates that when overall GPP increases carbon fluxes to all components increase as well, thus maintaining proportionality (Litton *et al.* 2007). When other limitations are present, or if environment changes, allocation patterns often reflect either the limitation or influence of environment. For example with temperature increased and with all other factors standard there is a prediction of increased allocation to belowground resources as limiting resources such as water and nutrients are expected to be in shorter supply (Litton and Giardina 2008). Belowground biomass may serve as a major source of carbon sequestration as CO₂ continues to rise and the rate of carbon storage in soils could go up with rising temperature (Litton and Giardina 2008). With water limitations the allocation response will likely relate to a species adaptive capacity to compensate for water shortages. In addition when scaling up and looking at forest ecosystem response, interaction among species may lead to greater competition for resources and necessitating rapid adjustment in partitioning for survival and maintenance of competitive fitness.

The overall biomass enhancement under elevated CO₂ is fairly consistent across vegetation types and environments (Curtis and Wang 1998; Nowak *et al.* 2004a). On the other hand the flux of carbon to various pools (aboveground, belowground) is less certain, with even less certainty regarding the partitioning of biomass to particular components (leaves, stems, fine and coarse roots). This uncertainty can lead to modelling projections of forest loss under differing environmental scenarios and requires better quantification in order to more accurately project the impact of climate change upon forests. With growth under elevated CO₂ and no other major limitations present, allocation patterns generally transfer to aboveground biomass, with woody species showing much greater gains in aboveground biomass compared with herbaceous species and trees amongst woody species displaying the greatest gains in aboveground biomass (Ainsworth and Long 2005; de Graaff *et al.* 2006).

When limitations develop allocation patterns often are altered in order to satisfy the limiting resource. For example, increasing allocation to fine roots when experiencing nitrogen or water limitation under elevated CO₂ (Norby and Iversen 2006; Iversen and Norby 2008). An increase in soil C sequestration is often witnessed in elevated CO₂ experiments, although the magnitude seems to depend on the availability of soil nitrogen (de Graaff *et al.* 2006). Globally, the flux of carbon to belowground (coarse and fine roots, mycorrhizae and root exudates) exceeds carbon emitted to the atmosphere through fossil fuel combustion by an order of magnitude (Giardina *et al.* 2005). How this changes under elevated CO₂ is a major uncertainty in terms of the potential for carbon sequestration and controls on the rate of climate change.

1.5 Drought

Drought is defined as a natural reduction in the amount of precipitation received over an extended period of time, usually a season or more, which is insufficient to meet the demands of humans, agriculture or vegetation (Wilhite 2000). The scale of drought covers a broad range of climates and ecosystems from a regional to sub-continental scale (van der Molen *et al.* 2011). The characterization of drought, such as intensity and duration, are typically region specific and may even differ between years and decades. Intensity typically refers to the precipitation shortfall and or the severity of the impacts associated with the shortfall. Duration refers to the length of time under which the shortfall occurs and in a large percentage of the cases a drought is undefined until approximately 2-3 months into the drought period, although this greatly depends on season and other environmental factors.

Water deficiency is considered as the greatest limitation to plant distribution and productivity (Woodward and Williams 1987). Drought is a considerable environmental stressor which limits forest productivity (McDowell *et al.* 2008; Allen *et al.* 2010) and the distribution of trees and forests globally. Climate models predict that the intensity and frequency of drought will increase in many regions of the globe (Raupach 1998; IPCC 2007), with the potential to cause large scale decline of many forested areas (Breda *et al.* 2006). Much of the impact depends on the intensity and severity of drought and of the adaptive capacity of species to tolerate or acclimate to drought. Many tree species have adapted over time to the occurrence of drought and to different types of drought. For example, in many seasonally dry rainforests, native tree species are drought deciduous and will drop leaves

when the dry season intensifies and put on new foliage prior to the beginning of the wet season (Mooney and Dunn 1970). Other woody plants in arid ecosystems avoid drought by producing roots with access to deep ground water (Lambers *et al.* 1998). Additional adaptive mechanisms to persist with drought include increasing root to shoot ratio, development of thick, impermeable leaves and development of dimorphic root systems (Lambers *et al.* 1998).

1.5.1 *Physiological responses to drought*

One of the first physiological responses to decreasing soil moisture is a reduction of g_s (Evans and Loreto 2000; Loreto and Centritto 2008). In the short term, responses to drought are typically at the level of leaf physiology, whereas drought persists or intensifies responses are more tied to plant growth. A reduction in g_s decreases water loss from leaves but also restricts CO_2 entry, which as a consequence decreases CO_2 assimilation. In the initial stages of drought the key limitation to assimilation is decreased CO_2 diffusion from the atmosphere to the site of carboxylation (Pinheiro and Chaves 2011), while as drought intensifies metabolic limitations may take precedence (Flexas and Medrano 2002b; Flexas *et al.* 2012). The limitations to diffusion include a stomatal and a mesophyll component (Flexas *et al.* 2008). The magnitude of the effect of mesophyll conductance (g_m) under mild to moderate drought conditions is still under debate, with methodological issues concerning estimates of the intercellular or the chloroplastic CO_2 concentration (Pinheiro and Chaves 2011). Limitations at the metabolic point include down regulation or impaired ATP synthesis (Lawlor 2002; Lawlor and Cornic 2002). Photoinhibition and damage to biochemical machinery eventually occur under very severe drought and with full stomatal closure (Flexas and Medrano 2002b). Along with guard cell influence on stomata conductance, chemical signals in the form of abscisic acid (ABA) are thought to influence stomatal closure. As roots dehydrate they transfer ABA through the xylem up to transpiring leaves in order to expedite stomatal closure (Chaves *et al.* 2003). Chemical signals mediate stomatal closure and provide another means to head off water shortages and avoid potential cavitation (Jones and Sutherland 1991). Stomata respond directly to the rate of water loss from the leaf due to changes in evaporative demand (Monteith 1995; Maroco *et al.* 1997), with species specific characteristics such as leaf water potential and hydraulic conductivity often determining the rate at which stomata close (Buckley and Mott 2002).

According to the optimal stomatal model physiological changes in g_s during drought are mainly related to changes in D , although as drought intensifies or progresses changes may

also occur to g_1 (Medlyn *et al.* 2011). The parameter g_1 is typically species specific and in theory should change only under extreme conditions, such as experienced during intense drought or during prolonged drought periods (Cowan 1986; Mäkelä *et al.* 1996). When drought intensifies or increases in duration, other growth or structural changes may take precedence over g_s , such as an increase in the root to shoot ratio or change in earlywood or latewood vessels (Sperry 2000).

1.5.2 Biomass allocation – Structural responses to drought

With increasing drought stress many changes in tree structure and function take place. Initial responses are at the biochemical and physiological level such as ABA signalling and reductions in g_s . Physical changes typically proceed from physiological response, especially as drought intensifies and physiological response is insufficient to limit drought stress. Physical changes include early leaf senescence, which helps to avoid further drought impacts by reducing the transpiring tree crown surface area (Breda *et al.* 2006). Additionally, changes in vessel diameter and size have also been recorded but these changes typically occur as trees acclimate to a longer term drought (Arend and Fromm 2007; Atwell *et al.* 2007). Allocation to roots typically goes up as drought progresses or for species growing in drought prone areas, allowing exploration of a greater soil volume. In addition to gaining access to more soil volume the addition of more biomass to fine roots carries the secondary benefit of access to more nutrients (Chaves *et al.* 2003). The root to shoot ratio of species experiencing drought typically goes up due to a greater allocation to roots under drought conditions (Nowak *et al.* 2004a; Breda *et al.* 2006), although this response is not universal and may change when other limitations or competition occur (Nowak *et al.* 2004).

As drought intensifies the response of trees to drought may include multiple avoidance mechanisms such as complete stomatal closure, changes in plant architecture, stunted leaf growth and early leaf senescence (Tardieu *et al.* 1996; Tardieu and Simonneau 1998; Warren *et al.* 2011). Reductions in overall productivity and growth also frequently occur, especially when drought conditions are of particularly long duration or of extreme intensity. Many observational studies over the last 20 years have shown an increase in forest dieback and reductions in overall growth (Allen *et al.* 2010) resulting from drought episodes. There are two key hypotheses commonly posited for this impact of drought on forest growth (Tardieu and Simonneau 1998 1998; McDowell *et al.* 2008). The first mechanism, carbon starvation, states that as drought progresses stomata close to prevent transpiration losses and maintain

leaf water potential above a critical point. Reductions in transpiration abetted by stomatal closure come at the cost of photosynthetic gain. As drought progresses and stomata remain closed, autotrophic respiration continues to use up stored carbon reserves. Once reserves are used up, or at least the most readily available (Sala *et al* 2010), metabolic processes may shut down and mortality may ensue. The second mechanism, hydraulic failure, states that under drought stomata remain open to allow photosynthetic carbon gain to continue but as drought intensifies and or progresses open stomata lead to stress on the water column somewhere along the soil plant atmosphere continuum. As drought intensifies or if a plant is unable to maintain efficient water absorption and transport of water there is the potential for air to enter vessels or tracheids leading to cavitation and loss of hydraulic conductance. With continued cavitation of vessels a complete loss of the water column may occur thus leading to hydraulic failure, tissue dehydration and mortality.

1.6 Interactions

1.6.1 Elevated CO₂ and Drought

Under climate change scenarios many forested regions of the world are expected to see an increase in the intensity and frequency of drought (IPCC 2007). As a result trees and forests are likely to encounter environments much different than during initial establishment and growth, even for many long lived trees. The response of trees and forests to the interaction of drought and elevated CO₂ plays out at many different levels, from leaf to the whole tree scale, with a caveat that increased efficiency at one scale does not guarantee greater efficiency at all scales. Much is dependent on species specific response patterns such as biomass allocation, physiology and ecological strategy. Additionally characteristics of drought such as timing, intensity and duration may impact species specific responses differentially. Much uncertainty exists in terms of forest response to the interacting influences of rising CO₂ and increasing occurrence of extreme weather events such as droughts and heat waves.

1.6.2 Physiological responses - Elevated CO₂ and drought

From experimental evidence it is apparent that g_s is consistently reduced when plants are grown under elevated CO₂ (Medlyn *et al.* 2001; Wullschleger *et al.* 2002; Ainsworth and

Long 2005; Ainsworth and Rogers 2007), with the exception of some conifer species (Ellsworth *et al.* 1995; Picon *et al.* 1996; Ellsworth 1999). Plants also close stomata when encountering water deficiency, high VPD or a combination of the two (Flexas and Medrano 2002a) under ambient CO₂. As g_s is typically one of the most rapid physiological responses to conditions of elevated CO₂ and to drought it is essential to understand if further changes take place and what mechanisms are involved when experiencing drought under elevated CO₂.

The response of photosynthesis to the interaction of elevated CO₂ and drought is also important to ascertain in climate change studies. With growth under elevated CO₂, rates of photosynthesis typically increase (Sage 1994; Drake *et al.* 1997; Medlyn *et al.* 1999; Ainsworth and Rogers 2007) and when experiencing drought conditions photosynthesis typically declines due to stomatal closure (Flexas and Medrano 2002a). Gains in photosynthesis however are maintained for species grown under elevated CO₂, even with reduced g_s , as a result of biochemical controls on photosynthesis (Farquhar *et al.* 1980; Long and Drake 1992; Ainsworth and Rogers 2007). In much of the experimental literature it appears that C_i/C_a remains fairly constant, for different functional types and related species, and that C_i/C_a is maintained at both ambient and elevated CO₂ (Drake *et al.* 1997). How the ratio changes as drought progresses under elevated CO₂ is less certain with some evidence of minor decreases in C_i/C_a under elevated CO₂ and drought, suggesting stomatal closure in relation to photosynthetic activity, thus decreasing C_i/C_a (Sage 1994; Herrick *et al.* 2004). As a result of limited change in C_i/C_a , leaves under elevated CO₂ will consistently see greater C_i compared with ambient grown plants and should therefore maintain higher photosynthetic rates regardless of drought duration or intensity, at least until g_s nears zero. With increasing drought severity stomatal closure may near a maximum, although the relative enhancement of photosynthesis under elevated CO₂ would still lead to stimulation of plant growth as the relative response of photosynthesis to C_a is higher at low g_s (McMurtrie *et al.* 2008; Duursma *et al.* 2011). Complications arise as drought intensifies or the duration of drought expands, as biochemical limitations may develop adding another complicating factor along with reduced g_s to limit photosynthetic performance under elevated CO₂ (Lawlor 2002).

Avoiding drought conditions by adjusting g_s , root to shoot ratio or some other physiological or morphological response could serve as an important strategy in dealing with reduced water availability under future climate. Reduction in g_s under elevated CO₂ decreases the rate of leaf-level water loss and soil water extraction, which as a result may delay water stress during drought (Morgan *et al.* 2004; Ainsworth and Rogers 2007). An

increase in photosynthetic rate and decrease in transpiration at the leaf level leads to an increase in ITE (Morison 1985; Drake *et al.* 1997; Saxe *et al.* 1998; Eamus 1991; Wullschleger *et al.* 2002; Barton *et al.* 2011). Decreased transpiration at the leaf level is a by-product of reduced g_s at high C_i , which in itself is an adaptive response to C_a whereby diffusional limitations to A are adjusted in response to biochemical limitations to A (Morison 1993b). An approximate doubling of ITE is observed across a large number of studies and is probably the most consistent and responsive effect in elevated CO₂ effects research (Saxe *et al.* 1998). Although useful for understanding leaf level response to elevated CO₂, ITE as a metric, is much less applicable to understanding whole plant response where measures such as hydraulic conductance, biomass allocation, and leaf area development are better identifiers of overall plant response (Wullschleger *et al.* 2002).

1.6.3 Productivity - Elevated CO₂ and drought

The combined increased in photosynthesis and reduction in g_s of plants grown under elevated CO₂ commonly leads to an increase in water use efficiency (unit carbon gain to unit water loss) at the whole plant scale (Farquhar *et al.* 1989; Eamus 1991; Field *et al.* 1995; Wullschleger *et al.* 2002). Of particular importance to our understanding of plant performance under elevated CO₂ and drought is whether water savings at the whole plant level, as represented through WUE, lead to soil water savings. Gains in WUE may delay or postpone drought beginnings and allow productivity to continue unabated, although much depends on ontogeny, allocation and other limitations (Field *et al.* 1997). At the whole plant scale an increase in WUE does not automatically mean that overall plant water use will be less, or in turn that soil moisture will be different between CO₂ treatments (Wullschleger *et al.* 2002). In fact, overall water use could be higher, especially if carbon gain is allocated disproportionally to leaf area (Picon *et al.* 1996; Centritto *et al.* 1999). Stage of growth is highly relevant to gains in WUE as closed canopy forests or mature forest trees may experience a stronger effect on transpiration savings but a decreased ability to increase biomass (Wullschleger *et al.* 2002). Alternatively, seedlings or young developing forest stands may display higher transpiration due to increased biomass gains, especially to aboveground biomass (Wullschleger *et al.* 2002).

Along with gains in WUE in relation to growth under elevated CO₂, partitioning may play a very important role in plant performance under the combination of elevated CO₂ and drought (Picon-Cochard and Guehl 1999; Wullschleger *et al.* 2002). For example, increased

allocation to fine roots versus leaf area may provide for the best opportunity to persist under drought and lead to the largest gains in WUE under elevated CO₂ and drought (Wullschleger *et al.* 2002). As leaf area is directly coordinated with xylem characteristics, an increase in leaf area at ambient CO₂ necessitates a concomitant increase in whole plant hydraulic conductance (Meinzer 2002). At elevated CO₂, even with a leaf area increase it may not be necessary to increase whole plant hydraulic conductance due to reductions in g_s and soil moisture savings (Hungate *et al.* 1997). Proportional changes in allocation to roots versus leaves or alteration to vessel diameters may strongly influence plant response to the combination of elevated CO₂ and potentially provide a better strategy for persisting through drought periods.

1.6.4 Biomass allocation

A broad range of studies have looked at plant response to elevated CO₂ (Arp 1991; Long and Drake 1992; Ceulemans and Mousseau 1994; Koch and Mooney 1996; Norby *et al.* 1999; Long *et al.* 2004) or drought (Breda *et al.* 2006; McDowell *et al.* 2008; Ditmarova *et al.* 2010; Allen *et al.* 2010; Galvez *et al.* 2011; Larcheveque *et al.* 2011) individually, but far fewer looking at the interaction of the two factors combined. Of the studies looking at the interaction, many have focused on ecosystems other than forests such as grasslands (Field *et al.* 1997; Niklaus *et al.* 1998; Owensby *et al.* 1999; Morgan *et al.* 2004), agricultural crops (Kimball *et al.* 1993; Hunsaker *et al.* 1994) and deserts (Nowak *et al.* 2004b; Housman *et al.* 2006).

Many have hypothesized that the water savings from increased WUE will lead to larger gains in productivity for arid ecosystems or for ecosystems experiencing a dry period (Strain and Bazzaz 1983; Nowak *et al.* 2004a; Morgan *et al.* 2004). This hypothesis is based on direct and indirect influences of elevated atmospheric CO₂ concentration on plant response. Direct influence is through enhanced photosynthesis, whereas the indirect influence is through reduced water consumption and hence soil moisture depletion (Ceulemans and Mousseau 1994; Norby *et al.* 2005). For arid ecosystems soil moisture savings under elevated CO₂ may extend the growing season and delay the impact of drought (Strain and Bazzaz 1983; Wullschleger *et al.* 2002).

The experimental evidence for this CO₂ effect on delaying drought impacts has been found for some grassland species (Morgan *et al.* 2004) with less evidence that the drought effect is enhanced for tree species (Centritto *et al.* 1999, 2002; Gunderson *et al.* 2002). Experiments looking at species from contrasting habitats show that xeric species increase

growth under drought conditions more than mesic species (Catovsky and Bazzaz 1999), although growth enhancements are often more pronounced for aboveground biomass with little to no change in belowground biomass (Niklaus *et al.* 2001; Nowak *et al.* 2004). In various other non-forest type experiments focusing on the CO₂ x H₂O interaction, the percentage growth response to elevated CO₂ is generally found to be higher under water stressed situations (Kimball *et al.* 1993; Field *et al.* 1997; Arp *et al.* 1998), although some experiments find no significant interaction (Derner *et al.* 2003; Nowak *et al.* 2004; Housman *et al.* 2006). Often, findings of limited interaction is the result of an additional limitation such as nutrients for grassland species (Field *et al.* 1997; Gill *et al.* 2002) or compensatory extraction of all available soil moisture regardless of CO₂ concentration, perhaps as a means to limit competitive interactions of other species (Nowak *et al.* 2004b).

In grassland systems canopy closure is reached much sooner than for forests and this may partly explain the increase in soil moisture savings found in grassland experiments, with the implications that as leaf area reaches a maximum transpiring surface area also reaches a maximum. Increases in leaf area as a compensatory mechanism for utilizing available water is thought to occur only when the potential for plant growth and available resources are closely matched (Field *et al.* 1997). When available resources do not closely match growth potential, allocation may proceed in a manner suitable to current conditions. Alternatively, increased water use efficiency for species growing under elevated CO₂ may predicate little need to allocated greater biomass to fine roots (Wullschleger *et al.* 2002), especially when drought is less severe.

From experimental evidence on tree species it is apparent that productivity gains occur when grown under non limiting resources and elevated CO₂ (Curtis and Wang 1998; Medlyn *et al.* 1999; Norby *et al.* 2005) and productivity generally goes down when experiencing drought at ambient conditions (Breda *et al.* 2006; McDowell *et al.* 2008; Allen *et al.* 2010). When including the combination of drought and CO₂ the outcome is much more variable. From most experiments it is found that ITE at the leaf level is increased under elevated CO₂ and drought (Eamus 1991; Barton *et al.* 2011), strongly tied to reductions in g_s . When scaling up to the whole tree or canopy level the results are less conclusive, as a result of changes in biomass occurring with growth under elevated atmospheric CO₂ (Centritto *et al.* 1999; Wullschleger *et al.* 2002). Additionally, conflicting findings on tree and forest response to the combination of elevated CO₂ and drought are often a consequence of factors such as length of experiment (Curtis and Wang 1998; Norby *et al.* 1999), seasonality (Ellsworth 1999), intensity of drought (Picon *et al.* 1996; Atwell *et al.* 2007), nutrient limitations (Field

et al. 1997) or temperature (Warren *et al.* 2011). Occasionally, findings of limited water savings are often the result of minor or non-significant reductions in g_s between ambient and elevated CO₂ grown plants (Ellsworth *et al.* 1995; Picon *et al.* 1996; Ellsworth 1999), particularly for coniferous species. In some instances, g_s may be higher rather than lower for some species under elevated CO₂ (Heath and Kerstiens 1997). Many studies display limited evidence for a more pronounced reduction in g_s under the combination of elevated CO₂ and drought (De Luis *et al.* 1999), partly as water savings at the leaf level due to lower g_s at high C_a may delay or negate the need for any further stomatal closure, at least until drought is more severe and leaf water potential reaches a critical point (Brodribb and Holbrook 2003).

Along with changes to physiology under the combination of drought and elevated CO₂, another potential plant mechanism is to alter biomass allocation or morphology (Morison 1993a; Wullschleger *et al.* 2002), although not all changes in allocation will positively benefit plants growing under elevated CO₂ when drought is introduced. For example, gains in productivity to above ground biomass could lead to similar or greater transpiration losses due to greater leaf area (Ainsworth and Long 2005). Conversely, greater allocation to fine roots and less to aboveground biomass can lead to lower transpiration losses and greater overall gains in WUE (Morgan *et al.* 2004).

Increased allocation of carbon to root growth may enable plants to exploit soil water in a deeper and wider range of soil and assist in delaying drought impacts when present (Wullschleger *et al.* 2002), although root response is also thought to depend on soil water depth and duration between rainfall events (Canadell *et al.* 1996; Castelli *et al.* 2000). Under drought conditions the duration between precipitation events is generally quite extensive and thus the need to increase root growth would be more of a priority (Castelli *et al.* 2000). Alternatively in situations where time between precipitation events is minimal the allocation to roots may not serve to aid overall water limitations, and any roots that do form may be nearer the soil surface (Kosola and Eissenstat 1994). In natural environments soil moisture conservation resulting from the CO₂ effect could delay drought and postpone tree stress, although experimental evidence in forest species is in short supply. Limited evidence for an effect of CO₂ delaying drought stress for tree species often relates to allocation patterns, with more carbon allocated to leaf area typically leading to similar overall patterns of water use (Picon *et al.* 1996; Centritto *et al.* 1999; Broadmeadow and Jackson 2000), whereas enhancements in stem architecture and mass appear to postpone inhibitory effects of drought on growth (Atwell *et al.* 2007). Increases in vessel numbers and reduction in vessel size

could improve conductance and optimal sapwood to leaf area ratio, mitigating greater leaf area by providing a better means of water transport (Carter and White 2009).

Changes in allocation patterns at the whole tree scale will greatly influence the response to drought, as will stage of development. Experimental length, and thus development stage may greatly influence forest response through interactions with environment. For example, in a study using FACE technology with deciduous tree seedlings Eguchi *et al.* (2005) found that soil moisture increased under elevated CO₂ even though water use at the whole tree level also increased. The basis for the soil moisture increase being an increased LAI of the young stand caused a decrease in soil evaporation (Eguchi *et al.* 2005). Soil moisture savings are often identified in elevated CO₂ and drought experiments, with the mechanism often soil shading caused by increased LAI (Cech *et al.* 2003; Eguchi *et al.* 2005) but also the result of decreased g_s (Picon-Cochard and Guehl 1999), increase in root:shoot ratio (Broadmeadow and Jackson 2000) or minor change in rooting depth (Norby and O'Neill 1989). When looking across multiple years or during brief periods of drought, an increase in soil moisture under elevated CO₂ during drought is less frequently observed (Gunderson *et al.* 2002; Nowak *et al.* 2004; Uddling *et al.* 2008). Regardless of drought intensity or duration, experiments often find that leaf area may compensate for a reduction in g_s , so that all available water is used no matter if growth occurs under ambient or elevated CO₂ (Picon *et al.* 1996).

Scaling up to look at species response based on ecological strategy suggests that drought tolerant species will outperform drought avoiding species under conditions of elevated CO₂ and drought (Strain and Bazzaz 1983). Such a response is thought to pertain to adaptive mechanisms which provide an advantage under dry conditions to become amplified under the combination of elevated CO₂ and drought. For example, deep roots, high root:shoot ratio, thick leaves, small vessels, reduced g_s and slow growth all may provide a means for drought tolerant species to persist prior to, during and throughout the occurrence of drought. In contrast, drought avoiding species identified by shallow roots, a low root:shoot ratio and large vessels may lead to low survivorship under water limitations (Polley *et al.* 2002). Adapting to a more mesic environment narrows the need to form structures to persist under low soil moisture conditions, whereas under arid environments an imperative exists to maintain preparedness for drought. Under climate change scenarios the potential exists for a variety of droughts, from rapid and severe to persistent and moderate, with forest response likely a combination of species specific adaptation and acclimation potential to varying drought trajectory.

1.7 Australian rainforests (Impact of rising temperature)

Eucalypt may be the most ubiquitous and widespread forest type across the Australian landscape but, surprisingly it does not comprise the original vegetation of the Australian continent (Webb *et al.* 1984). That title is held by rainforests within Australia, which many claim to be one of the oldest forest types in the world (Bowman *et al.* 2000). Historical extent of rainforest on the Australian continent is believed to have encompassed nearly a third of Australia (Bowman 2000). The extent of rainforest today is rather small compared to historical levels and when looking at the entirety of the Australian continent but very important in terms of the level of native biodiversity and as an index of climate variability (Stork *et al.* 2007). Recent analyses suggest that the extent of rainforest in Australia is increasing as a result of fire suppression activities and of increasing precipitation in north-eastern Australia (Tng *et al.* 2012). The long term outlook of rainforest expansion is uncertain as temperatures continue to rise and the long term outlook in response to temperature is unknown. Response of rainforest vegetation to temperature, regardless of geographical location is essential to understand in order to develop accurate predictions of rainforest utility as a source or a sink of carbon. As rainforest extent and productivity is so important to the global carbon cycle many are concerned that rising temperatures may lead to whole scale decimation of rainforest habitats and regions of the globe (Clark *et al.* 2010).

An increase in temperature over the next century is expected for tropical regions of the globe, corresponding with rising atmospheric CO₂ concentration (IPCC 2007). Tropical forests are estimated to account for nearly 35% of global net primary productivity (Saugier and Mooney 2001), and as such play a vital role in the global carbon cycle, storing a large fraction of terrestrial carbon in their vegetation and soils (Dixon *et al.* 1994; Clark *et al.* 2010). There is currently much uncertainty around whether tropical forests will amount to a larger or smaller proportion of NPP under future climate (Clark *et al.* 2003; Clark 2004; Wright 2005; Lloyd and Farquhar 2008; Lewis 2009; Phillips *et al.* 2009). Many tropical forests are currently near upper temperature limits and as temperature continues to increase, concern is tropical forests may convert from a net carbon sink to a net carbon source (Clark *et al.* 2003; Cox *et al.* 2004). For example, in recent analyses of tropical forests in Costa Rica it is suggested that some tropical forests are presently displaying declining rates of growth and increased mortality (Clark *et al.* 2003; Feeley *et al.* 2007; Clark *et al.* 2010). Alternatively, mounting evidence for increasing growth and productivity is available for parts of the

Amazon (Phillips *et al.* 2008), for tropical rainforests in Africa (Lewis *et al.* 2009) and for South America (Lewis *et al.* 2009). Conflicting findings in terms of overall growth may relate to different stages of forest succession following widespread past disturbance at each location (Lewis *et al.* 2004, 2006).

Temperature influences many forest processes from cellular expansion to overall rates of photosynthesis and productivity (Berry and Björkman 1980). One important mechanism thought to strongly control tropical forests becoming a carbon source is the direct impact of temperature on rates of photosynthesis and respiration (Doughty and Goulden 2008), and related influence on rates of growth and overall productivity (Cox *et al.* 2004; Clark *et al.* 2004). Temperature strongly influences many forest processes from controls on enzymatic rates, photosynthesis, cellular expansion, growth and productivity (Berry and Björkman 1980). Correspondingly, many physiological and physical processes display an ability to acclimate to growth temperature (Atkin and Tjoelker 2003), which when looking at the direct effect of temperature on forest response alone means that an increase in temperature may not automatically lead to a decline in growth and productivity (Lloyd and Farquhar 2008). When looking across tropical forests as a whole it appears that recent forest response has been for increasing growth and productivity (Lewis *et al.* 2009) and whether gains in productivity remain under future climate will depend on forest response to a continuing increase in temperature, atmospheric CO₂ and the occurrence of drought.

According to Lloyd and Farquhar (2008) the indirect effect of temperature on altering VPD may inhibit gains in photosynthesis to a greater degree than temperature alone. As a result the most detrimental combination of environmental influences on tropical forests may well be warmer and dryer conditions where the indirect impact of temperature (VPD) and direct impact, especially at extremes, will lead to declines in potential growth and productivity (Lloyd and Farquhar 2008). Gains in productivity expected with growth under elevated CO₂ may be negated due to increased stomatal closure at high VPD, which along with declining soil moisture may lead to more rapid stomatal closure when experiencing hot and dry conditions or the incidence of drought. Gaining a better understanding of the physiological response of tropical rainforests and the mechanisms responsible is of the utmost importance due to the large influence of tropical forests on climate. Changes in the proportion of NPP that tropical forests make up would not likely be filled by other vegetation types and could lead rapid rise in atmospheric CO₂ and an associated increase in warming (Malhi and Phillips 2005).

1.8 Objective

The aim of this thesis is to investigate the responses of eucalypt forests to changes in water availability and elevated CO₂, and the responses of Australian rainforests to warming temperatures. To achieve this, a series of studies were undertaken to investigate the dynamics of carbon gain and rates of water loss at the leaf and whole plant scale. Understanding forest response to the interactions of drought, elevated CO₂ and temperature at the leaf, whole plant and ecosystem scale will greatly assist with projections, planning and management of forests under climate change.

The specific aims of the study are:

1. (Chapter 2) To assess the impact of drought on eucalypt seedlings under elevated CO₂. There are multiple ecological strategies to deal with drought along with the potential for multiple drought trajectories under climate change. Species from xeric environments are projected to experience larger relative gains due to elevated CO₂ and drought compared with species from more mesic environments. To identify physiological and productivity gains according to ecological strategy we monitored the response of two eucalypt tree species, of opposing ecological strategy, in a glasshouse experiment under the treatment combination of ambient (380 ppm) or elevated (700 ppm) CO₂ and with (50% FC) or without (100% FC) drought. Particular emphasis in Chapter (2) was on whole plant biomass gain and leaf level physiology. Data collected throughout the experiment sets up Chapter (2) with the following hypotheses at the leaf level:

- Stomatal conductance (g_s) and leaf intercellular CO₂ (C_i) will be lower under drought (50% FC)
- Photosynthesis (A) and productivity will be lower under 50% FC
- Xeric species will have lower g_s and C_i , and as a result higher instantaneous transpiration efficiency (ITE) than mesic species.
- Response of photosynthesis (A) and productivity to elevated CO₂ will be higher for xeric species than mesic species.
- “CO₂ effect” on ITE will be proportional in all treatments.

And, at the whole plant scale:

- Xeric species will have higher water use efficiency (WUE).
- WUE will relate to ITE and will respond in the same direction as ITE for all treatments.

- Leaf area (LA) will be determined by water availability. At 50% FC plants will display 50% less LA than species grown at 100% FC.
 - “CO₂ effect” on leaf area will completely compensate for “CO₂ effect” on g_s such that total tree transpiration will be unaffected by CO₂.
- 2. (Chapter 3) To identify mechanisms controlling plant response to drought under elevated CO₂, particularly the impact of short term and intense drought.** Short, intense drought periods may be more consequential to forest response than longer term moderate drought. To investigate how severity impacts forest response, the same eucalypt species from Chapter (2) were subjected to a dry down, where water was withheld until plants neared a pre-determined critical point of g_s and leaf water potential. Of particular emphasis were physiological responses in the form of A , g_s , g_1 and Ψ_{leaf} along with measures of overall water loss and carbon gain. This chapter focuses on how severe water limitations impact forest response and the importance of plant size in determining drought impacts. Hypotheses at the leaf level developed for Chapter (3) are heavily influenced by the Medlyn *et al.* (2011) model of g_s and also based on potential for soil water savings at elevated CO₂. Data collected during the dry down make up Chapter (3) with the following hypotheses at the leaf level:
- Marginal cost of water (g_1) will be lower for xeric species.
 - Marginal cost of water (g_1) will increase with increasing drought stress.
 - Leaf water potential (Ψ_{leaf}) will be higher (less negative) for species in elevated CO₂ under all treatments.
 - Soil moisture will remain higher under elevated CO₂ under all treatments during dry down.
 - Time to drought stress, as portrayed in critical set points, will be less for species in elevated CO₂ under all treatments.
- 3. (Chapter 4) - To assess the impact of temperature and seasonality on the physiological response of Australian tropical rainforests.** Models of forest response to climate change often use the Farquhar *et al.* (1980) biochemical model of photosynthesis. Many of the parameters in the model show little variability between C₃ species with the exception of two main parameters V_{cmax} and J_{max} . These two parameters appear to vary considerably between functional groups and between habitats. Very little information is available concerning the biochemical aspects of photosynthesis for tropical forest species

and especially in regards to rising temperature and acclimation potential of tropical forest species. In this project we attempt to elucidate the potential temperature acclimation of tropical forest species by looking at two tropical tree species. Correspondingly, the role of stomatal limitations is uncertain in terms of photosynthetic response to temperature and VPD. The influence of temperature on physiological responses is most pronounced when nearing extremes. As tropical rainforests are thought to exist precariously close to upper temperature limits it is important to identify mechanisms influencing and controlling physiological response. Understanding and separating the biochemical from stomatal limitations to photosynthesis is integral for better modelling of tropical rainforest response to changing climate. Chapter (4) will cover the impact of temperature on photosynthesis and g_s of Australian rainforest tree species, focusing specifically on the impact of high temperatures on leaf level response and modelling the response of g_s to light, temperature, seasonality and VPD. Hypotheses tested in this chapter (4) include:

- The marginal cost of water g_1 will increase with growth temperature.
- Optimal temperature for photosynthesis will increase with an increase in growth temperature

4. (Chapter 5) – Discussion and Conclusions

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CHAPTER 2

Productivity and water use of two *Eucalyptus* species of contrasting drought tolerance under elevated CO₂ and moderate long-term drought

Summary The effect of long-term moderate drought (50% field capacity), elevated CO₂ and the interaction between these two factors (CO₂ x drought) was investigated for two *Eucalyptus* species (*E. pilularis* and *E. populnea*) of contrasting drought tolerance growing in large pots in ambient (380 ppm) or elevated (700 ppm) CO₂ glasshouses. Seedlings were sown from seed directly into 90-liter pots and maintained for four months at full field capacity. At the end of four months, one half of the pots for each species were allowed to dry down to 50% of field capacity, and maintained at that level for the remainder of the main experiment. To investigate effects of growth under elevated CO₂ and long-term moderate drought we measured leaf-level gas exchange (photosynthesis and stomatal conductance), whole-plant transpiration and at the conclusion of the experiment harvested seedlings to determine whole-plant biomass and allocation patterns both between and within species. Leaf-level gas exchange was not affected by growth under long-term moderate drought, whereas there were substantial reductions in whole-plant biomass and plant allocation (leaves, stems and roots) for both species. The effect of elevated CO₂ led to a sustained increase in A_{sat} and reduction in stomatal conductance (g_s), and consequently plants grown under elevated CO₂ were significantly larger at the end of the experiment. For both species, transpiration rate was similar for plants grown under elevated or ambient CO₂, reflecting an increase in leaf area to compensate for the CO₂ induced reduction in g_s . Water use efficiency (WUE) was increased with CO₂ enrichment by a greater degree for mesic *E. pilularis* under well-watered (100% field capacity) than drought (50% field capacity) conditions (58% vs. 44%), while the relative increase in WUE for xeric *E. populnea* was similar between well-watered and drought conditions (47% vs. 48%). These results suggest that under elevated CO₂ and long-term moderate drought both *E. pilularis* and *E. populnea* exhibit a capacity to adjust growth processes to match water availability in place of stronger physiological controls to avoid moderate drought stress.

2.1 Introduction

Water is one of the major limitations to forest productivity, health and overall extent (Hinckley *et al.* 1981; Boyer 1982; Nemani *et al.* 1993; Allen *et al.* 2010). Over the last decade the incidence of drought, along with rising temperatures, has led to forest dieback, habitat fragmentation and overall forest mortality in almost every biome on every vegetated continent

(McDowell *et al.* 2008; Allen 2009; van Mantgem *et al.* 2009; Allen *et al.* 2010). Projections for the next century suggest an increase in the incidence, intensity and duration of drought for many forested regions (Burke *et al.* 2006; IPCC 2007; Sheffield and Wood 2008). The effects of the substantial and continuing increase in atmospheric CO₂, as water becomes more limiting, may include both positive effects to forests represented by reduced stomatal conductance (Drake *et al.* 1997; Medlyn *et al.* 2001) and whole-tree water loss (Wullschleger and Norby 2001; Hungate *et al.* 2002), and negative effects such as amplification of drought and heat stress (Wullschleger *et al.* 2002; Allen *et al.* 2010). At present forest ecosystems constitute the largest terrestrial sink for carbon, accounting for nearly 45% of terrestrial net primary productivity (Bonan 2008), with growing concern that ongoing alteration of climate may push forests from being the largest terrestrial carbon sink towards one of the largest sources of emitted CO₂ (Clark 2004; Breshears *et al.* 2005; Allen and Breshears 2007; Kurz *et al.* 2008; Allen *et al.* 2010; Anderegg *et al.* 2012). One of the largest uncertainties regarding source or sink capacity is the potential role of elevated CO₂ in ameliorating drought impacts and reducing overall plant stresses when water availability declines (Wullschleger *et al.* 2002; van der Molen *et al.* 2011).

Mechanisms for CO₂ x drought interactions

Many forests are sensitive to water limitations, especially when nearing their range limits or on the margins of water availability (Allen and Breshears 1998; Johnson *et al.* 2002; Crawford 2008). It is often hypothesized that elevated CO₂ will impart the greatest relative benefit to forest ecosystems under water limitation, and therefore that elevated CO₂ will reduce the impact of drought (Gifford 1979; Idso and Idso 1994; Catovsky and Bazzaz 1999; Duursma *et al.* 2011). There are two main mechanisms commonly expressed in support of this hypothesis. The first results from a reduction in stomatal conductance under elevated CO₂, which causes a lowered transpiration rate and soil moisture savings, thus enabling trees under elevated CO₂ to continue to transpire longer into a drought episode (Eamus 1991; Morison 1993; Medlyn *et al.* 2001). Secondly, lower intercellular CO₂ (C_i) under drought conditions causes a larger enhancement of photosynthesis due to the non-linear response of photosynthesis (A) to C_i (Lloyd and Farquhar 1996; Grossman-Clarke *et al.* 2001; McMurtrie *et al.* 2008; Duursma and Medlyn 2012). At lower CO₂ concentrations the slope of the A-C_i curve is higher, which means that a drought-induced reduction in C_i leads to a larger reduction in A under ambient than under elevated CO₂ (Grossman-Clarke *et al.* 2001). In this thesis, I

explore the two mechanisms for CO₂ x drought interactions separately. Importantly, identification of soil moisture savings on delaying or limiting drought impacts under elevated CO₂ requires soil moisture to vary. In the experiment presented in this chapter, we removed this mechanism by maintaining soil moisture at two constant set levels during the main part of the overall experiment. Consequently, the current chapter will thus largely center on physiological and structural adjustments in response to long-term moderate drought and elevated CO₂, examining the mechanism of lower C_i. At the end of the experiment, plants were allowed to dry down from full field capacity, allowing an examination of the mechanism of soil moisture savings under elevated CO₂. The dry down period is examined in a subsequent chapter (Chapter 3).

Predicting CO₂ × drought interactions

The impacts of drought on forest productivity are commonly exhibited as a slowing of growth (Kozlowski and Pallardy 2002; Allen *et al.* 2010), tied to physiological adjustments in the face of plant water stress (McDowell 2011). In most forest process models, the initial physiological response to water limitation is a reduction in stomatal conductance (g_s) with declining soil moisture. This reduction in g_s causes a reduction in photosynthesis (A), because A is dependent on the supply of CO₂.

$$A = \frac{g_s}{1.6} (C_a - C_i) \quad (1)$$

A reduction in g_s (mol m⁻² s⁻¹) due to water limitation slows the rate of CO₂ diffusion to the sites of carboxylation inside a leaf, thus decreasing the ratio of intercellular to ambient CO₂ (C_i/C_a) and photosynthesis (A : μmol m⁻² s⁻¹). As drought progresses or intensifies, other biochemical limitations may ensue, but under mild to moderate drought it is widely held that a reduction in photosynthesis is primarily due to stomatal closure (Saliendra *et al.* 1995; Lawlor 2002; Chaves *et al.* 2002; Chaves and Oliveira 2004; Egea *et al.* 2011). Additional findings reflect that photosynthesis may be limited during periods of soil water deficit by mesophyll conductance (g_m) (see Flexas *et al.* 2008), but in general g_m is considered less sensitive to water stress than g_s (Bunce 2009; Pinheiro and Bates 2011).

The impact of elevated CO₂ on water relations is generally via a reduction in g_s (Eamus and Jarvis 1989; Medlyn *et al.* 2001; Ainsworth and Rogers 2007) even in well-watered conditions, and a related decline in transpiration per unit leaf area (Eamus and Jarvis

1989; Medlyn *et al.* 2001; Ainsworth and Rogers 2007). The reduction in g_s under elevated CO_2 is typically not associated with a comparable decline in the ratio of leaf intercellular to ambient CO_2 (C_i/C_a), but rather g_s operates in order to maintain a similar C_i/C_a regardless of growth C_a (Mott 1988; Sage 1994; Drake *et al.* 1997). As a result, C_i is higher under elevated CO_2 , which leads to an increase in photosynthetic rate (Long and Drake 1992; Koch and Mooney 1996; Curtis and Wang 1998; Norby *et al.* 1999). The increase in photosynthetic rate (A) with CO_2 enrichment tied together with reduced g_s leads to an increase in instantaneous transpiration efficiency (ITE; defined as the ratio of photosynthesis (A) to transpiration) at the leaf level and generally an increase in water use efficiency (WUE) at the whole tree scale (ratio of carbon gain to water loss) (Farquhar *et al.* 1989; Eamus 1991; Field *et al.* 1995; Drake *et al.* 1997; Centritto *et al.* 1999; Körner 2000; Barton *et al.* 2012).

These observations can be synthesized into a simple model which allows us to predict plant responses to CO_2 and drought. According to Cowan and Farquhar (1977) the optimal stomatal conductance is that which maximizes daily carbon gain (photosynthesis, A) for a given daily water loss (transpiration, E). Using the Cowan and Farquhar (1977) definition of optimal stomatal conductance, Medlyn *et al.* (2011) developed the following model of g_s .

$$g_s = g_0 + 1.6\left(1 + \frac{g_1}{\sqrt{D}}\right) \frac{A}{C_a} \quad (2)$$

where g_0 ($\text{mol m}^{-2} \text{s}^{-1}$) is the cuticular conductance and D (kPa) is the leaf to air vapor pressure deficit (Medlyn *et al.* 2011). The parameter g_1 is a constant that is inversely related to the marginal cost of water to the plant. Under drought conditions it is generally accepted that the cost of water to the plant will increase and as a result the g_1 parameter will decline (Mäkelä *et al.* 1996). When $g_s \gg g_0$ such that g_0 is negligible, equation (2) can be readily rearranged to obtain the following expression for the ratio of intercellular to atmospheric CO_2 concentration:

$$\frac{C_i}{C_a} = \frac{g_1}{g_1 + \sqrt{D}} \quad (3)$$

This equation predicts that the C_i/C_a ratio varies with vapor pressure deficit and g_1 , but will remain unchanged under elevated CO_2 . Similarly, the following expression is obtained for the ratio of assimilation (A) to transpiration (E), or the instantaneous transpiration efficiency (ITE):

$$\text{ITE} = \frac{A}{E} = \frac{C_a P}{1.6(D + g_1 \sqrt{D})} \quad (4)$$

where P (kPa) is total air pressure. Accordingly, when g_1 and D are unchanged between C_a treatments, ITE should be proportional to C_a . Conversely at a given C_a , ITE is reduced by high D or high g_1 .

This framework gives our first set of hypotheses to be addressed:

- 1) We predict that g_1 will not be affected by CO_2 . ITE at a constant D will therefore be proportional to CO_2 (eqn 4).
- 2) g_1 will be reduced in drought conditions, but there will still be no effect of CO_2 . ITE for droughted plants will remain proportional to CO_2 .
- 3) Droughted plants will operate at a lower C_i . The increase in A with CO_2 enrichment will therefore be higher for droughted plants and the reduction in g_s will be smaller.

Predicting species differences

Complicating our understanding of the effect of elevated CO_2 on limiting drought impacts are species-specific drought strategies. Overall, tree species responses in dealing with water limitations are commonly expressed as belonging to one of two drought strategies. In the first category are drought-avoiding species which close stomata before experiencing any change in leaf water potential (Ψ_{leaf}), while in the second category are drought-tolerant species that employ reductions in g_s once Ψ_{leaf} begins to decline (Guehl *et al.* 1991; Picon *et al.* 1996; Martínez-Ferri *et al.* 2000). These species differences can be characterized by differences in g_1 : drought-avoiding species will have high g_1 that decreases rapidly in drought; drought-tolerant species will have low g_1 that is relatively unresponsive to drought. These observations lead to our second set of hypotheses:

- 4) Xeric (drought-tolerant) species will have lower g_1 in well-watered conditions.
- 5) Photosynthesis will be most responsive to CO_2 enrichment for xeric, drought-tolerant species as they generally operate at lower C_i .
- 6) ITE will be proportional to CO_2 , regardless of drought strategy.

The above hypotheses make predictions about leaf-level responses to elevated CO₂. We hypothesize that these leaf-level responses can be used to predict whole-plant responses. We hypothesize:

- 7) Water use efficiency (WUE) will be proportional to CO₂ for both species and drought treatments.
- 8) Under elevated CO₂, relative gains in biomass will be higher for xeric species and within a given species (mesic or xeric) under drought conditions, whereas water use will display a larger relative reduction for mesic species and within a given species (mesic or xeric) under well-watered conditions.

At the whole-tree scale differences in species responses are often determined by inherent allocation strategies and adjustments that are made under stress conditions. A major determinant of plant response under elevated CO₂ and drought relates to biomass allocation, as allocation patterns are generally directed to meet limiting resources (Litton *et al.* 2007; Iversen and Norby 2008). In experiments focusing solely on water limitations, a general pattern is for an increase in rooting mass and area (Katterer *et al.* 1995; Metcalfe *et al.* 2008). Under elevated CO₂ a typical forest response is for the distribution of fine roots to extend deeper within the soil profile relative to ambient CO₂, typically related to nutrient limitation (Iversen 2010). When nutrient or other limitations are absent, biomass allocation is commonly directed towards aboveground biomass (Litton *et al.* 2007).

A common observation in elevated CO₂ experiments on trees is an increase in leaf area, which often results in equal or greater water loss at the canopy scale thus moderating transpiration savings at the leaf level (Guehl *et al.* 1994; Field *et al.* 1995; Picon *et al.* 1996; Heath and Kerstiens 1997; Centritto *et al.* 1999a; Kergoat *et al.* 2002; Wullschleger *et al.* 2002; Uddling *et al.* 2008). Conversely, allocation patterns under elevated CO₂ are often simply proportional, the result of an increase in tree size versus differences in allocation (Norby 1994; Tissue *et al.* 1997; Wullschleger *et al.* 2002). As a result, an increase in leaf area is often matched by an increase in rooting mass or area. Alternatively, a decrease to leaf area or canopy transpiration under elevated CO₂ may reduce the need for greater allocation to rooting mass or area (Hungate *et al.* 1997; Wullschleger *et al.* 2002). Further adjustments in hydraulic architecture under elevated CO₂ could additionally delay drought impacts when water limitations develop (Wullschleger *et al.* 2002). For example, increased investment of carbon in xylem of greater wood density with more cavitation resistant conduits could impart greater

drought resistance (Hacke and Sperry 2001; Sperry *et al.* 2002; Domec *et al.* 2009). Additionally, hydraulic adjustments with CO₂ enrichment such as reduction in vessel diameters, vessel numbers or an increase in sapwood to leaf area may beneficially alter xylem permeability and therefore capacity of water flux under drought conditions (Atwell *et al.* 2007; Domec *et al.* 2010).

Along with different physiological functioning in response to water limitations, species characterizing each particular drought strategy exhibit structural adjustments to environment. For example, at lower limits of water availability, species present typically produce small vessels, deep root systems and higher root to shoot ratios, while at the upper ends of water availability species present generally allocate more biomass to aboveground structures (Tilman 1988; Jackson *et al.* 1996; Chaves *et al.* 2003; Litton *et al.* 2007). A major questions that largely remains unaddressed, in regards to elevated CO₂, is if an how species specific strategies in responding to drought may differ physiologically at the leaf-level (i.e., gas exchange) or structurally at the whole-tree scale. Consequently at the whole-tree scale, we made the following predictions for changes in biomass allocation with growth under moderate long-term drought and elevated CO₂.

- 1) Leaf area will compensate for reduced stomatal conductance so that overall transpiration under elevated CO₂ will be similar to ambient conditions.
- 2) Leaf area will match water reduction so that halving the available water will halve leaf area.
- 3) Plant allocation patterns will shift towards root mass under drought conditions.

Experimental approach

To gain a better understanding of physiological and structural adjustments that tree species of opposing drought tolerance employ under elevated CO₂ and moderate long-term drought, we conducted a glasshouse experiment with two eucalypts of contrasting drought strategy. *Eucalyptus* serves as a particularly useful genus for testing the impact of drought under elevated CO₂ due to the prevalence of drought across the Australian continent (McAlpine *et al.* 2009) and based on the wide range of species native habitats, from extremely arid to highly mesic environments (Merchant *et al.* 2007, 2010; Warren *et al.* 2011a, b). A common hypothesis is that under elevated CO₂, xeric tree species under drought episodes will display greater relative gains in productivity and growth than species from more mesic

environments or when experiencing drought episodes (Strain and Bazzaz 1983; Miao *et al.* 1992; Morison 1993a; Catovsky and Bazzaz 1999; Wullschleger *et al.* 2002). To test the xeric versus mesic hypothesis we conducted a glasshouse experiment with xeric, drought-tolerant *Eucalyptus populnea* and mesic, drought-avoiding *Eucalyptus pilularis*. In order to mimic long-term moderate drought and to avoid pot constraints we used large 90-liter pots which were constantly re-wetted to maintain full field capacity (100% FC) or 50% of FC, thus avoiding frequent drying cycles common of many drought experiments (e.g., Guehl *et al.* 1994; Picon *et al.* 1996; Centritto *et al.* 1999, 2002). Throughout the experiment we focused on measurements of carbon gain, water loss and biomass allocation to better understand species specific responses to water limitations under elevated CO₂. We set out to test species specific responses under elevated CO₂ and long-term moderate drought with the following hypotheses at the leaf and whole-tree scale, which in review include:

- 1) Stomatal conductance (g_s) and leaf intercellular CO₂ (C_i) will be lower at 50% of field capacity, therefore the CO₂ effect on photosynthesis (A) and productivity should be higher
- 2) Xeric *E. populnea* will operate at a lower C_i and have a higher ITE and higher WUE
 - a. Therefore, photosynthesis and productivity of xeric *E. populnea* should be more responsive to CO₂ enrichment
- 3) CO₂ effect on ITE will be proportional to growth CO₂ for both species and in all treatments
 - a. Therefore, at low water availability, the reduction in g_s would be smaller
 - b. Reduction in g_s will also be smaller in the xeric species
- 4) WUE will be related to ITE and will respond to CO₂ and drought in the same way as ITE
- 5) Leaf area will be determined by water availability – halving water availability will halve leaf area, however leaf area will be lower in xeric species due to growth strategy
- 6) The CO₂ effect on leaf area will completely compensate for the CO₂ effect on g_s so that total tree transpiration will be unaffected by CO₂

2.2 Material / Methods

2.2.1 Plant Material

Two *Eucalyptus* species native to New South Wales and Queensland, Australia were selected for the study (Figure 2-1). The two species were chosen due to their perceived strategy in responding to drought (Hodgkinson 1979; Noble 1989). Xeric *Eucalyptus populnea* is a drought-tolerant species occupying eucalypt dry woodland of interior Queensland and New South Wales (Figure 2-1; Table 2-1). Conversely, mesic *Eucalyptus pilularis* is a drought-avoiding species occupying eucalypt tall open forests of the higher rainfall, coastal regions of Queensland and New South Wales (Figure 2-1; Table 2-1).

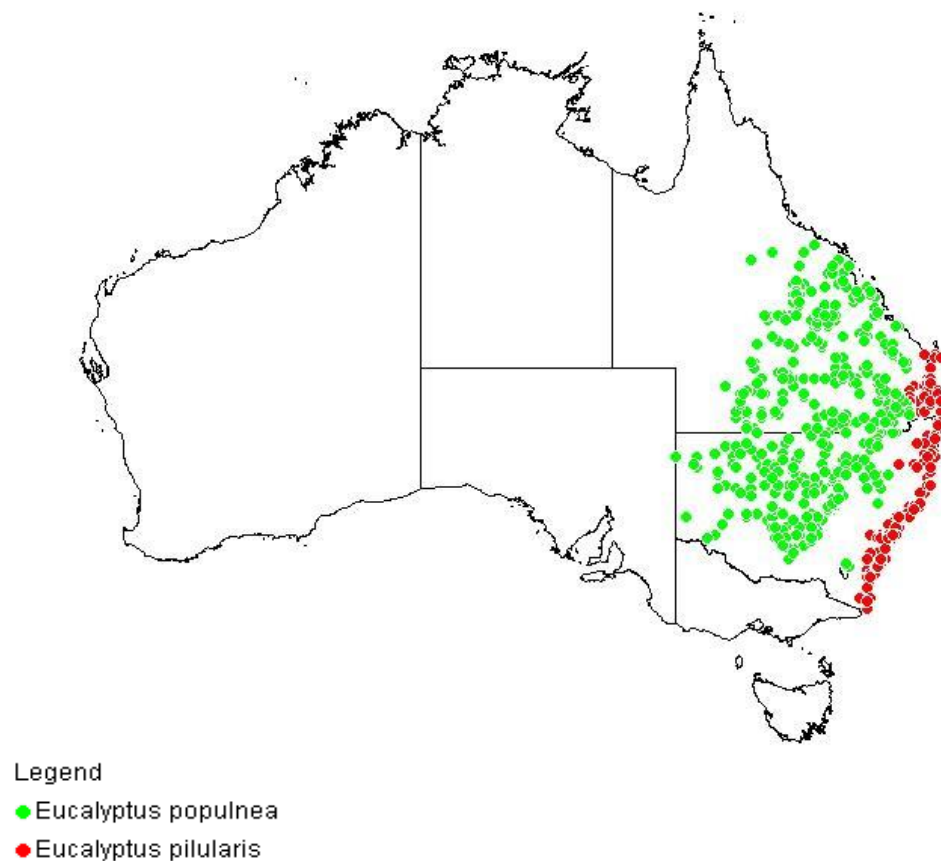


Figure 2-1. *Eucalyptus populnea* and *Eucalyptus pilularis* distribution within Australia. Source: Australia's Virtual Herbarium.

Table 2-1. Physical and environmental characteristics of *E. pilularis* and *E. populnea*. Source: Forest Trees of Australia, Boland *et al.* 1984.

Species	Max height (m)	Max dbh (m)	Rainfall (mm)	Max temp (°C)
<i>Eucalyptus pilularis</i>	70	3.0	900-1750	32
<i>Eucalyptus populnea</i>	20	0.8	350-1000	37

2.2.2 Experimental setup

A red silt loam soil was collected from Robertson, NSW Australia. The soil was sieved and then allowed to air dry, after which 80 kgs of nutrient amended dry soil was added to each of 48 pots (60 cm diameter and 48 cm height). Nutrient amendments were mixed in bulk for each 90 L pot at the following concentrations: CaCO_3 , MgCO_3 , $(\text{NH}_4)_2\text{SO}_4$, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, K_2CO_3 , CaSO_4 , MgSO_4 , at 7, 1.75, 2.83, 4, 2.5, 1, 1 g kg^{-1} dry soil and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, H_3BO_3 and EDTA-FeIII Na salt at 100, 19.6, 0.03, 0.55, 4.4 mg kg^{-1} of dry soil respectively. Supplementary KNO_3 , $\text{Ca}(\text{NO}_3)_2$, $\text{Mg}(\text{NO}_3)_2$, $(\text{NH}_4)_2\text{SO}_4$ and NH_4NO_3 in the ratio (by weight) 1:2:1:1:1 were added to the soil fortnightly throughout the experiment to ensure that nutrients were adequately supplied.

Experiments took place at Macquarie University, Sydney, Australia. Multiple seeds of *Eucalyptus pilularis* and *Eucalyptus populnea* acquired from CSIRO Tree Seed Center (Canberra, ACT Australia) were sown directly into large 90 L pots in February 2010 and grown for four months at full field capacity (100% FC) (approximate 32 % w/v). Trees were grown in four glasshouses, two of which had CO_2 partial pressure similar to ambient (380 ppm) and the other two of which had CO_2 partial pressure maintained at elevated (700 ppm) by releasing CO_2 gas from a high pressure tank when the CO_2 partial pressure declined below 700 ppm. Temperatures in the glasshouses were maintained by air conditioners at $\sim 24^\circ\text{C}$ during the day and $\sim 16^\circ\text{C}$ during the night. Air temperature and relative humidity were recorded in the four glasshouses every 15 minutes during experiments with a data logger (HOBO U12-013; Onset Computer Corporation). Average daytime air temperature was 25.1°C in the ambient glasshouses and 24.9°C in the elevated glasshouses, with average night-time air temperature of 16.2°C and 15.9°C in ambient and elevated glasshouses, respectively. Average daytime VPD was 1.32 kPa in both the ambient and elevated glasshouses, with an average night-time VPD of 0.47 kPa.

Four months after seedling emergence (early June 2010) a drought treatment was instituted where one half of the pots from each species (12 of 24 pots from each species) were subjected to a drought. In the context of the experiment presented in this chapter, the terms well-watered and drought are used throughout as a short-hand description for high water availability and low water availability, respectively. This short-hand description for soil water availability is in keeping in line with several comparable drought studies (see Guehl *et al.* 1994; Picon *et al.* 1996; Atwell *et al.* 2007; Cernusak *et al.* 2011). The drought treatment (low water availability) consisted of setting and maintaining soil moisture content at 50% of field capacity (50% FC). Pots not included in drought treatment were maintained at full field capacity (100% FC: i.e. high water availability or well-watered) throughout the experiment. The drought treatment (50% FC) and well-watered treatment (100% FC) continued and were constantly maintained throughout the remainder of the experiment (~6.5 months for *E. pilularis* and ~9 months for *E. populnea*). Maintenance of soil moisture content in pots was accomplished by direct measurement of soil moisture content with time domain reflectometry (TDR) waveguides placed at 15 cm soil depth (MiniTrase, Soilmoisture Equipment Corp., USA) and by weighing individual pots every 2-3 days. Daily re-watering of pots back to percent of field capacity was done manually to ensure water was distributed evenly and to avoid clumping of roots in the vicinity of the water source. Throughout the experiment pots were rotated fortnightly within each glasshouse to avoid any position effects.

2.2.3 Gas exchange

Light saturated net photosynthetic rates (A_{sat}), stomatal conductance (g_s) and leaf intercellular CO_2 (C_i) concentration were measured on fully formed leaves with an open-flow infrared gas analyzer equipped with a red-blue light source (LI-6400XT, Li-Cor, Lincoln NE, USA). Measurements were conducted on two separate occasions, the first at one month after instituting drought treatment (July 2010; both species) and the second just prior to final harvest in October 2010 for *E. pilularis* and January 2011 for *E. populnea*. Leaf cuvette conditions during each measurement period were maintained at a light level of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of 25°C and relative humidity $> 60\%$. The CO_2 concentration within the leaf cuvette during each measurement period corresponded to plant growth environment (ambient glasshouses: $380 \mu\text{mol m}^{-1}$; elevated glasshouses: $700 \mu\text{mol m}^{-1}$).

Photosynthesis to leaf intercellular CO_2 concentration ($A-C_i$) response curves were additionally measured for each species and treatment combination, one month after start of

drought treatment (July 2010) and again prior to final harvest (October 2010 for *E. pilularis* and January 2011 for *E. populnea*) on fully formed leaves with a Li-Cor 6400 portable photosynthesis system (Li-Cor, Lincoln NE, USA). Leaf cuvette conditions were maintained at a PAR level of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of 25°C and relative humidity $> 60\%$. Ambient CO_2 concentration (C_a) in the cuvette was maintained with a CO_2 mixer at the following concentrations: 1500, 1100, 700, 380, 200, 100, 50 and $0 \mu\text{mol mol}^{-1}$.

From these measured A- C_i curves, values of apparent V_{cmax} and J_{max} were determined using the formulation and parameterization of the original Farquhar *et al.* (1980) model as set forth by Medlyn *et al.* (2002a), and using parameters for Rubisco kinetics as described by Bernacchi *et al.* (2001). For all model fitting and parameter determination associated with the Farquhar *et al.* (1980) biochemical model of photosynthesis, we utilized a non-linear least squares fitting routine implemented as a Python package, available at <https://github.com/mdekauwe/FitFarquharModel>.

2.2.4 Watering treatment

To determine whole-tree transpiration, individual pots were weighed every 2-3 days using a standard floor scale (Avery Weigh-Tronix, Fairmont MN, USA). Direct evaporation from the soil surface was controlled by covering exposed soil evenly with white gravel $\sim 10\text{mm}$ diameter in size. Water lost through transpiration was added back to each individual pot to restore soil to percent of field capacity (50% or 100% FC).

2.2.5 Tree architecture

The three-dimensional arrangement of branch architecture and leaf arrangement of each tree was recorded using a 3D-digitizer (Polhemus FASTRAK, Colchester VT, USA) along with the software package FLORADIG (CSIRO Entomology, Brisbane, Australia). Measurements were conducted ~ 2 months prior to final harvest for each species. Collected 3-D spatial coordinates from FLORADIG were converted into YPLANT (Pearcy and Yang 1996) using a program written in C (Falster and Westoby 2003). We used the R package *YpTools* (Duursma 2011) to analyze and visualize the collected tree architectural data directly in R.

2.2.6 Tree growth and biomass allocation

The height and basal diameter of each tree were measured fortnightly throughout the experiment. Height was measured from soil surface to top of tree, while basal diameter was measured at 50 mm above soil surface. Crown width was measured at final harvest. Crown volume and leaf area at 2 months prior to final harvest were determined with YPLANT, and used to calculate leaf area density (LAD; ratio of total leaf area to total crown volume) and the ratio of displayed to total leaf area ($\overline{\text{STAR}}$).

Aboveground plant parts were destructively harvested on three separate occasions; on day 96, 110 and 124 (days after emergence) and a single final harvest of aboveground and belowground biomass taking place on day 301 for *E. pilularis* and day 377 for *E. populnea*. The earlier harvest date for *E. pilularis* (day 301 vs. day 377) was the result of these plants nearing size limits of the glasshouse environment. Aboveground plant parts were separated into leaves and stems and then oven-dried for 48 hrs. at 70°C, after which component parts were weighed with an electronic balance. At final harvest plant parts were further separated into leaves, stems (lateral and main) and coarse (> 2 mm) and fine (< 2 mm) roots. Roots were separated from soil by washing and sieved to limit the loss of fine roots. Leaf numbers were counted and area measured using a portable leaf area meter (LI-3100C, Li-Cor, Lincoln NE, USA). Component plant parts from final harvest were oven-dried for 48 hrs. at 70°C and weighed with an electronic balance. Water use efficiency (the ratio of total dry mass produced to total amount of water transpired) was estimated for each species and treatment combination. WUE was calculated as the ratio of the total dry-mass increase to total water loss between the harvests made on day 124 and 301 for *E. pilularis* and on day 124 and 377 for *E. populnea*. Leaf dry masses and area were used to calculate specific leaf area (SLA). Plant component dry mass was used to calculate leaf mass, stem mass, and root mass ratio (LMR, SMR and RMR); while root to shoot ratio (R:S) was calculated as the ratio of whole root dry mass to whole stem and leaf dry mass.

2.2.7 Statistical analysis

Statistical analyses were performed with R 2.14.1 (R Core Development Team, 2011). Linear mixed-effects models (package *nlme* in R) were used to test for main effects of CO₂ and drought treatment, along with the interaction between CO₂ and drought on gas exchange, water loss and carbon gain both within and between species. All data were tested for normality and log transformed when necessary.

2.3 Results

2.3.1 A-C_i curves – *E. pilularis* and *E. populnea*

There was no significant effect of elevated CO₂ on apparent V_{cmax} or J_{max} for either mesic *E. pilularis* or xeric *E. populnea*, on any date or for either watering regime. Thus, down-regulation of photosynthesis did not occur over the 301 days of CO₂ enrichment for *E. pilularis* or the 377 days of CO₂ enrichment for *E. populnea*.

Drought significantly reduced V_{cmax}, but not J_{max} for *E. pilularis* and only on the first measurement date in July (Table 2-2). This observed response in V_{cmax} for *E. pilularis* under drought conditions was consistent between CO₂ concentrations, with a reduction in July of 27% and 18% for elevated and ambient CO₂, respectively, compared with no significant change in October. The reduction in apparent V_{cmax} in July could potentially have been caused by a reduction in Rubisco content or activity, or by a reduction in mesophyll conductance. Comparatively for *E. populnea*, values of V_{cmax} and J_{max} were consistent over time and did not significantly differ with drought treatment.

Between species, values of V_{cmax} were significantly (p<0.01) larger for *E. pilularis* compared with *E. populnea* (Table 2-2), irrespective of CO₂ or drought treatment. However, for J_{max} there was no significant difference between the two species over time or treatment combinations.

Table 2-2. Apparent V_{cmax} (μmol m⁻² s⁻¹) and J_{max} (μmol m⁻² s⁻¹) of *E. pilularis* in July and October 2010, and *E. populnea* in July 2010 and January 2011. Values are means ± 1 SE. n=4(July); n=6(October, January).

<i>Eucalyptus pilularis</i>					
Parameter	Month	Ambient CO ₂		Elevated CO ₂	
		Well-watered	Droughted	Well-watered	Droughted
V _{cmax}	July	113.4 ± 7.9	96.3 ± 17.4	114.9 ± 2.5	90.7 ± 4.9
J _{max}	July	122.3 ± 3.4	122.8 ± 10.9	131.4 ± 1.2	122.7 ± 5.7
V _{cmax}	October	103.2 ± 7.5	113.9 ± 5.8	103.1 ± 5.3	105.2 ± 9.3
J _{max}	October	114.1 ± 7.1	124.8 ± 5.1	118.9 ± 5.3	122.1 ± 8.0

<i>Eucalyptus populnea</i>					
Parameter	Month	Ambient CO ₂		Elevated CO ₂	
		Well-watered	Droughted	Well-watered	Droughted
V _{cmax}	July	91.4 ± 3.1	93.9 ± 9.2	91.9 ± 2.2	98.6 ± 1.5
J _{max}	July	139.3 ± 11.8	134.3 ± 9.8	131.0 ± 4.9	138.6 ± 1.8
V _{cmax}	January	82.2 ± 9.8	91.0 ± 15.2	77.7 ± 14.6	79.6 ± 11.5
J _{max}	January	127.1 ± 4.3	123.7 ± 8.9	129.6 ± 9.3	127.1 ± 10.4

2.3.2 Leaf gas exchange

E. pilularis and *E. populnea*

There was a highly significant ($p < 0.001$) effect of elevated CO₂ on light saturated photosynthetic rates (A_{sat}), stomatal conductance (g_s) and instantaneous transpiration efficiency (ITE) for both *E. pilularis* and *E. populnea* on each measurement date, irrespective of watering regime (Table 2-3). This effect of elevated CO₂ on leaf gas exchange led to an increase in A_{sat} and ITE, and a reduction in g_s (Table 2-4; Fig. 2-3) under both well-watered and drought conditions. Elevated CO₂ also caused a significant reduction in both g_1 and C_i/C_a for both *E. pilularis* and *E. populnea* on both measurement dates at both well-watered and drought conditions (Table 2-3).

Notably, for both *E. pilularis* and *E. populnea*, nearly all of the measured gas exchange parameters were lower on the second measurement date, leading to a significant effect of measurement date on gas exchange (Table 2-4; Fig. 2-3). As height increases with time, we observed that there was a correlation between the parameter g_1 and plant height (Fig. 2.2) in both species. Importantly, this effect of measurement date did not alter, or lead to a significant interaction with the effect of elevated CO₂ on any of the measured gas exchange parameters. A sole exception to this effect of measurement date was for g_s of *E. pilularis*, which exhibited minimal variation over time (Table 2-4).

Separately there was no significant effect of drought on A_{sat} , g_s , g_1 , C_i/C_a or ITE for either *E. pilularis* or *E. populnea* at any time point or under either CO₂ concentration. Additionally, there were no significant CO₂ x drought interactions for any of the measured gas exchange parameters. This suggests, that for both species, the observed leaf-level increase in A_{sat} and ITE, and reduction in g_s , C_i/C_a and g_1 with CO₂ enrichment were relatively unaffected by long-term moderate drought conditions.

2.3.3 Leaf gas exchange

Xeric vs. *Mesic* species

Between species, there was a highly significant ($p < 0.001$) difference in A_{sat} , g_s , C_i/C_a , g_1 and ITE (Table 2-3). Xeric *E. populnea* displayed a larger overall A_{sat} , g_s and g_1 in all treatments, whereas mesic *E. pilularis* exhibited a higher overall ITE (Table 2-4). There was a significant decrease in all measured gas exchange parameters with time (Table 2-4), which

for the most part was not different between species. An exception was for A_{sat} and g_s , where was a significant species x date interaction (Table 2-3). This interaction for A_{sat} was the result of a large difference between species on the first measurement date, with a much larger A_{sat} exhibited by *E. populnea* compared with *E. pilularis*, while on the second measurement date A_{sat} was similar between species (Table 2-4; Fig. 2-3). The species x date interaction for g_s , on the other hand, was the result of little variation in g_s over time by *E. pilularis* and a substantial reduction over time by *E. populnea*, along with a larger overall difference between the two species in g_s (Table 2-4; Fig. 2-3).

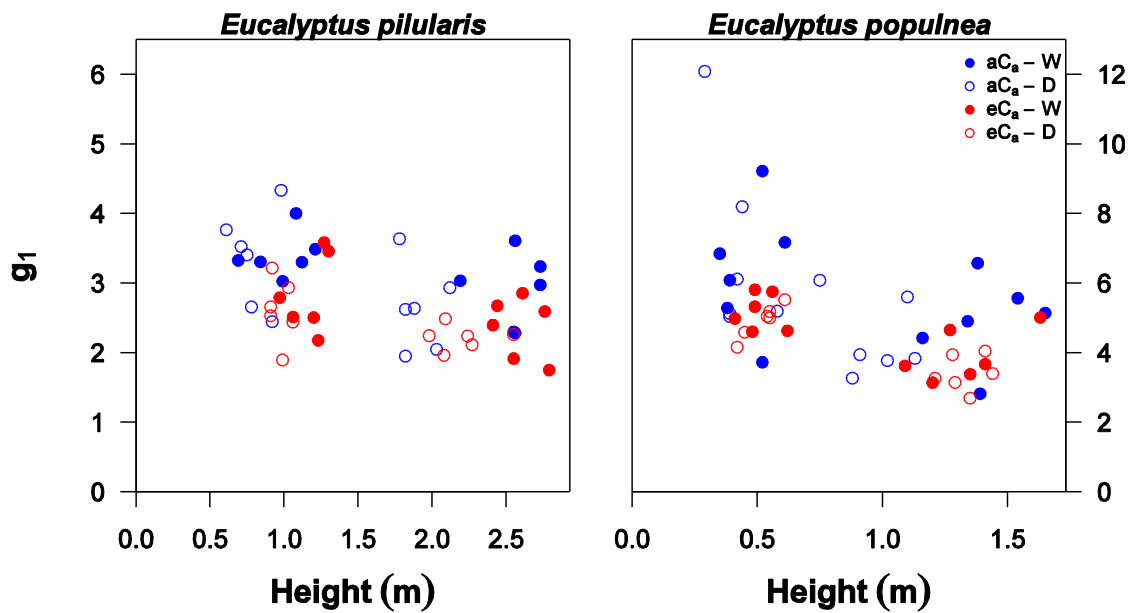


Figure 2-2. The parameter g_1 as a function of height. Symbols are the calculated g_1 from measured gas exchange parameters. The figure suggests a consistent decrease in the g_1 parameter with increasing plant size.
***Note scale difference between the two species.**

Table 2-3. Statistics of ecophysiological responses for *E. pilularis* and *E. populnea*.

Parameter	Main effects				Interactions
	Species	CO ₂	Drought	Date	Species x Date
Numerator df	1	1	1	1	1
Denominator df	40	40	40	40	40
Gas exchange					
A _{sat} (μmol m ⁻² s ⁻¹)	30.00***	307.21***	1.76	2.57	21.32***
g _s (mol m ⁻² s ⁻¹)	220.37***	36.59***	5.26*	67.80***	47.23***
g _i	175.45***	27.54***	2.68	40.59***	
VPD (kPa)	0.41	0.38	0.19	2.33	
C _i /C _a	109.11***	18.92***	2.21	21.98***	
ITE (μmol CO ₂ mmol H ₂ O ⁻¹)	84.50***	210.32***	0.75	33.15***	

F values and significance levels (**P*<0.05; ***P*<0.01; ****P*<0.001) for the main effects of species, elevated CO₂ (CO₂), drought and date; along with significant interactions of species x date on A_{sat} and g_s. Numerator and denominator df are the numerator and denominator degrees of freedom for the *F* tests. Note: Only interactions which were significant are displayed. *VPD is leaf to air vapor pressure deficit within the leaf cuvette.

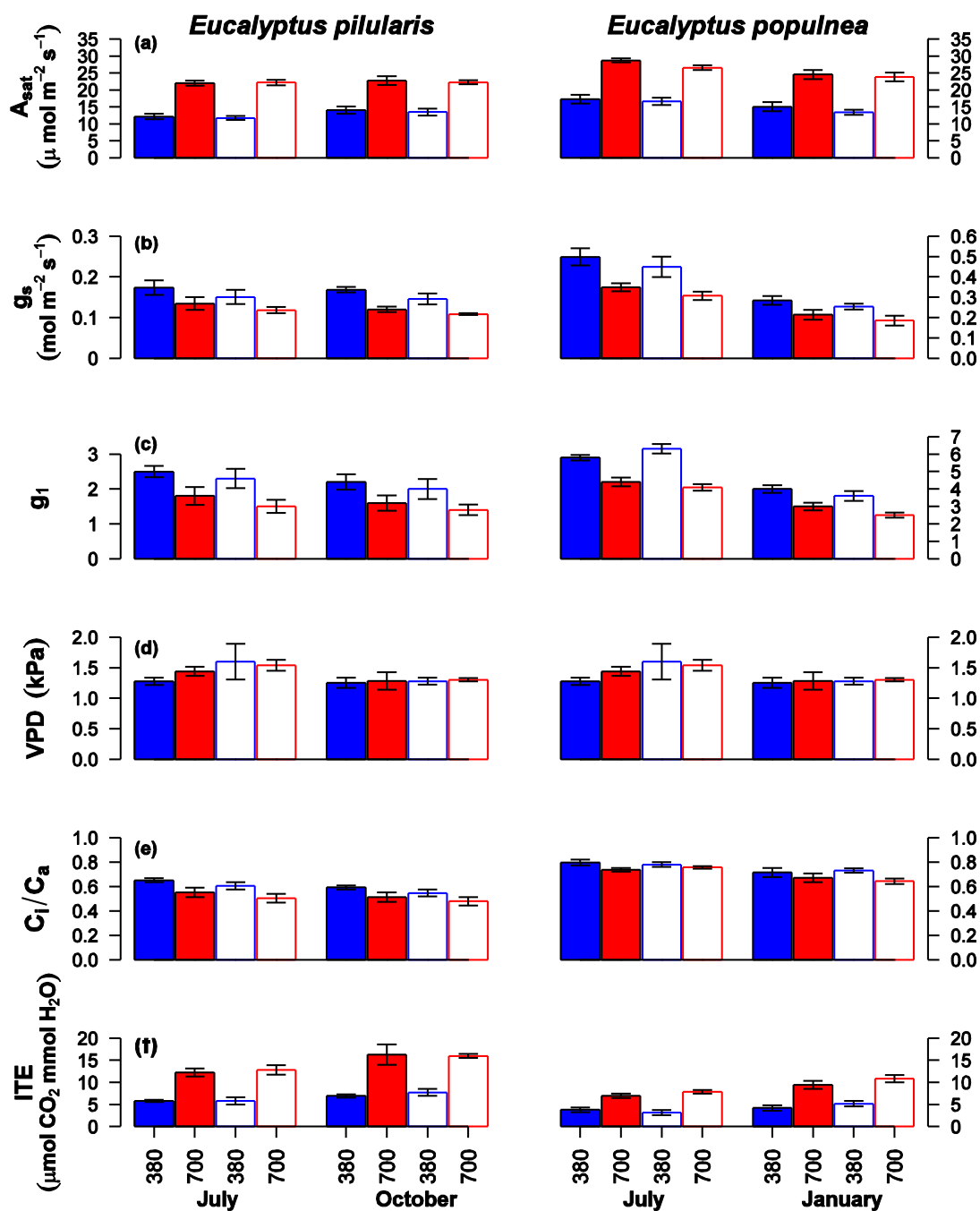


Figure 2-3. (a) Light saturated net photosynthetic rate (A_{sat}), (b) stomatal conductance (g_s), (c) g_1 , (d) Vapor pressure deficit (VPD), (e) Leaf intercellular to ambient CO₂ concentration (C_i/C_a) and (f) instantaneous transpiration efficiency (ITE) of *E. pilularis* in July and October 2010 and *E. populnea* in July 2010 and January 2011. Data are the means of 6 trees per treatment and measurement date; error bars are ± 1 SE. Filled bars – well-watered (100% FC); Open bars – Drought (50% FC). Blue bars – Ambient CO₂; Red bars – Elevated CO₂. The CO₂ concentration within the leaf cuvette during each measurement period corresponded to plant growth environment (ambient glasshouses: 380 $\mu\text{mol m}^{-1}$; elevated glasshouses: 700 $\mu\text{mol m}^{-1}$).

Table 2-4. Relative effect of elevated CO₂ and drought (50% field capacity) on measured leaf gas exchange parameters for *E. pilularis* and *E. populnea*. Control data are the means of 6 trees per treatment on each measurement date. Effect sizes are relative to well-watered conditions and ambient CO₂ (Baseline), and given as a percentage change from baseline values. The CO₂ concentration within the leaf cuvette during each measurement period corresponded to plant growth environment (ambient glasshouses: 380 $\mu\text{mol m}^{-1}$; elevated glasshouses: 700 $\mu\text{mol m}^{-1}$). *VPD is leaf to air vapor pressure deficit within the leaf cuvette.

		<i>Eucalyptus pilularis</i>					<i>Eucalyptus populnea</i>					
CO ₂ / Drought effect		aC _a - D	eC _a - W	eC _a - D	eC _a - D		aC _a - D	eC _a - W	eC _a - D	eC _a - D		
		aC _a - W	vs.	vs.	vs.		aC _a - W	vs.	vs.	vs.		
Parameter	Month	aC _a - W	aC _a - W	aC _a - D	eC _a - W	Month	aC _a - W	aC _a - W	aC _a - D	eC _a - W		
		Baseline					Baseline					
A _{sat} (μmol m ⁻² s ⁻¹)	July	12.1	-3	+82	+90	+1	July	17.3	-3	+66	+59	-8
g _s (mol m ⁻² s ⁻¹)	July	0.17	-12	-24	-20	-8	July	0.5	-10	-34	-31	-6
g _i	July	2.5	-8	-28	-35	-17	July	5.8	+9	-24	-35	-7
VPD (kPa)	July	1.3	+25	+13	-4	+2	July	1.1	+35	+27	-20	-6
C _i /C _a	July	0.65	-6	-15	-16	-7	July	0.79	-2	-7	-3	+3
ITE (μmol CO ₂ mmol H ₂ O ⁻¹)	July	5.8	0	+110	+121	+5	July	3.8	-16	+82	+144	+13
A _{sat} (μmol m ⁻² s ⁻¹)	Oct	14	-4	+62	+65	-2	Jan	15	-11	+64	+78	-3
g _s (mol m ⁻² s ⁻¹)	Oct	0.17	-12	-29	-27	-8	Jan	0.28	-11	-25	-28	-14
g _i	Oct	2.2	-9	-27	-30	-13	Jan	4	-10	-25	-31	-17
VPD (kPa)	Oct	1.3	+2	+2	+2	+7	Jan	1.5	-23	-7	+15	-15
C _i /C _a	Oct	0.59	-7	-14	-13	-6	Jan	0.71	3	-6	-12	+3
ITE (μmol CO ₂ mmol H ₂ O ⁻¹)	Oct	6.9	+12	+136	++108	-2	Jan	4.2	+24	+126	+108	+14

2.3.4 Whole- plant responses

2.3.4.1 Pre-drought treatment destructive harvests – *E. pilularis* and *E. populnea*

The first three destructive harvests on day 96, 110 and 124 (days after emergence) took place before a drought treatment was instituted, and as a result data for aboveground biomass and leaf area were only tested for the effect of elevated CO₂.

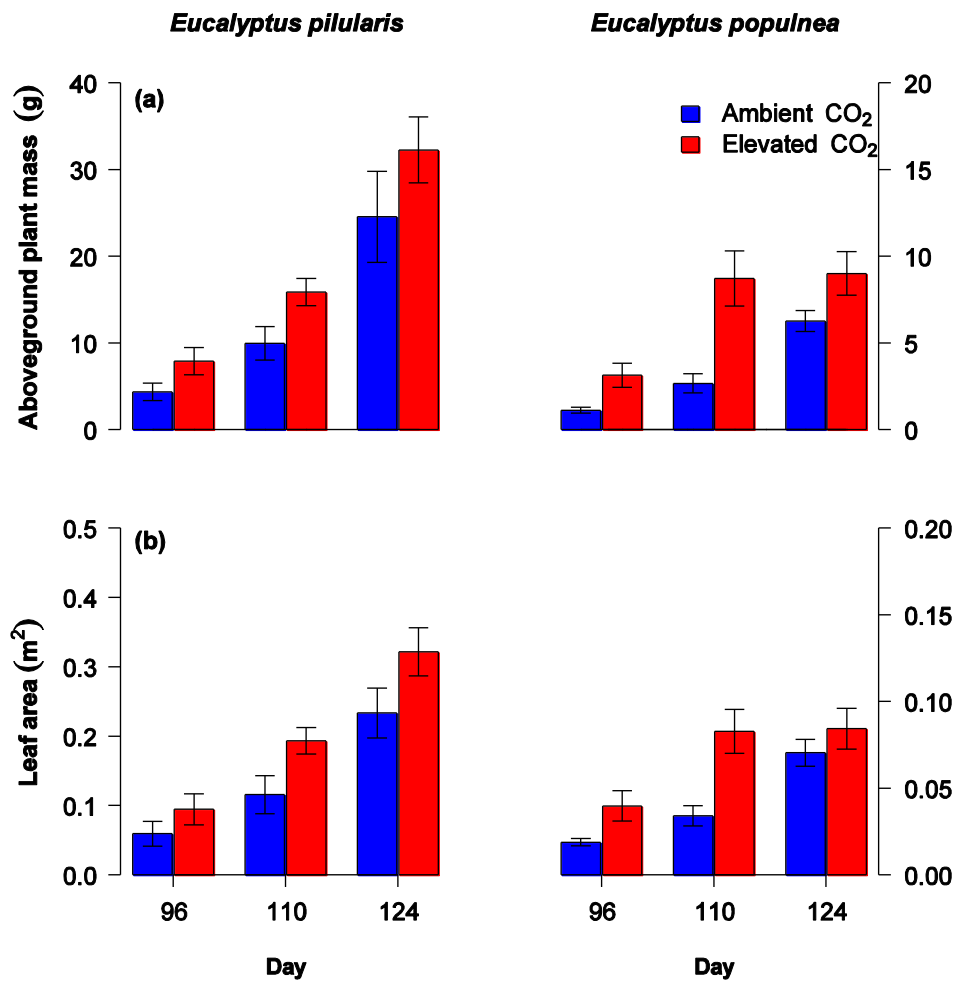


Figure 2-4. (a) Aboveground biomass and (b) leaf area from three destructive harvests conducted prior to drought treatment. Data are the means of 12 trees per treatment on each harvest date; error bars are ± 1 SE. Blue bars – Ambient CO₂; Red bars – Elevated CO₂.

For *E. pilularis*, on all pre-drought treatment harvest dates, there was an increase in both aboveground biomass and leaf area (Fig. 2-4) with CO₂ enrichment, although the effect of elevated CO₂ on the increase in biomass and leaf area was only significant ($p < 0.05$) on the

second harvest date, day 110. Similarly, *E. populnea* displayed an increase in aboveground biomass and leaf area with CO₂ enrichment. The effect of elevated CO₂ for *E. populnea* resulted in a significant increase on the first two harvest dates, day 96 and 110, but not on the third pre-drought treatment harvest date, day 124 (Fig. 2-4). Between the two species aboveground biomass and leaf area was significantly higher for *E. pilularis* on all pre-drought treatment harvest dates (Fig. 2-4). The difference between species in aboveground biomass and leaf area was not caused by a significant interaction with elevated CO₂ but rather by species-specific growth rates during the pre-drought treatment period.

2.3.4.2 Whole-plant responses – *E. pilularis*

CO₂ effect

Results of whole-plant responses to CO₂ and drought for both *E. pilularis* and *E. populnea* are summarized in Table 2-5; statistical results are presented in Table 2-6. For *E. pilularis*, water use efficiency (WUE) was significantly ($p < 0.001$) increased by growth at elevated CO₂. However, the relative increases in WUE were only about one half of the observed increases in ITE from gas exchange measurements, and only one half to two-thirds the increase in the CO₂ concentration (Table 2-5). The increase in WUE was higher for plants grown under well-watered than drought conditions (58% vs. 44%), although the CO₂ x drought (CO₂ x D) interaction was not statistically significant.

The imposed reduction of soil moisture content to 50% FC resulted in a highly significant ($p < 0.0001$) reduction in transpiration (58%) of a similar magnitude to the reduction in moisture availability (Table 2-5). There was no change in total transpiration under elevated CO₂, for either watering regime, suggesting that plant water use was strongly controlled by water availability (Fig. 2-5). Since transpiration was unchanged under elevated CO₂, the increases in plant biomass for *E. pilularis* are of the same magnitude as increases in WUE: 56% and 40% for well-watered and drought conditions, respectively.

Along with an increase in total plant biomass, individual biomass components (leaf, stem and root mass) were all significantly ($p < 0.05$) increased under elevated CO₂ (Fig. 2-7; Table 2-5). However, the effect of CO₂ enrichment on plant allocation patterns

Table 2-5. Relative effect of elevated CO₂ and drought on plant water relations, total biomass, plant dimensions and plant allocation for mesic *E. pilularis* and xeric *E. populnea*. Baseline data and effect sizes are based on the means of 6 trees per treatment. Effect sizes are relative to well-watered conditions and ambient CO₂ (Baseline), and given as a percentage change from baseline values.

CO ₂ / Drought effect	<i>Eucalyptus pilularis</i>					<i>Eucalyptus populnea</i>				
	aC _a - W	aC _a -D vs.	eC _a -W vs.	eC _a -D vs.	eC _a -D vs.	aC _a - W	aC _a -D vs.	eC _a -W vs.	eC _a -D vs.	eC _a -D vs.
	Parameter	aC _a -W	aC _a -W	aC _a -D	eC _a -W	Parameter	aC _a -W	aC _a -W	aC _a -D	eC _a -W
<i>Water relations</i>	Baseline					Baseline				
Total transpiration (l)	299	-58	-2	-3	-58	196	-50	-7	+3	-45
WUE (mmol C mol ⁻¹ H ₂ O)	4.11	+10	+58	+44	0	2.37	+15	+47	+48	+16
<i>Plant dry biomass</i>										
Total mass (g)	1657	-52	+56	+40	-57	633	-43	+38	+55	-36
Leaf mass (g)	673	-49	+65	+53	-53	141	-35	+60	+41	-43
Total stem mass(g)	722	-58	+40	+42	-57	196	-69	+16	+126	-39
Main stem mass (g)	417	-66	+34	+61	-59	66	-60	0	+83	-28
Lateral stem mass (g)	305	-48	+47	+26	-55	131	-73	+24	+158	-44
Total root mass (g)	262	-46	+77	+2	-69	295	-30	+41	+40	-30
Coarse root mass (g)	143	-59	+46	+9	-69	168	-30	+36	+25	-35
Fine root mass (g)	119	-30	+114	-3	-68	127	-30	+48	+60	-24
<i>Plant Dimensions</i>										
Leaf number (n)	2366	-42	+22	+9	-48	341	-55	+47	+99	-38
Leaf area (m ²)	6.47	-50	+44	+15	-60	0.84	-47	+33	+50	-40
Crown width (m)	1.65	-15	+4	+1	-18	1.42	-23	+6	+39	0
Height (m)	2.7	-20	+4	+14	-12	1.54	-31	-9	+40	+5
Basal diameter (mm)	35.2	-29	+19	+19	-28	18.9	-38	+13	+35	-27
SLA (m ² kg ⁻¹)	9.6	-2	-12	-24	-15	6.2	-17	-19	+8	+11
Mean leaf size (cm ²)	28.5	-16	+15	+4	-24	25.1	+18	-10	-27	-4
LAD	1.6	-2	+17	+10	-8	1.3	+69	+35	-36	-20
STAR	0.31	+11	-10	-7	+14	0.36	+1	-4	+5	+10
<i>Biomass Allocation</i>										
R:S (g g ⁻¹)	0.19	+18	+15	-33	-31	0.88	+51	+5	-15	+23
LMR (g g ⁻¹)	0.41	+7	+6	+10	+11	0.22	+14	+19	-9	-13
SMR (g g ⁻¹)	0.43	-12	-10	+2	0	0.32	-46	-19	+48	-1
RMR (g g ⁻¹)	0.16	+14	+13	-28	-27	0.46	+25	+4	-11	+7
Leaf lignin (%)	36.1	-1	-1	0	0	31.5	-4	-4	+7	+7
Leaf cellulose (%)	20.9	-31	-38	-18	-8	18.1	-22	-23	-1	0
Leaf lignin:cellulose	2	+27	+42	+18	+6	1.85	+17	+19	+12	+10
Stem lignin (%)	23.4	+9	-6	-9	+5	22.9	+1	+5	+3	-1
Stem cellulose (%)	36.7	-9	+1	+8	-3	37.2	-3	+2	0	-4
Stem lignin:cellulose	0.64	+20	-7	-15	+9	0.62	+3	+4	+4	+3

differed depending on watering regime, particularly for belowground biomass. For well-watered plants, CO₂ enrichment resulted in an increase in all biomass components, with the largest relative increases in leaf (65%) and root (77%) mass, and particularly fine root mass at 114% (Table 2-5). These shifts for plants under elevated CO₂ and well-watered conditions resulted in an increase in the root:shoot ratio, and increases in leaf mass ratio and root mass ratio (Table 2-5). Under drought conditions, CO₂ enrichment resulted in a relative increase in all biomass components except for fine root mass, where there was a small decrease (3%) rather than an increase (Table 2-5).

In respect to plant dimensions for *E. pilularis*, there was a significant ($p < 0.05$) effect of elevated CO₂ on total height, leaf area and basal diameter, whereas there was no significant difference between CO₂ treatments in crown width. In the case of total height the relative increase with CO₂ enrichment was higher under drought than well-watered conditions (14% vs. 4%), whereas the relative increase for basal diameter was similar (~19%) between watering regimes. Conversely, the relative increase in leaf area was considerably higher under well-watered than drought conditions (44% vs. 15%), although the interaction (CO₂ x D) was not statistically significant (Table 2-6).

For crown and leaf properties of *E. pilularis*, there was a significant decrease under elevated CO₂ in the ratio of displayed to total leaf area (\overline{STAR}) and specific leaf area (SLA), but no significant effect of CO₂ enrichment on the ratio of total leaf area to total crown volume (LAD), leaf number or mean leaf size. The relative decrease in SLA with CO₂ enrichment was larger under drought than well-watered conditions (12% vs. 24%) and conversely there was a larger decrease for \overline{STAR} under well-watered than drought (10% vs. 7%) conditions (Table 2-5).

Drought effect

Low water availability led to a highly significant ($p < 0.0001$) decrease in total biomass, transpiration and all individual biomass components (leaf, stem and root) of *E. pilularis* under both elevated and ambient CO₂. Drought caused a slightly larger relative decrease in total biomass under elevated than ambient CO₂ (57% vs. 52%), whereas the relative reduction in transpiration with drought was similar (58%) between CO₂ treatments (Table 2-5; Fig. 2-7). As a result the increase in WUE under drought conditions was higher at ambient than elevated CO₂ (10% vs. no change), although the interaction (CO₂ x D) was not statistically significant.

The relative reduction in aboveground biomass (leaf and stem mass) with drought was similar between CO₂ treatments (Table 2-5). Conversely for belowground biomass, drought led to a substantial decrease in root mass, with a larger relative decrease taking place under elevated than ambient CO₂ (69% vs. 46%). This effect of drought between CO₂ treatments was amplified for fine root mass, with a markedly higher decrease under elevated than ambient CO₂ (68% vs. 30%) resulting in a significant ($p < 0.05$) CO₂ x D interaction. This shift in partitioning for plants under drought conditions resulted in a significant ($p < 0.05$) increase in LMR, irrespective of CO₂ treatment. Conversely, there was a strong trend ($p = 0.05$) towards a CO₂ x D interaction for both RMR and R:S as a result of a relative increase in RMR and R:S with drought for plants under ambient CO₂ and a larger relative decrease for plants under elevated CO₂ (Table 2-5).

In respect to plant dimensions, drought caused a significant decrease in total height, leaf area, crown width and basal diameter (Table 2-5). There was a larger relative decrease in height under ambient than elevated CO₂ (20% vs. 12%), whereas the relative reduction in basal diameter and crown width was similar between CO₂ treatments (Table 2-5). Conversely, drought caused a larger relative reduction in leaf area under elevated than ambient CO₂ (60% vs. 50%; Fig. 2-9; Table 2-5).

For crown and leaf properties, drought led to a significant ($p < 0.05$) decrease in SLA, leaf number and mean leaf size, and a significant ($p < 0.01$) increase in \overline{STAR} ; whereas there was no significant effect of drought on LAD. Under drought conditions there was a much larger decrease in SLA and mean leaf size under elevated than ambient CO₂ (SLA: 15% vs. 2%; mean leaf size: 24% vs. 16%). In contrast, the effect of drought on the increase in \overline{STAR} and decrease in leaf number was similar between CO₂ treatments (Table 2-5).

2.3.4.3 Whole-plant responses – *E. populnea*

CO₂ effect

For xeric *E. populnea*, WUE was significantly ($p < 0.01$) increased by growth under elevated CO₂, with a comparable increase taking place under both well-watered and drought conditions (47 vs. 48%). As with *E. pilularis*, the relative increases in WUE for *E. populnea* were only around 40-50% of the observed increases in ITE from leaf-level gas exchange measurements (Table 2-4), and a just a little under 60% of the increase in the CO₂ concentration (Table 2-5).

Maintaining soil water content at 50% FC resulted in a highly significant ($p < 0.0001$) reduction in transpiration for *E. populnea* that closely matched moisture availability under both elevated and ambient CO₂ (45% vs. 50%). The effect of elevated CO₂ on transpiration differed between watering regimes. Under well-watered conditions, elevated CO₂ caused a decrease in transpiration of 7%, whereas under drought conditions there was a 3% increase in transpiration. The water savings with CO₂ enrichment for well-watered plants and lack of water savings for droughted plants was the result of relative differences in biomass gains with CO₂ enrichment. Under elevated CO₂, there was a larger relative increase in total biomass under drought than well-watered conditions (55% vs. 38%), suggesting that the effect of elevated CO₂ on overall plant water use for *E. populnea* was more strongly controlled by plant size than water availability (Fig. 2-5).

In addition to an increase in total biomass for *E. populnea*, there was also a significant ($p < 0.05$) increase with CO₂ enrichment in all individual biomass components (leaf, stem and root mass) with the exception of coarse root mass (Table 2-5; Table 2-6). However, patterns in aboveground biomass (leaf and stem) with CO₂ enrichment exhibited wide variation for *E. populnea* depending on watering regime. With CO₂ enrichment, there was a larger relative increase in leaf mass under well-watered than drought conditions (60% vs. 41%), and conversely for total stem mass the relative increase was greater under drought than well-watered conditions (126% vs. 16%). This exaggerated increase in total stem mass under drought conditions resulted in a significant ($p < 0.05$) CO₂ x D interaction. Similarly, there was also a significant ($p < 0.05$) CO₂ x D interaction for main and lateral stem mass as a result of a much larger increase under drought than well-watered conditions (main stem: 83% vs. no change; lateral stem: 126% vs. 16%). Conversely, the relative effect of elevated CO₂ on total belowground biomass (coarse and fine roots) was similar between watering regimes, while the relative increase in fine root mass was higher under drought than well-watered conditions (60% vs. 48%). These shifts for plants under elevated CO₂ and drought conditions resulted in a considerable increase in SMR, and a small decrease in R:S (Table 2-5).

In respect to plant dimensions for *E. populnea*, there was a significant ($p < 0.05$) effect of elevated CO₂ on total height, leaf area, crown width and basal diameter. Total height decreased by 9% with CO₂ enrichment under well-watered conditions, whereas under drought conditions there was a relative increase of 40% which resulted in a significant ($p < 0.01$) CO₂ x D interaction. Similarly, in the case of crown width the relative increase with CO₂ enrichment was higher under drought than well-watered conditions (39% vs. 6%) leading to a significant ($p < 0.05$) CO₂ x D interaction (Table 2-5). For leaf area and basal diameter the

relative increase with CO₂ enrichment was also higher under drought than well-watered conditions (leaf area: 50% vs. 33%; basal diameter: 35% vs. 13%), although the interaction (CO₂ x D) was not statistically significant.

For crown and leaf properties of *E. populnea*, there was a significant effect of elevated CO₂ on leaf number, but no significant effect of elevated CO₂ on the remaining leaf (SLA and mean leaf size) or crown properties (LAD and \overline{STAR}). The effect of elevated CO₂ led to a larger relative increase in leaf number under drought than well-watered conditions (99% vs. 47%).

Drought effect

Drought led to a highly significant ($p < 0.0001$) decrease in total biomass, transpiration and all individual biomass components for *E. populnea*, with the exception of fine root mass, under both elevated and ambient CO₂ (Table 2-5). For total biomass and transpiration the relative decrease due to drought was higher under ambient than elevated CO₂ (biomass: 43% vs. 35%; transpiration 50% vs. 45%), which consequently resulted in a similar increase in WUE between ambient and elevated CO₂ (15% vs. 16%).

The relative decrease in aboveground biomass (leaf and stem mass) due to drought was higher under ambient than elevated CO₂ (55% vs. 41%). This difference was largely the result of a much larger decrease in total stem mass under ambient than elevated CO₂ (69% vs. 39%), as the relative decrease in leaf mass was comparable between CO₂ treatments (Table 2-5). Drought also resulted in a larger relative decrease in main and lateral stem mass under ambient than elevated CO₂ (main: 60% vs. 28%; lateral: 73% vs. 44%). This pattern in total, main and lateral stem mass resulted in a significant ($p < 0.05$) CO₂ x D interaction. These considerable differences in biomass partitioning between CO₂ treatments resulted in a significant CO₂ x D interaction for SMR and LMR (Table 2-5).

In respect to plant dimensions, there was a significant ($p < 0.05$) effect of drought on total height, basal diameter, leaf area, and crown width. In the case of total height there was also a significant ($p < 0.01$) CO₂ x D interaction as a result of an increase with drought of 5% under elevated CO₂ and decrease of 31% under ambient CO₂ (Table 2-5). For crown width there was also a significant ($p < 0.05$) CO₂ x D interactions as a result of a relative decrease with drought of 23% under ambient CO₂ and no change in crown width under elevated CO₂. Conversely for basal diameter and leaf area, the effect of drought led to a larger decrease under ambient than elevated CO₂ (basal diameter: 38% vs. 27%; leaf area: 47% vs. 40%).

In the context of crown and leaf properties, the effect of drought caused a significant decrease in leaf number and an increase in $\overline{\text{STAR}}$; whereas there were no significant effects of drought on mean leaf size, SLA, LAD or any component of leaf or wood chemistry. However, there was a significant ($p < 0.01$) $\text{CO}_2 \times \text{D}$ interaction on LAD as a result of a contrasting response between CO_2 treatments; the effect of drought under elevated CO_2 resulted in a 20% relative decrease in LAD compared to a relative increase of 69% under ambient CO_2 . In the case of leaf number there was a larger decrease under ambient than elevated CO_2 (55% vs. 38%). Conversely, there was a larger relative increase in $\overline{\text{STAR}}$ under elevated than ambient CO_2 (10% vs. 1%).

2.3.4.4 Whole plant responses – Xeric vs. Mesic

There was a highly significant ($p < 0.001$) difference between species in total biomass, transpiration, WUE and in all individual biomass components (leaf, stem and root mass), with the exception of fine root mass (Table 2-6; Fig. 2-7). Of the two species, mesic *E. pilularis* exhibited higher total transpiration along with much greater gains in total biomass, WUE and all individual biomass components, save fine root mass, in all treatment combinations (Table 2-6; Fig. 2-7). In respect to fine root mass, absolute partitioning was similar between species even with the much smaller total biomass of *E. populnea* compared with *E. pilularis* (Table 2-5; Fig. 2-7).

For both total biomass and total transpiration there was a significant species \times D interaction, with a stronger effect of drought conditions on *E. pilularis* than *E. populnea* (Fig. 2-5, 2-7; Table 2-5). Notably, the larger relative effect of drought on transpiration rates for *E. pilularis* compared with *E. populnea* was sustained throughout the experiment (Fig. 2-5). These patterns in transpiration at the whole plant scale were compared with leaf level transpiration from gas exchange measurements, and were found to be consistent both within and between species (Fig. 2-6).

Between species, biomass allocation patterns most commonly differed as a result of watering regime. For total stem mass there was a significant species \times $\text{CO}_2 \times \text{D}$ interaction, which was strongly tied to the response of *E. populnea*; as the relative difference between well-watered and drought conditions was similar between CO_2 treatments for *E. pilularis* (Table 2-6). In contrast, for *E. populnea* the relative decrease in total stem mass with drought at ambient CO_2 was nearly double that under elevated CO_2 , while there was minimal difference between CO_2 treatments under well-watered conditions (Table 2-6).

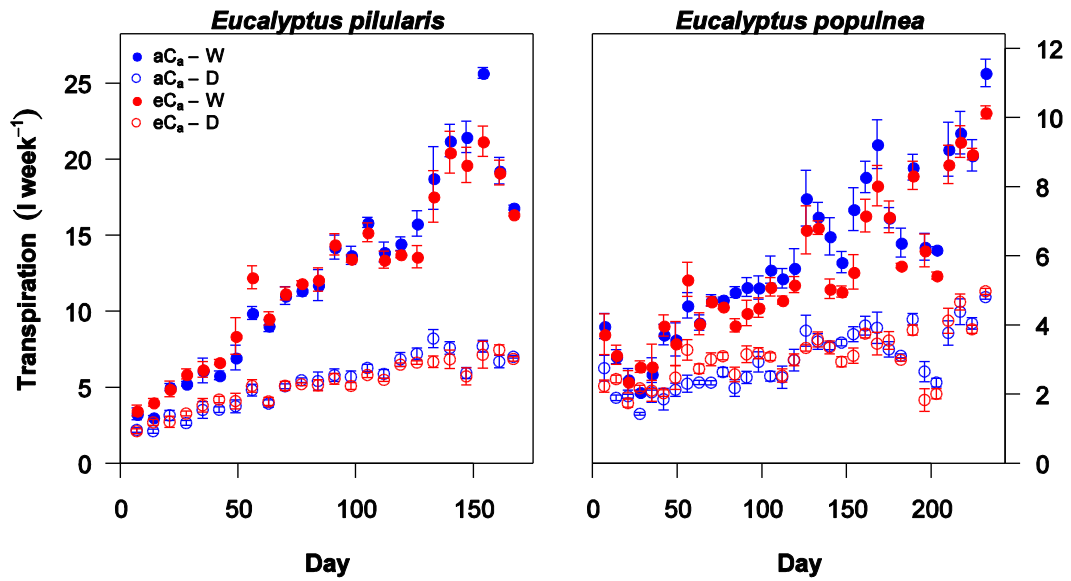


Figure 2-5. Mean weekly transpiration of *E. pilularis* and *E. populnea* from beginning of drought treatment (Day 0) to final harvest (Day 177 – *E. pilularis*; Day 253 – *E. populnea*). Symbols are the means of 6 trees per treatment; error bars are ± 1 SE. Filled symbols - well-watered (100% FC); Open symbols – Drought (50% FC). Blue symbols – Ambient CO₂; Red Symbols – Elevated CO₂. *Note scale difference between the two species.

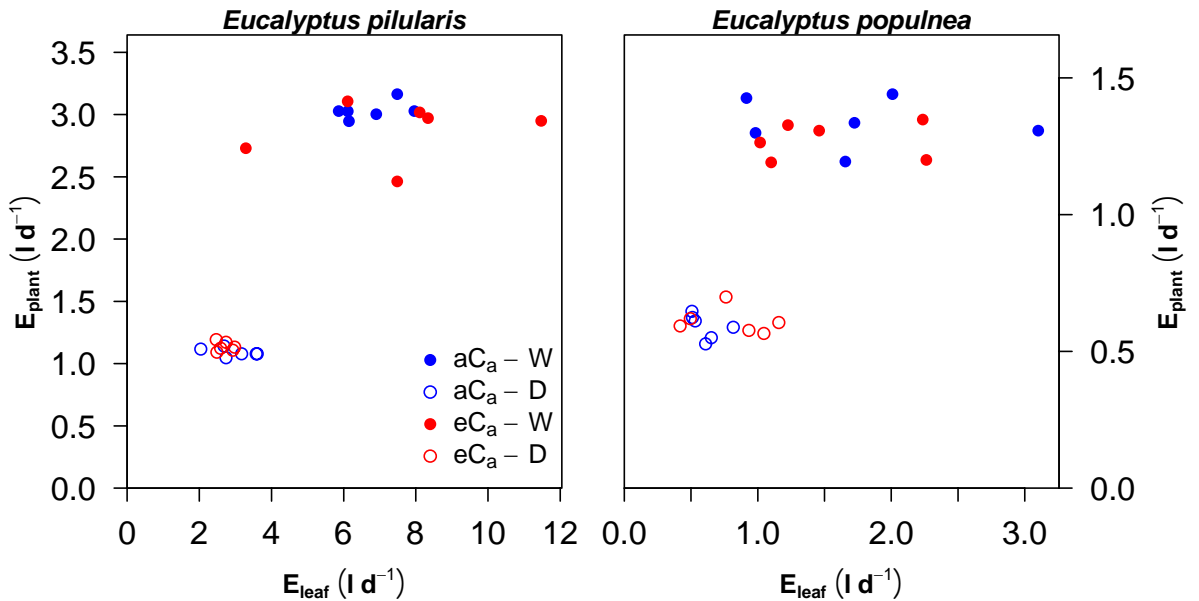


Figure 2-6. Relationship between daily tree water use (E_T , in liters d^{-1}) and leaf-level transpiration (E_{leaf}) over the final gas exchange measurement period. Leaf level transpiration (E_{leaf}) in this figure is calculated as transpiration (in, $mmol\ m^{-2}\ s^{-1}$) * leaf area (m^2) * a conversion factor. The conversion factor is given for H₂O, in this case, by the following (liter/gram * gram/mol * mol/mmol * second/day), assuming an 8 hour day. The figure suggests consistent patterns in water loss at the leaf and at the whole plant scale (e.g. Fig. 2-6). *Note scale difference between the two species.

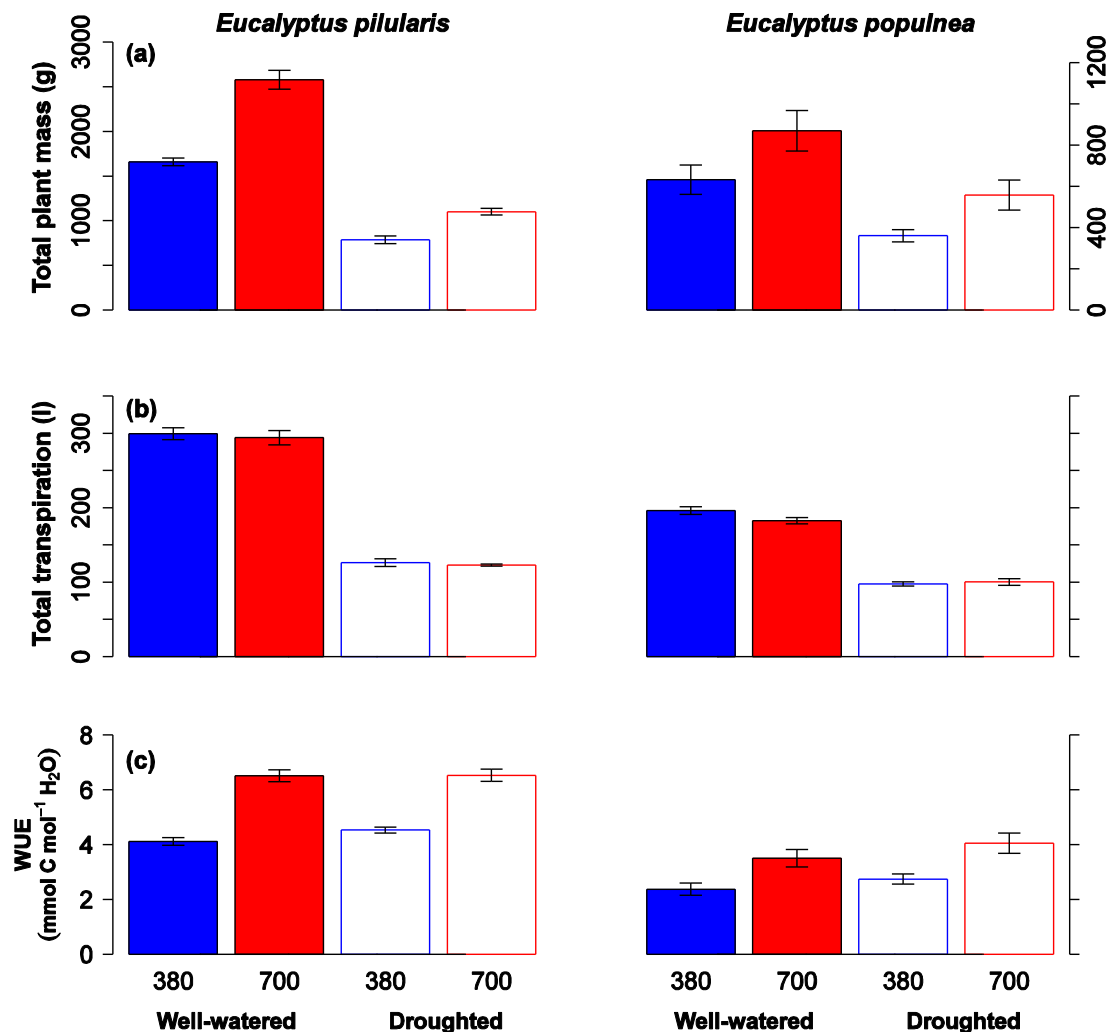


Figure 2-7. (a) Total biomass, (b) total transpiration and (c) water use efficiency of *E. pilularis* and *E. populnea*. Data are the means of 6 trees per treatment; error bars are ± 1 SE. Filled bars - well-watered (100% FC); Open bars - Drought (50% FC). Blue bars - Ambient CO₂; Red bars - Elevated CO₂.

For main stem mass there was significant species x D interaction, while for lateral stem mass there was a significant species x CO₂ x D interaction. In respect to main stem mass, the significant species x D interaction resulted from a considerably larger reduction in main stem mass between the two species for *E. populnea*, under drought compared to well-watered conditions (Table 2-5). Similarly, the significant species x CO₂ x D interaction for lateral stem mass was controlled by the marked decrease by *E. populnea* under ambient CO₂ and drought conditions, whereas in all other species and treatment combinations the relative reduction in lateral stem mass with drought was much less severe (Table 2-5). In contrast to

patterns in aboveground biomass, the species x D interaction for belowground biomass (total and coarse roots) was more strongly directed by *E. pilularis*. As exhibited by the much larger relative reduction in both total root and coarse root mass for *E. pilularis* under drought conditions, whereas for *E. populnea* there was a minimal difference in belowground and coarse root mass between watering regimes, irrespective of CO₂ concentration (Table 2-5).

Patterns in component biomass allocation (LMR, SMR, RMR and R:S) were highly species specific. Between species, there was a highly significant ($p < 0.0001$) increase in aboveground (LMR and SMR) for *E. pilularis* and a highly significant ($p < 0.0001$) increase towards belowground (RMR and R:S) component biomass allocation for *E. populnea* (Table 2-6; Fig. 2-8). Additionally, for both RMR and R:S there was a significant ($p < 0.05$) species x D interaction, as a result of a minor reduction in RMR and R:S for *E. pilularis* under drought conditions compared to a small increase for *E. populnea* (Table 2-6; Fig. 2-8).

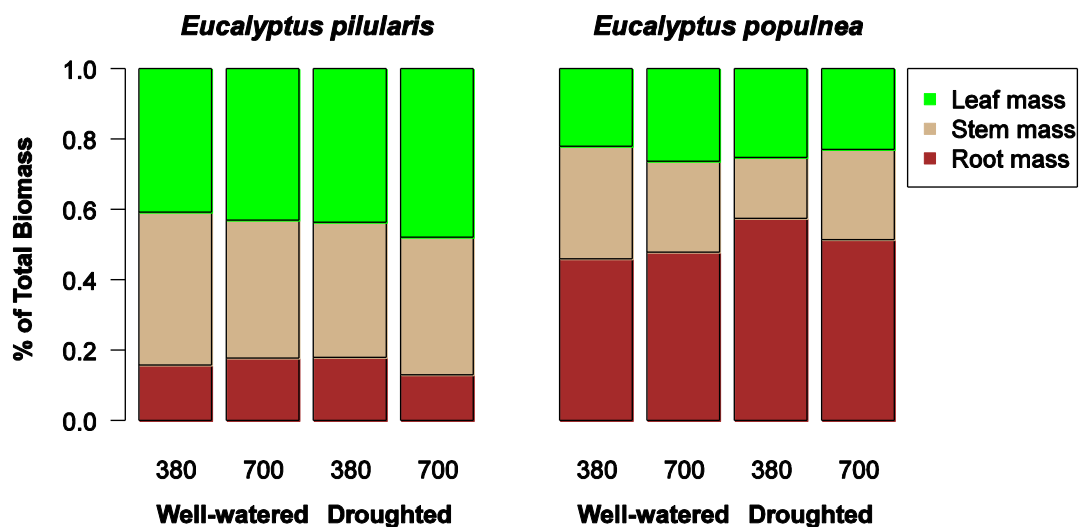


Figure 2-8. Percent of component biomass to leaves, stems and roots of *E. pilularis* and *E. populnea* at final harvest. Data are the mean ratio of 6 trees per species and treatment.

Between species, there was a highly significant ($p < 0.001$) difference in total height, leaf area, crown width and basal diameter. The faster inherent growth rate of mesic *E. pilularis* led to much greater overall gains of the two species in total height, leaf area, crown

width and basal diameter, irrespective of CO₂ concentration or watering regime (Table 2-5; Fig. 2-9). For both total height and crown width there was a significant species x CO₂ x D interaction, and also a species x CO₂ interaction (Table 2-6) for crown width, which was strongly directed by the response of xeric *E. populnea*. Under ambient CO₂ and drought conditions there was very considerable decrease for *E. populnea* in total height and crown width and minor differences with CO₂ enrichment, while for *E. pilularis* there was a similar relative decrease with drought in total height and crown width at both ambient and elevated CO₂ (Table 2-5). For leaf area, there was a significant ($p < 0.05$) species x D interaction (Table 2-6) as a result of a much larger reduction in leaf area, of the two species, for *E. pilularis* in response to drought conditions (Table 2-5; Fig. 2-9).

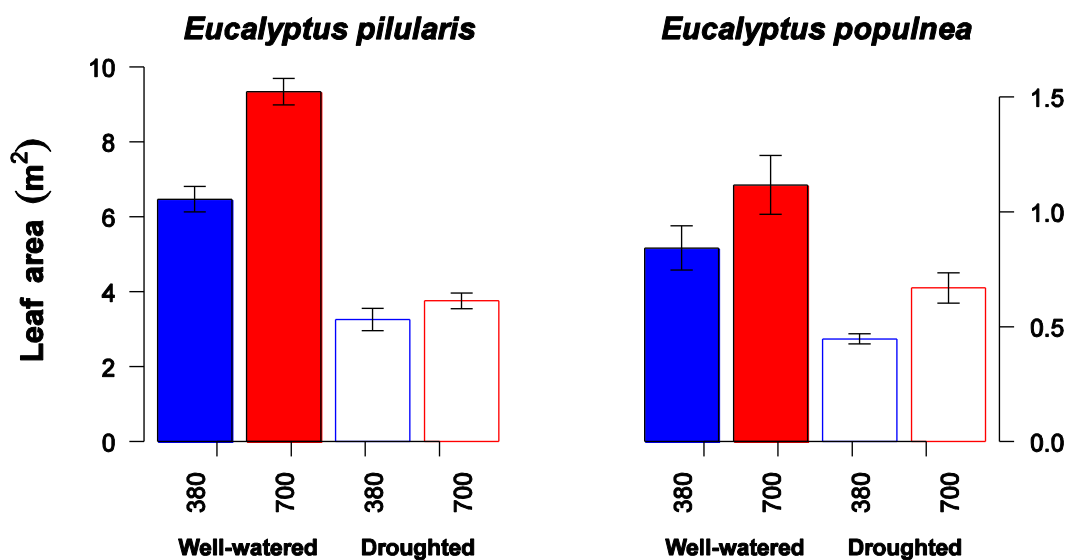


Figure 2-9. Total leaf area of *E. pilularis* and *E. populnea* at final harvest. Data are the means of 6 trees per treatment; error bars are ± 1 SE. Filled bars - well-watered (100% FC); Open bars – Drought (50% FC). Blue bars – Ambient CO₂; Red bars – Elevated CO₂.

For crown and leaf properties there was a highly significant ($p < 0.0001$) difference between species in leaf number, SLA, mean leaf size and \overline{STAR} , whereas there was no significant difference in LAD. *E. pilularis* had larger leaf number and SLA, whereas *E. populnea* had a larger overall mean leaf size. In addition, there were several significant

interactions for crown and leaf properties. There was a significant ($p < 0.01$) species x CO₂ interaction for leaf number as a result of much larger relative gains in leaf number with CO₂ enrichment for *E. populnea* compared to *E. pilularis* (Table 2-5). Additionally there was a significant ($p < 0.05$) species x D and species x CO₂ interaction for mean leaf size, and a significant ($p < 0.05$) species x CO₂ x D interaction for SLA. In the case of mean life size the species x CO₂ interaction was the result of an increase in mean leaf size for *E. pilularis* and decrease for *E. populnea* with CO₂ enrichment. Conversely, the species x D interaction for mean leaf size resulted from little variation in leaf size with drought for *E. populnea* and much wider divergence in mean leaf size between watering regimes for *E. pilularis* (Table 2-6). For SLA, the species x CO₂ x D interaction developed due to a reduction in SLA under elevated CO₂ and drought for *E. pilularis* and a contrasting pattern in SLA for *E. populnea*, which exhibited an increase and a decrease in SLA under elevated and ambient CO₂, respectively.

At the canopy scale, \overline{STAR} was consistently higher for xeric *E. populnea* which also corresponded to a pattern of declining \overline{STAR} with increasing plant size (Table 2-5). There was additionally a significant ($p < 0.05$) species x CO₂ interaction for \overline{STAR} , and a species x CO₂ x D interaction on LAD. For \overline{STAR} , the species x CO₂ interaction resulted from a much larger decrease by *E. pilularis* with CO₂ enrichment, whereas a much smaller decrease was exhibited by *E. populnea* between CO₂ treatments (Table 2-5). Conversely, the significant species x CO₂ x D interaction on LAD was strongly influenced by the very wide variation between watering regimes at ambient CO₂ for *E. populnea*, which directly contrasted with all other species and treatment combinations (Table 2-5).

Table 2-6. Statistics of plant water relations, biomass, plant dimensions, and biomass allocation.

Parameter	Main effects			Interactions		
	Species	CO ₂	D	Species x CO ₂	Species x D	Species x CO ₂ x D
Numerator df	1	1	1	1	1	1
Denominator df	40	40	40	40	40	40
<i>Water relations</i>						
Total transpiration (l)	242.18***	1.07	1206.56***	0.01	24.61***	1.25
WUE (mmol C mol ⁻¹ H ₂ O)	140.60***	72.50***	4.68*	0.09	1.12	0.24
<i>Plant dry biomass</i>						
Total mass (g)	233.01***	39.93***	120.30***	0.07	6.56*	0.6
Leaf mass (g)	439.12***	35.46***	72.16***	0.26	2.09	0.19
Total stem mass(g)	448.61***	34.40***	156.26***	0.37	0.3	5.49*
Main stem mass (g)	649.40***	24.39***	128.10***	0.44	6.64*	2.14
Lateral stem mass (g)	181.01***	24.98***	99.08***	1.18	0.77	6.89*
Total root mass (g)	7.92**	10.90**	44.22***	0.03	8.01**	1.36
Coarse root mass (g)	26.09***	8.23**	75.70***	0.02	14.53**	0.14
Fine root mass (g)	0.77	11.56**	20.16***	0.04	3.62	2.76
<i>Plant Dimensions</i>						
Leaf number (n)	879.84***	30.31***	94.43***	8.91**	0.04	2.35
Leaf area (m ²)	1172.78***	26.52***	139.06***	0.34	4.71*	1.92
Crown width (m)	17.77***	15.54**	31.13***	9.06**	0.5	7.62**
Height (m)	456.51***	12.82**	33.62***	0.55	0.03	9.05**
Basal diameter (mm)	373.07***	30.50***	110.30***	0.27	0.79	1.56
SLA (m ² kg ⁻¹)	122.62***	9.44**	2.73	2.69	0.25	6.79*
Mean leaf size (cm ²)	36.76***	0.17	5.60*	4.16*	6.05*	0.01
LAD	1.65	0	0.15	2.49	1.88	7.08*
STAR	40.46***	4.59*	15.88**	4.45*	2.37	0.51
<i>Biomass Allocation</i>						
R:S (g g ⁻¹)	414.62***	1.2	1.28	0.07	5.58*	0.37
LMR (g g ⁻¹)	295.70***	3.43	2.64	0.99	2.94	3.46
SMR (g g ⁻¹)	85.36***	0.04	9.54**	0.79	2.26	2.18
RMR (g g ⁻¹)	422.69***	1.13	3.43	0.03	6.80*	0.02
Leaf lignin (%)	5.59***	9.73	5.07	1.18	0.18	0.21
Leaf cellulose (%)	0.14	10.38**	5.26*	1.76	0.37	0.01
Leaf lignin:cellulose	5.59*	9.73**	5.07*	1.18	0.18	0.21
Stem lignin (%)	0	0.51	0.88	2.51	0.87	0.06
Stem cellulose (%)	5.23*	4.18*	14.04**	1.5	1.24	2.24
Stem lignin:cellulose	0.39	1.34	3.35	2.59	1.13	0.39

F values and significance levels (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) for the main effects of species, elevated CO₂ (CO₂) and drought and their interactions on biomass, plant dimensions, biomass allocation and water relations. Numerator and denominator df are the numerator and denominator degrees of freedom for the *F* tests.

2.4 Discussion

Leaf-level

Drought did not significantly affect any measured gas exchange parameter (A_{sat} , g_s , ITE, C_i/C_a or g_1) in either species or at either CO_2 level. Conversely, CO_2 enrichment caused a significant reduction in g_s and an increase in A_{sat} for both species, irrespective of watering regime. This photosynthetic enhancement with CO_2 enrichment was maintained throughout the experiment with no evidence of photosynthetic down-regulation for either *E. pilularis* or *E. populnea* (Table 2-2). These results contradicted several of our initial hypotheses regarding the marginal cost of carbon gain (g_1).

For example, we hypothesized that 1-) the marginal water cost of carbon gain (g_1) would be unaffected by CO_2 concentration, that 2-) g_1 would be reduced under drought conditions and that 3-) xeric *E. populnea* would exhibit the lowest g_1 of the two species under well-watered conditions. Instead, there was a significant decrease in g_1 with CO_2 enrichment for both species (*E. pilularis* and *E. populnea*), but no significant reduction in g_1 under drought conditions. In addition, mesic *E. pilularis* rather than xeric *E. populnea* exhibited the lowest g_1 under well-watered conditions. These results are important for the insights that they give us into stomatal behavior.

We found that g_1 , both within and between species, was closely related to overall plant size (Fig. 2-2). A decrease in g_1 with increasing plant size suggests that water is becoming relatively more expensive to the plant and therefore larger plants may be less likely than smaller plants to give up water to gain carbon (Buckley and Roberts 2006). Partly, this may be a characteristic of ontogeny versus size per se, and thus plants outside of the seedling stage may display less variation in g_1 as they increase in overall size (H  roult *et al.* 2012). During the early stages of growth as seedlings are rapidly developing there is often a scaling of root and shoot growth, whereas once rooting volume becomes stable a larger allocation to shoot growth may take place (Villar-Salvador *et al.* 2012). Thus an increase to shoot growth and especially transpiring leaf area without a concomitant increase in rooting volume during the later stages of seedling growth could effectively lead to a decrease in the marginal water cost of carbon gain (g_1). Thus, we suggest that the observed reduction in g_1 with CO_2 was related to the effect of CO_2 on plant size. We further suggest that *E. populnea* may have had a higher g_1 than *E. pilularis*, in opposition to our hypotheses, because they were smaller plants (Fig. 2-2; Table 2-5).

The parameter g_1 strongly influences both ITE and C_i/C_a (eqns 3 – 4). Because of the decrease in g_1 with CO_2 enrichment, the increase in ITE under elevated CO_2 was larger than predicted for both *E. pilularis* and *E. populnea*, irrespective of watering regime. We predicted that the relative increase in ITE with CO_2 enrichment would scale proportionally to C_a at approximately 84%, whereas for *E. pilularis* the relative increase in ITE ranged from 108-136% and for *E. populnea* from 82-144%. In a recent meta-analysis of FACE experiments, Ainsworth and Long (2005) reported a similar result, where an average ITE for C_3 species was reported as slightly above the proportional increase expected with C_a .

Between species we predicted that xeric *E. populnea* would display a higher ITE of the two species under well-watered conditions due to operating at a lower C_i and a lower g_1 . In contrast, *E. pilularis* consistently operated at a significantly lower C_i and a lower g_1 of the two species and correspondingly exhibited a significantly larger ITE. This difference between species ties back to the larger g_1 of *E. populnea* compared to *E. pilularis* on both measurement dates (Table 2-3).

In addition, and counter to our initial expectations, there was no significant effect of long-term moderate drought on ITE for either species (Table 2-3). A drop in g_1 as drought progresses or intensifies has been shown by numerous researchers (Delucia and Heckathorn 1989; Barton *et al.* 2012; Zhou *et al.* "In press"). However, in these studies, the drought conditions are short-term and relatively intense, which contrasts with the long-term moderate drought that we maintained in our experiment. Our data demonstrate that under long-term low water availability, plants may adjust structurally in order to endure the impact of moderate drought in place of stronger physiological controls to avoid stress.

Numerous studies have also identified mesophyll conductance (g_m) as a major limitation to CO_2 diffusion during drought (Flexas *et al.* 2002; Warren *et al.* 2008; Niinemets *et al.* 2009; Flexas *et al.* 2012). In many of these studies, the impact of g_m on CO_2 diffusion is often greater under severe and prolonged drought stress conditions (Flexas *et al.* 2002; Warren *et al.* 2004; Peeva and Cornic 2009), as a result of changes in mesophyll structure with persistent water stress (Loreto and Centritto 2008). Mesophyll conductance affects the apparent V_{cmax} , so if g_m had changed in response to drought in our experiment, we would have observed a change in apparent V_{cmax} . However, we only observed a reduction in apparent V_{cmax} in early (July) measurements in *E. pilularis*, indicating that long-term structural adjustments in response to low water availability allowed plants to maintain g_m as well as J_{max} and V_{cmax} at unstressed levels.

At the leaf scale, the non-significant reduction in all measured gas exchange parameters observed under moderate drought conditions for both species reflects a stronger effect of structural (growth) rather than physiological adjustments to avoid water stress under long-term moderate drought conditions (see next section). However, we note that the minimal physiological reductions under drought conditions could also partly result from the time of day at which gas exchange measurements were conducted. During our experiment, gas exchange measurements were conducted prior to midday, whereas re-watering of pots to a percent of field capacity took place each evening so that gas exchange measurements conducted in the morning may have missed periods of peak daily water stress happening later in the day (e.g., Singaas *et al.* 2000; Spunda *et al.* 2005). Centritto *et al.* (1999) found a significant effect of drought on declining photosynthetic rate and a reduction in g_s of cherry (*Prunus avium*) under conditions of growing drought intensity and when measurements were made throughout the day. Similarly, Kets *et al.* (2010) found a consistent diurnal pattern of reduced A and g_s under elevated CO_2 for *Populus tremuloides*, which was exacerbated under drought conditions. However, in both cases drought intensity was much greater than the percent of field capacity which was maintained throughout our experiment. In a similar study where soil water content was maintained at 50% field capacity, Atwell *et al.* (2007) found minor diurnal variation in Ψ_{leaf} for *Eucalyptus tereticornis*; supporting our argument that that species adjust more structurally than physiologically under long-term moderate drought conditions, whereas under drought of increasing intensity further mechanisms to avoid water stress may take precedence.

Whole tree scale

Carbon gain and water loss

Our initial hypotheses at the whole-plant scale were based on supported hypotheses for the parameter g_1 at the leaf-level. As our hypotheses at the leaf-level surrounding g_1 were rejected, we needed to revise our hypotheses at the whole-plant scale.

Our initial hypotheses for WUE were that 1-) xeric *E. populnea* would exhibit a higher absolute WUE of the two species, 2-) the increase in WUE for both species would be proportional to CO_2 , and 3-) the largest relative increase in WUE for both species would occur under drought conditions. Our revised hypotheses, taking into account the observed changes in leaf-level g_1 , are that 1-) the absolute WUE will be larger for mesic *E. pilularis*, 2-

) that the increase in WUE for both species will be more than proportional to CO₂, and 3-) the relative increase in WUE for both species will remain the same under both well-watered and drought conditions.

In support of our revised hypothesis (1) we observed a larger overall WUE for mesic *E. pilularis* rather than xeric *E. populnea*. A similar observation involving eucalypts was reported by Searson *et al.* (2004) with higher absolute leaf and whole-tree WUE for *Eucalyptus* species native to mesic rather than xeric environments under ambient CO₂. In contrast, high CO₂ experiments have displayed conflicting results at the leaf and whole-tree scale. Delucia and Heckathorn (1989) found a higher leaf WUE under elevated CO₂ for mesic opposed to xeric species, whereas Guehl *et al.* (1994) observed a larger increase in whole-tree WUE with CO₂ enrichment for a drought-tolerant versus a drought-avoiding species.

Counter to our revised within species hypothesis (2), the relative increase in WUE for both species was less than proportional to C_a (eC_a/aC_a, ~84%). Mesic *E. pilularis* exhibited a relative increase in WUE of 58% and 44% for well-watered and drought conditions, respectively, while for xeric *E. populnea* the relative increase was similar between watering regimes of 47% under well-watered and 48% with drought conditions. Similar results of WUE being less than proportional to C_a have been reported for temperate forest trees (Guehl *et al.* 1994; Picon *et al.* 1996; Centritto *et al.* 1999, 2002) and for tropical forest trees (Cernusak *et al.* 2011).

Xeric *E. populnea* did conform to our revised hypothesis (3), with a similar relative increase in WUE under well-watered and drought conditions (47% vs. 48%). However, mesic *E. pilularis* did not support this hypothesis, with a considerably larger increase under eC_a-W, although this interaction (CO₂ x drought) was not statistically significant. In general the increase in WUE with CO₂ enrichment is found to be larger under drought conditions (Field *et al.* 1997; Wullschleger *et al.* 2002). In contrast, for *E. pilularis*, WUE was larger under well-watered conditions, similar to observations by Picon *et al.* (1996) for *Quercus robur*. The larger increase in WUE, for *E. pilularis*, under well-watered conditions was likely tied to the substantial gains in leaf area and fine root mass over the growth period (Fig. 2-9) and also to a sustained reduction in g_s under well-watered conditions, which was closely aligned with observations of g_s under drought conditions (Table 2-4).

The discrepancy between prediction and observations, for WUE, has been attributed by various authors to factors such as increased respiration, high VPD, down-regulation of photosynthesis, nutrient limitation, and limited leaf and plant coupling to environment

(Eamus 1991; Guehl *et al.* 1994; Picon *et al.* 1996; Huxman *et al.* 1998; Saxe *et al.* 1998; Vu *et al.* 2002; Cernusak *et al.* 2011; De Kauwe *et al.* 2013). For example, Guehl *et al.* (1994) suggested an increase in respiration rate with CO₂ enrichment was responsible for a less than proportional increase in WUE for drought-avoiding *P. pinaster*; while in an elevated CO₂ study with several tropical tree species Cernusak *et al.* (2011) suggested that a reduction in g_s with CO₂ enrichment caused an increase in leaf temperature and associated increase in VPD, ultimately resulting in an increase in transpiration (e.g., Kimball and Bernacchi 2006). Likewise, for both *E. pilularis* and *E. populnea*, there was a significant and sustained reduction in g_s with CO₂ enrichment and also at the leaf-level an increase in leaf temperature and corresponding increase in VPD. Such an increase for both *E. pilularis* and *E. populnea* under elevated CO₂ likely triggered an increase in transpiration and/or respiration, thus leading to a less than proportional increase in WUE to C_a as was observed (Table 2-5). In contrast, there was no evidence of photosynthetic down-regulation or nutrient limitation for either *E. pilularis* or *E. populnea* at any point during the experiment (Table 2-2). The appearance of photosynthetic down-regulation is often the result of nutrient limitation (especially nitrogen), lack of active sinks or limited rooting volume (Eamus and Jarvis 1989; Tissue *et al.* 1993; Sage 1994; Curtis and Wang 1998; Tissue *et al.* 2001; Sholtis *et al.* 2004); all of which were avoided in our study by providing an optimal balance of nutrients, utilizing rapidly growing seedlings and large pots to avoid root restriction.

Additionally, a less than proportional increase in WUE to C_a is often tied to decoupling between the leaf and plant canopy to the environment (De Kauwe *et al.* 2013). At the leaf and canopy scale, water loss is controlled by boundary layer conductance (g_b) and stomatal conductance (g_s) (Martin *et al.* 1999). When leaves and plant canopy are poorly coupled to environment, transpiration and CO₂ assimilation are more strongly controlled by g_b and net radiation rather than g_s (McNaughton and Jarvis 1983; Jarvis and McNaughton 1986). Along with leaf size and leaf morphology, the major control on g_b is wind speed (Monteith and Unsworth 1990; Nobel 1991; Schuepp 1993; Daudet *et al.* 1999; Martin *et al.* 1999). Wind speed is considered relatively stable in glasshouse environments due to the use of forced and natural ventilation (Pasgianos *et al.* 2003; Baille *et al.* 2005; Katsoulas *et al.* 2007), although even within a glasshouse environment there is variation with plant canopy height such that inside wind speed and turbulence increases with height aboveground (Baille *et al.* 2005; Katsoulas *et al.* 2007). Additionally, boundary layer conductance, at least within a glasshouse environment, has been found to increase with increasing leaf area index (LAI) (Baille *et al.* 2005; Katsoulas *et al.* 2007).

An artifact in our experiment that may have contributed to the higher WUE of *E. pilularis* under eC_a-W, and comparatively smaller WUE in all other cases, was the height of fans within glasshouses. Forced fans were located approximately 2.5 meters above the floor of each glasshouse, thereby more strongly directed at the taller plants. Consequently, the larger (both in height and leaf area (Table 2-5; Fig. 2-9)) CO₂ enriched and well-watered *E. pilularis* likely experienced higher wind speeds and increased boundary layer conductance; and consequently a decrease in water vapor concentration at the leaf surface, thus increasing VPD and decreasing g_s (Jarvis and McNaughton 1986; Albertson *et al.* 2001). As a result, CO₂ enriched and well-watered *E. pilularis* likely experienced reduced transpirational water loss at the leaf scale, with a limited impact towards productivity gains at the whole-plant scale, and ultimately resulting in a larger relative increase in WUE than in all other cases (Table 2-5).

Our hypotheses for whole-plant biomass and transpiration also had to be revised. We initially hypothesized that the relative increase in total biomass with CO₂ enrichment would be higher under drought than well-watered conditions and for xeric rather than mesic species. This hypothesis was based on our expectations that drought would reduce g_1 , and that g_1 would be higher in mesic *E. pilularis*, which in both cases was not substantiated by the data. Therefore, our revised hypotheses were that 1-) the effect of elevated CO₂ on total biomass will remain the same under well-watered (100% FC) or drought (50% FC) conditions, and 2-) because g_1 is lower for mesic *E. pilularis* the CO₂ effect on total biomass will be higher for *E. pilularis*.

At the whole-tree scale, CO₂ enrichment significantly enhanced total biomass for both *E. populnea* and *E. pilularis* in well-watered and drought conditions (Table 2-6; Fig. 2-7). However, our revised hypothesis of similar relative effect of elevated CO₂ under well-watered or drought conditions did not always hold. For both xeric *E. populnea* and mesic *E. pilularis*, the strongest growth responses to CO₂ enrichment occurred under conditions where inherent drought tolerance strategy (drought-tolerant or drought-avoiding) was most effectively matched to environment. For *E. populnea* this meant a larger relative biomass increase with CO₂ enrichment of 55% under drought compared with 40% at well-watered conditions. Conversely, the relative effect of CO₂ enrichment for *E. pilularis* was a larger increase of 56% under well-watered compared to 40% under drought conditions. A similar observation was reported for two birch species of contrasting drought tolerance (Catovsky and Bazzaz 1999), and for sweetgum (drought-tolerant) and maple (drought-avoiding) species

(Tschaplinski *et al.* 1995) with the largest relative gains in productivity under elevated CO₂ occurring when species were under treatment conditions most alike their native environment.

In contrast to the biomass gains for both species with CO₂ enrichment, water loss was not significantly different for *E. pilularis* or *E. populnea* at either well-watered or drought conditions between elevated and ambient CO₂ (Fig. 2-5, 2-7; Table 2-5). At the whole-plant scale, tied to the gains in productivity with CO₂ enrichment and the aforementioned similar water loss at a given watering regime (Fig. 2-5, 2-7), there was a significant increase in water use efficiency (WUE; ratio of carbon gain to water loss) for both *E. populnea* and *E. pilularis* under well-watered and drought conditions (Fig. 2-7; Table 2-5, 2-6).

Leaf area

An increase to leaf area is a common observation of many elevated CO₂ experiments, even when species are grown under drought conditions (Guehl *et al.* 1994; Picon *et al.* 1996; Centritto *et al.* 1999; Wullschleger *et al.* 2002). Picon *et al.* (1996) argued that the absence of a CO₂ effect on transpiration was a result of increased leaf area compensating for a reduction in stomatal conductance with CO₂ enrichment, with the magnitude of an increase in leaf area dependent on both initial leaf area and on the degree to which canopy transpiration is under stomatal control (Wullschleger *et al.* 2002). We observed an increase in leaf area for both *E. populnea* and *E. pilularis* with CO₂ enrichment, irrespective of watering regime, which corresponded with a sustained reduction in g_s . This CO₂ induced reduction in g_s resulted in similar rates of water loss at the whole-tree scale (Fig. 2-7; Table 2-5), thus providing support for the compensatory leaf area hypothesis (Gifford 1988; Picon *et al.* 1996). An exception was for xeric *E. populnea* under the combination of elevated CO₂ and drought where water loss was ~3% higher than at ambient CO₂, although in addition the relative increase in leaf area was ~50% greater and was larger than for any other species and treatment combination (Fig. 2-7, 2-9; Table 2-5). Guehl *et al.* (1994) observed a similar result for xeric *Q. petraea* under elevated CO₂ and well-watered conditions, with a considerable increase (112%) in leaf area tracked by an increase (38%) in transpiration. For *E. populnea* the increase in leaf area under elevated CO₂ and drought conditions was met by a consistent reduction in g_s , although highly variable between measurement dates; suggesting that the increased water loss may have resulted from poor coupling of leaf, plant and canopy to the glasshouse environment.

At the canopy scale, we hypothesized that the effect of drought (50% of field capacity) would lead to a reduction in leaf area by ~50% for both species at elevated and at ambient

CO₂. In ambient CO₂ the relative reduction in leaf area was close to our expectations at 47% for *E. populnea* and 50% for *E. pilularis*. In contrast, under elevated CO₂ the relative reduction was larger than expected at 60% for *E. pilularis* and under our prediction at 40% for *E. populnea*. Similar observations of a greater than 50% reduction in leaf area have been reported for both mesic and xeric species grown under elevated CO₂ and comparable drought conditions (~50% FC) (Guehl *et al.* 1994; Atwell *et al.* 2007). The discrepancy between prediction and observations for *E. pilularis* and *E. populnea* under elevated CO₂ and drought conditions may partly reflect overall plant size and hence water availability. In the experiment all pots in the drought treatment were consistently maintained at 50% of field capacity, whereas due to natural variation between the two species *E. populnea* was much smaller with lower daily average transpiration (Fig. 2-5). Therefore, the time spent each day with soil moisture content below 50% FC was less for *E. populnea* and leaf growth was thus not as limited by water stress. Conversely, for *E. pilularis* due to the much larger gains in total biomass and leaf area (Table 2-5; Fig. 2-8) under elevated CO₂ and drought, the length of time each day below 50% FC was more pronounced.

Biomass allocation

Along with leaf area, patterns in biomass allocation both within and between species were an important determinant of both water loss and carbon gain at the whole-plant scale. Between species, mesic *E. pilularis* preferentially allocated biomass gains with CO₂ enrichment aboveground, specifically to leaf mass and leaf area, irrespective of watering regime (Table 2-5; Fig. 2-7, 2-8). Conversely, xeric *E. populnea* allocated a greater proportion of biomass gains with elevated CO₂ belowground under both well-watered and drought conditions (Fig. 2-7). Notably, the general pattern in biomass allocation for each species was not altered by growth CO₂. Rather, the effect of elevated CO₂ for both species was towards an increase in overall plant size, thus individual biomass components (leaf, stem and root mass) typically increased in tandem with overall plant size (Fig. 2-7; Table 2-5). Similar observations of faster tree growth under elevated CO₂ without a concomitant change in allocation patterns have been reported from multiple elevated CO₂ experiments on different functional types including conifers (Tissue *et al.* 1997), tropical tree species (Reekie and Bazzaz 1989) and eucalypts (Atwell *et al.* 2007). Conversely, many reports suggest an increase in allocation to either aboveground or belowground biomass in response to elevated

CO₂ (Curtis and Wang 1998; Kimball *et al.*2002; de Graaff *et al.*2006; Ferguson and Nowak 2011).

From the literature it is widely observed that biomass allocation is extremely variable within vegetation types (Jackson *et al.*1996) and even among individuals of the same species (King *et al.*2007; Poorter *et al.*2012). This variability is commonly attributed to ontogeny, as increases in plant allocation are often just the result of the many morphological and physiological changes that occur under the normal course of plant growth and development (McConnaughay and Coleman 1999). For both *E. pilularis* and *E. populnea*, there were individual cases under elevated CO₂ where allocation diverged from the generally observed pattern. For *E. pilularis*, this divergence was represented by an increased allocation belowground, specifically to fine root mass, under elevated CO₂ and well-watered conditions (Table 2-5; Fig. 2-7), whereas for *E. populnea* the divergence was exhibited by an increased allocation aboveground, to both main and lateral stem mass, under elevated CO₂ and drought conditions. The relative increase in biomass allocated to fine root mass for *E. pilularis* under elevated CO₂ and well-watered conditions was an exceptionally large 114%, whereas under drought conditions there was a surprising relative decrease of 3% under elevated compared to ambient CO₂. An increase to fine root mass is predicted to operate under drought rather than well-watered conditions (Norby *et al.*1989; Morison 1993), although recent observations from elevated CO₂ experiments suggest an increase in fine root mass and at depth is a common response regardless of water limitation (Iversen 2010). The increase in fine root mass under elevated CO₂ is thought to relate to an increase in the carbon available to allocate towards belowground biomass and separately, gradual nutrient limitation in response to CO₂ enrichment versus a search for water (Finzi *et al.*2007; Iversen 2010), although in essence the development of fine roots distributed deeper within the soil profile would fulfill both needs (Hanson and Weltzin 2000; Wullschlegel *et al.*2002; Uddling *et al.*2008). In the case of *E. pilularis* the relative increase in fine root mass under elevated CO₂ and well-watered conditions was not strictly proportional (Fig. 2-7), suggesting that the increase was due to more than just greater carbon availability. Rather, as all pots were initially prepared with the same bulk nutrients and fortnightly addition of supplemental nitrogen was maintained at similar quantities for all treatment combinations, the relative increase in fine root mass for *E. pilularis* under elevated CO₂ and well-watered conditions was likely to meet declining nutrient availability for the much larger trees under well-watered conditions.

For *E. populnea* there was a considerable increase with CO₂ enrichment of 83% to main stem and an even larger increase of 158% to lateral stem mass under drought conditions

(Table 2-5; Figure 2-7). An increased allocation to stem mass with CO₂ enrichment has often been reported for both main stem mass (Duff *et al.* 1994) and for branch (lateral stem) mass (Berryman *et al.* 1993; Liberloo *et al.* 2006; Atwell *et al.* 2007). Atwell *et al.* (2007) similarly found a larger relative increase in lateral stem mass for a eucalypt woodland species (*Eucalyptus tereticornis*) under elevated CO₂ and drought conditions, which was accompanied by an increase in vessel numbers and vessel area. Greater allocation to both main and lateral stem mass for *E. populnea* occurred with a concomitant increase in leaf mass, leaf numbers and leaf area, thus potentially acting to compliment the reduction in g_s for *E. populnea* under elevated CO₂ and drought conditions by increasing xylem conductivity. An increase in xylem conductivity would assist with greater water transport through both main and lateral stems (Maherali *et al.* 1997; Atwell *et al.* 2007); while an increase in the number of lateral stems could potentially increase safety by limiting whole plant damage, in essence by sacrificing a single lateral branch during a cavitation event rather than to the more costly main stem (Tyree and Zimmermann 2002).

Notably, within species there were no significant interactions between elevated CO₂ and drought for total biomass, plant dimensions or individual biomass components, with the exception of fine root mass for *E. pilularis* and crown width, height and main and lateral stem mass for *E. populnea*. The lack of an interactive effect implies that elevated CO₂ did not ameliorate the impact of long-term moderate drought on growth or productivity but rather sped up ontogeny (Guehl *et al.* 1994; Centritto *et al.* 1999). In essence, allocation patterns changed due to the impact of growth under elevated CO₂ or growth under drought conditions but not as a direct result of elevated CO₂ alleviating any inhibitory effects of drought on growth, productivity or allocation. Conversely, between species patterns in allocation matched perceived drought strategies; for xeric *E. populnea* this entailed greater allocation to roots, whereas for mesic *E. pilularis* this involved greater allocation to aboveground biomass (Fig. 2-7). Even when there was a significant CO₂ x D interaction on growth or productivity it was opposite to most predictions of an increased allocation to fine roots or R:S under drought conditions (Morison 1993; Wullschleger *et al.* 2002). In the case of *E. pilularis* the effect of elevated CO₂ enhanced fine root mass to a much greater degree under well-watered rather than drought conditions, whereas for *E. populnea* the effect of elevated CO₂ led to an increase in aboveground versus belowground biomass and hence lower R:S under drought conditions. In all other cases, the effect of elevated CO₂ led to faster plant development and larger gains in productivity for both species but not increased drought resistance.

In summary, measured gas exchange parameters were not significantly different for either species under drought conditions, even though watering regime led to discernibly different biomass gains for both mesic *E. pilularis* and xeric *E. populnea*. Minor variation in leaf gas exchange under long-term moderate drought suggests an adjustment of growth and hydraulic architecture to maintain an optimal balance at the leaf scale between carbon gain and water loss, whereas a more rapid and explicit reduction in gas exchange parameters is commonly observed when the intensity of drought increases under elevated or ambient CO₂ (Heath and Kerstiens 1997; Clifford *et al.* 2000; Albert *et al.* 2011). In many elevated CO₂ and drought experiments, the intensity of drought is much greater than the percent of field capacity which we maintained throughout the experiment, frequently entailing complete dry down cycles where watering is temporarily, or completely stopped (e.g., Clifford *et al.* 2000; Duursma *et al.* 2011; Albert *et al.* 2011). In an associated experiment presented in Chapter (3) we evaluate the impact of drought of greater intensity but shorter duration, to identify diurnal adjustments in gas exchange along with changes in daily transpiration and leaf water potential as drought intensifies.

Conclusion

The results of the present study suggest that under long-term moderate drought, both mesic *E. pilularis* and xeric *E. populnea* exhibit a capacity to adjust growth processes to match water availability in place of stronger physiological controls under ambient or elevated CO₂. However, CO₂ enrichment provided a benefit to plant growth for both species during long-term moderate drought through a CO₂ induced reduction in g_s and increase in A_{sat} , thus enhancing productivity and whole-plant growth. These whole-plant increases in productivity with CO₂ enrichment were substantial and accompanied by a considerable decrease in transpiration at the leaf scale. Remarkably, transpiration rates were relatively unchanged at the whole-plant scale between plants grown under elevated or ambient CO₂ for *E. pilularis* or *E. populnea*, connected in both cases to an increase in leaf area compensating for the reduction in g_s with CO₂ enrichment. Between species, the gains in productivity and water savings at the leaf-level with CO₂ enrichment led to a larger relative increase in WUE for xeric *E. populnea* under drought conditions and for mesic *E. pilularis* under well-watered conditions. However, the CO₂ effect on WUE for mesic *E. pilularis* was likely the result of better plant coupling to the glasshouse environment for the larger CO₂ enriched and well-watered plants as the relative increase in WUE for *E. pilularis* grown under drought

conditions was considerably less. Conversely, the relative increase in WUE with CO₂ enrichment for xeric *E. populnea* was similar between well-watered and drought conditions, suggesting greater potential for xeric *Eucalyptus* species to adjust structurally and physiologically to growth environment. Consequently, the effect of elevated CO₂ may be more apparent under short-term drought episodes of greater intensity, where tree size and allocation patterns at time of drought may influence species response to a greater degree than species' inherent drought strategy. We plan to address the impact of increasing drought intensity on physiological responses and potential for water savings with CO₂ enrichment in the next chapter. The results of the present study show that physiological controls may not be the only factor at play during prolonged moderate drought episodes. Further, projected gains in WUE with CO₂ enrichment depend upon strong plant coupling to environment and suggest a strong role of plant allocation and respiratory responses in determining the magnitude of gains under elevated CO₂.

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CHAPTER 3

Water Savings under elevated CO₂ and drought for two *Eucalyptus* species of contrasting drought tolerance

Summary Elevated CO₂ is expected to reduce stomatal conductance and hence lower leaf level transpiration rates, with a potential to delay the onset or impact of drought at the whole plant scale. We investigated the effect of a rapid dry down on leaf-level gas exchange, and whole plant and soil water relations for two *Eucalyptus* species (*E. pilularis* and *E. populnea*) of contrasting drought tolerance at the end of a long-term (~9-11 months) experiment where soil moisture was maintained at well-watered (100% field capacity) or drought (50% field capacity) conditions in elevated (700 ppm) or ambient CO₂ (380 ppm) glasshouses. Over the dry down period, the effect of elevated CO₂ was to delay water stress, through a CO₂ induced reduction in stomatal conductance, for both mesic *E. pilularis* and xeric *E. populnea*. Additionally, there was a sustained increase in photosynthetic rate with CO₂ enrichment over the dry down period for both species. However, the impact of long-term growth under elevated or ambient CO₂ and well-watered or drought conditions led to markedly different patterns in biomass allocation for each species, which strongly contributed to their response to increasing water stress. Larger plants (both within and between species) grown under well-watered conditions experienced earlier water stress during respective dry down events than smaller plants grown under drought conditions. Long term growth under elevated CO₂ led to considerably larger plants for both species at well-watered (100% field capacity) and drought (50% field capacity) conditions. *E. pilularis* grown under elevated CO₂ and well-watered conditions experienced the largest combined increase in total biomass and leaf area, and consequently did not experience a significant delay in water stress at the leaf or whole plant scale during its dry down. In contrast, for all other species and treatment combinations, the CO₂ induced reduction in g_s during respective dry down events, along with structural adjustments, delayed the impact of water stress for plants grown under elevated compared to ambient CO₂. Xeric *E. populnea* exhibited a consistent pattern of greater allocation towards belowground biomass and in the ratio of fine root mass to leaf area (FR:LA), with larger yet proportional gains taking place under elevated CO₂. A relative reduction rather than an increase in fine root mass and FR:LA was exhibited by mesic *E. pilularis* grown under elevated CO₂ and drought (50% field capacity) conditions leading to a greater dependence on physiological mechanisms to avoid progressive drought stress rather than adjustments in the plant vascular system under elevated CO₂. These results suggest that xeric *E. populnea*, with a greater inherent adaptive capacity to adjust structurally to varying water availability, may experience a greater benefit with CO₂ enrichment, through a complementary reduction in g_s and related water savings, than mesic *E. pilularis* which exhibits a limited ability to adjust structurally to endure drought of increasing intensity and instead relies upon rapid stomatal closure to avoid water stress.

3.1 Introduction

Drought greatly impacts the productivity and health of forests in most every region of the world (Allen *et al.* 2010). An increase in both drought frequency and drought intensity is projected over the next century as a result of the continuing rise in atmospheric CO₂ concentrations and rising temperatures (Meehl *et al.* 2005; IPCC 2007; Bates *et al.* 2008). Recent reports have documented wide scale forest dieback and forest mortality in response to more frequent and intense drought episodes, and increasing temperatures (Allen *et al.* 2010; Carnicer *et al.* 2011; Linares *et al.* 2011). The longer-term outlook on forest productivity and health in response to drought is much less certain due to important interactions between elevated CO₂ and water availability (Wullschleger *et al.* 2002; Duursma *et al.* 2011). Understanding forest response to an increase in drought frequency and intensity under future climate is particularly important to arid and semi-arid regions of the world which presently experience substantial negative impacts associated with drought (Adams *et al.* 2009; Breshears *et al.* 2009; Aranda *et al.* 2012). Importantly, the effect of elevated CO₂ is expected to be most pronounced for water limited regions as a result of potential water savings at the leaf and whole plant scale with CO₂ enrichment (Gifford 1979; Idso and Idso 1994; Catovsky and Bazzaz 1999). Key issues to better ascertain include identifying mechanisms contributing to water savings at the leaf and whole plant scale with CO₂ enrichment; identifying whether potential water savings under elevated CO₂ can delay drought initiation or lessen water stress at the leaf and whole tree scale once drought is underway; and determining how this may vary between forest tree species native to xeric or mesic environments, or species which have adjusted physiologically or structurally to drought episodes during their life history.

A perceived benefit of elevated CO₂ on tree growth under water limitation is a delay in drought impacts; either by postponing drought occurrence or lessening the stresses once drought begins (Wullschleger *et al.* 2002; Morgan *et al.* 2004; Keel *et al.* 2007). There are two main mechanisms thought to account for this benefit. The first relates to the non-linear response of photosynthesis (A) to ambient CO₂ (C_a). Photosynthetic rate (A) exhibits a saturating response to intercellular CO₂ (C_i) such that the relative response of A to C_a is higher at low stomatal conductance (Lloyd and Farquhar 1996; Grossman-Clarke *et al.* 2001; McMurtrie *et al.* 2008; Duursma *et al.* 2011). This mechanism (non-linear response of A to C_i) was a major focus of Ch. (2) and will not subsequently be covered in this chapter.

However, it is important to point out that in order to identify and focus wholly on this first mechanism we maintained soil water content at two constant set levels (see Ch. 2 for details) throughout the duration of the main experiment, thus simulating long-term moderate drought. The experiment presented in this chapter carries on from the end of the main experiment and simulates shorter-term high intensity drought in order to focus on the second of the two main mechanisms. The second mechanism thought to delay drought impacts under elevated CO₂, and hence maintain plant growth, operates through a feedback via soil moisture. Under elevated CO₂, stomatal conductance (g_s) is typically reduced (Eamus 1991; Medlyn *et al.* 2001; Ainsworth and Rogers 2007) leading to a lowered transpiration rate and corresponding soil moisture savings, thus enabling plants grown at high CO₂ to continue to transpire as drought progresses or intensifies. To identify soil moisture savings, importantly, requires soil moisture to vary. Consequently, in the experiment presented in this chapter, we permitted plants to dry down in order to focus on the mechanism of soil moisture savings under elevated CO₂.

At the leaf scale, initial responses to drought typically entail a reduction in g_s followed closely by a decline in photosynthetic rate (Lawlor 2002; Chaves *et al.* 2002; Chaves and Oliveira 2004). The reduction in A and g_s under drought conditions is a direct response to decreasing relative water content (RWC) and water potential (Ψ) (Chaves 1991; Cornic 1994; Kramer and Boyer 1995; Lawlor 1995; Cornic and Massacci 1996; Lawlor 2002). During initial stages of drought or under moderate water stress the main limitation to photosynthesis is considered to be g_s (Cornic 2000). As drought progresses or intensifies, further limitations at the leaf scale develop. These additional limitations are controlled by mesophyll conductance to CO₂ (Lawlor 2002; Flexas and Medrano 2002; Chaves *et al.* 2003; Flexas *et al.* 2004; Flexas *et al.* 2012) and by biochemical limitations represented by the maximum carboxylation rate of Rubisco (V_{cmax}) and the maximum electron transport rate (J_{max}) (Lawlor 2002). The relative effect of stomatal and non-stomatal limitations to CO₂ varies widely between, and often within species, as a result of a strong dependence on the degree of drought stress and ambient environmental conditions (Flexas *et al.* 2007; Warren 2008). In addition, elevated CO₂ further moderates the relative effect of stomatal and non-stomatal limitations and may moderate the rate at which the two key limitations develop under drought (Lawlor 2002).

The effect of drought differs at the leaf and whole plant scale for species from xeric and mesic environments. At the leaf scale, mesic species commonly close their stomata before experiencing any change in leaf water potential (Ψ_{leaf}), whereas for xeric species a

reduction in g_s under drought conditions operates in coordination with a decrease in Ψ_{leaf} (Guehl *et al.* 1991; Picon *et al.* 1996; Martínez-Ferri *et al.* 1999). A recently developed model based on the theory of optimal stomatal conductance (Cowan and Farquhar 1977; Medlyn *et al.* 2011) may help us to separate out the impacts of elevated CO_2 and drought both within and between species. According to Cowan and Farquhar (1977), the optimal stomatal conductance is that which maximizes daily carbon gain (A) for a given daily water loss (E). Utilizing the Cowan and Farquhar (1977) definition of optimal stomatal conductance, Medlyn *et al.* (2011) developed the following model of g_s .

$$g_s = g_0 + 1.6(1 + \frac{g_1}{\sqrt{D}}) \frac{A}{c_a} \quad (1)$$

where g_0 is the cuticular conductance, g_1 is a constant that reflects the marginal cost of water to the plant and D is the leaf to air vapor pressure deficit (Medlyn *et al.* 2011). Over an average interval between successive rains the g_1 parameter is considered to be constant (H  roult *et al.* 2012). However, under drought conditions and ambient CO_2 it is thought that the g_1 parameter will decline as drought progresses (M  kel   *et al.* 1996); whereas the effect of elevated CO_2 may initially delay the decline in g_1 under moderate drought conditions. To facilitate growth under conditions of limited water supply, xeric species generally construct more cavitation resistant woody tissues, in order to endure periods of drought (Hacke and Sperry 2001). Conversely, mesic species maintain stronger control over leaf scale mechanisms when water availability declines. Consequently, construction costs are generally higher for xeric species (Givnish 1988; Pockman and Sperry 2000; Villar and Merino 2001; Hacke *et al.* 2001) and in relation the parameter g_1 is considered lower. A lower g_1 for xeric species suggests a more conservative water use strategy and hence smaller return (C) on investment (H_2O), so that as drought progresses g_1 is expected to minimally change for xeric species. In contrast, mesic species are considered to exhibit a higher g_1 that decreases rapidly as drought progresses (H  roult *et al.* 2012). Further, as drought progresses, additional limitations other than g_s typically develop (Pinheiro and Chaves 2011), and similar to g_s these additional limitations often exhibit a slower decline in response to drying conditions when under elevated CO_2 . At the leaf scale, water savings connected to CO_2 enrichment may result in a delayed appearance of the key stomatal and biochemical limitations to carbon gain and may represent potential for sustained growth during periods of limited water availability.

The hypothesis that water savings at the leaf level will ameliorate drought is important when considering increased incidence and intensity of drought under future climate (Kimball

et al. 2002; Wullschleger and Norby 2001; Wullschleger *et al.* 2002; Nowak *et al.* 2004b; Leuzinger and Körner 2007; Barton *et al.* 2012). However, to date, experimental evidence on the water saving potential of species under the combination of elevated CO₂ and drought is not entirely clear (Wullschleger *et al.* 2002). Numerous authors have reported increases in biomass under elevated CO₂ and drought but do not observe a reduction in water use (Guehl *et al.* 1994; Picon *et al.* 1996; Roden and Ball 1996; Heath and Kerstiens 1997; Centritto *et al.* 1999, 2002). A common observation in glasshouse experiments with potted seedlings is an increase in leaf area with CO₂ enrichment which compensates for the reduction in g_s so that total transpiration is similar between ambient and elevated CO₂ grown trees (Guehl *et al.* 1994; Roden and Ball 1996; Centritto *et al.* 1999, 2002), although in some cases transpiration is even greater under elevated CO₂ (Heath and Kerstiens 1997). In field experiments with trees and shrubs the evidence is also quite varied, with soil water savings in some experiments in topmost soil layers (Leuzinger and Körner 2007), water savings and increased WUE on a seasonal basis (Wullschleger and Norby 2001) and no clear water savings in others (Gunderson *et al.* 2002; Nowak *et al.* 2004a; Li *et al.* 2007; Duursma *et al.* 2011). In grassland experiments soil water savings are often witnessed under the combination of elevated CO₂ and drought (Knapp *et al.* 1993; Owensby *et al.* 1997; Field *et al.* 1997; Morgan *et al.* 2004; Nelson *et al.* 2004). The reason for the discrepancy in water savings for various species and functional types under elevated CO₂ and drought is crucial to better understand in light of increasing drought occurrence and intensity (Huntington 2006).

In the present study, our principal objective was to determine if water savings at the leaf level with CO₂ enrichment would provide for greater water savings at the whole tree scale. A second objective was to determine if relative water savings under elevated CO₂ and progressive drought are greater for xeric versus mesic species or for species experiencing limited water availability during their life history. Our final objective was to separate out the effect of tree size on water savings, or lack thereof, under elevated CO₂. In this chapter we set out to test both within and between species responses to elevated CO₂ and progressive drought with the following hypotheses at the leaf and whole plant scale: 1-) g_1 will decline along with leaf water potential, with mesic species exhibiting a faster decline than xeric species; 2-) At comparable sizes drought stress will be reached much sooner for mesic versus xeric species, and for either species under ambient rather than elevated CO₂; 3-) Plants grown under elevated CO₂ will use less water than plants grown under ambient CO₂; 4-) Water savings will depend on overall tree size (i.e., larger trees will lose more water).

3.2 Material / Methods

3.2.1 Plant Material and experimental treatments

Two *Eucalyptus* species native to New South Wales and Queensland, Australia were sown directly from seed into large 90 L pots in February 2010, and grown for four months at full field capacity (~32% w/v). The two species were chosen as representative of xeric and mesic native habitats (Hodgkinson 1979; Noble 1989; Merchant *et al.* 2007, 2010). Xeric *Eucalyptus populnea* is a drought-tolerant species occupying eucalypt dry woodland, while mesic *Eucalyptus pilularis* is a drought-avoiding species occupying eucalypt tall open forests of the higher rainfall, coastal regions of eastern Australia. The soil medium within each pot (48 pots in total; 24 pots per species) was a nutrient amended, red silt loam soil collected from Robertson, NSW. Replicate pots containing seedlings of *E. populnea* and *E. pilularis* were grown in two ambient CO₂ (380 ppm) and two elevated CO₂ (700 ppm) glasshouses located at Macquarie University, North Ryde, NSW. Full details of nutrient amendments and growing conditions within glasshouses are provided in Chapter 2. Four months after seedling emergence (early June 2010) a drought treatment was instituted where one half of the pots from each species (12 of 24 pots from each species) were subjected to a drought treatment while the other half remained well-watered at full field capacity. The drought treatment consisted of setting and maintaining soil moisture content at 50% of field capacity (50% FC). These two watering treatments (100% and 50% of field capacity) were maintained until November 2010 for mesic *E. pilularis* (~9 months after seedling emergence) and January 2011 for xeric *E. populnea* (~11 months after seedling emergence).

Approximately four weeks prior to final harvest (final harvest in December 2010 for *E. pilularis* and February 2011 for *E. populnea*) we instituted a separate dry down cycle for both species. The dry down cycle involved re-watering all pots for a given species back to full field capacity (100% FC) whereupon they were allowed to dry down until reaching a predetermined physiological stress point at the leaf level, which we characterized as a measured stomatal conductance (g_s) prior to 0900 of ~50 mmol m⁻² s⁻¹. Replicate seedlings of either species reaching this set stress point were then re-watered to their respective percent of field capacity (50% or 100% FC) until final harvest.

3.2.2 Gas exchange

Leaf gas exchange was measured on fully formed leaves of each species with an open-flow infrared gas analyzer equipped with a red-blue light source (LI-6400XT, Li-Cor, Lincoln NE, USA). Diurnal measurements were conducted every 1-3 days during the dry down depending on species; twice daily for *E. pilularis* (0730-1000; 1200-1430) and every three days (twice each day) for *E. populnea* (0730-1000; 1200-1430). Leaf cuvette conditions during each measurement period were maintained at an irradiance of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of 25°C , and relative humidity $> 60\%$. The CO_2 concentration within the leaf cuvette during each measurement period corresponded to plant growth environment (ambient glasshouses: $380 \mu\text{mol m}^{-1}$; elevated glasshouses: $700 \mu\text{mol m}^{-1}$).

Both apparent V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and the marginal cost of water parameter (g_1) were estimated over the dry down period from the gas exchange measurements. The parameter g_1 was calculated from measured values of g_s and A by re-arranging eqn. (1). Apparent V_{cmax} was calculated from measured A and C_i by re-arranging equation (2):

$$A = V_{\text{cmax}} \left(\frac{C_i - \Gamma^*}{C_i + K_m} \right) \quad (2)$$

where, Γ^* is the CO_2 compensation point in the absence of mitochondrial respiration, and K_m is the effective Michaelis-Menten coefficient for CO_2 . The apparent V_{cmax} is determined in the leaf by Rubisco content, Rubisco activity, and delivery of CO_2 to the mesophyll from the intercellular spaces. The effect of water stress on a daily (*E. pilularis*) or every third day (*E. populnea*) basis for apparent V_{cmax} and g_1 was calculated as the difference from the value of apparent V_{cmax} or g_1 when pre-dawn leaf water potential (Ψ_{pd}) was nearest to zero (i.e., Day 1 of each dry down). For both apparent V_{cmax} and g_1 , the response curves were developed as a function of pre-dawn leaf water potential (Ψ_{pd}). This permitted an estimate of cuticular conductance (g_0), which was taken as the minimum g_s for each plant and can be interpreted as the leaf water vapor conductance when photosynthesis is zero.

3.2.3 Plant water relations

Water relations were evaluated through pre-dawn and midday leaf water potential (Ψ_{pd} and Ψ_{md} , respectively) measured daily during the dry down for *E. pilularis* and every third day during the dry down for *E. populnea*. Leaf water potential (Ψ_{pd} and Ψ_{md}) was determined using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA;

Scholander *et al.* 1965). Measurements for xeric *E. populnea* were less frequent due to smaller overall plant size and an associated limited number of leaves.

To determine whole-tree transpiration, we weighed individual pots each day during the dry down for *E. pilularis* and every 1-3 days during the dry down for *E. populnea* using a standard floor scale (Avery Weigh-Tronix, Fairmont MN, USA). Direct evaporation from the soil surface was controlled by evenly covering exposed soil with a thin layer of white gravel ~10mm diameter in size.

3.2.4 Statistical analysis

Linear mixed-effects models (package *nlme* in R) were used to test for main effects of growth CO₂ treatment (CO₂), growth watering treatment (W) and time (T), and interactions between main effects on all dependent variables measured in elevated and ambient CO₂ glasshouses. Where necessary, data were transformed prior to analyses to meet assumptions of normality and homogeneity of variance. All statistical analyses were performed with R 2.14.1 (R Core Development Team, 2011).

3.3 Results

3.3.1 *Eucalyptus pilularis*

Gas Exchange

The time course of daily water stress at the leaf (A_{sat} , g_s , Ψ_{pd} and Ψ_{md}) and whole tree scale (transpiration) for *E. pilularis* is shown in Fig. 3-1 and statistical results displayed in Table 3-1. During the dry down the overall effect of both growth CO₂ (CO₂) and growth watering treatment (W) was highly significant ($p < 0.001$) for A_{sat} , whereas for g_s the only significant overall effect was growth watering treatment (Fig. 3-1a & d). This overall effect represents the average daily response of A_{sat} or g_s . Plants grown at elevated CO₂ (eC_a) exhibited consistently higher rates of photosynthesis than plants grown at ambient CO₂ (aC_a). Similarly, plants grown under drought conditions maintained significantly higher rates of A_{sat} throughout the dry down compared with plants grown under well-watered conditions. There was a highly significant ($p < 0.0001$) decline in both A_{sat} and g_s as the dry down progressed, with additional significant interactions developing over the dry down period. For A_{sat} , these

included a W x T and CO₂ x W x T interaction that developed as plants grown under elevated CO₂ and drought conditions (eC_a-D) exhibited a markedly slower decline in A_{sat} as the dry down progressed (Fig. 3-1a) than for any other treatment combination.

There was a highly significant ($p < 0.0001$) difference between diurnal measurements for *E. pilularis*, with a considerably lower average daily A_{sat} and g_s measured at midday compared to the morning (Fig. 3-1). The main effect of growth watering treatment (W) was not significant for average daily A_{sat} or g_s measured in the morning, although there was a significant ($p < 0.01$) W x T interaction. This interaction was the result of a higher A_{sat} and g_s in the morning, particularly during the latter stages of the dry down, for plants grown under drought than well-watered conditions. Additionally, for g_s measured in the morning, there was a significant CO₂ x T interaction as the decline in g_s was markedly slower for plants grown under eC_a, particularly towards the latter half of the dry down (Fig. 3-1e; Table 3-1).

By comparison a strong and persistent midday depression was observed in both A_{sat} and g_s, with a significant main effect of growth watering treatment (W) as plants grown under drought conditions displayed a smaller depression throughout the dry down. The effect of growth CO₂ was also significant for A_{sat} measured midday due to consistently higher rates of photosynthesis for plants grown under eC_a (Table x-x; Fig. 3-1c). Lastly for both A_{sat} and g_s measured midday there was a significant growth CO₂ x W x T interaction as the decline in both parameters was much slower for plants grown eC_a-D, throughout the dry down (Fig. 3-1c & f; Table 3-1).

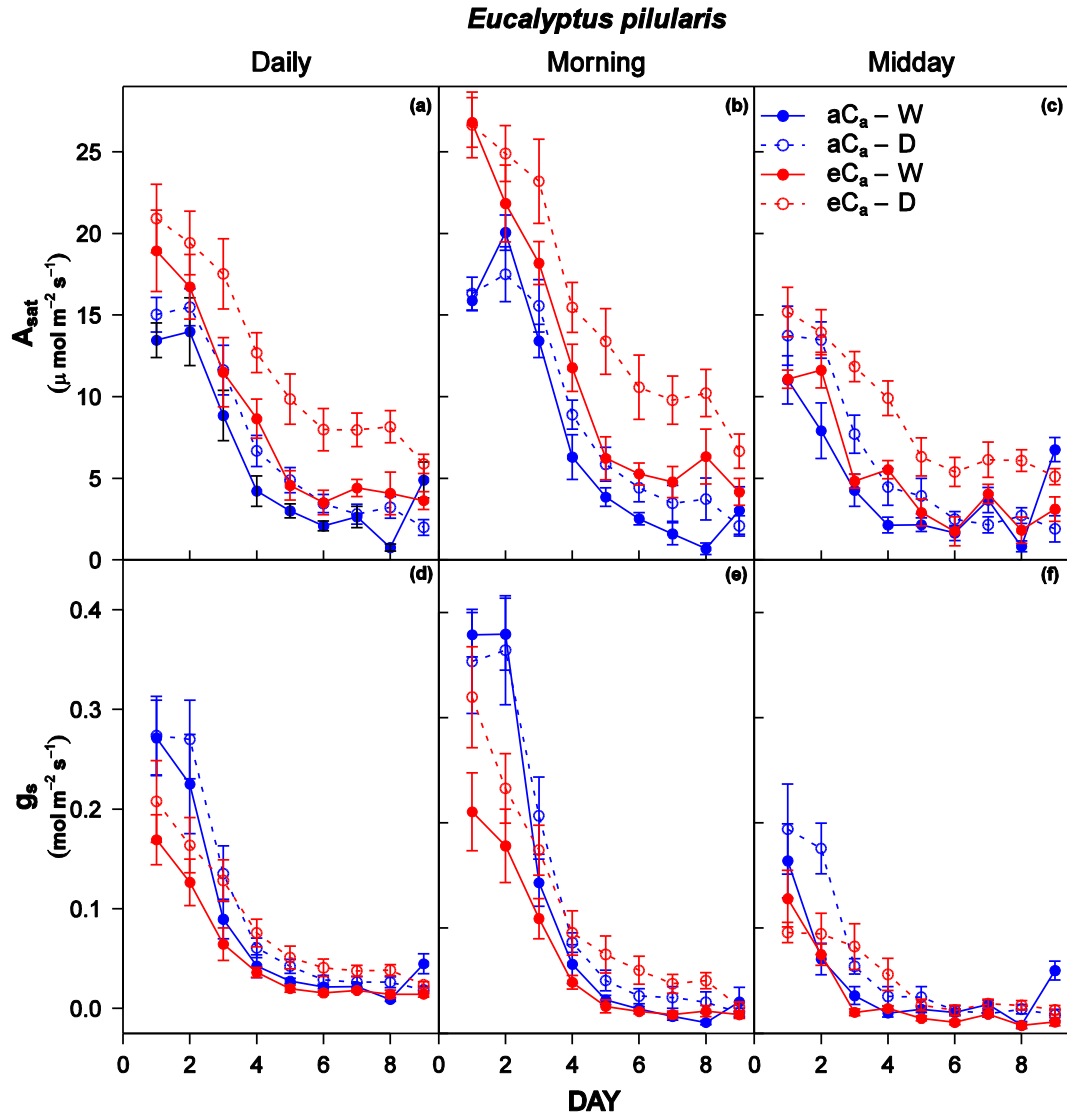


Figure 3-1. (a-c) Light saturated net photosynthetic rate (A_{sat}) measured daily ($n=12$), morning and midday ($n=6$), (d-f) stomatal conductance (g_s) measured daily ($n=12$), morning and midday ($n=6$) of *E. pilularis* during a dry down in November 2010. Measurement CO_2 corresponds to growth CO_2 (ambient CO_2 : $380 \mu\text{mol m}^{-1}$; elevated CO_2 : $700 \mu\text{mol m}^{-1}$). Data are the means of 12 trees per treatment (a & d) and 6 trees per treatment (b-c & e-f); error bars are ± 1 SE (*Exception on final days of dry down where $n=1-3$). Filled symbols – grown under well-watered conditions (100% FC); Open symbols – grown under drought conditions (50% FC). Blue symbols – grown under ambient CO_2 ; Red symbols – grown under elevated CO_2 .

Table 3-1. Statistics of ecophysiological responses for *E. pilularis* during respective dry down.

Parameter	Main effects			Interaction			
	CO ₂	W	T	CO ₂ x W	CO ₂ x T	W x T	CO ₂ x W x T
Numerator df	1	1	1	1	1	1	1
Denominator df	20	20	169	20	169	169	169
Leaf gas exchange (Morning)							
A _{sat} (μmol m ⁻² s ⁻¹)	53.25***	2.47	605.66***	2.38	0.85	11.39**	0.83
g _s (mol m ⁻² s ⁻¹)	0.71	0.14	758.12***	3.91	13.97**	7.88**	0.29
Leaf gas exchange (Midday)							
A _{sat} (μmol m ⁻² s ⁻¹)	12.81**	11.92**	247.88***	2.71	0.50	0.03	7.43**
g _s (mol m ⁻² s ⁻¹)	0.83	5.31*	249.99***	1.60	0.22	0.90	11.34**
Plant water relations							
Daily transpiration (l day ⁻¹)	1.56	290.21***	1346.74***	0.09	19.78***	66.82***	0.00
Pre-dawn Ψ _{leaf} (mPa)	1.82	19.21**	2036.59***	1.04	5.71*	14.30**	2.30
Midday Ψ _{leaf} (mPa)	5.68*	16.89**	1362.72***	3.58	1.46	25.20***	0.65
Soil water content (m ³ m ⁻³)	0.71	24.86**	2982.12***	0.00	0.04	122.61***	5.73*

F values and significance levels (**P*<0.05; ***P*<0.01; ****P*<0.001) for the main effects of growth CO₂ (CO₂), growth watering treatment (W) and time (T), and their interactions on leaf level gas exchange and plant water relations. Numerator and denominator df are the numerator and denominator degrees of freedom for the *F* tests.

Water relations

The change in leaf water potential (Ψ_{pd} and Ψ_{md}), soil moisture content and daily transpiration during the imposed dry down for *E. pilularis* is shown in Figure 3-2. All parameters displayed a highly significant ($p < 0.0001$) decline as the dry down progressed. There was a significant ($p < 0.01$) main effect of growth watering treatment (W) on Ψ_{pd} but no significant effect of growth CO_2 , whereas both main effects of CO_2 and W were significant ($p < 0.05$) for Ψ_{md} . However, for Ψ_{pd} there was a significant $CO_2 \times T$ and $W \times T$ interaction as a result of a slower decline as drought progressed for plants grown under eC_a and equally for plants grown under drought conditions (Table 3-1; Fig. 3-2a). Similarly, Ψ_{md} for plants grown under drought conditions displayed a much slower decline over the dry down compared to plants grown under well-watered conditions, resulting in a highly significant ($p < 0.0001$) $W \times T$ interaction (Fig. 3-2b).

During the dry down for *E. pilularis*, soil water content was significantly affected by growth watering treatment (W) but not by growth CO_2 (Table 3-1; Fig. 3-2c). Additionally, there was a significant $W \times T$ and $CO_2 \times W \times T$ interaction as a result of slight variation in soil moisture content between treatments near the end of the dry down (Table 3-1; Fig. 3-2c). As drought progressed there was a crossover in soil water content for plants grown under well-watered conditions. Plants grown under eC_a -W experienced higher soil water content until ~day 5, after which plants grown under aC_a -W experienced a greater soil water content (Fig. 3-2c). In contrast, for plants grown under drought conditions, soil water content was similar under eC_a or aC_a until about day 7. From day 7 onwards soil water content was slightly higher for plants grown under eC_a -D (Fig. 3-2c).

Growth watering treatment (W) significantly ($p < 0.01$) affected daily transpiration, with much greater rates of transpiration throughout the dry down for plants grown under well-watered compared to drought conditions (Fig. 3-2d). In contrast, there was no significant effect of growth CO_2 on daily transpiration. However, there was a significant $CO_2 \times T$ and $W \times T$ interaction on daily transpiration. The $CO_2 \times T$ interaction developed as a result of an initial lower rate of transpiration for plants grown under eC_a , followed by a crossover on ~day 5 where plants grown at eC_a displayed greater daily transpiration rates than plants grown under aC_a , irrespective of watering treatment (Fig. 3-2d). Conversely, the $W \times T$ interaction resulted from a very large

difference in transpiration rates in the first half of the dry down between plants grown at well-watered and drought conditions, followed by similar rates in the latter stages of the dry down.

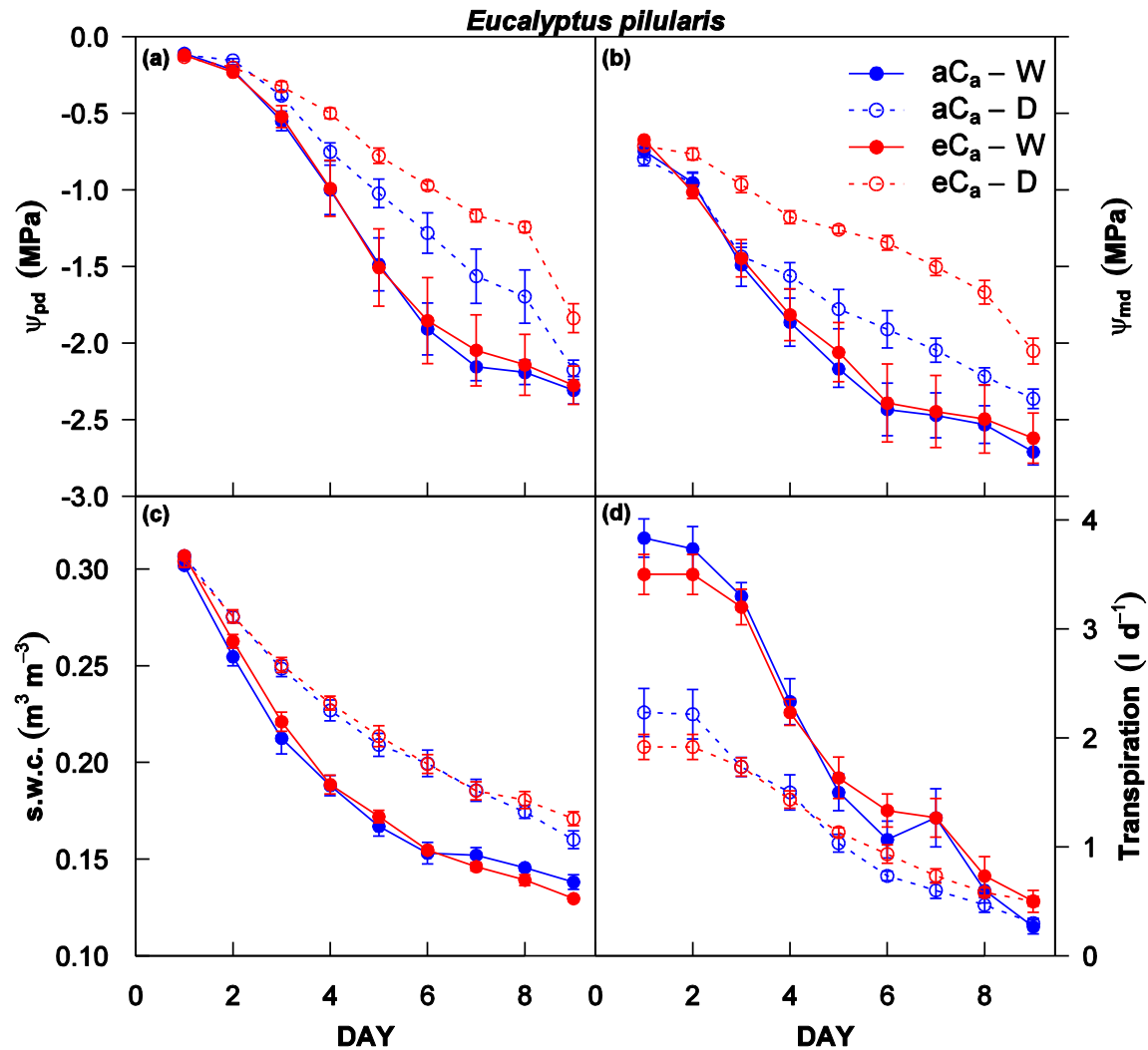


Figure 3-2. (a) Pre-dawn leaf water potential (Ψ_{pd}), (b) Midday leaf water potential (Ψ_{md}), (c) Soil water content and (d) Daily transpiration of *E. pilularis* during a dry down in November 2010. Data are the means of 6 trees per treatment; error bars are ± 1 SE (*Exception on final days of dry down where $n=2-3$). Filled symbols – grown under well-watered conditions (100% FC); Open symbols – grown under drought conditions (50% FC). Blue symbols – grown under ambient CO_2 ; Red symbols – grown under elevated CO_2 .

3.3.2 *Eucalyptus populnea*

Gas Exchange

The overall effect of growth CO₂ was highly significant ($p < 0.0001$) on A_{sat} and g_s for *E. populnea* during the dry down, whereas there was no significant overall effect of growth watering treatment (W) (Table 3-2). Rates of photosynthesis were consistently higher for plants grown under eC_a throughout the dry down for *E. populnea* (Fig. 3-3a), while g_s was more variable between treatment combinations as the dry down progressed (Fig. 3-3d). There was a significant decline in both A_{sat} and g_s as the dry down progressed. Additionally, there was a significant CO₂ x T and W x T interaction for both parameters, as a result of a more rapid decline in A_{sat} and g_s for plants grown under well-watered conditions and smaller variation in A_{sat} and g_s midway through the dry down between plants grown under elevated and ambient CO₂ (Fig. 3-3a & d).

There was no significant difference between diurnal measurements of leaf gas exchange for *E. populnea* during the imposed dry down (Fig. 3-3). The main effect of growth CO₂ on average daily g_s was not significant ($p = 0.06$) when evaluated for midday measurements (Table 3-2), although there was a significant ($p < 0.01$) CO₂ x T interaction as a result of a much larger difference between CO₂ treatments in the first half, compared to the second half, of the dry down (Fig. 3-3). Similarly, the significant CO₂ x T interaction was not present for g_s measured in the morning or for A_{sat} measured midday. In both cases however, the main effect of growth CO₂ was sustained with only slight variation on a daily basis as the dry down progressed (Fig. 3-3c & e; Table 3-2).

Water relations

Figure 3-4 shows the response of leaf water potential (Ψ_{pd} and Ψ_{md}), soil water content and daily transpiration during the imposed dry down for *E. populnea*. All parameters displayed a highly significant ($p < 0.0001$) decline as the dry down progressed. The main effect of growth CO₂ was not significant for Ψ_{pd} , whereas there was a significant ($p < 0.05$) effect of growth watering treatment (W) as plants grown under drought conditions maintained a consistently higher Ψ_{pd} throughout the dry down (Fig. 3-4a). Additionally, there was a significant W x T and CO₂ x W x T interaction for *E. populnea* as a result of the much more rapid decline in Ψ_{pd} for

plants grown under ambient CO_2 and well-watered conditions ($aC_a\text{-W}$) compared to $eC_a\text{-W}$ and a highly delayed decline in Ψ_{pd} of plants grown under $eC_a\text{-D}$ (Fig. 3-4a).

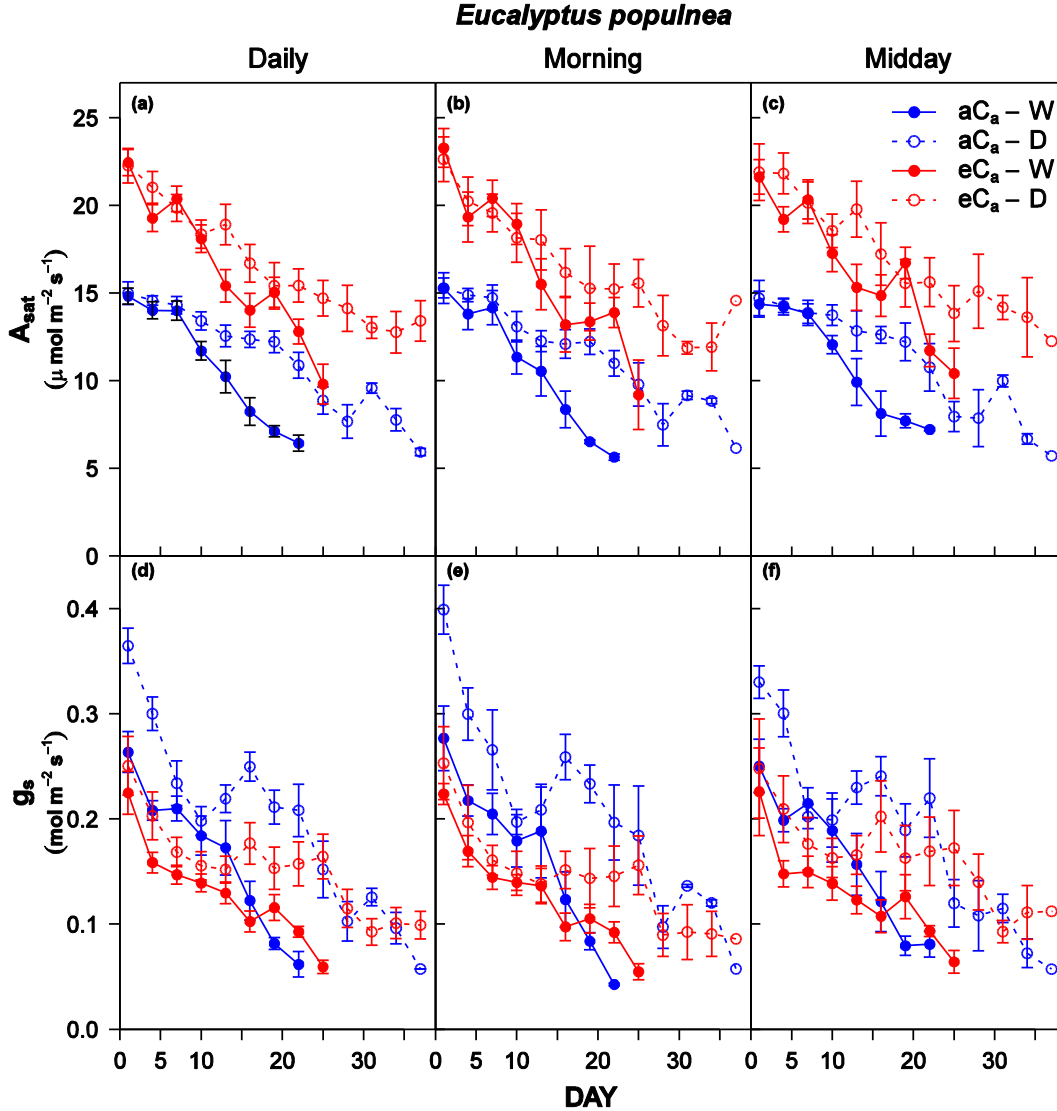


Figure 3-3. (a-c) Light saturated net photosynthetic rate (A_{sat}) measured daily ($n=12$), morning and midday ($n=6$), (d-f) stomatal conductance (g_s) measured daily ($n=12$), morning and midday ($n=6$) of *E. populnea* during a dry down in January 2011. Measurement CO_2 corresponds to growth CO_2 (ambient CO_2 : $380 \mu\text{mol m}^{-1}$; elevated CO_2 : $700 \mu\text{mol m}^{-1}$). Data are the means of 12 trees per treatment (a & d) and 6 trees per treatment (b-c & e-f); error bars are ± 1 SE (*Exception on final days of dry down where $n=1-3$). Filled symbols – grown under well-watered conditions (100% FC); Open symbols – grown under drought conditions (50% FC). Blue symbols – grown under ambient CO_2 ; Red symbols – grown under elevated CO_2 .

Table 3-2. Statistics of ecophysiological responses for *E. populnea* during respective dry down.

Parameter	Main effects			Interaction			
	CO ₂	W	T	CO ₂ x W	CO ₂ x T	W x T	CO ₂ x W x T
Numerator df	1	1	1	1	1	1	1
Denominator df	20	20	187	20	187	187	187
Leaf gas exchange (Morning)							
A _{sat} (μmol m ⁻² s ⁻¹)	43.28***	0.08	395.45***	0.37	7.83**	26.18***	0.45
g _s (mol m ⁻² s ⁻¹)	9.66**	2.19	313.80***	1.64	3.60	16.85***	1.97
Leaf gas exchange (Midday)							
A _{sat} (μmol m ⁻² s ⁻¹)	50.44***	0.24	429.61***	0.12	3.70	10.00**	1.71
g _s (mol m ⁻² s ⁻¹)	4.09	4.02	247.80***	0.01	12.08**	10.05**	0.17
Plant water relations							
Daily transpiration (l day ⁻¹)	7.43*	147.33***	530.82***	1.25	2.44	88.30***	3.86
Pre-dawn Ψ _{leaf} (mPa)	3.73	8.41**	1938.82***	0.00	1.03	104.43***	8.80**
Midday Ψ _{leaf} (mPa)	7.51*	4.75*	1098.36***	0.97	3.90*	100.76***	0.11
Soil water content (m ³ m ⁻³)	0.25	26.93***	5711.55***	2.90	9.47**	558.64***	26.24***

F values and significance levels (**P*<0.05; ***P*<0.01; ****P*<0.001) for the main effects of growth CO₂ (CO₂), growth watering treatment (W) and time (T), and their interactions on leaf level gas exchange and plant water relations. Numerator and denominator df are the numerator and denominator degrees of freedom for the *F* tests.

The decrease over time in Ψ_{md} was significantly ($p < 0.05$) affected by growth CO_2 and by growth watering treatment (W) (Table 3-2). Additionally, there was a significant $CO_2 \times T$ and $W \times T$ interaction on Ψ_{md} (Table 3-2). Both interactions resulted from a much larger difference in Ψ_{md} , as the dry down progressed, between plants grown at elevated and ambient CO_2 and separately between plants grown at well-watered and drought conditions (Fig. 3-4b).

During the dry down, there was a highly significant ($p < 0.0001$) effect of growth watering treatment (W) on soil water content as plants grown under drought conditions maintained much higher soil water content throughout the dry down (Fig. 3-4c), whereas there was no significant effect of growth CO_2 (Table 3-2). However, there were multiple significant interactions in respect to soil water content involving growth CO_2 (Table 3-2). Over the course of the dry down there was a significant $CO_2 \times T$, $W \times T$ and $CO_2 \times W \times T$ interaction (Table 3-2). The $CO_2 \times T$ interaction was the result of minor variation in soil water content between CO_2 treatments at the beginning of the dry down (Fig. 3-4c) followed by larger differences as the dry down progressed. Conversely, the remaining interactions were largely the result of a contrasting pattern between watering treatments. Under well-watered conditions plants grown under eC_a maintained higher soil water content throughout the dry down, whereas under drought conditions plants grown under eC_a initially displayed higher soil water content but towards the end of the dry down soil water content dropped below plants grown under aC_a -D (Fig. 3-4c). Additionally, plants grown under well-watered conditions experienced a much more rapid decrease in soil moisture content, which was largely responsible for the $W \times T$ interaction and strongly influenced the $CO_2 \times W \times T$ interaction (Table 3-2; Fig. 3-4c).

There was a highly significant ($p < 0.0001$) effect of growth watering treatment (W), and a significant effect of growth CO_2 on daily transpiration, although uniquely for the effect of CO_2 a considerably different pattern was exhibited between plants grown under well-watered or drought conditions. Under well-watered conditions, daily transpiration was initially lower for plants grown under elevated CO_2 followed by a crossover on ~day 10 whereby daily transpiration was higher for plants grown under elevated CO_2 than plants grown under ambient CO_2 . In contrast, plants grown under elevated CO_2 and well-watered conditions maintained slightly higher rates of transpiration than plants grown under ambient CO_2 throughout the dry down, with this pattern being more pronounced in the later stages of the dry down. Additionally, there was a highly significant $W \times T$ interaction as plants grown under drought conditions arrived at pre-set water

stress points much later and therefore continued to transpire over the interim of the dry down (Table 3-2; Fig. 3-4d).

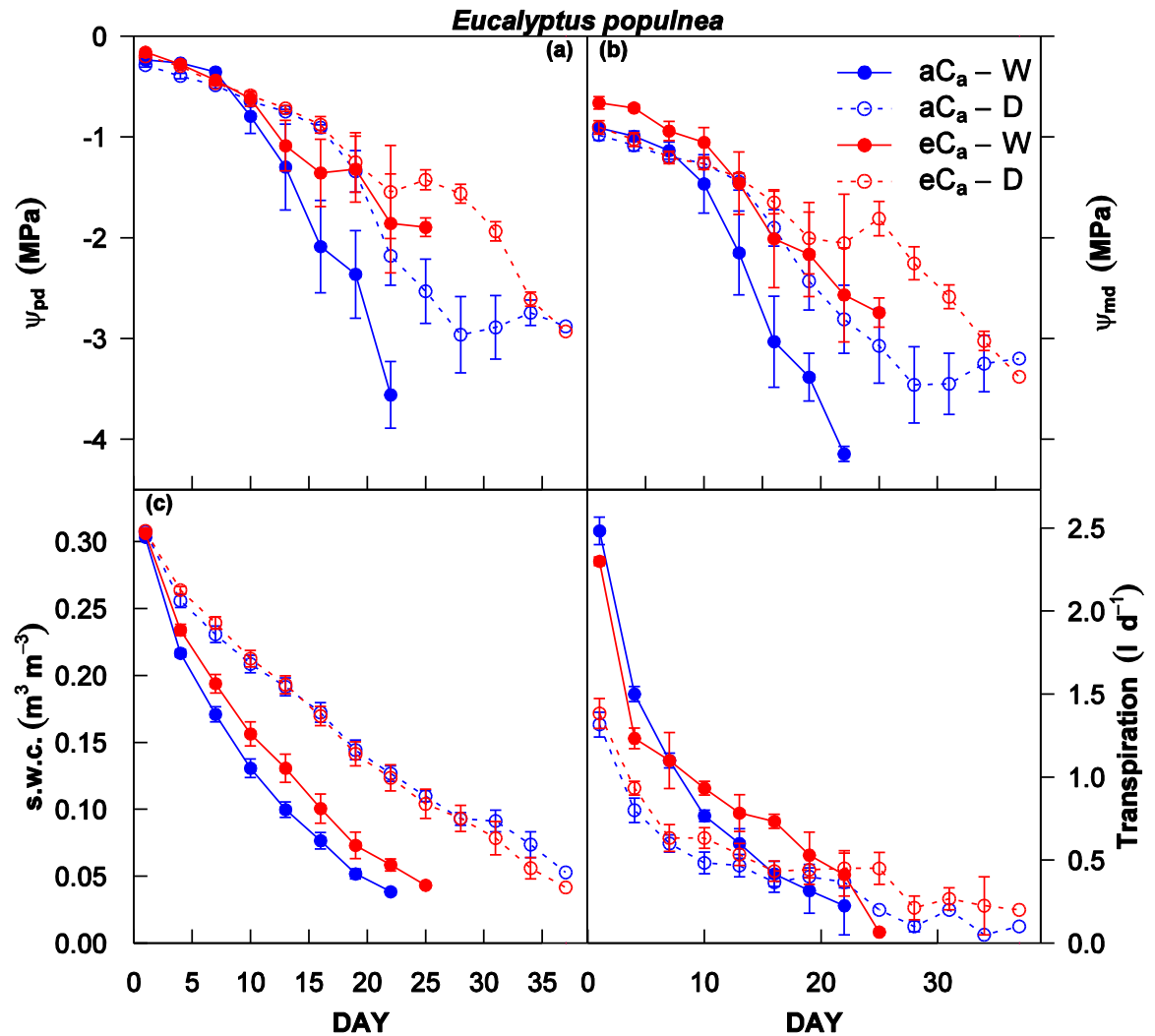


Figure 3-4. (a) Pre-dawn leaf water potential (Ψ_{pd}), (b) Midday leaf water potential (Ψ_{md}), (c) Soil water content and (d) Daily transpiration of *E. populnea* during a dry down in January 2011. Measurement CO_2 corresponds to growth CO_2 (ambient CO_2 : $380 \mu\text{mol m}^{-2}$; elevated CO_2 : $700 \mu\text{mol m}^{-2}$). Data are the means of 6 trees per treatment; error bars are ± 1 SE (*Exception on final days of dry down where $n=1-3$). Filled symbols – grown under well-watered conditions (100% FC); Open symbols – grown under drought conditions (50% FC). Blue symbols – grown under ambient CO_2 ; Red symbols – grown under elevated CO_2 .

3.3.3 Tree size – *Eucalyptus pilularis* and *Eucalyptus populnea*

To separate out the effect of tree size on carbon gain and water loss during the dry down period we replaced time (T) with pre-dawn leaf water potential (Ψ_{pd}) as the covariate in our analyses. Using Ψ_{pd} as the covariate allows us to evaluate the impact of drought stress on leaf level physiology, independent of plant size. Table 3-3 represents the size difference, from final harvest (see Ch. 2 for details), for plants grown under elevated or ambient CO₂ (growth CO₂) and well-watered or drought conditions (growth watering treatment).

Further, for gas exchange (A_{sat} and g_s) and daily transpiration we normalized the data for each plant. The data were normalized based on the maximum values measured (A_{sat} , g_s and daily transpiration) for each replicate when Ψ_{pd} was nearest to zero (i.e., Day 1 of each dry down). This normalization procedure was carried out to allow us to directly compare rates of decline in these parameters in response to the drying treatment, independent of their initial values.

Table 3-3. Biomass and leaf area data for mesic *E. pilularis* and xeric *E. populnea* grown under elevated or ambient CO₂, and well-watered or drought conditions. Data are the mean of 6 trees per treatment \pm 1 SE. Final harvest was conducted at completion of dry down for each species (Day 301 for *E. pilularis* and Day 377 for *E. populnea* of larger experiment).

<i>Eucalyptus pilularis</i>				
Parameter	Ambient CO ₂		Elevated CO ₂	
	Well-watered	Droughted	Well-watered	Droughted
Total plant mass (g)	1657 \pm 44	787 \pm 42	2578 \pm 105	1101 \pm 37
Total leaf area (m ²)	6.5 \pm 0.3	3.3 \pm 0.3	9.3 \pm 0.4	3.8 \pm 0.2
FR/LA (g m ⁻²)	18.5 \pm 3.2	26.3 \pm 4.6	26.8 \pm 3.7	21.5 \pm 2.8

<i>Eucalyptus populnea</i>				
Parameter	Ambient CO ₂		Elevated CO ₂	
	Well-watered	Droughted	Well-watered	Droughted
Total plant mass (g)	633 \pm 71	361 \pm 30	870 \pm 98	557 \pm 73
Total leaf area (m ²)	0.84 \pm 0.10	0.45 \pm 0.02	1.12 \pm 0.13	0.67 \pm 0.07
FR/LA (g m ⁻²)	147.5 \pm 16.0	198.6 \pm 18.9	166.2 \pm 14.2	212.7 \pm 30.9

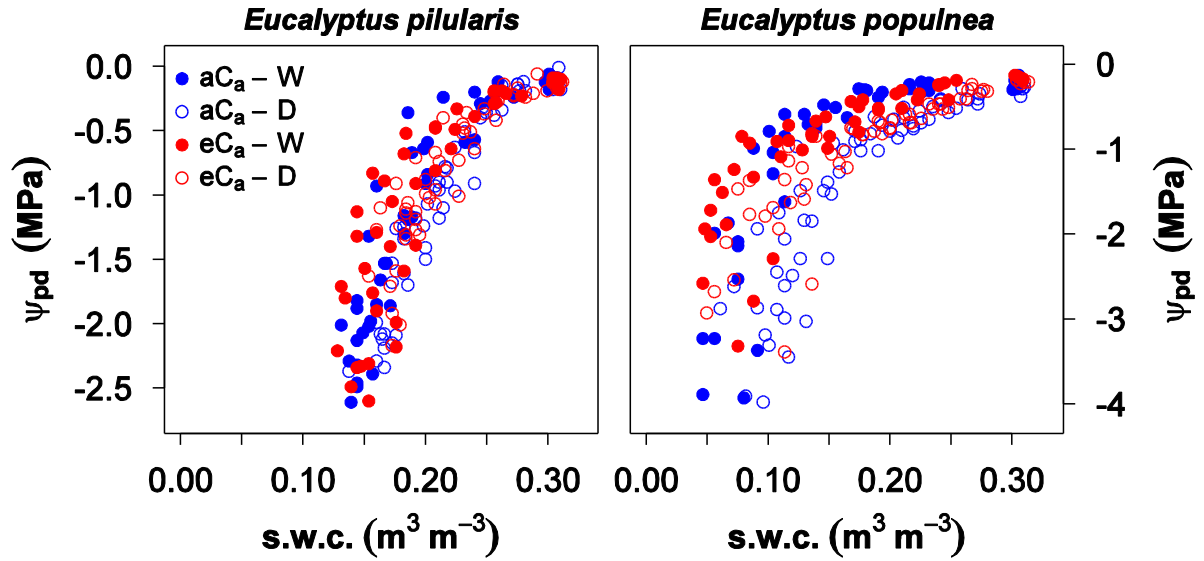


Figure 3-5. Relationship of pre-dawn leaf water potential (Ψ_{pd}) to soil water content (s.w.c.) for *E. pilularis* and *E. populnea* over their dry downs. Filled symbols – grown under well-watered conditions (100% FC); Open symbols – grown under drought conditions (50% FC). Blue symbols – grown under ambient CO_2 ; Red symbols – grown under elevated CO_2 . *Note scale difference between the two species.

Gas exchange (*E. pilularis* and *E. populnea*)

The response of normalized A_{sat} and g_s to growth CO_2 , growth watering treatment (W) and Ψ_{pd} for *E. pilularis* and *E. populnea* is presented in Table 3-4 and shown in Fig. 3-6. There was a highly significant ($p < 0.0001$) effect of increasing water stress (Ψ_{pd}) on the decline in normalized A_{sat} and g_s for *E. pilularis*. However, after accounting for changes in plant size in *E. pilularis*, there was no significant effect of growth CO_2 or growth watering treatment (W) on the rate of decline in A_{sat} or g_s , suggesting that for a given plant size, the time to peak water stress for both A_{sat} and g_s was little changed for plants grown under elevated or ambient CO_2 and well-watered or drought conditions (Fig. 3-6a & b).

Similarly for *E. populnea*, there was a highly significant ($p < 0.0001$) effect of increasing water stress (Ψ_{pd}) on the rate of decline in normalized A_{sat} and g_s . Additionally when accounting for the impact of plant size for *E. populnea*, there was no significant effect of growth CO_2 or

growth watering treatment (W) on the rate of decline in A_{sat} or g_s . However, there was a significant ($p < 0.05$) $\text{CO}_2 \times \Psi_{\text{pd}}$ interaction on A_{sat} for *E. populnea* as the larger plants (Table 3-3) grown under eC_a experienced a faster rate of decline from a maximum, particularly when nearing peak water stress, than plants grown under aC_a , irrespective of growth watering treatment (W) (Fig. 3-6c).

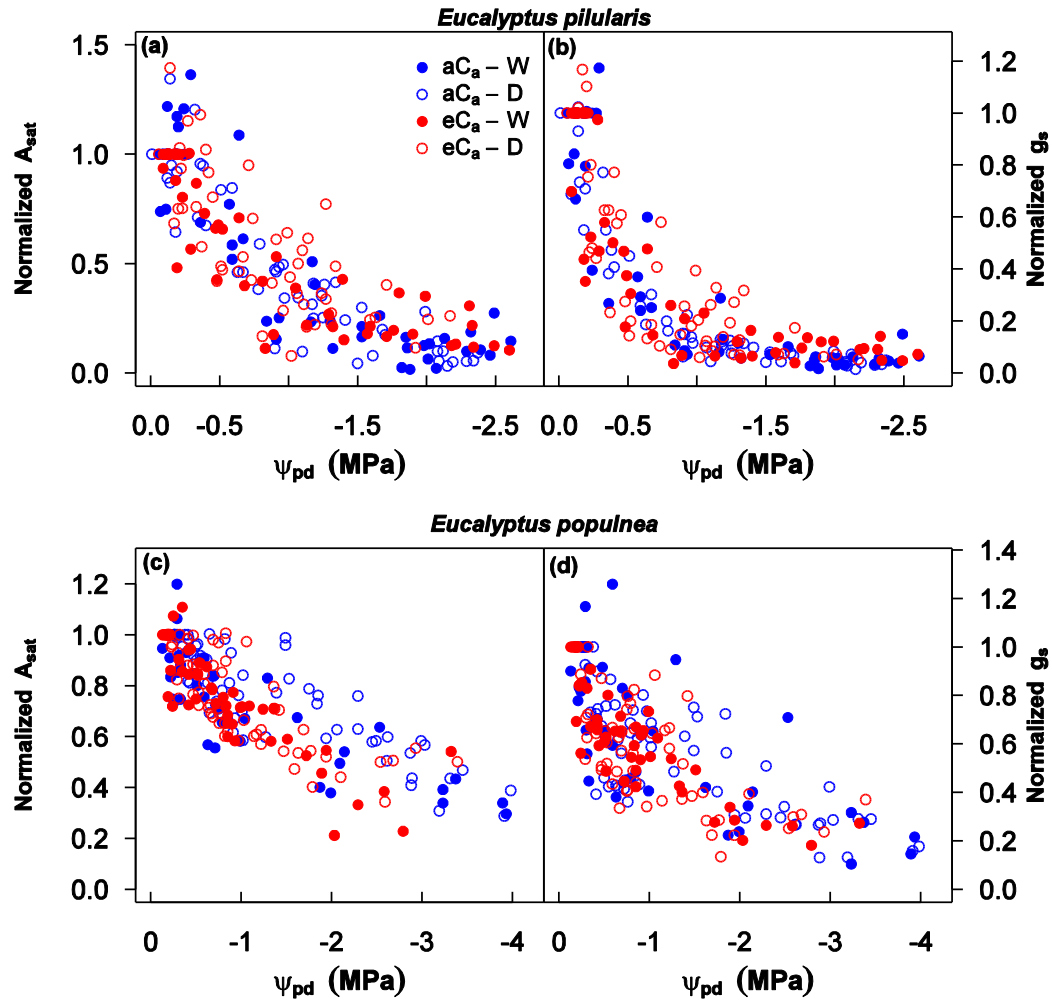


Figure 3-6. Normalized response of light saturated photosynthetic rate (A_{sat}) for *E. pilularis* (a) and *E. populnea* (c), and stomatal conductance (g_s) of *E. pilularis* (b) and *E. populnea* (d) to increasing water stress (Ψ_{pd}). The CO_2 concentration within the leaf cuvette during each measurement period corresponded to plant growth environment (ambient glasshouses: $380 \mu\text{mol m}^{-1}$; elevated glasshouses: $700 \mu\text{mol m}^{-1}$). Data represent normalized gas exchange measurements conducted during respective dry downs. Filled symbols – grown under well-watered conditions (100% FC); Open symbols – grown under drought conditions (50% FC). Blue symbols – grown under ambient CO_2 ; Red symbols – grown under elevated CO_2 . *Note scale difference between the two species.

Table 3-4. Linear mixed-effects model results (significance levels: * $P<0.05$; ** $P<0.01$; *** $P<0.001$) for normalized A_{sat} , g_s and daily transpiration of *E. pilularis* and *E. populnea* during respective dry down events. Numerator and denominator df are the numerator and denominator degrees of freedom for the F tests.

<i>Eucalyptus pilularis</i>	Main effects			Interactions			
Parameter	CO ₂	W	Ψ_{pd}	CO ₂ x W	CO ₂ x Ψ_{pd}	W x Ψ_{pd}	CO ₂ x W x Ψ_{pd}
Numerator df	1	1	1	1	1	1	1
Denominator df	20	20	169	20	169	169	169
Leaf gas exchange (Normalized)							
A_{sat} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	1.42	1.73	558.51***	0.59	2.27	2.22	1.61
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	1.13	0.12	329.35***	0.23	0.00	3.71	2.97
Plant water relations (Normalized)							
Daily transpiration (l day^{-1})	6.86*	1.61	1640.43***	0.50	0.20	11.09**	2.89

<i>Eucalyptus populnea</i>	Main effects			Interactions			
Parameter	CO ₂	W	Ψ_{pd}	CO ₂ x W	CO ₂ x Ψ_{pd}	W x Ψ_{pd}	CO ₂ x W x Ψ_{pd}
Numerator df	1	1	1	1	1	1	1
Denominator df	20	20	187	20	187	187	187
Leaf gas exchange (Normalized)							
A_{sat} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.09	0.12	486.86***	0.98	6.73*	3.31	0.11
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	0.04	3.42	293.00***	0.19	3.20	1.73	0.06
Plant water relations (Normalized)							
Daily transpiration (l day^{-1})	2.33	0.21	345.23***	0.08	9.77**	0.13	0.14

Table 3-5. Linear mixed-effects model results for apparent V_{cmax} of *E. pilularis* and *E. populnea* during respective dry down events. Num DF and Den DF are numerator and denominator degrees of freedom for the F -tests.

<i>Eucalyptus pilularis</i>				
	Num DF	Den DF	F value	P value
Intercept	1	152	996.72	<0.0001
CO ₂	1	20	0.75	0.40
W	1	20	1.62	0.22
Ψ_{pd}	1	152	216.78	<0.0001
CO ₂ x W	1	20	0.60	0.45
CO ₂ X Ψ_{pd}	1	152	3.53	0.06
W x Ψ_{pd}	1	152	0.16	0.69
CO ₂ x W x Ψ_{pd}	1	152	1.01	0.32

<i>Eucalyptus populnea</i>				
	Num DF	Den DF	F value	P value
Intercept	1	186	1904.89	<0.0001
CO ₂	1	20	5.63	<0.05
W	1	20	0.07	0.80
Ψ_{pd}	1	186	301.08	<0.0001
CO ₂ x W	1	20	0.63	0.44
CO ₂ X Ψ_{pd}	1	186	2.08	0.15
W x Ψ_{pd}	1	186	3.86	0.05
CO ₂ x W x Ψ_{pd}	1	186	0.33	0.56

Mesophyll limitations (apparent V_{cmax})

For *E. pilularis*, apparent V_{cmax} declined rapidly in response to water stress in all treatment combinations (Fig. 3-7). There was a highly significant ($p < 0.0001$) effect of Ψ_{pd} on the decline in apparent V_{cmax} for *E. pilularis*, whereas there was no significant effect of growth CO₂ or growth watering treatment (Table 3-5). However, there was a trend ($p = 0.06$) towards a CO₂ x Ψ_{pd} interaction for *E. pilularis* as the rate of decline in apparent V_{cmax} was progressively slowed as water stress increased for plants grown under elevated compared to ambient CO₂.

The decline in apparent V_{cmax} was much more conservative for *E. populnea* in response to increasing water stress (Fig. 3-7), although the effect of Ψ_{pd} was still highly significant ($p < 0.0001$) in respect to the rate of decline in apparent V_{cmax} (Table 3-5). Additionally for *E. populnea*, there was a significant ($p < 0.05$) effect of growth CO₂ on apparent V_{cmax} as the larger plants (Table 3-3) grown under eC_a exhibited a consistently lower

apparent V_{cmax} than plants grown under aC_a , irrespective of growth watering treatment (Fig. 3-7; Table 3-5). However, there was a strong trend ($p=0.05$) for *E. populnea* towards a $W \times \Psi_{\text{pd}}$ interaction as plants grown under well-watered conditions exhibited a lower apparent V_{cmax} as water stress intensified than plants grown under drought conditions (Fig. 3-7; Table 3-5).

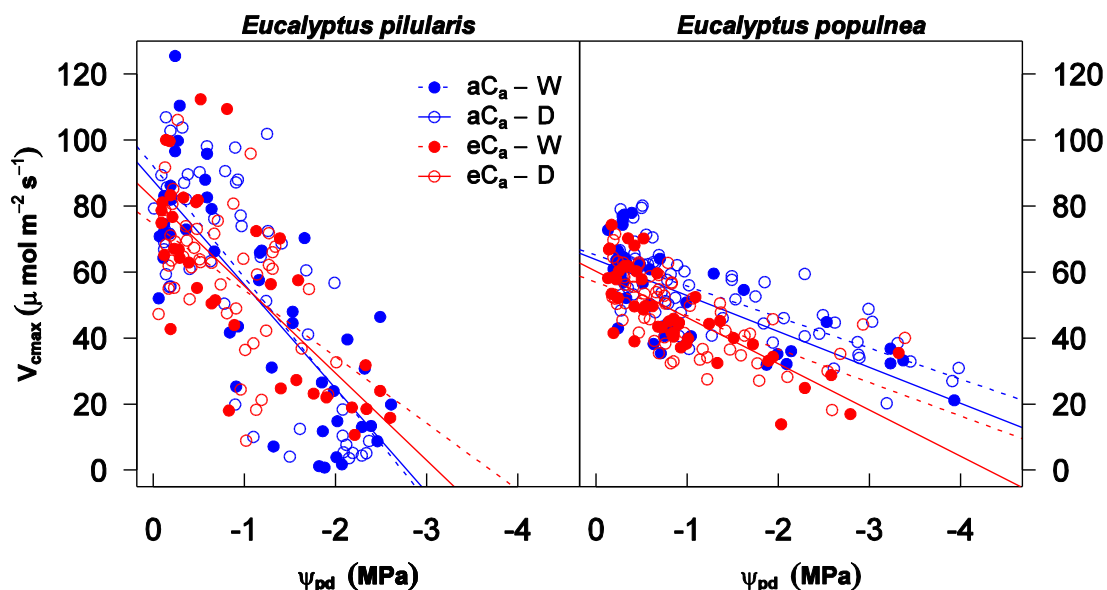


Figure 3-7. Response of apparent V_{cmax} to increasing water stress for *E. pilularis* and *E. populnea*. Data are calculated V_{cmax} from gas exchange measurements conducted during morning hours throughout the dry down. The CO_2 concentration within the leaf cuvette during each measurement period corresponded to plant growth environment (ambient glasshouses: $380 \mu\text{mol m}^{-2}$; elevated glasshouses: $700 \mu\text{mol m}^{-2}$). Filled symbols – grown under well-watered conditions (100% FC); Open symbols – grown under drought conditions (50% FC). Blue symbols – grown under ambient CO_2 ; Red symbols – grown under elevated CO_2 . *Note scale difference between the two species.

Marginal water cost of carbon gain (g_1)

For *E. pilularis*, the effect of the dry down on g_1 was very rapid, with a severe drop in g_1 corresponding to a small decrease in Ψ_{pd} (Fig. 3-8). There was a distinct contrast between early and late stage water stress on the response of g_1 for *E. pilularis* (Fig. 3-8).

Consequently, we chose to analyze the response of g_1 to Ψ_{pd} in two separate bins (Early stage: $\Psi_{\text{pd}} > -0.5 \text{ MPa}$; Late stage: $\Psi_{\text{pd}} < -0.5 \text{ MPa}$). During the initial stages of water stress ($\Psi_{\text{pd}} > -0.5 \text{ MPa}$) there was a highly significant ($p < 0.0001$) effect of Ψ_{pd} on the rate of decline in g_1 . Further, during the early stage of water stress there was a significant ($p < 0.05$) effect of growth CO_2 as a result of a slower decline in g_1 and separately an initial lower value of g_1 during the

initial stages of water stress for plants grown under elevated than ambient CO₂, irrespective of growth watering treatment (Table 3-6a; Fig. 3-8).

After the initial highly precipitous decline in g_1 in response to a small decrease in Ψ_{pd} for *E. pilularis*, there was a much more constrained response of g_1 to Ψ_{pd} throughout the remainder of the dry down period (Fig. 3-8). During the late stage of water stress ($\Psi_{pd} < -0.5$ MPa) there remained a significant ($p < 0.01$) main effect of growth CO₂ as plants grown under eC_a maintained consistently lower g_1 during the late stage of water stress than plants grown at aC_a (Table 3-6b; Fig. 3-8). In addition, with increasing water stress ($\Psi_{pd} < -0.5$ MPa) there was a significant growth CO₂ x Ψ_{pd} and growth CO₂ x W x Ψ_{pd} interaction. Both interactions were largely attributable to a less severe water stress experienced by plants grown under eC_a-D, and secondly a lower overall g_1 for plants grown under eC_a (Fig. 3-8).

Table 3-6. Linear mixed-effects model results for g_1 of *E. pilularis* during the dry down period. Num DF and Den DF are numerator and denominator degrees of freedom for the *F*-tests. (a) Represents binned values of $\Psi_{pd} \geq (-0.5$ MPa) and (b) represents binned values of $\Psi_{pd} < (-0.5$ MPa). Num DF is the same for both (a) and (b).

(a)					(b)		
	Num DF	Den DF	<i>F</i> value	<i>P</i> value	Den DF	<i>F</i> value	<i>P</i> value
Intercept	1	41	743.39	<0.0001	97	463.99	<0.0001
CO ₂	1	20	6.23	<0.05	20	10.58	<0.01
W	1	20	1.04	0.32	20	0.19	0.67
Ψ_{pd}	1	41	48.31	<0.0001	97	0.98	0.32
CO ₂ x W	1	20	2.55	0.13	20	4.50	<0.05
CO ₂ x Ψ_{pd}	1	41	0.76	0.39	97	3.56	0.06
W x Ψ_{pd}	1	41	0.52	0.48	97	0.24	0.63
CO ₂ x W x Ψ_{pd}	1	41	1.20	0.28	97	7.00	<0.01

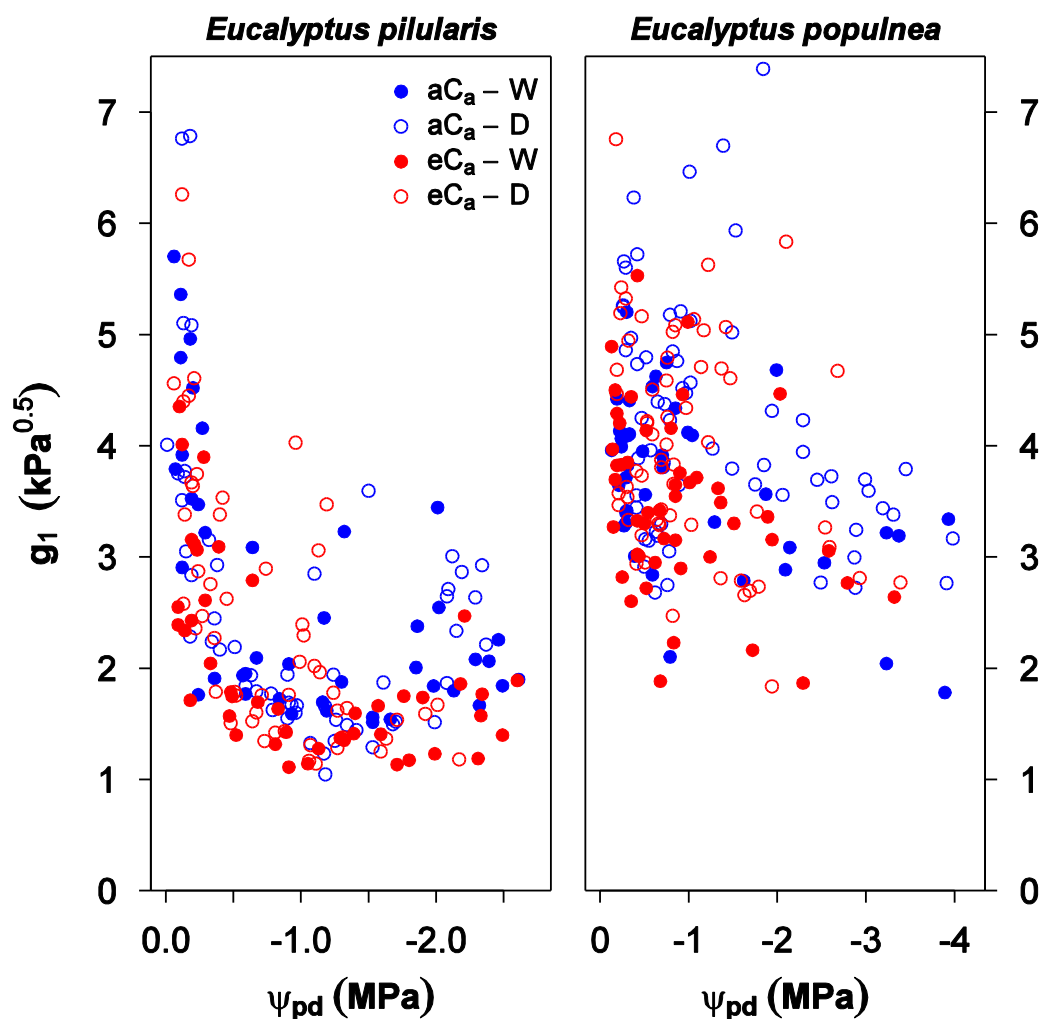


Figure 3-8. g_1 response to increasing water stress for *E. pilularis* and *E. populnea*. Data are calculated g_1 (eqn. 1) from gas exchange measurements conducted during morning hours throughout the dry down. The CO_2 concentration within the leaf cuvette during each measurement period corresponded to plant growth environment (ambient glasshouses: $380 \mu\text{mol m}^{-1}$; elevated glasshouses: $700 \mu\text{mol m}^{-1}$). Filled symbols – grown under well-watered conditions (100% FC); Open symbols – grown under drought conditions (50% FC). Blue symbols – grown under ambient CO_2 ; Red symbols – grown under elevated CO_2 . ***Note scale difference between the two species.**

For *E. populnea*, the effect of the dry down on g_1 was delayed, with a fairly conservative overall decline in response to decreasing Ψ_{pd} (Fig. 3-8). In contrast to *E. pilularis* there was not a distinct sharp decline in g_1 for *E. populnea* in response to a small decrease in Ψ_{pd} (Fig. 3-8). As a result we analyzed g_1 for *E. populnea* in response to the full range of Ψ_{pd} , rather than binned at Ψ_{pd} intervals as for *E. pilularis*. Across the full range of Ψ_{pd} for *E. populnea*, there was a significant ($p < 0.01$) effect of growth watering treatment (W)

as g_1 for plants grown under drought conditions remained slightly above and declined less quickly than for plants grown under well-watered conditions (Fig. 3-8). In addition, there was a highly significant ($p < 0.0001$) effect of Ψ_{pd} on the decline in g_1 for *E. populnea*, irrespective of growth CO_2 or growth watering treatment (Fig. 3-8; Table 3-7).

Table 3-7. Linear mixed-effects model results for g_1 of *E. populnea* during the dry down period. Num DF and Den DF are numerator and denominator degrees of freedom for the F -tests.

	Num DF	Den DF	F value	P value
Intercept	1	187	3093.85	<0.0001
CO_2	1	20	1.36	0.26
W	1	20	6.82	<0.05
Ψ_{pd}	1	187	45.07	<0.0001
$CO_2 \times W$	1	20	0.13	0.72
$CO_2 \times \Psi_{pd}$	1	187	0.59	0.44
$W \times \Psi_{pd}$	1	187	0.48	0.49
$CO_2 \times W \times \Psi_{pd}$	1	187	0.13	0.72

Water relations

For both species, transpiration rates over the dry downs were normalized based on the maximum rate of transpiration when Ψ_{pd} was nearest to zero (i.e., Day 1 of each dry down). The response of normalized daily transpiration to growth CO_2 , growth watering treatment (W) and Ψ_{pd} for *E. pilularis* and *E. populnea* is shown in Fig. 3-9 and Table 3-4. There was a highly significant ($p < 0.0001$) effect of Ψ_{pd} on the rate of decline in daily transpiration for *E. pilularis*. There was also a significant ($p < 0.05$) effect of growth CO_2 on the rate of decline in daily transpiration for *E. pilularis*. However, the effect of CO_2 was represented differentially for *E. pilularis* depending on watering regime; under eC_a -W there was a moderately slower rate of decline in daily transpiration than plants grown under aC_a -W, whereas under eC_a -D there was a quicker rate of decline in daily transpiration than plants grown under aC_a -D, particularly as water stress intensified (Fig. 3-9), although importantly the interaction ($CO_2 \times W$) was not statistically significant. However as a result of the contrasting responses in daily transpiration with growth watering treatment (W), particularly at peak water stress, there was a significant ($p < 0.01$) $W \times \Psi_{pd}$ interaction.

For *E. populnea*, there was a similar highly significant ($p < 0.0001$) effect of Ψ_{pd} on the rate of decline in normalized daily transpiration (Table 3-4; Fig. 3-9). Conversely, there was

no significant effect of growth CO_2 or growth watering treatment (W). However there was a significant ($p < 0.05$) $\text{CO}_2 \times \Psi_{\text{pd}}$ interaction as a result of a slower decline in normalized daily transpiration for eC_a plants during the early stages of water stress, followed by a comparable decline between plants grown at elevated or ambient CO_2 as water stress intensified (Table 3-4; Fig. 3-9).

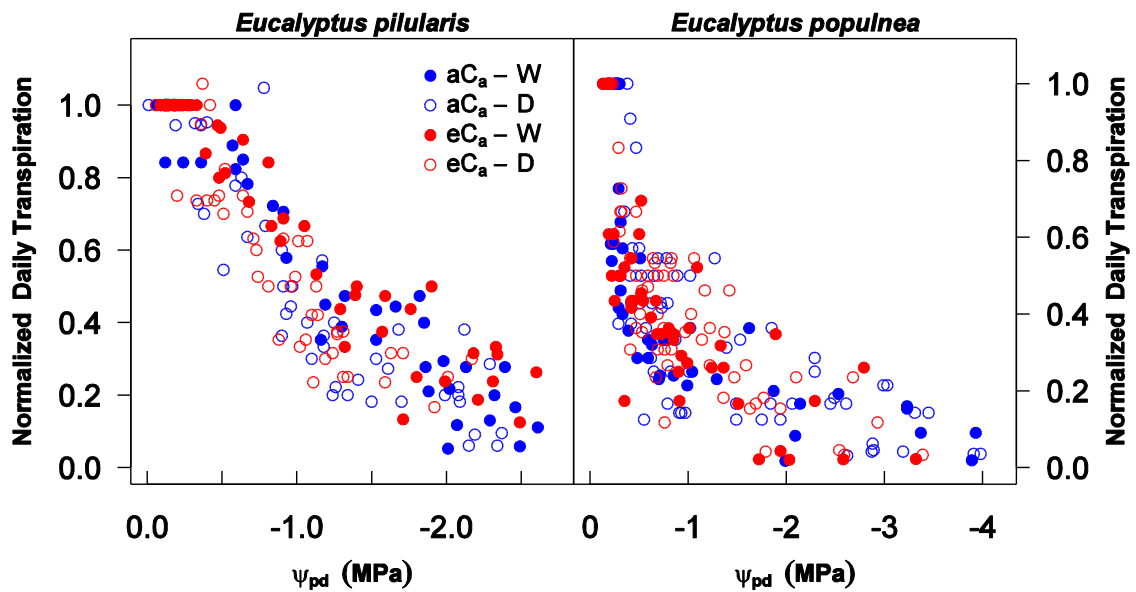


Figure 3-9. Normalized response of daily transpiration to increasing water stress for *E. pilularis* and *E. populnea*. For both species, transpiration rates over the dry down were normalized based on the maximum rate of transpiration when Ψ_{pd} was nearest to zero (i.e., Day 1 of each dry down). Filled symbols – grown under well-watered conditions (100% FC); Open symbols – grown under drought conditions (50% FC). Blue symbols – grown under ambient CO_2 ; Red symbols – grown under elevated CO_2 .

3.4 Discussion

Leaf level

At the leaf scale, there was a significant effect of elevated CO_2 on photosynthesis throughout the dry downs in both well-watered and droughted plants, leading to a consistently higher A_{sat} for both mesic *E. pilularis* and xeric *E. populnea*. A sustained increase in photosynthesis, even under drought conditions, is a common observation of many elevated CO_2 experiments in grassland and crop species (Morgan *et al.* 2004; Robredo *et al.* 2007), and forests (Leuzinger and Körner 2007).

Droughted plants also had significantly higher A_{sat} compared to well-watered plants, particularly in the latter stages of the dry downs (Fig. 3-1a, 3-3a). This drought effect was the result of a large difference in overall plant size. Plants of both species grown under drought conditions were considerably smaller than plants grown under well-watered conditions (Table 3-3). The smaller plants had lower daily transpiration rates, particularly in the early stages of their dry downs, slowing the rate of soil drying and thus maintaining a higher A_{sat} for both species.

In contrast to the effect on A_{sat} , a sustained effect of elevated CO_2 on g_s during the drydown was only observed for xeric *E. populnea*. In *E. pilularis* g_s was reduced significantly only during the early stages of its dry down. Similar findings of a limited CO_2 effect on reduced g_s and concomitant increase in A_{sat} during drought, as was observed for *E. pilularis*, have been reported by numerous authors for temperate and tropical tree species (Catovsky and Bazzaz 1999; Centritto *et al.* 1999, 2002; Cernusak *et al.* 2011). Notably, between *E. pilularis* and *E. populnea* there was a markedly different diurnal pattern in g_s , with mesic *E. pilularis* displaying a larger reduction in g_s at midday, while xeric *E. populnea* maintained similar g_s regardless of time of day. However, the diurnal pattern in g_s exhibited by each species was not affected by growth CO_2 or growth watering treatment, but was rather a species specific response to daily water stress.

Elevated CO_2 delayed the impact of pre-dawn leaf water stress only during the latter stages of respective dry down events for both *E. pilularis* and *E. populnea*. However, for *E. pilularis* grown under $\text{eC}_a\text{-W}$ the effect of elevated CO_2 on a delay in water stress was not statistically significant. In the initial stages of soil drying for *E. pilularis*, Ψ_{pd} was similar for plants grown under elevated or ambient CO_2 and well-watered or drought conditions. However, there was a large difference in the timing of water stress between growth watering treatments (W) for *E. pilularis*, with plants grown under $\text{eC}_a\text{-D}$ experiencing a delay in pre-dawn water stress from ~day 4 onwards compared to plants grown under $\text{aC}_a\text{-D}$, whereas plants grown under $\text{eC}_a\text{-W}$ did not exhibit a delay until ~day 6 (Fig. 3-2a). In contrast, for *E. populnea* a delay in pre-dawn water stress was more pronounced for plants grown under $\text{eC}_a\text{-W}$ with a delay developing from ~ day 15, whereas plants grown under $\text{eC}_a\text{-D}$ did not exhibit a delay compared to plants grown under $\text{aC}_a\text{-D}$ until ~day 22 (Fig. 3-4a). Similar results have been reported by numerous authors, with higher leaf water potentials (less negative), under elevated CO_2 , most often observed during the latter stages of imposed drought stress or when natural drought events are most severe (Rogers *et al.* 1994; Tognetti *et al.* 2000). In contrast for Ψ_{md} there was a strong effect of elevated CO_2 for both species throughout their dry downs.

However, the CO₂ effect for *E. pilularis* was overwhelmingly directed by Ψ_{md} of plants grown under eC_a-D, which maintained a much higher (less negative) Ψ_{md} than aC_a-D plants throughout the dry down, whereas little difference in Ψ_{md} was observed between plants grown under eC_a-W or aC_a-W (Fig. 3-2b).

At the leaf scale there was a rapid reduction over the dry down in apparent V_{cmax} and g_1 for *E. pilularis* (Fig. 3-6, 3-7). An effect of elevated CO₂ was only present in the latter stages of the dry down for *E. pilularis* and only for g_1 (Table 3-6). However, the effect of elevated CO₂ on g_1 for *E. pilularis* was linked to tree size, as larger plants (Table 3-3) grown under eC_a displayed a lower g_1 with increasing water stress than plants grown under aC_a. Between the two species (*E. pilularis* and *E. populnea*), the impact of tree size was even more explicit in regards to the rate of decline in both apparent V_{cmax} and g_1 to increasing water stress (Fig. 3-6, 3-7). Of the two, mesic *E. pilularis* is much faster growing and consequently was considerably larger, under elevated and ambient CO₂, at the beginning of its respective dry down than *E. populnea*. Accordingly, the rate of decline in apparent V_{cmax} and g_1 was much more rapid for mesic *E. pilularis*, with xeric *E. populnea* displaying a comparatively conservative decline in both parameters (Fig. 3-6, 3-7). H  roult *et al.* (2012) found a similar result with humid zone *Eucalyptus* species exhibiting a faster decline in g_1 during drought than sub-humid zone *Eucalyptus* species. Partly, this slower decline in g_1 with increasing water stress for sub-humid or xeric species is accounted for by physiological responses to increasing water stress but also likely ties in to species inherent biomass allocation strategies and life history.

Whole tree level - Daily

Growth under elevated CO₂ did not delay the overall rate of soil drying for *E. pilularis*, as represented by similar soil water content for plants grown under elevated or ambient CO₂ and well-watered or drought conditions (Fig. 3-2c). A similar result was reported by Centritto *et al.* (2002) for cherry seedlings, with a negligible effect of elevated CO₂ on slowing the rate of soil drying during a dry down event. For *E. pilularis*, however, there was a slight and significant ($p < 0.05$; CO₂ x W x T interaction) increase in soil water content as the dry down progressed (~day 7 onwards) for plants grown under eC_a-D, whereas the opposite occurred under eC_a-W with a decrease from ~day 7 forward (Fig. 3-2c). For *E. populnea*, an overall effect of elevated CO₂ was similarly not present, although there was a significant ($p < 0.01$; CO₂ x T interaction) and sustained delay in soil drying (higher soil water

content) from ~day 3 onwards for plants grown under eC_a-W, whereas plants grown under eC_a-D exhibited similar soil water content to plants grown under aC_a-D throughout the dry down (Fig. 3-2c).

The overall effect of elevated CO₂ did not alter rates of daily transpiration for *E. pilularis*. However, there was a distinct change in the response of daily transpiration to CO₂ enrichment over time; during the early stages of the dry down there was a lower rate of daily transpiration (although not statistically significant) for plants grown under eC_a compared to aC_a, followed by a significantly higher rate from ~day 5 onwards, irrespective of growth watering treatment (Fig. 3-2d). A similar result was observed by Picon *et al.* (1996) for *Quercus robur* and Centritto *et al.* (1999) for *Prunus avium*. Conversely, for *E. populnea* there was a significant overall effect of elevated CO₂ on daily transpiration throughout its respective dry down. However, the effect of elevated CO₂ on daily transpiration for *E. populnea* was exhibited differentially depending on growth watering treatment (Fig. 3-4d); resulting in a sustained increase in daily transpiration for plants grown under eC_a-D compared to aC_a-D, and conversely an initial lower rate of daily transpiration for plants grown under eC_a-W followed by an increase as the dry down progressed (~day 7).

Whole tree level – Overall

On a leaf level basis there was a noticeable effect of elevated CO₂ on delaying water stress for *E. populnea* grown under well-watered and drought conditions, and *E. pilularis* grown under drought conditions, exhibited by a sustained increase in A_{sat}, and a higher (less negative) pre-dawn Ψ_{pd} in the latter stages of their dry downs. However, at the whole tree scale responses were much more nuanced. This was strongly tied to differences in plant size and biomass allocation (Table 3-3). At a physiological level, mesic *E. pilularis* exhibited very tight controls on g_s as water stress increased, nearing complete stomatal closure when water stress neared a species-specific minimum Ψ_{pd} (Fig. 3-1d). In contrast, xeric *E. populnea* displayed a more markedly delayed reduction in g_s to increasing water stress on both a diurnal and longer basis (Fig. 3-3d-f). For xeric *E. populnea* this rather weak control of g_s , with increasing water stress, was coordinated with inherent biomass allocation strategies. Between the two species, xeric *E. populnea* displayed a much greater relative allocation to rooting mass and additionally a much larger increase in the ratio of fine root mass to leaf area (FR:LA) (Table 3-3). A higher FR:LA could presumably lead to a more favorable water balance (Wullschleger *et al.* 2002), and has been identified as a common

morphological response under elevated CO₂ in place of, or rather than, an adjustment in g_s (Norby and O'Neill 1991; Norby *et al.* 1999). Closely in line with that, *E. populnea* grown under eC_a displayed an even greater increase in FR:LA than under aC_a (Table 3-3), which consequently resulted in a more favorable water balance over the dry down for plants grown under eC_a. However for *E. populnea*, this structural acclimation to long-term growth under eC_a was complemented by a CO₂ induced reduction in g_s during the dry down period. These results suggest both a positive long-term (i.e., structural adjustment) and short-term physiological effect of elevated CO₂ on xeric *E. populnea* during periods of varying drought intensity.

In addition, the impact of plant size was highly important in determining the time to water stress for both species. For example, the relative increase in total biomass and leaf area was considerably less for *E. populnea* grown under eC_a-W than eC_a-D (Table 3-3). This resulted in a stronger delay in the time to water stress at the leaf (Ψ_{pd}) and whole plant scale (soil water content) for plants grown under eC_a-W compared to eC_a-D. Conversely, for *E. pilularis* there was a greater relative increase in total biomass and leaf area for plants grown under eC_a-W than eC_a-D, with similar consequences towards the time to peak water stress. Due to the much larger plant size and transpiring leaf area (Table 3-3), *E. pilularis* grown under eC_a-W did not experience a significant delay in water stress at the leaf or at the whole plant scale (Fig. 3-2a & c; Table 3-1). In comparison, plants grown under eC_a-D, with 60% less leaf area than plants grown under eC_a-W, experienced a considerable slowing in the time to peak water stress at the leaf scale (Fig. 3-2a). This occurred without a concomitant increase in FR:LA (Table 3-3) for *E. pilularis* under eC_a-D, suggesting a reduction in leaf area with long-term growth under moderate drought conditions (50% FC) was sufficient to delay water stress at the leaf scale during drought of greater intensity.

An additional mechanism with the potential to delay the time to peak water stress, such as was observed for mesic *E. pilularis* grown under eC_a-D, is a lowering of osmotic potential. A delay in water stress under elevated CO₂ is often accounted for by increased rates of carbon assimilation providing greater substrate for osmotic adjustment, thereby lowering osmotic potential at full turgor (π_o) (Wullschleger *et al.* 2002). This adjustment with CO₂ enrichment has been found to operate under both well-watered and drought conditions for grassland and tree species (Morse *et al.* 1993; Ferris and Taylor 1994; Vivin *et al.* 1996; Johnson *et al.* 2002), although it is often found to be minimal (Polley *et al.* 1996, 1999; Picon-Cochard and Guehl 1999) or not present when sink demand for soluble carbohydrates is high (Tschaplinski *et al.* 1995; Wullschleger *et al.* 2002). In the early stages of water

stress, growth declines more than photosynthesis, leading to conservation, or even an increase, of carbohydrate reserves (McDowell 2011), thus providing the necessary substrate towards osmotic adjustment. Throughout its dry down, *E. pilularis* grown under eC_a-D maintained a considerably higher A_{sat}, while also exhibiting similar rates of transpiration and soil drying as plants grown under aC_a-D (Fig. 3-1, 3-2). This parallel pattern in soil drying was not matched by a parallel decline in Ψ_{pd} (Fig. 3-2a and c), suggesting an additional mechanism, such as osmotic adjustment, was in operation in order to sustain a higher Ψ_{pd} as drought progressed. An enhanced osmotic adjustment could additionally compensate for the reduced allocation to fine roots of *E. pilularis* grown under eC_a-D, as was observed by Vivin *et al.* (1996) for *Quercus robur*, however the consequences of enhanced osmotic adjustment for whole plant growth during periods of increasing water stress is uncertain (Vivin *et al.* 1996; Wullschleger *et al.* 2002).

Conclusion

Elevated CO₂ delayed water stress during the dry down for xeric *E. populnea* grown under both well-watered and drought conditions, as a result of a CO₂ induced reduction in g_s . For mesic *E. pilularis* there was also a CO₂ induced reduction in g_s during the dry down which resulted in a delay in water stress at the leaf scale for plants grown under drought conditions. However, the considerable increase in biomass and leaf area for *E. pilularis* grown under elevated CO₂ and well-watered conditions cancelled out leaf level water savings, resulting in no statistically significant delay in water stress at the leaf or whole plant scale. For both species, these results were strongly tied to differences in allocation and physiological responses. A rapid decline in g_1 for mesic *E. pilularis* was linked to tighter controls on g_s with increasing water stress, a response enacted as the cost of water becomes relatively more expensive in respect to both acquisition and transport. Conversely, a comparatively conservative decline in g_1 as represented by xeric *E. populnea* ties into a pattern of increased biomass allocation to the plant vascular system in lieu of rapid stomatal closure under increasing water stress. These results suggest that xeric *E. populnea* with a greater inherent adaptive capacity to adjust structurally to varying water availability may experience a greater benefit with CO₂ enrichment, through a complementary reduction in g_s and associated water savings, than mesic *E. pilularis* which exhibits a limited ability to adjust structurally to endure drought of increasing intensity and instead relies upon rapid stomatal closure to avoid increasing water stress. Consideration of these findings should assist with modelling

outcomes of *Eucalyptus* species response to changing climate in xeric and mesic environments, where projected increases in temperature will lead to greater evaporative demand and potentially more negative consequences for species with limited capacity to adjust both physiological and hydraulic functioning (Warren *et al.* 2011).

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CHAPTER 4

Response to temperature at the leaf scale of Australian tropical rainforest species; Stomatal versus biochemical limitations

Summary Understanding the effect of temperature on tropical rainforests is an issue of ever growing importance due to the continuing rise in atmospheric CO₂ concentration and associated rise in temperature. Many models of forest response to climate change utilize general biochemical parameters for C₃ species, which may lead to exaggerated estimates on the effect of temperature on photosynthetic carbon gain. In this experiment I measured the biochemical and stomatal limitations to leaf level photosynthesis in response to temperature on two canopy species at the Australian Canopy Crane Research Station (ACCRS) in Cape Tribulation, Queensland Australia. Data were used to perform a sensitivity analysis of a coupled photosynthesis-stomatal model, comparing rainforest parameter values with two parameter sets commonly used for modeling from Leuning (2002) and Kattge and Knorr (2007). We found that the generally used parameters for C₃ species may lead to an under prediction of T_{opt} and in relation an under prediction of maximum photosynthetic rate, for tropical rainforest species. The biochemical factors most strongly contributing to this disparity was the activation energy (H_a) of V_{cmax} and J_{max}. The analysis showed that general parameters for C₃ species commonly used in global scale models of forest responses to climate change under predict the optimum temperature of photosynthesis for tropical forest species, even when temperature acclimation is taken into account. The parameter values obtained in this study will prove useful for improving global vegetation models.

4.1 Introduction

Global scale models looking at the impact of rising temperature, linked to climate change, often predict increasing dieback of tropical forests (Cox *et al.* 2000, 2004; Betts *et al.* 2004), and numerous researchers have hypothesized that increasing temperatures will have an exceedingly negative impact on tropical forest health and productivity (Clark *et al.* 2003; Clark 2004; Feeley *et al.* 2007). A release of carbon from tropical forests, due to increasing dieback, could potentially exacerbate future climate change (Cox *et al.* 2013). However, key uncertainties remain in current Earth system models regarding the magnitude of the effect on tropical forests (Huntingford *et al.* 2013), with a particular uncertainty on the effect of thermal

acclimation and adaption of tropical forests to warming (Kattge and Knorr 2007; Lloyd and Farquhar 2008). A key component directing the impact of climate change on tropical forests is the strength of the warming effect on leaf level physiology (Loescher *et al.* 2003). However, little is known concerning the biochemical response to temperature of tropical forest species (Kattge and Knorr 2007), even though these forests account for over 30% of global terrestrial primary production (Malhi *et al.* 2006). Similarly, the effect of rising temperatures on leaf level physiological processes such as stomatal conductance and respiration is highly uncertain for tropical forests (Doughty 2011). Determining the biochemical response of tropical forests *in vivo* to temperature may provide greater insight into the direct causes for reductions in photosynthesis and growth, such as for example the irreversible damage to biochemical machinery at extreme temperatures (Berry and Björkman 1980). Additionally, the findings of limited impact of temperature on biochemical properties may suggest a greater focus on the indirect causes for reductions in photosynthesis and growth, such as high vapor pressure deficit (D) causing stomatal closure or the impact of increasing temperature on respiration (Lloyd and Farquhar 2008; Doughty and Goulden 2008).

Models of forest response to climate change often use the Farquhar, von Caemmerer and Berry (1980) biochemical model of photosynthesis. In the Farquhar *et al.* (1980) model, leaf photosynthesis is considered to be limited by either the maximum Rubisco carboxylation capacity (V_{cmax}) or the maximum RuBP regeneration capacity (J_{max}). Importantly, there is considerable variation in the key parameters of this model (V_{cmax} and J_{max}) between C_3 plants and environmental conditions (Berry and Björkman 1980; Wullschlegel 1993), with a particular sensitivity to temperature (Medlyn *et al.* 2002a; Kattge and Knorr 2007). Much of this variation between species and environments is linked to both adaptation (i.e. evolutionary changes to environment) and acclimation (i.e. short to long-term adjustments at the organismal level) to growth environment (Berry and Björkman 1980; Hikosaka *et al.* 2006). Hence in an adaptation sense species from cold environments are expected to display a lower temperature optimum for photosynthesis than species from warmer environments (Berry and Björkman 1980). Acclimation on the other hand is typically related to seasonal adjustments in the temperature optimum of photosynthesis (e.g. Slatyer 1977a; Slatyer and Morrow 1977; Ferrar *et al.* 1989) or in the activation energy of V_{cmax} and J_{max} (Battaglia *et al.* 1996; Medlyn *et al.* 2002b; Han *et al.* 2004; Gunderson *et al.* 2010).

Rising temperatures associated with climate change may additionally impact carbon uptake through effects on stomatal conductance (g_s) (Bunce 2000; Doughty and Goulden

2008; Lin *et al.* 2012). The impact of temperature on g_s occurs through the relationship of g_s with leaf to air vapor pressure deficit (D). An increase in temperature typically increases D which leads to stomatal closure, unless relative humidity increases along with temperature. Because the absolute humidity of the air is more or less stable throughout the day in rainforest environments (Shuttleworth *et al.* 1985), daily variation in temperature serves as the major driver of variation in D in these environments (Lloyd and Farquhar 2008). Consequently, identifying stomatal sensitivity to parallel increases in temperature and D is also a key component process to better understand in regards to photosynthetic response to temperature of tropical rainforests.

The objective of this current study was threefold; the first was to provide some of the first *in situ* measures of photosynthetic response to temperature for tropical rainforest species. The second was to evaluate these measures to determine the relative influence of photosynthetic biochemistry and stomatal control on the response of leaf net photosynthesis (A_{net}) to temperature, using a coupled photosynthesis-stomatal (A_n-g_s) model. The third primary objective was to compare our collected measures for tropical rainforest tree species with current parameters of leaf biochemistry utilized in many process-based models in order to determine the generality of parameters or to identify areas where more data is needed. The initial step in this process was conducting a sensitivity analysis of the relevant biochemical and stomatal parameters from Leuning (2002) and Kattge and Knorr (2007); followed by a comparison of these parameters with our collected measures to quantify the relative sensitivity of the optimum temperature (T_{opt}) of net photosynthesis (A_{net}) to biochemical and stomatal processes. Lastly, to gain a better understanding of the mechanistic causes leading to differences in the temperature response of A_{net} , we applied the A_n-g_s model to *in situ* photosynthetic data collected in two field experiments. These two separate field experiments covered tropical rainforest trees in Cape Tribulation, Queensland, Australia and a temperate *Eucalyptus* species (temperate broadleaved evergreen) in south-eastern NSW, Australia. Between the two plant functional types (tropical rainforest and temperate broadleaved evergreen) there was considerable difference in the leaf net photosynthesis to temperature responses. As a final step in our overall analysis, the A_n-g_s model was applied in a stepwise fashion to determine the component processes (biochemical and stomatal) which most contributed to the observed differences in the temperature response between the two plant functional types.

4.2 Materials and Methods

4.2.1 Site and study species

A field study was carried out using the Australian Canopy Crane at the Daintree Rainforest Observatory (DRO) (www.jcu.edu.au/canopycrane/) in Cape Tribulation, Queensland, Australia (16°17' S, 145°29' E, 40 m a.s.l.), approximately 140 km North of Cairns in North Australia. The Australian Canopy Crane is a 45 m tall tower crane (Liebherr model 91EC) that provides access to ~1 hectare of lowland tropical rainforest (complex mesophyll vine forest type 1a (Tracey 1982), while surrounding the canopy crane are extensive lowland and upland rainforests of the Daintree National Park and Wet Tropics World Heritage Area (Wardhaugh *et al.* 2012). Within the ~1 hectare covered by the canopy crane there are 745 individual trees from 82 species (Laidlaw *et al.* 2007; Wardhaugh *et al.* 2012). At the crane site there is large variation in individual tree heights both within and between species, ranging from ~10 to 35 m, resulting in a highly discontinuous canopy. Two mature rainforest tree species (*Acmena graveolens* (F.M. Bailey) L.S. Smith and *Argyrodendron peralatum* (Bailey) Edlin.) were selected for the study from a site survey list first completed in 2005 (Laidlaw *et al.* 2007) and later updated in 2009. Our selection criteria were based on presence in the upper canopy and overall abundance on site.

4.2.2 Gas exchange

During the field study, with the canopy crane, leaf gas exchange measurements were conducted on attached, fully formed leaves of mature, field grown trees of both *A. graveolens* and *A. peralatum*. Measurements were made in April 2011 on three individuals each of the two species. For both species, we selected the most recent, fully formed leaves high up in the canopy and experiencing a similar light environment.

Leaf gas exchange measurements were made with an open-flow gas analyzer equipped with a red-blue light source (LI-6400XT, Li-Cor, Lincoln, NE, USA). Measurements were made at four temperature combinations (25, 30, 35 and 40 °C) in a random order. Measurement procedure entailed placing a selected leaf inside of the leaf cuvette at a given temperature, whereupon said leaf was allowed to equilibrate to conditions before measurements proceeded. At each temperature, photosynthesis was measured in a stepwise fashion at the following CO₂ concentrations: 1500, 1100, 700, 380, 200, 100, 50 and 0 µmol

mol⁻¹. During measurements leaf cuvette conditions were maintained at an irradiance of 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

4.2.3 Coupled $A_n\text{-}g_s$ model

A coupled photosynthesis-stomatal conductance ($A_n\text{-}g_s$) model was used in our sensitivity analysis. This model married the Farquhar *et al.* (1980) biochemical model of photosynthesis with the model of stomatal conductance proposed by Medlyn *et al.* (2011). Numerous formulations and parameterizations of the original Farquhar *et al.* (1980) biochemical model of photosynthesis have been described. In this study we refer to the formulation and parameterization of the original Farquhar *et al.* (1980) model as set forth by Medlyn *et al.* (2002a), and using parameters for Rubisco kinetics as described by Bernacchi *et al.* (2001).

The temperature dependence of apparent V_{cmax} and J_{max} can be modeled by two related functions (see Medlyn *et al.* 2002a). The first is the standard Arrhenius function

$$f(T_k) = k_{25} \exp \left[\frac{H_a(T_k - 298)}{298RT_k} \right] \quad (1)$$

where H_a is the activation energy and k_{25} is the apparent V_{cmax} or J_{max} at 25 °C. R is the universal gas constant (8.314 J mol⁻¹ K⁻¹) and T_k is leaf temperature in K. The activation energy (H_a) describes the exponential rate of rise in enzyme activity to an increase in temperature. The second function is a modified form of the Arrhenius function, which provides a peaked function (Harley *et al.* 1992), and is given by

$$f(T_k) = k_{25} \exp \left[\frac{H_a(T_k - 298)}{298RT_k} \right] \frac{1 + \exp(298\Delta S - H_d/298R)}{1 + \exp(T_k\Delta S - H_d/T_kR)} \quad (2)$$

where H_d is the deactivation energy and ΔS is an entropy term. H_d describes the rate of decrease of the function above the temperature optimum.

We utilized both the standard Arrhenius and peaked functions in this study to model the temperature response of apparent V_{cmax} and J_{max} . In order to avoid over-parameterization of the model, the deactivation energy (H_d) of J_{max} was assumed as a constant of 200 kJ mol⁻¹ for the model fitting (Medlyn *et al.* 2002a; Kattge and Knorr 2007; Lin *et al.* 2012). Maintaining the deactivation energy (H_d) as a constant reduces the number of free parameters and allows for a more streamlined comparison of results between studies, and is standard

procedure in most analyses of the temperature response of photosynthesis (see Dreyer *et al.* 2001; Medlyn *et al.* 2002a; Kattge and Knorr 2007; Lin *et al.* 2011).

To identify the limiting biochemical and stomatal processes as temperature rises, we coupled the Farquhar *et al.* (1980) model with the model of stomatal conductance proposed by Medlyn *et al.* (2011). In this model, stomatal conductance is a function of net photosynthetic rate (A_n), ambient atmospheric CO₂ concentration (C_a) and leaf to air vapor pressure deficit (D), derived as

$$g_s = g_0 + 1.6 \left(1 + \frac{g_1}{\sqrt{D}} \right) \frac{A}{C_a} \quad (3)$$

where g_0 is cuticular conductance and g_1 is related to the marginal carbon cost of water to the plant, and is predicted to increase with growth temperature (Medlyn *et al.* 2011).

Following Lin *et al.* (2012) we adapted the simultaneous solution of photosynthetic and stomatal models proposed by Leuning (1990) for use in this study.

4.2.4 Sensitivity analysis

We conducted a sensitivity analysis to determine the relative contribution of each temperature dependent parameter (biochemical and stomatal) in the overall temperature dependence of net photosynthesis, based on the aforementioned coupled A_n - g_s model. To do so, we simulated the A_n - T response curve for leaf temperature from 15 to 45 °C. As our baseline, we used combined biochemical and stomatal parameters from our PFT of tropical rainforest (*A. graveolens* and *A. peralatum*), and held D constant at 1 kPa. Following development of our baseline values for biochemical and stomatal processes, we then changed each parameter one at a time, using reported biochemical parameters from Leuning (2002) and Kattge and Knorr (2007), and stomatal parameters from Leuning (1990).

Sensitivity analysis of our baseline values with the Leuning (2002) parameter set required estimation of g_s , as presented by Leuning (1990). In the semi-empirical stomatal model of Leuning (1990), stomatal conductance is given as the following.

$$g_s = g_0 + \frac{a A_n}{C_a \left(1 + \frac{D}{D_0} \right)} \quad (4)$$

where g_0 is the value of g_s at the light compensation point, A_n is the net leaf CO₂ assimilation rate, C_a is ambient CO₂ concentration, D is the leaf to air vapor pressure deficit, and a and D_0 are empirical coefficients.

Similarly, sensitivity analysis of our baseline values with the Kattge and Knorr (2007) parameter set required calculation of a temperature acclimation function of V_{cmax} and J_{max} . This function for the temperature acclimation of ΔS of V_{cmax} and J_{max} (Kattge and Knorr 2007) is given by the following.

$$\Delta S = a + bT_{\text{growth}} \quad (5)$$

where a and b are the slope and intercept, respectively, defined separately for each T_{growth} relationship. T_{growth} represents the average growth temperature of the previous 30 days.

4.2.5 Field data

We extended our analysis with the coupled model in order to better understand which processes most influence A_n - T responses by comparing temperature response data from two field experiments focused on two separate plant functional types (PFT's). The first temperature response data set (PFT – temperate broadleaved evergreen) comes from unpublished data for *Eucalyptus delegatensis* from the Tumbarumba eddy flux site at the Bago State Forest in south-eastern, NSW, Australia (35°39'S, 148°09'E, 1200 m a.s.l.); while the second data set (PFT – tropical rainforest) comes from the aforementioned field study using Australian Canopy Crane (see section 4.2.1 for details). For each study, the response of photosynthetic biochemistry to temperature was quantified by measuring leaf photosynthesis to leaf intercellular CO_2 (A - C_i) curves at a range of temperatures (see section 4.2.2 for details). Climate conditions at the two field sites are considerably different. For example, mean annual precipitation at the Tumbarumba site is ~1380 mm, compared with ~3500 mm at the canopy crane site in Cape Tribulation. There is a large seasonal difference in temperature for the Tumbarumba site with a mean daily maximum and minimum in summer of 22.7 °C and 9.5 °C, respectively, while in the winter there is a mean daily maximum of 5.9 °C and the mean daily minimum of -1.1 °C (Keith *et al.* 2012). In contrast, the canopy crane site in Cape Tribulation exists in a markedly more thermally stable environment with a mean daily maximum and minimum of 31.2 °C and 23.1 °C, respectively from November to April (wet season), and a mean daily maximum of 28.2 °C and mean daily minimum of 21.8 °C from May to October (dry season).

4.2.6 Model application to data

Data from both field studies (A-C_i curves for PFT of tropical rainforest and temperate broadleaved evergreen) were fitted to the Farquhar *et al.* (1980) model as described in Medlyn *et al.* (2002a), giving responses of the model parameters apparent V_{cmax} and J_{max} to leaf temperature. The parameters for the temperature responses of apparent V_{cmax} and J_{max} (H_a and ΔS) were then fitted based on Equation (1) and (2). For all model fitting and parameter determination associated with the Farquhar *et al.* (1980) biochemical model of photosynthesis, we utilized a package (<https://github.com/mdekauwe/FitFarquharModel>) developed in Python. Conversely, parameters for the stomatal model were obtained by fitting Eq. (3) using the R package *fitBBOpti* to focused measurements of stomatal conductance collected at the Tumberumba site for *E. delegatensis* in (November 2001, and February and May 2002) and at the canopy crane site for *A. graveolens* and *A. peralatum* in November 2011. In both cases, these g_s measurements were conducted over a range of temperatures and at saturating light (1800 μmol m⁻² s⁻¹) and ambient atmospheric CO₂ concentration.

After determining biochemical and stomatal parameters from each field data set, we then input parameters for each PFT (tropical rainforest and temperate broadleaved evergreen) into the coupled model to simulate the A_n-T response for temperatures ranging from 15 to 45 °C. Following the initial simulation for each PFT, we then changed individual sets of parameters (biochemical and stomatal) from one PFT to the other, one set of parameters at a time, to evaluate the relative contribution of each component process in the overall temperature dependence of leaf net photosynthesis. In this way, we were able to identify component processes which most strongly contributed to the observed differences in the temperature optimum between the two plant functional types.

4.3 Results

4.3.1 A-C_i curves (Tropical rainforest)

An example of the data used to fit the parameters apparent V_{cmax} and J_{max} for *A. graveolens* and *A. peralatum* is shown in Fig. 4-1. Values of apparent V_{cmax} and J_{max} at 25 °C fitted from the Farquhar *et al.* (1980) biochemical model of photosynthesis are shown in Table 4-1 for both species. At the reference temperature of 25 °C, there was no significant difference between species in values of V_{cmax} or J_{max}. In fact, for J_{max}, mean values at 25 °C

were identical between species (Table 4-1). The temperature response of V_{cmax} and J_{max} for *A. graveolens* and *A. peralatum* is shown in Fig. 4-2. The values of J_{max} across the temperature range varied quite minimally, with an increase from 25 °C to 30 °C, followed by a slight decline beyond 35 °C.

Table 4-1. Mean values for apparent V_{cmax} and J_{max} at 25 °C of *A. graveolens* and *A. peralatum*. Data are the means of 3 trees \pm 1 SD.

Parameter	Units	Species	
		<i>Acmena graveolens</i>	<i>Argyrodendron peralatum</i>
V_{cmax}^{25}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	47.0 ± 7.1	37.8 ± 1.9
J_{max}^{25}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	78.7 ± 4.1	78.7 ± 5.4

Conversely, the increase for V_{cmax} was much more rapid. Values of V_{cmax} , recorded across the whole range of temperatures, varied from 40 to 140 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The steepest increase in V_{cmax} for both species occurred from 30 °C to 35 °C. Equally, there was minimal difference between species in V_{cmax} and J_{max} across the measured temperature range (Fig. 4-2). Therefore, we chose to fit the temperature optimum (T_{opt}) of V_{cmax} and J_{max} for the two species (*A. graveolens* and *A. peralatum*) together. Combined biochemical parameters for V_{cmax} and J_{max} of PFT (tropical rainforest) are shown in Table 4-3.

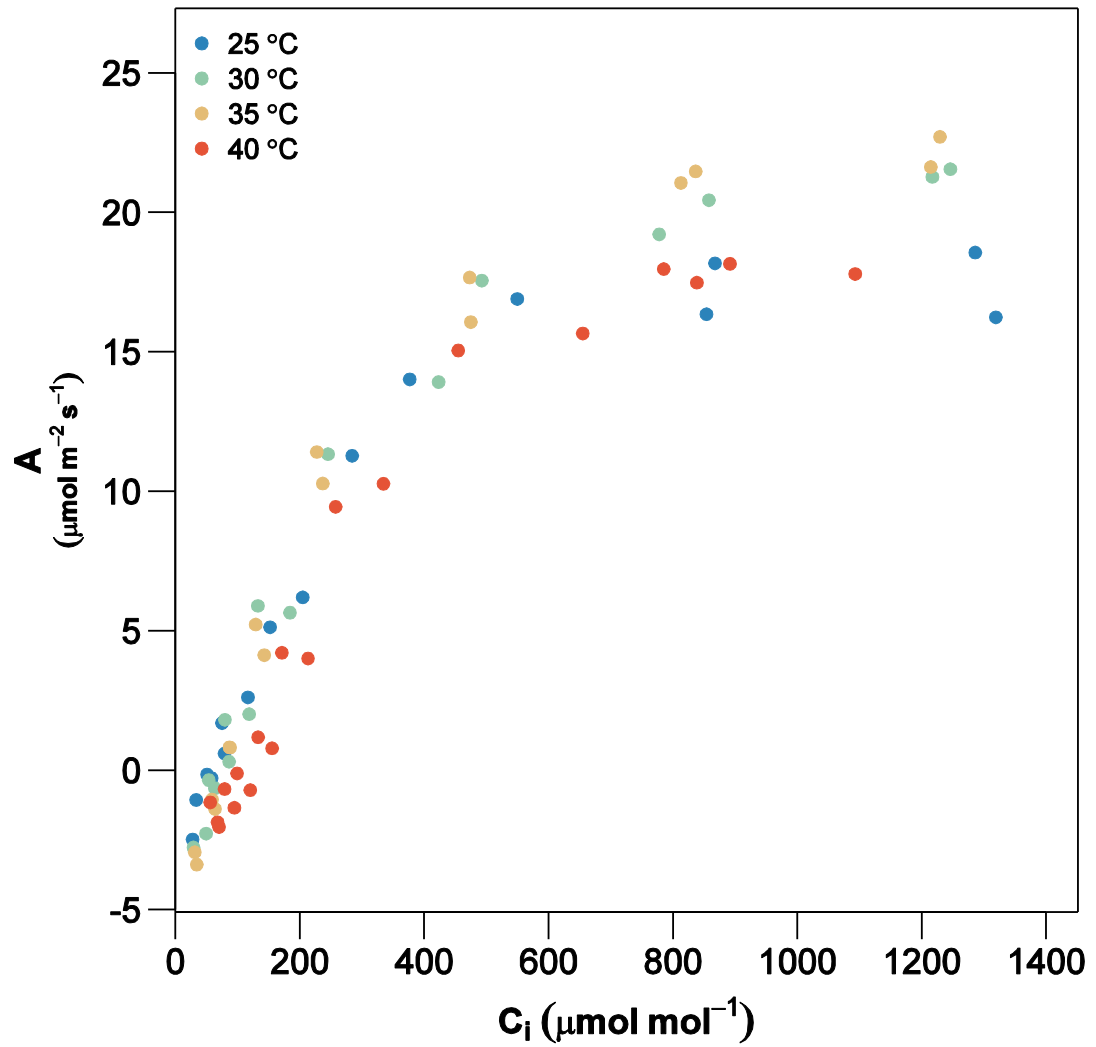


Figure 4-1. Typical set of A - C_i curves measured at four different temperatures (25, 30, 35 and 40 °C) from one individual of each species (*A. graveolens* and *A. perlatum*) representing PFT of tropical rainforest.

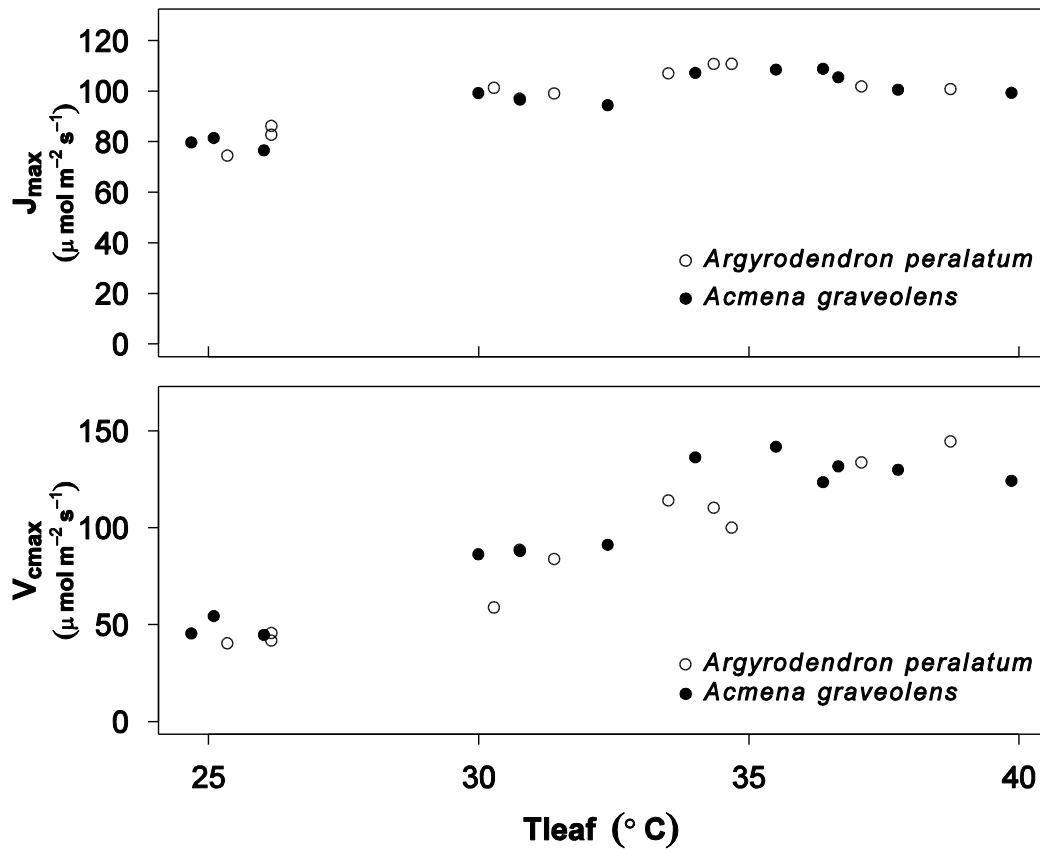


Figure 4-2. Estimated values of the maximum rate of electron transport (J_{max}) and the maximum rate of carboxylation (V_{cmax}) measured across a range of temperature for *A. graveolens* and *A. peralatum*.

Marginal cost of water (g_1) parameter - (Tropical rainforest)

Data used to fit the parameter g_1 for our PFT of tropical rainforest (*A. graveolens* and *A. peralatum*) are shown in Fig. 4-3 and values for g_1 are given in Table 4-3. The data for PFT of tropical rainforest were fit assuming cuticular conductance (g_0) was zero. However, for the analysis of field grown trees presented in Table 4-3 and Fig. 4-5, g_0 was estimated in order to assess its impact on the temperature response of photosynthesis. Over the measurement period (average growth temperature: 26.4 $^{\circ}\text{C}$), the measured g_1 for PFT of tropical rainforest was 3.7, which is in the mid-range of reported values of g_1 (Medlyn *et al.* 2011). This is also consistent with the observed increase in g_1 with growth temperature, with species from cold climates generally exhibiting a lower g_1 than species from warm climates (Medlyn *et al.* 2011).

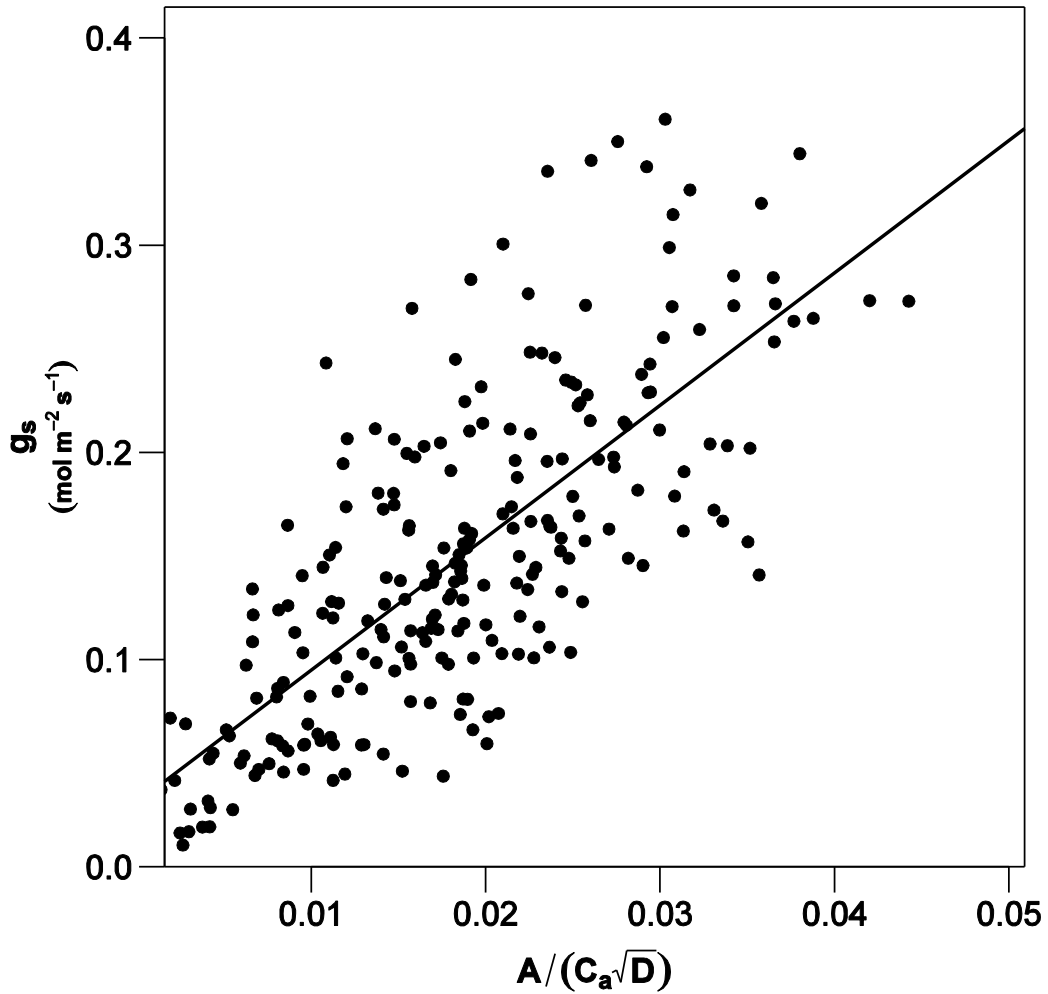


Figure 4-3. Measured values of stomatal conductance (g_s) as a function of $A/(C_a\sqrt{D})$ for PFT of tropical rainforest (*A. graveolens* and *A. peralatum*).

4.3.2 Sensitivity analysis

Biochemical and stomatal: H_a , ΔS and g_l

The parameters from Leuning (2002) and Kattge and Knorr (2007) included in our sensitivity analysis are shown in Table 4-2, and results from the sensitivity analysis in Table 4-2 and Figure 4-4. The largest effects on T_{opt} occurred through changing parameters for the activation energy (H_a) of V_{cmax} , and changing parameters for ΔS and H_d for V_{cmax} and J_{max} from the Leuning (2002) parameter set. However, the directional effect on T_{opt} of each of these parameter changes varied. For the activation energy (H_{av}) of V_{cmax} the parameter sets

were lower than our quantified values for tropical rainforests and consequently T_{opt} exhibited a decrease from 30.5 °C to ~28.0 °C (Fig. 4-4a; Table 4-2). Conversely, the activation energy (H_{aj}) of J_{max} from the two parameter sets was higher than our quantified values for tropical rainforests and as a result the T_{opt} displayed an increase from 30.5 °C to 32.7 °C (Table 4-2; Fig. 4-4b). The effect of changing the entropy term (ΔS) and deactivation energy (H_d) of J_{max} led to a considerable decrease of 1.6 to 3.1 °C in the T_{opt} (Fig. 4-4d).

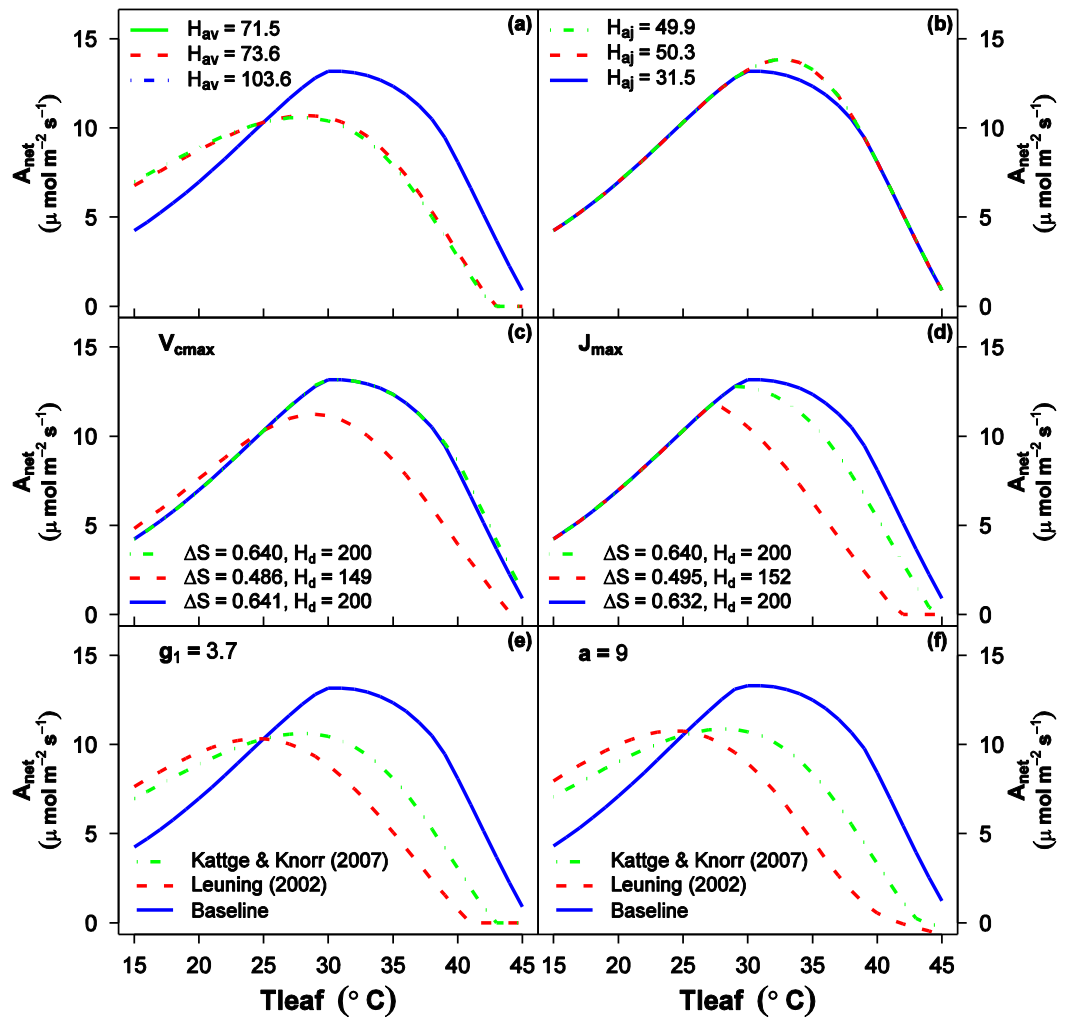


Figure 4-4. Simulated temperature dependence of light-saturated net leaf photosynthesis (A_{net}) under different ranges of biochemical (a-d) (V_{cmax} : H_a , ΔS and H_d ; J_{max} : H_a , ΔS and H_d) and stomatal (e-f) (a) component processes. Parameter ranges for sensitivity analysis are given in Table 4-1 (Leuning 2002; Kattge and Knorr 2007). Baseline values (blue line) for the model simulation are given in Table 4-3 for the PFT of tropical rainforest with a constant $D = 1$ kPa.

Inputting the Leuning (2002) parameters for ΔS and H_d of V_{cmax} caused a decrease in the T_{opt} from 30.5 °C to 28.9 °C, whereas inputting ΔS and H_d of V_{cmax} from Kattge and Knorr (2007) led to no change in T_{opt} (Fig. 4-4c). Swapping the marginal carbon cost of water parameter (g_1) with the stomatal parameter (a) from Leuning (1990) led to a very minor increase in the T_{opt} for our baseline PFT (tropical rainforest) from 30.5 °C to 30.6 °C and a minor increase in the maximum rate of A_{net} from 13.2 to 13.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 4-4e-f).

Importantly, along with changes in the T_{opt} for A_{net} when changing individual parameters, there was additionally a very strong effect of changing H_{av} on the maximum rate of A_{net} . Both parameter changes (Table 4-2) of H_{av} led to a 2.2 to 2.7 °C reduction in the T_{opt} which corresponded to an approximate 19% reduction in the maximum rate of A_{net} . Similarly, there was a large reduction in both the T_{opt} for A_{net} , and in the maximum rate of A_{net} , when inputting the ΔS and H_d for V_{cmax} and J_{max} from Leuning (2002). The decrease in A_{net} from our baseline maximum (13.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was larger when inputting ΔS and H_d for V_{cmax} than J_{max} (11.22 vs. 11.86 $\mu\text{mol m}^{-2} \text{s}^{-1}$), even though the decrease in the T_{opt} was larger when inputting ΔS and H_d for J_{max} (3.1 °C vs. 1.6 °C). All other parameter changes (biochemical and stomatal) led to a much smaller change from the baseline (PFT-tropical rainforest) maximum rate in A_{net} , even in conditions where there was a large change in the T_{opt} of A_{net} (Table 4-2; Fig. 4-4).

A large difference was exhibited when maintaining parameter sets together (H_a , ΔS and H_d of V_{cmax} and J_{max}) from Leuning (2002), Kattge and Knorr (2007) and baseline (PFT-tropical rainforest), while utilizing V_{cmax}^{25} and J_{max}^{25} from our baseline (Fig. 4-4e; Table 4-3). The T_{opt} changed from 30.5 °C at baseline to 24.5 °C and 28.0 °C when switching to the Leuning (2002) and Kattge and Knorr (2007) parameter sets, respectively. This change in the T_{opt} of A_{net} , additionally resulted in a 20 to 30% decrease from the baseline (PFT-tropical rainforest) maximum rate of A_{net} (Fig. 4-4e). These differences in T_{opt} and in the maximum rate of A_{net} remained when altering the stomatal model (i.e., using the “ a ” parameter from Leuning (1990, 1995)) (Fig. 4-4e-f). However, the differences from the baseline in the maximum rate of A_{net} and for T_{opt} were smaller, particularly the T_{opt} for the Leuning (2002) parameter set (Fig. 4-4e-f).

Table 4-2. Sensitivity analysis of the response of A_n to changes in biochemical (H_a , ΔS , and H_d) and stomatal (a) parameters. Parameters or parameter sets were changed one at a time and the effect on simulated T_{opt} was observed. Reference data are from (1) Leuning (2002) and (2) Kattge and Knorr (2007). For the Kattge and Knorr (2007) parameters, a growth temperature of 26.4 °C (average growth temperature of PFT-tropical rainforest) was utilized for calculation of ΔS (V_{cmax} and J_{max}). *Units for ΔS are in $\text{kJ mol}^{-1} \text{ } ^\circ\text{C}^{-1}$.

Parameter	Value	Units	Reference	Topt (°C)			Change in T_{opt} of A_n (°C)
				A_{vc}	A_i	A_n	
H_a (V_{cmax})	73.6	kJ mol^{-1}	1	28.3	30.5	28.3	-2.2
	71.5		2	27.8	30.5	27.8	-2.7
H_a (J_{max})	50.3	kJ mol^{-1}	1	32.7	34.8	32.7	+2.2
	49.9		2	32.7	34.7	32.7	+2.2
ΔS , H_d (V_{cmax})	0.486	149	1	28.9	30.5	28.9	-1.6
	0.64	200		32.9	30.5	30.5	0
ΔS , H_d (J_{max})	0.495	152	1	32.7	23.1	27.4	-3.1
	0.64	200		32.7	28.1	28.9	-1.6
a	9	Unit less	1	32.7	30.6	30.6	+0.1

4.3.3 Field data – Comparison of component processes

In the second component of this sensitivity analysis we compared the temperature response of field grown trees, representative of two plant functional types (PFT - tropical rainforest and temperate broadleaved evergreen). The biochemical and stomatal parameters, quantified from field measurements, for each plant functional type (PFT) are shown in Table 4-3.

From Table 4-3 it is clear that numerous differences in individual parameters exist between the two plant functional types, with resulting consequences towards the T_{opt} for each PFT (Fig. 4-5; Table 4-4). The baseline T_{opt} of A_{net} was 26.1 °C and 30.1 °C for PFT of temperate broadleaved evergreen and tropical rainforest, respectively (Table 4-4). The biggest difference and of greatest consequence to simulated A_{net} -T relationships was exhibited through the activation energy (H_{av}) of V_{cmax} (Fig. 4-5; Table 4-4). There were, similarly, large differences amongst the remaining biochemical (H_{aj} and ΔS of V_{cmax} and J_{max}) parameters, but none resulting in as great a change in T_{opt} between the two PFT's (Fig. 4-5). Switching H_{av} and $\Delta S(V_{cmax})$ between the two PFT's resulted in a decrease in T_{opt} for tropical rainforests from 30.1 °C to 25.6 °C, and conversely T_{opt} for the PFT of temperate broadleaved evergreen increased from 26.1 °C to 31.9 °C (Fig. 4-5; Table 4-4). When

switching only H_{av} the difference in T_{opt} was even greater with a decrease of 5.5 °C for tropical rainforests and an increase of 7.3 °C for PFT of temperate broadleaved evergreen, displaying the overriding influence of the activation energy (H_{av}) of V_{cmax} in the A_{net} - T response of each PFT.

Table 4-3. Parameters used in sensitivity analysis: biochemical (H_a , ΔS), stomatal (g_1) and vapor pressure deficit (D). Parameters were changed one at a time and the effect on simulated T_{opt} was observed.

Parameter	Units	Plant functional types (PFT)	
		Tropical rainforest	Temperate broadleaved evergreen
V_{cmax}^{25}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	41.5 ± 4.3	90.8 ± 3.9
H_{av}	kJ mol^{-1}	103.6 ± 17.3	62.1 ± 8.5
$\Delta S (V_{cmax})$	$\text{J mol}^{-1} \text{°C}^{-1}$	640.8 ± 4.1	630.7 ± 31.1
J_{max}^{25}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	79.2 ± 1.8	219.4 ± 11.0
H_{aj}	kJ mol^{-1}	31.5 ± 3.7	42.5 ± 9.2
$\Delta S (J_{max})$	$\text{J mol}^{-1} \text{°C}^{-1}$	632.2 ± 1.8	645.1 ± 8.6
g_1	$\text{kPa}^{0.5}$	3.7 ± 0.2	3.3 ± 0.1
$g_1 \text{ w/ } g_0$	$\text{kPa}^{0.5}$	2.9 ± 0.5	3.6 ± 0.6
g_0	$\text{mol m}^{-2} \text{s}^{-1}$	0.03 ± 0.02	-0.01 ± 0.01
D	kPa	$D = 0.0085 \times \exp(0.1656 \times T)$	$D = 0.1428 \times \exp(0.0974 \times T)$

There was a small increase in T_{opt} for both PFT's when swapping the stomatal (g_1) parameter between the two. Changing g_1 from 3.7 to 3.3 for tropical rainforests led to an increase in the T_{opt} of A_{net} from 30.1 °C to 30.5 °C, and similarly switching g_1 from 3.3 to 3.7 for the PFT of temperate broadleaved evergreen caused a 0.2 °C increase in T_{opt} of A_{net} (Fig. 4-5; Table 4-4). Adding cuticular conductance (g_0) (from Table 4-3), along with g_1 in the analysis, resulted in a similar change in the T_{opt} of A_{net} for each PFT as that for swapping g_1 alone (Table 4-4). Lastly, switching D between PFT's resulted in an increase in T_{opt} of A_{net} . This increase was of greater magnitude for tropical rainforests where T_{opt} of A_{net} increased from 30.1 °C to 31.6 °C when switching D, compared to only a 0.2 °C increase of T_{opt} for the PFT of temperate broadleaved evergreen (Fig. 4-5; Table 4-4).

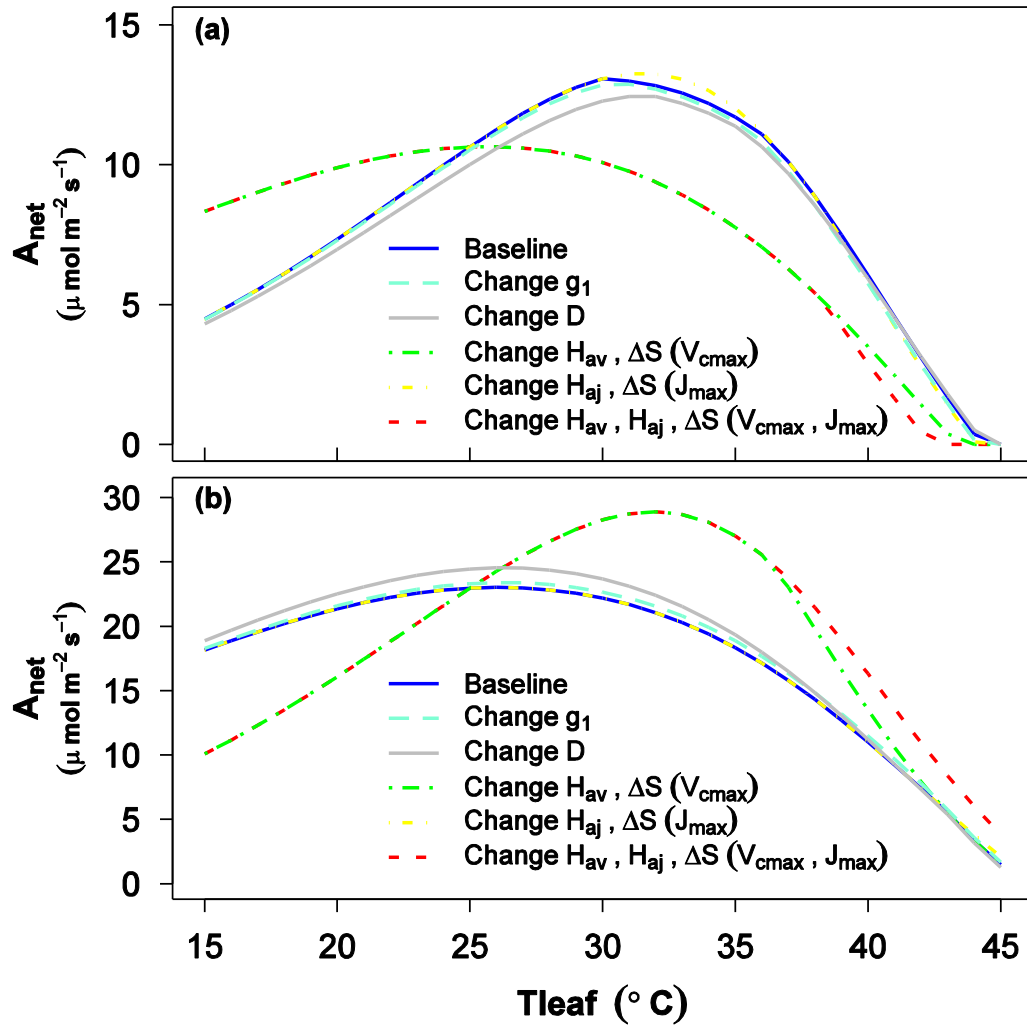


Figure 4-5. Simulated temperature dependence of light-saturated net leaf photosynthesis (A_{net}) under different ranges of biochemical (V_{cmax} : H_a and ΔS ; J_{max} : H_a and ΔS) and stomatal (g_1) component processes, and vapor pressure deficit (D) relationships. Parameter ranges for sensitivity analysis are given in Table 4-3 for PFT: (a) tropical rainforest (*A. graveolens* and *A. peralatum*) and (b) temperate broadleaved evergreen (*E. delegatensis*). Baseline values (blue line) for each model simulation are given in Table 4-3 for again (a) tropical rainforest and (b) temperate broadleaved evergreen. Additional lines represent simulation results when component processes (biochemical and stomatal) and D relationships are changed from one PFT to the other.

Table 4-4. Sensitivity analysis of the response of A_{net} and component processes (biochemical, stomatal and vapor pressure deficit (D)) for two sets of field data (PFT: tropical rainforest (*A. graveolens* and *A. peralatum*) and temperate broadleaved evergreen (*E. delegatensis*)). For each PFT, individual parameters or parameter sets were switched to the value or values for the other PFT (Table 4-3) and the effect on simulated T_{opt} was observed.

Plant functional type (PFT)	Simulated from the A_n - g_s model			Changed parameters	T_{opt}			Change in T_{opt} of A_n (°C)
	T_{opt} A_v (°C)	A_j (°C)	A_n (°C)		A_v (°C)	A_j (°C)	A_n (°C)	
Tropical rainforest	31.5	29.3	30.1	$H_{\text{av}}, H_{\text{aj}}, \Delta S(V_{\text{cmax}} \text{ and } J_{\text{max}})$	25.6	28.1	25.6	-4.5
				H_{av} and $\Delta S(V_{\text{cmax}})$	25.6	29.2	25.6	-4.5
				H_{av}	24.6	29.3	24.6	-5.5
				$\Delta S(V_{\text{cmax}})$	34.4	29.3	29.2	-0.9
				H_{aj} and $\Delta S(J_{\text{max}})$	31.5	28.1	29.6	-0.5
				H_{aj}	31.5	32.1	31.5	+1.4
				$\Delta S(J_{\text{max}})$	31.5	25.9	28.2	-1.9
				g_1	31.4	29.1	30.5	+0.4
				g_1 and g_0	31.4	29.0	30.4	+0.3
				D	31.6	29.1	31.6	+1.5
Temperate broadleaved evergreen	26.1	28.5	26.1	$H_{\text{av}}, H_{\text{aj}}, \Delta S(V_{\text{cmax}} \text{ and } J_{\text{max}})$	31.9	30.1	31.9	+5.8
				H_{av} and $\Delta S(V_{\text{cmax}})$	31.9	28.5	31.9	+5.8
				H_{av}	35.2	28.5	33.4	+7.3
				$\Delta S(V_{\text{cmax}})$	24.9	28.5	24.9	-1.2
				H_{aj} and $\Delta S(J_{\text{max}})$	26.1	30.1	26.1	0
				H_{aj}	26.1	26.3	26.1	0
				$\Delta S(J_{\text{max}})$	26.1	32.8	26.1	0
				g_1	26.3	28.6	26.3	+0.2
				g_1 and g_0	26.4	28.7	26.4	+0.3
				D	26.3	28.5	26.3	+0.2

4.4 Discussion

The impact of temperature on leaf level physiology is an important determinant in modeling forest response to climate variability. However, there is wide variation amongst studies and species in A_{net} -T responses (Wullschleger 1993; Leuning 2002; Medlyn *et al.* 2002a; Hikosaka *et al.* 2006; Kattge and Knorr 2007). A clear understanding of the mechanisms responsible for this variability amongst studies and species has been slow to emerge (Dreyer *et al.* 2001; Medlyn *et al.* 2002a; Kattge and Knorr 2007). With the continuing rise in temperature associated with climate change, it is imperative to cover a wide range in plant functional types and growth temperatures in order to better understand the role of component processes on the A_{net} -T response. Many forest process models utilize general biochemical and stomatal parameters for C_3 species (Leuning 2002; Kattge and Knorr 2007), which cover a wide range of species but are noticeably limited at temperature extremes and for particular plant functional types. In this study, we evaluated the biochemical and stomatal component processes affecting the A_{net} -T response of tropical rainforest species against different parameter sets (Fig. 4-4, 4-5; Table 4-2, 4-3, 4-4) in order to gauge the applicability of general C_3 species parameters (Leuning 2002; Kattge and Knorr 2007) to tropical environments.

Stomatal (g_1) processes and VPD

The role of stomatal conductance (g_s) in the temperature response of photosynthesis emerges through the relationship of g_s with D. As D increases, along with temperature, there is a reduction in g_s enacted to limit water loss, which in relation leads to a decline in leaf intercellular CO_2 (C_i) and ultimately a reduction in A_{net} . Many researchers have hypothesized that g_s may acclimate to temperature and thus potentially mitigate reductions in productivity as temperature increases (Mooney *et al.* 1978; Šantrůček and Sage 1996; Bunce 2000). A particularly useful stomatal parameter for looking at the impacts of changing environmental conditions is the marginal carbon cost of water (g_1) parameter (Medlyn *et al.* 2011). Observations have shown that the parameter g_1 may increase with growth temperature (Leuning 1990; Medlyn *et al.* 2011), and conversely that a higher g_1 may result in an increase in T_{opt} (Lin *et al.* 2012). In terms of growth temperature, we observed an increase in g_1 from 3.3 to 3.7 for the PFT of broadleaved temperate evergreen to tropical rainforest, respectively, which corresponded to an average growth temperature for tropical rainforest of 26.4 °C and

for temperate broadleaved evergreen of 20.6 °C. However, this difference in g_1 between PFT's did not lead to a consistently higher T_{opt} in our sensitivity analysis, with a higher T_{opt} exhibited by the PFT of tropical rainforest when decreasing g_1 from 3.7 to 3.3 (Table 4-4). Notably, this pattern appeared to relate to the D relationship quantified from field data. When maintaining a constant D of 1 kPa the pattern from the coupled A_{net} - g_s model followed along with expectations; with an increase in T_{opt} of 0.1 °C when increasing g_1 from 3.3 to 3.7 and mirroring that, a decrease of 0.1 °C in T_{opt} when decreasing g_1 . These results show the strong effect that D can impose upon T_{opt} , irrespective of biochemical and stomatal acclimation to temperature, and highlight the importance of monitoring and quantifying D-T relationships (Lin *et al.* 2012) in order to more accurately assess the relative contribution of each factor to the A_{net} -T response.

Biochemical (H_a , ΔS) processes

In the Farquhar *et al.* (1980) biochemical model, photosynthetic rate is determined by the minimum of two component processes; namely the maximum rate of RuBP carboxylation (V_{cmax}) and the maximum rate of RuBP regeneration (J_{max}). Thus changes in the temperature dependence of photosynthesis are partly attributed to the temperature dependence of V_{cmax} or J_{max} , as represented through the activation energy (H_a) and the entropy term (ΔS) of both component processes (Hikosaka *et al.* 2007). In this study the largest difference in T_{opt} of A_{net} for tropical rainforests emerged when changing the activation energy (H_{av}) of V_{cmax} and the activation energy (H_{aj}) of J_{max} between parameter sets (Fig. 4-4, 4-5; Table 4-2, 4-4). An increase in the activation energy of V_{cmax} with increasing growth temperature has been observed by numerous authors (Medlyn *et al.* 2002a; Onoda *et al.* 2005; Hikosaka *et al.* 2006; Hikosaka *et al.* 2007). This temperature related increase in H_{av} is commonly linked to changes in mesophyll conductance (g_m) to CO₂ (Bernacchi *et al.* 2002; Warren and Dreyer 2006). However in several studies, particularly those focused on temperature acclimation of photosynthesis, there is limited evidence for changes in H_{av} with growth temperature (Medlyn *et al.* 2002b; Kattge and Knorr 2007). Under current conditions, temperature acclimation of photosynthesis may play a limited role for tropical rainforests due to the high thermal stability of tropical environments (Wood *et al.* 2012). However, as temperature continues to rise along with atmospheric CO₂ concentrations it is highly imperative that we gain a more thorough

understanding of not only the adaptive capacity but also of the acclimation potential of tropical rainforests to temperature.

Conclusion

Understanding the effect of temperature on tropical rainforests is an issue of ever growing importance due to the continuing rise in atmospheric CO₂ concentration and associated rise in temperature. Many models of forest response to climate change utilize general biochemical parameters for C₃ species, which may lead to exaggerated estimates on the effect of temperature on photosynthetic carbon gain. In this study, we directly quantified biochemical and stomatal parameters for tropical rainforest species and compared these quantified values with general values for C₃ species using a coupled photosynthesis-stomatal conductance model. We found that the generally used parameters for C₃ species may lead to an under prediction of T_{opt} and in relation an under prediction of maximum photosynthetic rate, for tropical rainforest species. The biochemical factors most strongly contributing to this disparity was the activation energy (H_a) of V_{cmax} and J_{max}. An increase in the activation energy of V_{cmax} with growth temperature is often considered an acclimation response to changing environmental conditions. As our measurements for tropical rainforests were conducted at one time, our data provide support for adaptive capacity of tropical rainforests to growth temperature. We recommend that further studies on A_{net}-T responses for tropical rainforests should be conducted over multiple seasons in order to better identify the potential for biochemical and stomatal acclimation of tropical rainforests to increasing temperature.

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CHAPTER 5

Discussion and Conclusions

Climate change is likely to impact heavily on Australian forests. In this thesis, I investigated key environmental factors projected to impact Australian forests under future climate. In Chapters 2 and 3 the focus was upon the effect of elevated CO₂ and drought, and the interaction between these two factors on growth and physiology of two eucalypt species. Chapter 4 represented an investigation into the impact of temperature on leaf level biochemical and physiological attributes of Australian tropical rainforest species.

Elevated CO₂ and Drought

Experiments presented in chapters 2 and 3 were predicated on the hypothesis that elevated CO₂ will ameliorate the impacts of drought on forest growth and productivity. Two main mechanisms, associated with this hypothesis, were tested separately and constitute the framework of each chapter. Chapter 2 represented a test of the first proposed mechanism, that a low intercellular CO₂ (C_i) occurring under drought conditions will cause a larger enhancement of photosynthesis due to the non-linear response of photosynthesis to C_i. This mechanism was tested by maintaining soil moisture content at two set levels (well-watered: 100% and droughted: 50% field capacity) throughout the duration of the main experiment. In contrast to initial expectations, leaf level gas exchange measurements showed that the low C_i mechanism was not in operation under the simulated moderate drought conditions (50% field capacity) for either mesic *E. pilularis* or xeric *E. populnea*. Results showed that rather than reducing stomatal conductance and hence lowering C_i when water was limiting, plants grown under long-term moderate drought conditions adjusted growth at the leaf and whole plant scale.

Hypotheses at the whole plant scale were structured around plant water relations and leaf level responses. These hypotheses included that water-use efficiency would be proportional to CO₂ for both drought treatments and species, but that lower C_i in the droughted plants and xeric species would lead to relatively larger CO₂ effects on photosynthesis and biomass growth, and smaller CO₂ effects on transpiration, than in the well-watered plants and the mesic species. In contrast, the CO₂ effect on whole-plant water

use efficiency (WUE) was considerably less than the increase in CO₂. For both species, transpiration rate was similar for plants grown under elevated or ambient CO₂, reflecting an increase in leaf area to compensate for the CO₂-induced reduction in g_s . These results suggest that under elevated CO₂ and long-term moderate drought both mesic *E. pilularis* and xeric *E. populnea* exhibit a capacity to adjust growth processes to match water availability in order to avoid moderate drought stress.

Results at the leaf and whole plant scale correspond with the general observation that during water stress growth slows before any reductions in photosynthesis (Pinheiro and Chaves 2011; McDowell *et al.* 2011). However, the mechanism leading to this delay in growth and how it differs between species of opposing ecological strategy is less certain, particularly in relation to leaf expansion and changes in leaf area as drought progresses (Fender *et al.* 2011). The data presented in chapter 2 suggest strong controls on leaf area by water availability, for both xeric and mesic species. However, leaf expansion is affected by not only water availability but also carbon metabolism (Pantin *et al.* 2011, 2012). To further elucidate the impact of drought on xeric and mesic species, future research should additionally emphasize the role of changes in carbon metabolism along with the impact of water availability on leaf expansion and leaf area (McDowell *et al.* 2008; Pantin *et al.* 2011). This is particularly relevant in lieu of the findings of a less than proportional increase in WUE to CO₂ enrichment for both xeric *E. populnea* and mesic *E. pilularis*, with implications towards an increase in respiration rate as a potential cause for the lower than expected WUE for both species.

Chapter 3 constituted a test of the second proposed mechanism for elevated CO₂ mitigating the impacts of drought on forest growth and productivity. This mechanism is related to a reduction in stomatal conductance under elevated CO₂, thus lowering transpiration rate and conserving soil moisture and enabling trees under elevated CO₂ to continue to transpire longer into a drought episode. A test of this second mechanism involved bringing all pots of *E. pilularis* and *E. populnea* back to full field capacity and allowing plants to dry down to predetermined physiological stress levels. During dry downs, there were clear differences among species and antecedent watering treatments in the effect of CO₂ on water stress. Elevated CO₂ strongly delayed water stress in well-watered *E. populnea* but had no effect on progress of water stress in well-watered *E. pilularis*. Plants of both species grown under low water availability showed some reduction in water stress with elevated CO₂. These responses can be understood from the perspective of individual species ecological strategies: under well-watered conditions *E. pilularis* grows rapidly in response to elevated CO₂, making

it vulnerable to future drought, whereas *E. populnea* responds conservatively to elevated CO₂, allowing soil moisture savings when drought occurs.

Results from the dry down experiment show the strong role that leaf area plays in determining overall rates of transpiration in well-watered and drought conditions. An increase to leaf area under elevated CO₂ resulted in comparable declines in water stress and water use over dry downs as plants grown under ambient CO₂. These results suggest that larger plants with CO₂ enrichment may experience a lack of soil moisture savings. However, this is dependent on species specific growth patterns with CO₂ enrichment. For example, the xeric species within our experiment (*E. populnea*) exhibited relatively small gains in leaf area under elevated CO₂ and well-watered conditions and consequently experienced a delay in water stress at the leaf level and soil water savings at the whole plant scale. In contrast, the mesic species (*E. pilularis*) under the same conditions (elevated CO₂ and well-watered conditions) displayed a considerable increase in plant size and leaf area and consequently experienced no delay in water stress at the leaf or whole plant scale. These results suggest that patterns of ecological strategy (i.e., drought tolerant or drought-avoiding) may strongly influence the overall response under elevated CO₂, with drought-tolerant species likely experiencing a greater benefit of CO₂ enrichment particularly as drought intensifies.

Taken together, results from the two elevated CO₂ and drought experiments presented in this thesis suggest a potential for positive gains, of both mesic and xeric species, to CO₂ enrichment under variable drought conditions. Under long-term moderate water stress, both species adjusted growth to match water availability, suggesting a delay in growth versus stronger physiological controls in the early stages of water stress for mesic and xeric *Eucalyptus* species. As drought intensity increases, the impact of gains in leaf area and overall plant size with long-term CO₂ enrichment may take precedence over CO₂ induced reductions in stomatal conductance, leading to comparable declines in physiological functioning with increasing water stress (Wullschleger *et al.* 2002). To separate out the effect of leaf area, modelling efforts should evaluate the amount of leaf area that a given tree or LAI that a forest can support, given the CO₂ induced reduction in stomatal conductance, before experiencing similar rates of transpiration and water stress as plants under ambient conditions. Future experiments could complement this modelling work by elucidating the controls on leaf expansion and leaf area, particularly as it relates to future atmospheric CO₂ and drought conditions. In addition, future experiments should also focus upon changes in hydraulic architecture with CO₂ enrichment and in particular how it varies for species from xeric or mesic environments.

Lastly, a better understanding of the effect of warming on leaf level physiology for tropical rainforests is needed to assist with better parameterization of global scale models of forest response to climate change. Understanding the effect of temperature on tropical rainforests is an issue of ever growing importance due to the continuing rise in atmospheric CO₂ concentration and associated rise in temperature. Many models of forest response to climate change utilize general biochemical parameters for C₃ species, which may lead to exaggerated estimates on the effect of temperature on photosynthetic carbon gain.

Chapter 4 presented an experiment in which I measured the biochemical and stomatal limitations to leaf level photosynthesis in response to temperature on two canopy species at the Australian Canopy Crane Research Station (ACCRS) in Cape Tribulation, Queensland Australia. Data were used to perform a sensitivity analysis of a coupled photosynthesis-stomatal model, comparing rainforest parameter values with two parameter sets commonly used for modelling from Leuning (2002) and Kattge and Knorr (2007). Results showed that the generally used parameters for C₃ species may lead to an under prediction of T_{opt} and in relation an under prediction of maximum photosynthetic rate, for tropical rainforest species. The biochemical factors most strongly contributing to this disparity was the activation energy (Ha) of V_{cmax} and J_{max}. An increase in the activation energy of V_{cmax} with growth temperature is often considered an acclimation response to changing environmental conditions. The analysis showed that general parameters for C₃ species commonly used in global scale models of forest responses to climate change under predict the optimum temperature of photosynthesis for tropical forest species, even when temperature acclimation is taken into account. The parameter values obtained in this study will prove useful for improving global vegetation models.

Many experiments suggest that tropical forests are very near their upper temperature threshold (Doughty and Goulden 2008; Wood *et al.* 2012). Results from chapter 4 showed that the temperature optimum for the two Australian tropical rainforest species studies is ~30.5 °C. This is in the mid-range of reported optimum temperatures (26 °C to 34 °C) for photosynthesis of tropical rainforest species (Doughty and Goulden 2008), and above the mean annual temperature of 27 °C to 29 °C for tropical lowland rainforest (Jaramillo *et al.* 2010). As the measurements for Australian tropical rainforests were conducted at one time, the data provide support for adaptive capacity of tropical rainforests to growth temperature. Further studies on A_{net}-T responses for tropical rainforests should be conducted over multiple

seasons in order to better identify the potential for biochemical and stomatal acclimation of tropical rainforests to increasing temperature.

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